



Nanoparticules magnétiques et structures auto-assemblées avec (ou sans) polymères

Olivier Sandre

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Nanoparticules magnétiques et structures auto-assemblées avec (ou sans) polymères

Manuscrit présenté en vue de l'obtention de
l'Habilitation à Diriger les Recherches de l'UPMC

Olivier Sandre,
chargé de recherche CNRS

Soutenue le 18/1/2012,
devant le jury composé de :

- Sylvie Bégin-Colin, Professeur à l'Université de Strasbourg (Rapporteur)
- Robert N. Muller, Professeur à l'Université de Mons, Belgique (Rapporteur)
- Dominique Ausserré, Directeur de Recherche au CNRS (Rapporteur)
- Henri Cramail, Professeur à l'Université de Bordeaux (Examineur)
- Laurent Fontaine, Professeur à l'Université du Mans (Examineur)
- Ladislau Vekas, Directeur de Recherche au CFATR à Timisoara, Roumanie (Examineur)
- Valérie Cabuil, Professeur à l'Université Pierre et Marie Curie (Présidente)
- François Tournilhac, Directeur de Recherche au CNRS (Examineur)

Abrégé : Ce manuscrit décrit dix années de recherche effectuées à l'université Pierre et Marie Curie puis à l'université de Bordeaux sur des matériaux composites à base de nanoparticules d'oxyde de fer magnétique dispersées dans une matrice, le plus souvent polymère, mais aussi parfois cristal liquide, de tailles et de formes variées (gels, films, billes micrométriques, micelles et vésicules...). Les projets s'inscrivent dans le domaine de l'imagerie médicale et la délivrance d'un actif pharmaceutique contrôlée magnétiquement.
Abstract: This manuscript describes ten years of research performed at the Pierre & Marie Curie University (Paris 6) then at the University of Bordeaux on composite materials based on magnetic iron oxide nanoparticles dispersed in a matrix, most often of polymer, but also sometimes of liquid crystal, with various sizes and shapes (gels, films, micron scale beads, micelles or vesicles...). The projects belong to the domain of medical imaging, and to pharmaceutical active delivery triggered magnetically.

*à ma compagne Valérie et mon fils Pierre-Gil,
qui m'ont apporté leur soutien durant la rédaction de ce mémoire*

Nanoparticules magnétiques et structures auto-assemblées avec (ou sans) polymères

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A. Curriculum Vitae of Dr. Olivier Sandre

39 years old, born in Lyon, France on October 18th 1972,

Under civil union pact, 1 child (3 years old)

Professional address: Laboratoire de Chimie des Polymères Organiques (LCPO) ENSCBP 16 avenue Pey Berland 33607 Pessac Cedex France

<http://www.lcpo.fr>

gsm: +33(0)617584044 email : olivier.sandre@ipb.fr

tel: +33(0)540003695 fax: +33(0)540008487



Experience

April 2010-: **CNRS researcher** at « Laboratoire de Chimie des Polymères Organiques » (joint research unit 5629 Univ Bordeaux / CNRS / IPB, head Pr. Henri Cramail) to develop nano-composite magnetic colloids for theranostic applications in the team "Polymer nanotechnology for life sciences".

2001-2010: **CNRS researcher** at « Physicochimie des Electrolytes, Colloïdes et Sciences Analytiques », head Pr. Valérie Cabuil (formerly called LI2C, joint research unit 7195 UPMC Univ Paris 6 / CNRS / ESPCI ParisTech). Main topics: materials (gels, films) and dispersed multiresponsive colloids by incorporating magnetic nanoparticles in matrices (polymers, surfactants, liquid crystals...).

2000-2001: **Laureate of Lavoisier's fellowship** of French Ministry of Foreign Affairs for a post-doctoral study in USA, in Pr. David J. Pine's group at University of California – Santa Barbara (UCSB, Chemical Engineering / Materials Research Laboratory) in collaboration with Pr. Deborah K. Fygenson and Pr. Daniel Morse. Measurements of physical properties of biological microtubules made of a recombinant protein from a deep sea hyperthermophilic organism, *Pyrodictium Abyssii* (funded by Diversa Inc, San Diego CA).

1996-1997: **National service as military scientist** at « Laboratoire des Milieux Désordonnés et Hétérogènes » (former joint unit of CNRS and Paris 6 et 7 universities), supervised by Pr. Jean-Claude Bacri (University Paris 7) and Ronald E. Rosensweig (Blaise Pascal's invited chair) to study hydrodynamical instabilities of ferrofluids.

Education

1997-2000: **Ph. D.** (Doctorat) of UPMC (University Paris 6) in physical chemistry speciality supervised by Pr. Françoise Brochard-Wyart at « Laboratoire Physico-Chimie Curie » (joint unit 168 CNRS / Curie Institute / UPMC) on "Transient Pores, Adhesion and Fusion of Giant Vesicles", which results were advertised in several large audience journals: Science News, French edition of Scientific American, Science&Vie, Science&Avenir, Archimède, Biotech Info...

1995-1996: **M.Sc.** (DEA) at UPMC Univ Paris 6 obtained with highest grade (2nd rank of promotion), research study at Curie Institute with Dr. Pascal Silberzan about "force-free motion of droplets on asymmetrical surfaces".

1992-1996: Ecole Supérieure de Physique et de Chimie Industrielles de Paris (ESPCI ParisTech), Chemist, 1st rank of 111th promotion.

Research grants

2011-2014 **IDS FunMat**: Erasmus Mondus PhD fellowship on "Magnetic Micelles as Therapy and Diagnostic Agents" (<http://www.idsfunmat.u-bordeaux1.fr/>) co-directed with Pr. Mario Gauthier (Univ Waterloo, ON, Canada), in collaboration with Bertin Pharma incorporation.

2008-2011 **FP7 NMP-2007 SMALL-1 NANO3T**: "Biofunctionalized Metal and Magnetic Nanoparticles for Targeted Tumor Therapy" (<https://projects.imec.be/nano3t/>)

2008-2012 **FP7 CP-IP 213631-2 NANOTHER**: "Integration of novel nanoparticle based technology for therapeutics and diagnosis of different types of cancer" (www.nanother.eu)

2008-2010 **ANR Blanche** (leader Dr. Jean-François Berret): "IsoThermal Calorimetry as a Probe of Interactions between Magnetic Nanoparticles, Plasma Proteins and Cells"

2005-2008 **ACI Jeune Chercheur** (leader Pr. Sébastien Lecommandoux): "Adaptative and Multifunctional Nano-Objects based on Copolymers"

Comitments: Administrative Paritary Commission of CNRS researchers (CAP2, 2009-2011), UPMC Paris 6 University Scientific Council (2006–2010), ILL Scientific Sub-committee 9 (2010-2012), French Substitute Member of Management Committee of European COST Action TD1007 on PET/MRI.

49 peer-reviewed articles in journals or proceedings, 2 book chapters and 1 patent (see full list below).

Total number of citations > 1200, h-index = 18 (source: Web of Science)

B. Résumé des travaux: articles [1-42], actes de congrès [43-49] et chapitres d'ouvrages [50-51]

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C. Activités d'enseignement, dont encadrements

I. Enseignements

Formation initiale					
Discipline	Niveau	Nature	Etablissement	Années	Volume annuel
Chimie Minérale (V. Darthail)	1A IUT « mesures physiques »	TP de dosages potentiométriques et pH-métries	IUT Paris Jussieu (Paris 7)	2002	72h
Physique Thermique (M. Djabourov)	L3 (1 ^{ère} année d'école d'ingénieurs)	TP de calorimétrie différentielle (fusion des alcanes et de l'eau dans un mésoporeux)	ESPCI ParisTech	2008 2009 2010	75h 75h 45h
Chimie Industrielle (J-F. Le Meins)	L2 (Licence Professionnelle)	TP sur les propriétés thermo-mécaniques des matériaux polymères (rhéologie)	Université Bordeaux 1	2010	16h
Formation permanente					
Stage	Niveau	Intitulé	Etablissement	Années	Volume annuel
Colloïdes et Nanoparticules (F. Brochard-Wyart)	Stagiaires industriels (Guerbet, Sanofi Aventis...)	Nanoparticules magnétiques, propriétés physiques et applications au diagnostic en biologie	UPMC / CNRS	2009 2011	2h 2h

II. Encadrements de stagiaires et de jeunes chercheurs

Nom(s)	Cursus	Année (s)	Co-encadré(e) avec	Sujet
Mohammed KOUAR	Maîtrise Physiologie Cellulaire et Moléculaire, Univ. Paris 13	2002	-	Préparation de liposomes magnétiques de tailles calibrées pour une étude physico-chimique de l'interaction lipides-protéine LTP
Marguerite d'OLCE et Léa TRICHET	Maîtrise de Physique Univ. Paris 7, projet de "matière molle"	2003	-	Les vésicules magnétiques
Delphine EL KHARRAT	DEA "matière condensée", UPMC	2003	Valérie CABUIL	Mise en évidence expérimentale de l'interaction nanoparticules – polymère dans les ferrogels de polyacrylamide
Cristina DA CRUZ	ATER UPMC	2004	Valérie CABUIL	Diagramme de phases de nanoparticules magnétiques dans des cristaux liquides nématiques thermotropes
Tiphaine CHATELAIN	Master 2 chimie UPMC spécialité PCA	2005	Valérie CABUIL	Synthèse et fonctionnalisation de microsphères magnétiques thermosensibles en vue d'applications biomédicales

Sana TABNAOUI	Licence 3 chimie UPMC	2005	Siham DOUADI-MASROUKI	Dépôt de films lamellaires de copolymères di-blocs
Julien DESEIGNE	3A ESPCI	2006	-	Gouttelettes de cristal liquide nématique dopées fortement en nanoparticules magnétiques
Virginie WATEL	3A ESPCI	2006	-	Coprécipitation d'un mélange de Fer II - Fer III par alcalinisation en conditions millifluidiques
Maéva SERROR	Licence 3 chimie UPMC	2006	Siham DOUADI-MASROUKI	Polymérisation radicalaire contrôlée de polystyrène sur des nanoparticules magnétiques
Joffrey LEFRERE	Master 1 chimie UPMC	2006	Philippe MOUREAUX (Sté CF Gomma)	Contrôle de la raideur de matériaux antivibratoires dédiés à l'industrie automobile (caoutchoucs magnétiques)
Jean-Baptiste DELSUC et Kathy SIMOES	TEP Licence 3 chimie UPMC	2007	-	Synthèse de nano-bâtonnets de goethite αFeOOH et leur dispersion en solvant aqueux ou organique
Romain PINTAT	1A IUT Lyon	2007	-	Synthèse par polymérisation radicalaire par transfert d'atome de nanoparticules de ferrofluide enrobées de polystyrène
Delphine EL KHARRAT	Thèse de l'UPMC	2004-2007	Valérie CABUIL	Gels de polymères dopés par des nanoparticules de maghémite pour l'élaboration de matériaux magnétiques et thermosensibles
Siham DOUADI-MASROUKI	Thèse de l'UPMC	2004-2007	Valérie CABUIL et Maud SAVE	Synthèse et caractérisation de films lamellaires dopés par des nanoparticules magnétiques
Christelle VATRY	3A ESPCI	2008	Vincent Dupuis	Etude de l'effet d'un champ magnétique sur l'orientation d'un cristal liquide nématique thermotrope (5CB) ou lyotrope (goethite) : une étape vers l'obtention d'un nématique hybride organique / inorganique
Jérôme FRESNAIS	Post-doc ANR ITC-Nanoprobe	2008	Jean-François BERRET	Cocervation contrôlée de copolymères doubles hydrophiles et de nanoparticules magnétiques
Jérémy GUEDJ et Michaël GONZALEZ DIAZ	Licence 3 Physique UPMC (stage découverte L3PC)	2009	Vincent Dupuis	Etude de l'effet d'un champ magnétique sur l'orientation d'un cristal liquide nématique
Ali ABOU HASSAN	Thèse de l'UPMC	2007-2009	Valérie CABUIL	Contribution au développement de microréacteurs pour la synthèse de nanoparticules inorganiques à base d'oxydes de fer
Maud CHEMIN	Master chimie Bordeaux/3A ENSCBP	2011	Jean-François LE MEINS	Développement de vésicules hybrides copolymères à blocs/phospholipides pour la délivrance contrôlée de médicaments
Damien HABAULT	Master chimie Bordeaux/3A ENSCBP	2011	Jean-François LE MEINS	Incorporation de nanoparticules magnétiques dans la membrane de polymersomes à partir de doubles émulsions obtenues par microfluidique et preuve de concept d'hyperthermie magnétique en émulsion d'huile fluorée en système microfluidique
Marie DUFFIET	Licence 1 "stage d'excellence"	2011	Sébastien LECOMMANDOUX	Greffage de dopamine et dérivés du catéchol sur des nanoparticules magnétiques
Laure BRIEN et Anais COGNACQ	TIPE (classe prépa lycée Montaigne)	2011	Jean-François LE MEINS	Dégradation enzymatique de vésicules

D. Diffusion de la culture scientifique

J'ai participé plusieurs fois à des forums et salons pour les lycéens et les étudiants : aux « Rencontres Science et Citoyens » (au Futuroscope de Poitiers en 2003), au « Village de la Chimie » (à Issy-les-Moulineaux en 2004), au « Salon des Formations et des Métiers Scientifiques » (à Paris, à la Cité des Sciences et de l'Industrie en 2004), et à la Fête de la Science (en 2009 à l'UPMC).

E. Transfert technologique, relations industrielles et valorisation

1 brevet :

Titre : « Vésicules formées à partir d'un copolymère à blocs amphiphile, comprenant des nanoparticules magnétiques et au moins une molécule thérapeutique »

Pays : France **N° Dépôt/publication** : 10/04789 **Date de dépôt** : 08/12/2010

Déposants : IPB et CNRS **Inventeurs** : Sébastien Lecommandoux et Olivier Sandre

F. Tâches collectives : Animation et gestion de la recherche

Je me suis investi dans l'action syndicale en tant qu'élu du SGEN-CFDT au Conseil Scientifique de l'UPMC (titulaire depuis 2006 et membre du bureau), suppléant en Commission Administrative Paritaire des chargés de recherche CNRS pour le SGEN-CFDT Recherche EPST, ainsi qu'à la Commission de Réforme des chargés de recherche et au Comité Spécial Hygiène et Sécurité de la délégation régionale du CNRS Paris-B. Après ma mutation à Bordeaux en avril 2010, je n'ai gardé bien entendu que mon mandat national à la CAP2.

J'ai été rapporteur de nombreux articles dans des revues à comité de lecture (Polymer International, Materials Science and Engineering C, Journal of Magnetism and Magnetic Materials, Physical Review E, Journal of Polymer Science Part B : Polymer Physics, Journal of Applied Polymer Science, Journal of Physical Chemistry, ACS Nano, Langmuir, Macromolecules, Nanotechnology, Nanomaterials and Nanotechnology, European Polymer Journal, Journal of Colloid and Interface Science, Journal of Physics: Condensed Matter, Materials Chemistry and Physics...). Toujours dans le domaine de l'expertise scientifique, j'ai participé en tant que membre du conseil scientifique de l'UPMC à l'évaluation de projets BQR-Recherche et BQR-Equipements et à la refonte de ces appels d'offre en 2009 en projets « Emergence UPMC ». J'ai été sollicité aussi comme rapporteur par l'Agence Nationale de la Recherche (9 rapports rendus pour 16 sollicitations depuis 2006), par le Conseil Régional des Pays de Loire, par le CEFIPRA (organisme de coopération scientifique entre l'Inde et la France) et par la Direction Générale de la Recherche et l'Innovation du Ministère de la Recherche pour évaluer des projets de collaboration internationale « Hubert Curien ». J'ai été nommé membre du sous-comité n°9 "Structure and dynamics of soft condensed matter" de l'Institut Laue-Langevin à Grenoble depuis novembre 2010 et jusqu'en juin 2012 pour évaluer les propositions d'expériences de réflectivité et de diffusion des neutrons déposés deux fois par an à l'ILL. Je suis aussi suppléant représentant la France dans l'action européenne COST TD1007 sur l'imagerie bimodale IRM et PET, plus particulièrement dans le développement des agents de contraste multi-modaux, et dans une tâche analogue pour le projet national "Life-Bioimaging" au sein de l'institut de bioimagerie de Bordeaux (LabEx TRAIL). J'ai enfin été coorganisateur de deux mini-colloques aux Journées de la Matière Condensée de la SFP en 2008 (« Particules colloïdales et nano-objets : auto-organisation et inclusions dans les fluides complexes complexes ») et 2010 (« Polymères Biominétiques Auto-Assemblés »).

G. Historique de mes pérégrinations scientifiques (synthèse d'activité)

Persuadé comme feu le Professeur Jean Jacques que la « sérendipité » est à l'origine d'au moins autant de découvertes scientifiques en chimie que les projets préétablis, je m'efforcerai dans cette partie de montrer que mes recherches ont rebondi plusieurs fois grâce à des rencontres décisives avec des collègues. Néanmoins, le fil conducteur de mes recherches depuis mon recrutement au CNRS en octobre 2001 a toujours été donné par le dopage de divers milieux ou fluides complexes par des nanoparticules magnétiques, dont la synthèse constitue le savoir faire de l'équipe Cabuil au laboratoire LI2C de l'UPMC devenu PECSA en 2009, de même que leur dispersion – au moyen du greffage de ligands ou de tensioactifs adéquats – dans différents solvant aqueux ou organiques. L'objectif visé était l'obtention de matériaux nano-composites de tailles et formes très diverses (matrices macroscopiques, films minces, objets dispersés...), mais dont les propriétés puissent toujours être modulées par des paramètres extérieurs, notamment un champ magnétique. Puisqu'il faut bien les classer, ces systèmes s'inscrivent chacun dans l'une des lignes de la liste suivante :

- I. élastomères magnétiques, en particulier hydrogels magnétiques et thermosensibles
- II. vésicules et micelles magnétiques avec des copolymères diblocs amphiphiles "rod-coil"
- III. films lamellaires avec des copolymères diblocs symétriques synthétisés par ATRP
- IV. agrégats électrostatiques (coacervats) avec des copolymères diblocs neutres-chargés
- V. dispersions de nanoparticules magnétiques dans des cristaux liquides (ferronématiques)
- VI. synthèses assistées par l'outil microfluidique : des nanoparticules aux vésicules magnétiques

Parmi les différentes études qui constituent les parties de ce manuscrit, plusieurs ont été l'objet de thèses codirigées avec le Pr. Valérie Cabuil, à commencer par celle d'Alberto Galicia soutenue en 2003, puis de Delphine El Kharrat et Siham Douadi-Masrouki en 2007 et d'Ali Abou-Hassan en 2009. Dans le même temps, plusieurs thèses ont été soutenues à Bordeaux (Frédéric Chécot en 2005, Willy Agut en 2008, Charles Sanson en 2010) sous la direction de Sébastien Lecommandoux, avec qui je n'ai pas effectué de co-encadrement, mais plutôt une collaboration suivie sur le long terme, qui se poursuit aujourd'hui par le fait que j'ai rejoint son groupe au LCPO depuis le 1^{er} avril 2010.

Les derniers achèvements de certaines thématiques, concernant notamment la synthèse des aiguilles magnétiques ou celle des nanoparticules magnétiques en canal microfluidique, ne seront pas développés dans ce mémoire puisqu'elles constituent maintenant le sujet de recherche principal de jeunes chercheurs que j'ai eu le plaisir d'encadrer, respectivement Jérôme Fresnais et Ali Abou-Hassan, qui ont été depuis lors recrutés au laboratoire PECSA.

Mais pour revenir au commencement, c'est bien l'environnement du laboratoire LI2C (UMR 7612) de l'Université Pierre et Marie Curie, devenu PECSA (UMR 7195) en 2009, qui m'a permis d'acquérir un savoir faire en synthèse de nanoparticules, principalement d'oxydes et d'oxyhydroxydes de fer. Par ailleurs l'étude physico-chimique de leur état colloïdal m'a été grandement facilitée par l'accès aux techniques de diffusion des rayonnements, de la lumière au laboratoire et des rayons X et des neutrons sur de grands instruments (LURE puis SOLEIL pour les X, Laboratoire Léon Brillouin pour les neutrons). A noter aussi, et c'est loin d'être anecdotique, que l'écriture des « proposals » en vue de l'obtention des temps d'expériences a été un exercice très formateur, qui m'a donné de bonnes habitudes pour l'écriture de « grants » dans la course aux crédits que nous connaissons tous désormais! Mon insertion dans une équipe de chimistes (autour de Valérie Cabuil) comportant aussi quelques physiciens (autour de Régine Perzynski) a bien correspondu à l'ancrage de celle-ci dans les thématiques de la matière molle au sein d'une UMR de physicochimie historiquement plus tournée vers l'électrochimie, la simulation et les sciences analytiques. Depuis

2001, l'équipe Cabuil a grandi et s'est renforcée par le recrutement de plusieurs maîtres de conférences (en synthèse organique et inorganique) ainsi que d'un chercheur CR2 (sur un projet original de microfluidique de surface). De nouvelles thématiques sont apparues, comme la synthèse inorganique en microfluidique à laquelle j'ai participé en co-encadrant la thèse d'Ali Abou Hassan (soutenue en octobre 2009) ou l'utilisation des particules magnétiques pour l'extraction de polluants, tandis que d'autres se sont renforcées comme les applications biologiques avec par exemple le greffage de peptides ou d'enzymes sur des nanoparticules. Mes propres aspirations se situent assurément dans le domaine des matériaux nano-structurés pour des applications d'abord technologiques (actionneurs, capteurs, revêtements optiques...), et maintenant plus biotechnologiques (agents de contraste pour l'imagerie photonique ou l'IRM, objets stimulables pour la libération contrôlée de principes actifs...). Afin de poursuivre des recherches sur de tels matériaux – gels, films ou objets colloïdaux dispersables en milieu biologiques – j'ai jusqu'à présent combiné plusieurs stratégies pour appréhender la chimie des polymères: d'une part en effectuant moi-même des synthèses par polymérisation radicalaire libre pour les gels (partie I) qui conduit certes à une grande dispersité dans la taille des chaînes et la distribution des nœuds de réticulation, mais que l'on peut néanmoins utiliser pour créer des nanostructures en recourant au concept des réseaux (semi)-interpénétrés; d'autre part en faisant appel à des collègues pour disposer de copolymères à blocs synthétisés par une technique de polymérisation contrôlée conduisant à des indices de polymolécularité beaucoup plus réduits. Plusieurs rencontres de collègues de la communauté de « la matière molle » ont été, à ce titre, déterminantes dans mes recherches. Peu après mon recrutement, j'ai été envoyé par le département Sciences Chimiques à un « young scientists workshop » du réseau des organismes de recherche européens (CERC3) où j'ai fait la connaissance de Sébastien Lecommandoux, physico-chimiste au LCPO à Bordeaux étudiant des copolymères dont l'un des deux blocs est un polypeptide présentant une transition « rod-coil » avec le pH : j'ai débuté avec lui un travail fructueux sur les vésicules polymériques (polymersomes) répondant à un champ magnétique (partie II), qui se poursuit plus activement encore avec ma mutation dans son équipe.

Peu de temps après cette rencontre, Valérie Cabuil m'a invité à codiriger avec elle une thèse en collaboration avec Bernadette Charleux et Maud Save du laboratoire de Chimie Macromoléculaire de l'UPMC, qui se proposait de repartir d'une étude effectuée 10 ans plus tôt lors d'une précédente collaboration impliquant des physiciens des surfaces du Mans (B. Hamdoun, D. Ausserré) – des chimistes inorganiciens de Paris (V. Cabuil, S. Lefébure) – des polyméristes de Strasbourg (P. Lutz, Y. Gallot) et des opticiens de Saint Etienne (J-J. Rousseau) sur des films multi-lamellaires magnétiques (partie III). L'idée était de réaliser des échantillons analogues, mais avec de nouvelles techniques de synthèse et de varier les conditions d'élaboration et les paramètres (taille des nanoparticules, taille des blocs, taux de dopage...) pour optimiser les propriétés optiques. L'objectif était d'obtenir des structures de type « bandes interdites photoniques » réfléchissant les ondes électromagnétiques (visibles ou micro-ondes) par la combinaison des propriétés physiques des nanoparticules magnétiques (indice optique, bandes d'absorption) avec l'ordre induit par la répétition des couches de copolymères di-blocs, respectivement poly(styrène) et poly(n-butylméthacrylate) incompatibles. La recherche sur les matériaux nano-structurés a été relancée dernièrement par la course aux « métamatériaux », pouvant devenir transparents aux ondes sous certaines conditions (le dopage étant alors effectué plutôt par des nanoparticules d'or). Plutôt que de synthétiser ces copolymères par voie anionique, l'idée était de profiter du progrès fulgurant de la synthèse radicalaire contrôlée, aux conditions beaucoup moins drastiques. Mais nous nous sommes rendus compte finalement que ce choix de la technique de synthèse des copolymères n'était pas anodin pour les propriétés finales, puisque d'après nos mesures, en particulier de réflectivité des neutrons, les terminaisons des

chaînes qui dépendent de l'initiateur employé pour la polymérisation (anionique vs. radicalaire) ont modifié l'ordre d'empilement des couches PS et PBMA sur des substrats silicium. Par ailleurs nous avons aussi modifié la stratégie pour insérer les nanoparticules inorganiques dans les couches, en les enrobant au préalable de chaînes PS non pas adsorption, mais par polymérisation initiée à la surface des nanoparticules encore appelée « grafting from », un sujet aussi en plein essor.

Ma troisième rencontre fut avec Jean-François Berret revenu de l'unité mixte CNRS/Rhodia aux USA en 2005 pour développer à Paris (dans le laboratoire MSC de l'université Denis Diderot) des systèmes auto-assemblés par voie électrostatique (partie IV : coacervats) entre des nanoparticules magnétiques et des copolymères diblocs double-hydrophiles (DHBC) – synthétisés eux aussi par une technique radicalaire contrôlée développée par la société Rhodia (MADIX). Loin de prendre l'arrivée de J-F. Berret comme une concurrence sur un sujet proche de celui pour lequel le CNRS m'avait recruté, j'ai opté pour une intense collaboration avec lui profitant de son volontarisme naturel et sans doute aussi hérité de son expérience à Rhodia US. Nous nous sommes vite trouvés des complémentarités et avons signé ensemble de nombreux articles et engagé des projets (ANR blanche 2007 « ITC-Nanoprobe » et projet européen FP7-NMP-2007-SMALL « Nano3T »), de même que nous avons co-encadré le post-doctorat de Jérôme Fresnais qui a depuis été recruté comme CR2 au laboratoire PECSA sur une thématique tout à fait originale de microfluidique de surface.

Le prochain sujet exploré concernant des matrices de type non plus polymère mais cristal liquide (partie V) a été initié par Cristina da Cruz, ATER dans l'équipe Cabuil en 2004 après une thèse sur les cristaux liquides avec Huu Tinh Nguyen au CRPP à Bordeaux, qui avait noté la bonne dispersion de nanoparticules magnétiques recouvertes de certains surfactants dans des cristaux liquides en phase isotrope et au contraire leur brutale démixtion en gouttelettes au passage dans la phase nématique. Ayant étudié ce phénomène avec elle par diffusion des rayons X, j'ai eu l'occasion de présenter les résultats au laboratoire Van't Hoff à Utrecht célèbre dans le monde des colloïdes devant Henk Lekkerkerker, spécialiste des transitions de phase des cristaux liquides lyotropes. La discussion m'a incité à tenter de disperser dans des nématiques thermotropes des nanoparticules de symétrie non plus sphérique mais allongée, comme les molécules mésogènes. Cette idée m'a donné l'occasion d'étudier plus en détails, avec Vincent Dupuis physicien dans l'équipe Cabuil et Emmanuelle Lacaze de l'INSP, les propriétés magnéto-optiques des nanobâtonnets de goethite, éventuellement dopés par d'autres métaux paramagnétiques que le fer, prolongeant ainsi les résultats de P. Davidson au LPS à Orsay à un grand nombre d'échantillons synthétisés.

La dernière section de ce manuscrit (partie VI) concerne l'utilisation de la microfluidique pour la synthèse de nanoparticules puis la formulation de vésicules. Il y a quelques années, Valérie Cabuil a eu l'idée novatrice d'approprier l'outil micro-fluidique, un défi un peu osé pour des physico-chimistes sans accès privilégié aux techniques pointues de micro-fabrication lithographique en salle blanche ! Pourtant initiée par les chimistes des sciences séparatives et analytiques (Andreas Manz) et perfectionnée par les hydrodynamiciens (Steven Quake), c'était jusqu'alors un outil plutôt à l'interface avec la biophysique (Jean-Louis Viovy, David Weitz) qu'avec la chimie, même si Georges Whitesides s'y est intéressé très tôt, mais plutôt pour des réactions chimiques de surface de type thiols/or. Or la synthèse ou la formulation en micro-canal ouvre d'immenses potentialités, grâce à un contrôle précis et une plus grande homogénéité des paramètres physico-chimiques (pH, température, concentrations...) en comparaison de la même réaction ou procédé effectué en verrerie classique. La thèse d'Ali Abou-Hassan sur la synthèse en microréacteur de nanoparticules à base d'oxydes de fer a été saluée dans la communauté puisqu'elle a obtenu le prix de la meilleure thèse soutenue en 2009 de la division de Chimie-Physique des Sociétés Françaises de Physique et de Chimie. Il est intéressant de noter que durant la même période 2008-2011, mes collègues

actuels de l'équipe Lecommandoux, en particulier Jean-François Le Meins, ont développé l'approche tout à fait comparable de développer des puces de flux coaxiaux (sur le modèle de l'équipe Weitz à Harvard) suffisamment simples pour être reproduites dans un laboratoire de chimie, dans le but non plus de d'effectuer une réaction mais de formuler des vésicules à base de copolymères amphiphiles. Je présenterai dans ce mémoire le résultat d'un stage de master que nous avons codirigé sur la formulation de polymersomes magnétiques, et qui a aussi bénéficié de l'aide précieuse de notre collègue Jacques Leng de l'unité mixte CNRS/Rhodia à Bordeaux (LOF).

Sur les différentes thématiques exposées ci-dessus, les gels (I) ont donné lieu à deux thèses que j'ai co-encadrées (A. Galicia en 2003 et D. El kharrat en 2007), dirigées par V. Cabuil comme celle d'A. Abou-Hassan en 2009 sur la synthèse en microréacteurs (VI). Les polymersomes (II) ont été l'objet de trois thèses dirigées par S. Lecommandoux (F. Chécot en 2005, W. Agut en 2008 et C. Sanson en 2009), pour lesquelles j'ai contribué aux expériences de structure, les films lamellaires (III) une thèse que j'ai codirigée avec Valérie Cabuil et Maud Save (S. Douadi-Masrouki en 2007) tandis que les coacervats (IV) ont permis à J-F. Berret de lancer autour de lui une équipe de jeunes thésards et post-docs qui étudient des aspects comme l'assimilation de ces objets magnétiques micrométriques par des cellules biologiques (M. Safi qui soutiendra sa thèse fin 2011).

Ayant atteint un tournant de ma carrière, après neuf années à l'Université Paris 6 et à un âge où certains de mes anciens collègues de DEA avaient déjà été nommés professeurs d'université, j'ai ressenti le besoin de me rendre plus visible à l'international, dans un domaine – les nanomatériaux magnétiques à visée biomédicales – alors que le projet de mon laboratoire se tournait désormais plus vers les sciences analytiques, incluant des aspects tout à fait excitants tels la microfluidique. Afin de formuler de nouveaux matériaux originaux, je me suis posé la question de monter au laboratoire PECSA les installations nécessaires à la synthèse radicalaire qui est relativement légère (sous rampe à vide de synthèse classique), mais je me suis confronté à la question des techniques de caractérisation spécifique des polymères (SEC, RMN, ATG...). Acquérir de tels équipements et les entretenir représente un investissement trop coûteux pour un laboratoire qui n'est pas spécialisé dans les polymères. Par ailleurs les polyméristes de l'UPMC avec qui je collaborais et par lesquelles je pouvais avoir un contact précieux avec la synthèse des polymères ont toutes les deux quitté Paris. Associées à un désir personnel de prendre un nouveau départ, ces raisons m'ont conduit à la conclusion que je ne pouvais continuer dans la voie des nanoparticules hybrides polymères qu'en rejoignant une UMR où je pouvais trouver non seulement l'environnement technique, mais aussi et surtout la présence de chimistes polyméristes avec lesquels je puisse travailler en étroite collaboration et leur apporter de mon côté mes compétences en physico-chimie, liées à mon parcours personnel qui a toujours été à parts égales entre la physique et la chimie. Désirant aussi quitter Paris comme lieu de travail et de vie où je suis arrivé pour mes études en 1992, il m'est apparu que le Laboratoire de Chimie des Polymères Organiques à Bordeaux avec lequel je collaborais depuis 2004 se prêtait tout à fait à ce projet de mutation. D'une part Bordeaux possède un campus résolument tourné vers les matériaux (pôle aéronautique, GIS matériaux Aquitaine et maintenant LabEx Advanced Materials by Design), d'autre part le LCPO a toujours été un centre fort de chimie des polymères qui affiche désormais un projet plus global vers la synthèse et l'étude des matériaux fonctionnels tournés vers la chimie verte, le biomédical et l'électronique organique. Ma première année au LCPO m'a permis de faire le bilan de mes neuf premières années de recherche au CNRS, en finissant d'abord mes rédactions d'articles en cours (même s'il m'en reste encore quelques unes sur les gels thermosensibles d'une part et les cristaux liquides d'autre part...), puis bien entendu en préparant ce manuscrit en vue de l'obtention du titre d'Habilitation à Diriger des Recherches de l'UPMC. Cette étape est aussi nécessaire pour être officiellement directeur de thèse de Mlle An Vo-Thu Nguyen, qui débute sa thèse au 1^{er} octobre 2011 au sein de l'école doctorale

internationale IDS-FunMat sur la réalisation de micelles magnétiques à partir de copolymères arborescents, en cotutelle avec le Pr. Mario Gauthier de l'université de Waterloo au Canada.

Bien que toujours orientés dans plusieurs voies, mes projets de recherche ont pris aujourd'hui une connotation plus marquée vers les applications biologiques. Derrière leur apparenté unité de structure, les vésicules polymères magnétiques présente une grande diversité de fonctionnalités : chauffage par radiofréquences des nanoparticules associée à la thermo-sensibilité des polymères, contraste en imagerie de fluorescence ou en IRM, ciblage de récepteurs cellulaires tumoraux, délivrance contrôlée d'anticancéreux... Sous l'impulsion de Sébastien Lecommandoux, le LCPO s'est donné les moyens d'aborder ces sujets à la frontière des domaines pharmaceutiques et médicaux. Depuis que j'ai rejoint son groupe, j'ai codirigé avec J-F Le Meins deux projets de master consacrés pour l'un à la préparation et l'étude de vésicules hybrides lipides-polymères, pour l'autre à celle de polymersomes magnétiques géants ($\approx 150 \mu\text{m}$) grâce à une méthode d'émulsion double en canaux microfluidiques coaxiaux (avec ses avantages de contrôle des tailles de gouttes, de "haut débit"...). Dans les projets en élaboration figure la réticulation de polymersomes sous champ magnétique, une voie que nous avons envisagée dès le début de notre collaboration pour figer des structures anisotropes, mais que nous n'avons pas pu explorer du fait de l'éloignement. Revenant vers la thématique de ma thèse avec F. Brochard à l'Institut Curie sur les membranes artificielles, je souhaite creuser aussi l'analogie des propriétés physiques des polymersomes avec les membranes protéiques de certains virus (capsides) dont on pourrait chercher à reproduire les formes très géométriques (icosaèdres) avec des membranes polymériques non plus simplement sous forme de coquilles sphériques, mais facettées du fait de contraintes physiques (inclusions solides, flux hydrodynamiques en micro-canaux, champs magnétiques croisés...), avec l'idée sans doute audacieuse de tester le pouvoir immunisant de telles structures artificielles. Ne voulant pas toujours pas trancher entre la chimie et la physique, je continuerai de mener de front à la fois des études plutôt physiques comme la mise en évidence de l'hyperthermie à l'échelle des vésicules et des problématiques de synthèse comme le greffage de polymères à la surface des nanoparticules magnétique ou la nucléation de celles-ci au sein de micelles unimoléculaires de polymères arborescents (thèse en cotutelle avec Mario Gauthier de l'université de Waterloo au Canada), sujets qui seront développés dans la dernière partie de ce mémoire. Je me suis impliqué par ailleurs dans les applications des nanoparticules pour l'imagerie biomédicale, d'abord l'IRM sur laquelle je m'apprête à suivre une formation destinée aux chercheurs et médecins pour acquérir un savoir-faire sur les séquences employées sur les imageurs de recherche et médicaux, mais aussi peut-être vers d'autres types d'imagerie comme la tomographie par émission des positrons (via un réseau COST et ma participation au projet LifeBioimaging de la plateforme bordelaise, toujours bien entendu du côté de la chimie et du développement de nouveaux agents de contraste nano-particulaires).

Mais pour revenir au travail accompli durant ces dix dernières années, le prochain chapitre se propose de décrire un peu plus chacune des six grandes classes de matériaux hybrides entre lesquelles j'ai réparti mes sujets de recherche. Comme ce manuscrit n'a pas vocation à rentrer trop dans les détails, j'ai sélectionné pour chaque partie un article pour lequel j'ai été, si ce n'est l'auteur correspondant, du moins l'un des principaux rédacteurs, et dont le tiré à part est donné en annexe.

H. Activités scientifiques détaillées sur la période 2001-2011

Depuis mon entrée au CNRS dans l'équipe "Colloïdes Inorganiques" de Valérie Cabuil au LI2C (devenu PECSA en 2009), j'ai développé plusieurs types de matériaux composites à base de ferrofluides. Ces derniers sont des suspensions aqueuses ou huileuses de **nanoparticules d'un oxyde magnétique**, de type ferrite (de fer, cobalt, nickel, manganèse...). La méthode de synthèse élaborée il y a une trentaine d'année fait appel à une « chimie douce », basée sur les polycondensations en milieu alcalin aqueux de mélanges de sels métalliques.¹ Par delà sa simplicité, son grand avantage est aussi d'obtenir des quantités importantes de nanoparticules, de diamètres compris entre 5 et 15 nm, stables dans de grands domaines de pH et de force ionique et dans divers solvants. Le savoir-faire du physico-chimiste consiste à choisir des greffages adéquats des surfaces d'oxyde, soit par des charges (répulsion électrostatique pour les ferrofluides « ioniques »), soit par des tensioactifs (répulsion stérique pour les ferrofluides « surfactés ») afin d'éviter l'agrégation des colloïdes sur des périodes pouvant atteindre plusieurs années. Une fois leur stabilité colloïdale bien contrôlée, ces ferrofluides demeurent parfaitement fluides et mono-phasiques quelle que soit l'intensité du champ magnétique appliqué. Ils réagissent à des champs magnétiques même très faibles (dès 0.01 Tesla), par différents effets sous champ qui sont aujourd'hui bien compris : biréfringence induite, déformation de gouttes, instabilités hydrodynamiques des interfaces...

En associant un ferrofluide à un autre fluide complexe – polymère, cristal liquide ou tout type d'espèces amphiphiles formant des agrégats auto-assemblés – on peut réaliser des **systèmes mixtes organiques/inorganiques**. Mes recherches ont consisté à formuler de tels **nanocomposites**, à m'assurer de la dispersion homogène des nanoparticules dans la matrice ou au contraire de leur démixtion (et confinement dans certaines zones de la structure auto-assemblée), et à déceler des propriétés originales sous champ magnétique. Mes moyens d'étude ont été principalement les microscopies optique et électronique, la diffusion statique et dynamique de la lumière, la diffusion aux petits angles des neutrons (SANS/DNPA) et des rayons X (SAXS/DXPA), ainsi que certaines méthodes spécifiques des ferrofluides comme la magnétométrie ou la relaxation de biréfringence, qui permet de mesurer les propriétés rhéologiques dans le voisinage local des nanoparticules.² Je me suis intéressé ainsi à plusieurs systèmes auto-organisés dopés par des nanoparticules magnétiques, détaillés ci-après, dans le but d'élaborer des systèmes complexes réalisant **plusieurs fonctions** ou répondant à **divers stimuli** (champ magnétique continu ou alternatif, pH, température, ...).

I. Elastomères magnétiques et hydrogels thermosensibles

Association de réseaux polymères et de nanoparticules magnétiques

J'ai d'abord co-encadré avec **Valérie Cabuil**, **Régine Perzynski** et **Emmanuelle Dubois** la thèse d'**Alberto Galicia** (2000-2003), puis véritablement codirigé celle de **Delphine El kharrat** (2003-2007). Les études structurales ont été réalisées au Laboratoire Léon Brillouin (UMR12 CEA-Saclay) avec comme contacts locaux **Fabrice Cousin** et **François Boué**.

A la fin des années 80', les travaux de Y. Osada³ sur le phénomène d'électrostriction des hydrogels (gels polymères hydrophiles, neutres ou chargés), c'est à dire leur déformation par un champ électrique, ont ouvert de nouvelles perspectives pour la réalisation d'actionneurs mécaniques mous (« muscles artificiels »). Très vite on s'est rendu compte de l'intérêt des hydrogels composites, dopés par des charges inorganiques, de permittivité diélectrique élevée, dispersées dans le réseau.^{4,5}

¹ J.-P. Jolivet, R. Massart and J.-M. Fruchart, *Nouv. J. Chim.* 1983, 7, 325

² E. Hasmonay, E. Dubois, S. Neveu, J.-C. Bacri, R. Perzynski, *Eur. Phys. J. B* **2001**, 21, 19-29.

³ Osada Y., *Advances in Polymer Science* **1987** 82 1; Osada Y., Okuzaki H., Hori H., *Nature* **1992** 355 16 Jan

⁴ Starodoubstev S.G., Churochkina N.A., Kholkhlov A.R., *Langmuir* **2000** 16 1529-1534

⁵ Philipova O.E., Rulkens R., Kovtunen B.I., Abramchuck S.S., Khokhlov, Wegner G., *Macromolecules* **1998** 31 1168-1179

Comme des particules solides jouent aussi un rôle de renforcement mécanique du réseau, leur concentration dans le matériau composite doit être ajustée pour que l'hydrogel reste aussi suffisamment mou pour se contracter dans un champ E. Le même équilibre à trouver entre le dopage et le renforcement se rencontre avec un nouveau type d'hydrogels hybrides organiques/inorganiques introduit dans les années 90' par M. Zrínyi à Budapest⁶ : les « ferrogels ». Ces hydrogels qui contiennent des nanoparticules d'oxyde de fer de perméabilité magnétique élevée présentent quant à eux une magnéto-striction dans un champ magnétique. En ce qui me concerne, j'ai choisi d'utiliser les réseaux polymères à base de Poly(acrylamide) (PAM) réticulé par la N,N'-méthylènebis-acrylamide (MBA), formant des gels neutres fortement hydrophiles. On les prépare en polymérisant le monomère directement dans un ferrofluide aqueux à pH7, stabilisé par une espèce chargée et fortement complexante du fer, le citrate de sodium. On obtient un gel mou, de module élastique faible (autour de 10^3 - 10^4 Pa) magnétique (car emprisonnant une grande partie des nanoparticules introduites), et pouvant absorber un volume d'eau important (gonflement). L'incorporation d'autres monomères dans le réseau permet d'obtenir une sensibilité au pH comme avec l'acide acrylique (AAc) ou méthacrylique (MAAc), ou à la température comme avec le N-isopropylacrylamide (NIPAM). En variant les méthodes de préparation, des tailles et des propriétés d'échantillons très variées ont été atteintes :

Etude d'échantillons macroscopiques de « ferrogels »

Durant sa thèse dirigée par Valérie Cabuil, soutenue fin mars 2003, Alberto Galicia a montré la compatibilité chimique entre les ferrofluides aqueux stabilisés à pH7 par le citrate de sodium et les gels de PAM réticulés avec la MBA. Ces ferrogels neutres servent de référence à l'étude entamée sur des ferrogels plus complexes, comme les copolymères statistiques avec un co-monomère chargé (AAc) ou thermo-sensible (NIPAM), ou encore avec des blocs polyoxyéthylènes (PEG₁₀ ou PEG₄₀₀).

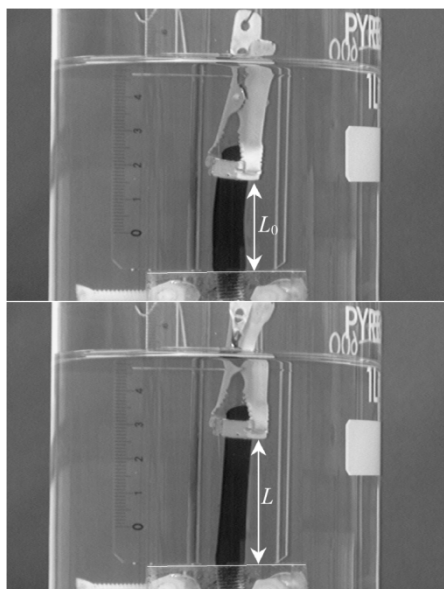


Fig. 1a : Expérience d'étirement par des poids calibrés d'un ferrogel maintenu en équilibre avec son bain de gonflement (synthèse avec $\Phi^0=4\%$ de particules, réticulation à 1% de MBA).

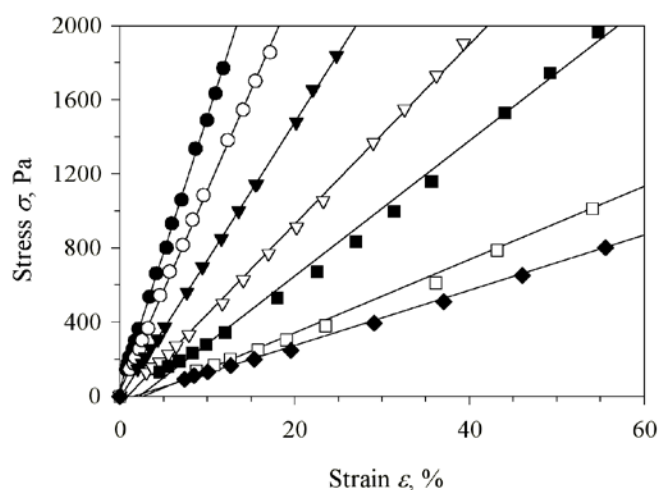


Fig. 1b : Tracé contrainte – déformation pour des ferrogels réticulés avec MBA/AM=1% de fraction volumique en particules variable : $\Phi^0=0\%$ (losanges noirs), 1% (carrés blancs), 2% (carrés noirs), 4% (triangles blancs), 5% (triangles noirs), 6% (cercles blancs) et 7% (cercles noirs). Ces échantillons ont été synthétisés et gonflés dans $[\text{Na}_3\text{Cit}]=8 \cdot 10^{-3} \text{ mol.L}^{-1}$.

⁶ Zrínyi M., Barsi L., Büki, A, *J. Chem. Phys.* **1996** 104, 8750

On a étudié la variation de grandeurs mesurables comme le **taux de gonflement** (masse d'eau absorbée sur poids sec de polymère), le **taux de relargage** (fraction de particules qui s'échappent du réseau pendant le gonflement) et le **module élastique** avec les paramètres pertinents : pourcentage molaire du réticulant, fraction volumique initiale de nanoparticules prises dans le gel, concentration en ions citrates de sodium dans la solution aqueuse.⁷ Pour relier ces propriétés macroscopiques avec la structure microscopique du réseau hybride polymère–nanoparticules, nous avons réalisé avec Régine Perzynski, Emmanuelle Dubois, Fabrice Cousin et Francois Boué des mesures de DNPA⁸, pour lesquelles les nanoparticules servent de **sondes structurales** en raison de leur fort contraste par rapport au polymère et au solvant. Deux modèles de distribution des grains magnétiques dans le gel ont pu être confrontés : d'un côté des **poches liquides de ferrofluide** si les particules sont expulsées dans des défauts du réseau, de l'autre des **chaînes polymères décorées** dans le cas d'une adsorption du polymère sur les grains d'oxyde. En examinant conjointement les expériences de DNPA et des mesures de relaxation de biréfringence sous champ magnétique pulsé, qui donnent quant à elles des informations sur le blocage des nanoparticules dans le réseau (fraction de particules mobiles et distribution des temps caractéristiques de rotation), nous avons pu montrer que les deux mécanismes sont présents : il existe un nombre limité de sites d'adsorption dans le réseau, les particules excédentaires étant expulsées dans des poches fluides. L'existence de ces poches a été attribuée à des **défauts dans le maillage dus à la pression osmotique exercée par le colloïde** au moment de la synthèse du réseau, lorsque celle-ci se trouve être plus élevée que la pression osmotique du polymère (en l'occurrence la pression de gonflement pour un hydrogel). Cette étude structurale des ferrogels a été publiée dans Journal of Magnetism and Magnetic Materials et Soft Matter.⁹

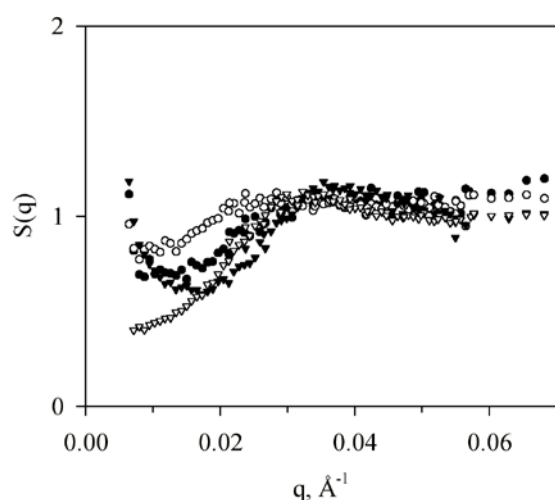


Fig. 2a : Facteur de structure des nanoparticules de fraction volumique $\Phi^0=3\%$. Répulsif dans le ferrofluide (triangles blancs), il devient attractif tandis que le pic disparaît après mélange dans l'acrylamide monomère (cercles noirs) ou après polymérisation sans réticulant (cercles blancs). Dans le ferrogel réticulé (triangles noirs) le pic de structure est réapparu et s'est déplacé à un vecteur de diffusion q plus élevé que celui du ferrofluide de départ.

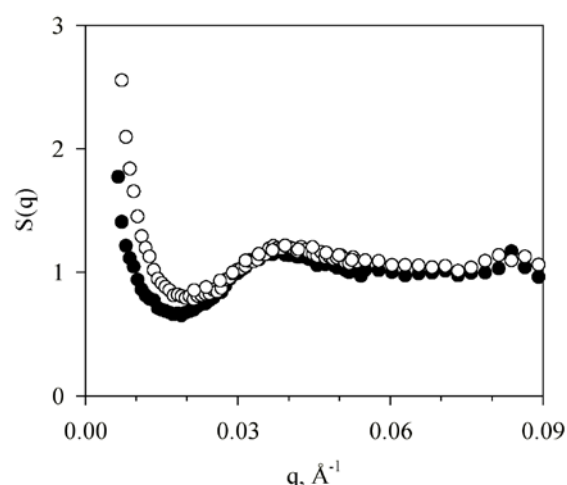


Fig. 2b : Facteur de structure de ferrogels gonflés dans 8mM de citrate de sodium. Les fractions volumiques à l'état initial sont respectivement $\Phi^0=5\%$ (cercles noirs) et $\Phi^0=7\%$ (cercles blancs) en accord avec la position des pics de structure donnant la distance moyenne entre particules. Les fractions volumiques moyennes à l'état gonflé étant $\Phi=0.75\%$ et 0.82% , les particules sont donc localisées dans des poches plus concentrées.

⁷ J.A. Galicia, O. Sandre, F. Cousin, D. Guemghar, C. Ménager, V. Cabuil, *J. Phys. Cond. Mat.* **15**, S1379 (2003)

⁸ Expérience du 8 ou 15 avril 2002 sur le spectromètre anisotrope PAXY au Laboratoire Léon Brillouin, CEA-Saclay. Longueur d'onde des neutrons : 8 Å ; à deux distances échantillon–détecteur : 2 et 5 m.

⁹ J. A. Galicia, F. Cousin, E. Dubois, O. Sandre, V. Cabuil, R. Perzynski, *Soft Matter* **5**, 2614-2624 (2009) ; *J. Mag. Mat.* **323**, 1211-1215 (2011).

Pour corroborer l'hypothèse de l'effet osmotique sur le gonflement des gels, Delphine El kharrat lors de son stage de DEA précédant sa thèse a évalué quantitativement la pression initiale de gonflement (juste après synthèse) d'hydrogels non dopés et de ferrogels, en comparant leur cinétique de gonflement dans des bains de pression osmotique étalonnée par différentes concentrations de Dextran. Ces résultats ont donné des valeurs de la **pression de gonflement entre 1000 Pa à 3000 Pa**, qui diminue quand le taux de réticulation augmente (en accord avec la théorie de Flory-Rehner du gonflement des réseaux) et qui baisse aussi avec l'introduction des nanoparticules magnétiques. Une telle contribution négative à la pression osmotique due aux particules témoigne d'une **interaction attractive entre le polymère et l'oxyde de fer**.

Elaboration et étude d'échantillons macroscopiques magnétiques et thermosensibles

De nombreuses équipes recherchent de nouveaux vecteurs pour le *drug-delivery*, qui libèrent un principe actif sous l'effet d'un stimulus externe. Le Poly(N-isopropylacrylamide) (PNIPAM) et ses dérivés sont des polymères très étudiés pour leur transition en température: solubles dans l'eau à froid, ils deviennent insolubles au dessus de la LCST (32°C pour le NIPAM pur). Des microbilles de PNIPAM rétrécissant par chauffage ont été décrites par R. Pelton dès 1986.¹⁰ Bien que ces billes soient prometteuses, il n'est pas envisageable de les utiliser telles quelles dans des échantillons biologiques qui ne peuvent être chauffés macroscopiquement au-dessus de 37°C. Pour parvenir à un chauffage uniquement à un niveau très local, on peut utiliser la forte bande d'absorption des nanoparticules magnétiques soumises à une onde radio-fréquence (entre 100 kHz et MHz), due à l'excitation de leurs moments magnétiques (relaxation de Néel). Ce chauffage à distance par induction magnétique pourrait ainsi servir à contrôler la transition d'un gel nanocomposite, combinant des propriétés de **perméabilité**, de **thermosensibilité** et de **superparamagnétisme**. Une telle technique d'hyperthermie a déjà été mise à profit pour tuer des cellules cancéreuses,¹¹ mais à l'époque où j'ai entrepris cette étude, très peu de systèmes artificiels pouvant être chauffés à distance par induction magnétique avait été développés, si ce n'est avec un polymère à LCST autre que le PNIPAM.¹² Depuis lors d'autres systèmes ont été publiés,^{13,14,15,16} y compris sur l'idée des gels semi-interpénétrés détaillée ci-dessous, dont j'ai manqué de célérité pour la publier avant les autres.

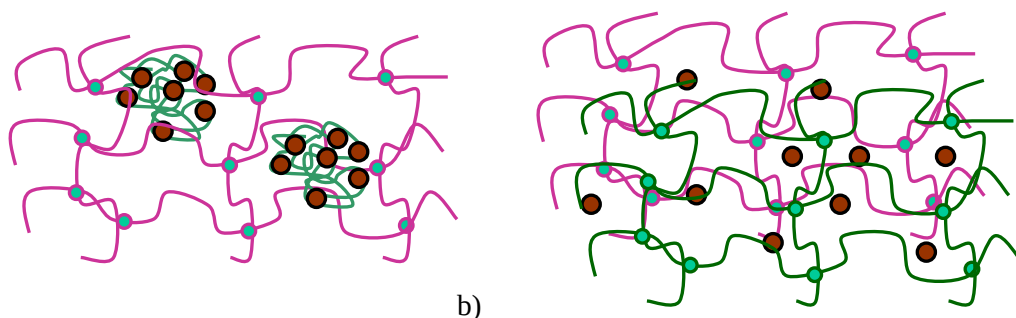


Fig. 3 : Représentations schématiques d'un réseau semi-interpénétré (a) et d'un réseau interpénétré (b). Les chaînes de PNIPAM apparaissent en violet, et celles de PAM en vert, décorées par des nanoparticules magnétiques (en marron).

¹⁰ R. H. Pelton, P. Chibante, *Colloids Surf* **20** (1986), 247.

¹¹ M. Chastellain, A. Petri, A. Gupta, K. Venkat Rao, H. Hofmann, *Adv. Eng. Mat.* **6** (2004), 235-241.

¹² A. Pich, S. Bhattacharya, Y. Lu, V. Boyko, H.-J. P. Adler, *Langmuir* **20** (2004), 10706-10711.

¹³ G. Q. Zhang, L. S. Zha, M. H. Zhou, J. H. Ma, B. R. Liang, *J. App. Polym. Sci.* **97**, 1931-1940 (2005)

¹⁴ N. Shamim, L. Hong, K. Hidajat, M.S. Uddin, *J. Coll. and Interf. Sci.* **304** (2006) 1-8

¹⁵ N.S. Satarkar, J. Zach Hilt, *Acta Biomaterialia* **4** (2008) 11-16; *J. Controlled Release* **130** (2008) 246-251.

¹⁶ P. Papaphilippou, L. Loizou, N. C. Popa, A. Han, L. Vekas, A. Odysseos, T. Krasia-Christoforou, *Biomacromol.* **10** (2009) 2662-2671; P. Papaphilippou, A. Pourgouris, O. Marinica, A. Taculescu, G. Athanasopoulos, L. Vekas, A. Odysseos, T. Krasia-Christoforou, *J. Mag. Mag. Mat.* **323** (2011), 557-563.

On s'est ainsi intéressé aux nanocomposites formés à partir de PAM et de PNIPAM réticulés en présence de nanoparticules d'oxyde de fer magnétique. L'association de ces deux polymères dans un premier temps comme simple copolymère statistique s'est révélée très peu efficace pour réaliser des ferrogels en comparaison des réseaux de PAM pur. On l'a expliqué du fait des interactions défavorables entre les grains magnétiques et le réseau de PNIPAM peu polaire (donc faiblement complexant de l'oxyde de fer). Par ailleurs, la transition en température perdait de son amplitude par rapport à celle du PNIPAM pur, à cause de la dilution des groupements isopropyles du NIPAM par les chaînons hydrophiles de PAM, contrariant leur association en micelles au-dessus de la LCST.

Afin de remédier au relargage des nanoparticules et de retrouver une transition plus marquée en température, j'ai conçu des « copolymères topologiques » de PNIPAM et de PAM. En variant les conditions, on a préparé des gels de deux topologies différentes : soit des réseaux PNIPAM **semi-interpénétrés** par des chaînes linéaires PAM (Fig. 3a), soit un réseau PNIPAM totalement **interpénétré** par un réseau de PAM chimiquement disjoint (Fig. 3b). A l'échelle macroscopique, on a observé que ces gels se contractent bien au-dessus de 32°C, valeur de la LCST du PNIPAM pur (Fig. 4 et 5). Alors que les copolymères statistiques relarguent quasiment 100% des nanoparticules introduites au départ, les réseaux (semi)interpénétrés permettent d'en conserver au minimum 50%.

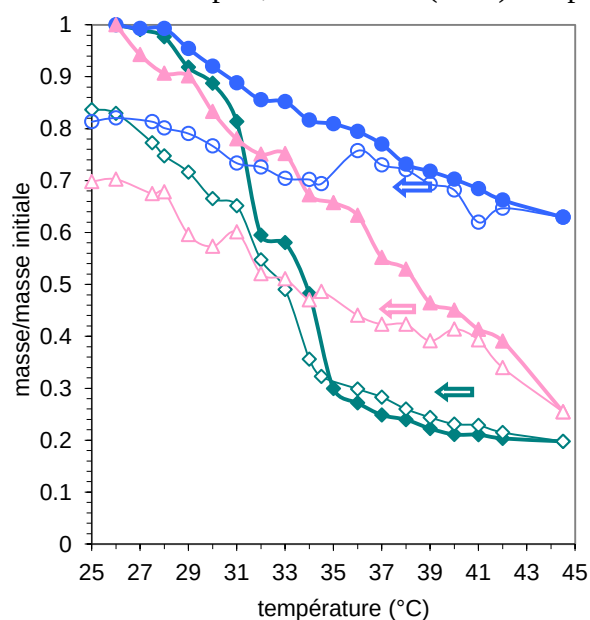


Fig. 4 : Transition du degré de gonflement de gels (en forme de cylindres de diamètre 8 mm) réalisés par copolymérisation statistique PNIPAM-PAM (80%–20% ronds bleus ou 90%–10% triangles roses) et par insertion de particules enrobées de PAM dans un réseau PNIPAM disjoint (90%–10% réseau semi-interpénétré, losanges verts), qui aboutit à une transition plus marquée.

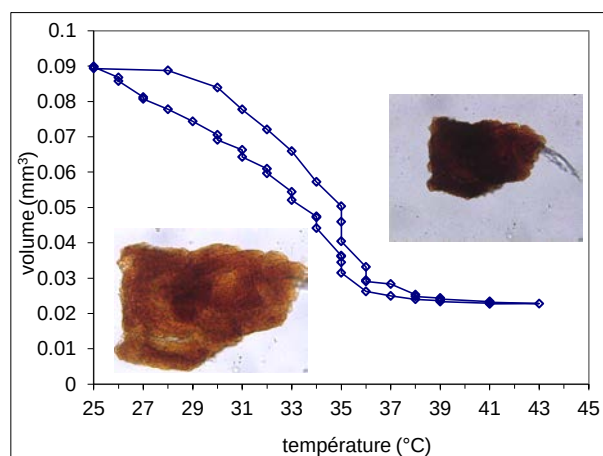


Fig. 5 : Observation optique de la transition d'un morceau de gel semi-interpénétré (environ 0.5 mm). Sa taille diminue fortement et sa couleur devient plus foncée à cause de l'élévation de la concentration des nanoparticules magnétiques. La cinétique de diffusion est assez rapide (5 minutes entre chaque point). On observe sur la courbe retour un hystérésis (la transition en volume des hydrogels est du 1^{er} ordre).

Puis en utilisant la diffusion des neutrons aux petits angles (DNPA)¹⁷, on a pu suivre la transition de volume des gels nanocomposites « de l'intérieur », en utilisant les nanoparticules inorganiques comme sondes structurales internes. Au cours de cette expérience, on a étudié quatre échantillons de gels semi-interpénétrés, pour deux rapports molaires PAM/PNIPAM de 5% et 10% et pour deux masses molaires des chaînes PAM 1000kD et 5000kD, ainsi qu'un échantillon réalisé en interpénétrant le réseau de NIPAM avec un second réseau de PAM (20% mol/mol). Comme prévu, le

¹⁷ Expérience du 21 au 24 mars 2005 sur le spectromètre isotrope PACE au Laboratoire Léon Brillouin, CEA-Saclay. Configurations : 12 Å – 5m et 6 Å – 2m.

signal des nanoparticules magnétiques a varié fortement quand la température passait la LCST du PNIPAM, aussi bien pour les gels semi-interpénétrés (Fig. 6) que pour les réseaux interpénétrés (Fig. 7), avec néanmoins quelques différences liées à leur structure mésoscopique respective. La courbe $S(q)$ d'un gel semi-interpénétré avec 10% de PAM de masse 5000kD (Fig. 6) présente dès la température ambiante un pic de structure autour de $2.5 \cdot 10^{-2} \text{ \AA}^{-1}$, correspondant à une distance de corrélations entre les nanoparticules sur environ quatre fois leur diamètre (250 Å). Lorsque la température passe au-dessus de 32°C, on observe une augmentation de l'intensité diffusée aux grands angles, témoignant d'une concentration moyenne en nanoparticules plus élevée qu'à l'état du gel gonflé. Cette contraction du réseau de PNIPAM provoque aussi la disparition du pic de structure, qui réapparaît à 24°C quand le gel regonfle.

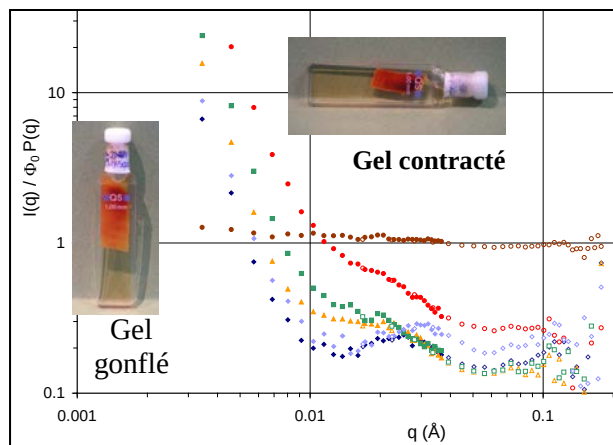


Fig. 6 : Intensité diffusée par les nanoparticules dans un réseau PNIPAM semi-interpénétré à 10% PAM 5000kD, à plusieurs températures: 24°C (bleu foncé), 32°C (orange), 34°C (vert), 39°C (rouge) et retour à 24°C (bleu clair). Fraction initiale : $\Phi_0=0.53\%$. La courbe en marron proche de $S(q)=1$ correspond à la solution de nanoparticules sans PNIPAM, faiblement agrégées par les chaînes de PAM.

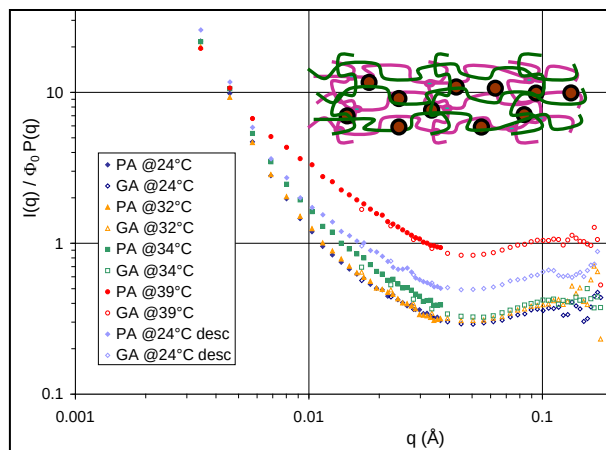


Fig. 7 : Intensité diffusée par les nanoparticules dans des réseaux interpénétrés PNIPAM-PAM (20%) à plusieurs températures: 24°C (bleu foncé), 32°C (orange), 34°C (vert), 39°C (rouge) et retour à 24°C. Fraction initiale : $\Phi_0=1.47\%$. La pente de la portion linéaire qui s'étend quand T augmente est très proche de -1 , correspondant à des chaînes décorées de moins en moins flexibles.

Avec des réseaux interpénétrés (Fig. 7), l'augmentation de la concentration moyenne en nanoparticules au-dessus de la LCST a été aussi observée. Mais contrairement au cas précédent, les intensités apparaissent très linéaires en échelle log-log lorsque T augmente, avec une pente proche de -1 . On en déduit un effet de confinement des nanoparticules par la contraction du réseau de PNIPAM, qui a pour conséquence de rigidifier les chaînes entre les nœuds du réseau (augmentation de leur longueur de persistance). Dans les mesures de DNPA qui suivirent (détaillées plus loin), on a renforcé encore les interactions entre les nanoparticules et les chaînes de PAM avant leur insertion dans le réseau thermosensible. Pour cela, on a bénéficié des avancées concomitantes sur les agrégats électrostatiques contrôlés de nanoparticules (voir chapitre IV sur les « coacervats »), qui purent être aussi bloqués dans la maille de PNIPAM.

Adsorption de ferrofluides sur des polymères linéaires (et vice-versa)

Thèse de D. El kharrat, en collaboration avec R. Perzynski et V. Dupuis

La recherche au LI2C sur la stabilisation en milieu aqueux des colloïdes magnétiques nanométriques a donné lieu à de nombreux travaux sur le greffage d'espèces moléculaires chargées à la surface de l'oxyde de fer : ligands citrate principalement, mais aussi tartrate, gluconate, acide

dimercaptosuccinique (ADMS) ou hydroxamate d'arginine.¹⁸ Le principe est d'avoir pour ces molécules d'une part une ou plusieurs fonctions complexantes des ions fer de surface (ligands bidendates ou tridendates), d'autre part des groupements chargés pour assurer aux colloïdes une barrière électrostatique répulsive et éventuellement un groupement réactif pour une fonctionnalisation future (cas des protéines avec l'ADMS). Dans cette partie on propose comme autre voie d'association des nanoparticules à des molécules organiques : **l'adsorption de polymères linéaires**, soit des homopolymères, soit des copolymères di-blocs dont l'un des blocs est électrostatiquement chargé. A la différence des liaisons covalentes ou électro-covalentes mises en jeu dans le greffage des molécules courtes évoquées ci-dessus, avec les polymères on peut utiliser des liaisons plus faibles (de type liaisons hydrogène ou électrostatique), car on bénéficie de l'adsorption coopérative d'un grand nombre de groupements polaires de la chaîne polymère.

Au commencement de cette étude, je me suis intéressé en premier lieu aux cas limites de l'interaction entre des nanoparticules et des polymères linéaires : d'une part des **pelotes très grosses de PAM pouvant interagir avec un grand nombre de particules**, d'autre part des **chaînes courtes pouvant stabiliser des colloïdes individuellement**, en apportant des interactions répulsives de nature à la fois stérique et électrostatique. Mon objectif initial était de tracer un **diagramme de phase exhaustif du système ferrofluides/chaînes linéaires** faisant intervenir des paramètres comme la longueur des chaînes, l'intensité des interactions entre les fonctions polaires du polymère et l'oxyde de fer, le rapport en nombre particules / polymère, ... De tels diagrammes mettant en évidence des **phases dispersées** et des **phases gels** ont déjà été construits dans la littérature avec des systèmes comme les nanoparticules d'oxyde de cérium ou d'oxyde de titane, et des polymères comme le polyacrylamide¹⁹ ou le polyacrylate de sodium.²⁰ L'idée était d'améliorer la compréhension générale des systèmes mixtes polymères / nanocolloïdes inorganiques en bénéficiant des outils spécifiques des ferrofluides (fort contraste en diffusion de rayonnements et biréfringence magnéto-optique) qui en font de bonnes **sondes locales de structure et de micro-rhéologie**. Or l'arrivée en 2005 de Jean-François Berret au laboratoire MSC de l'Université Denis Diderot (avec lequel le LI2C possédait une longue tradition de collaboration sur la physique des ferrofluides) a considérablement accéléré cette voie de recherches. Jean-François venant de l'UMR CNRS / Rhodia à Cranbury (NJ) aux USA où il avait travaillé sur le greffage de nanoparticules inorganiques de CeO₂ par des polymères, il possédait une très bonne connaissance de la physico-chimie des associations colloïdes/polymères, et désirait justement développer leurs applications dans le cas des colloïdes magnétiques.

Longues chaînes de d'homo-polymères linéaires décorées par des nanoparticules

L'adsorption des polymères à la surface des oxydes métalliques est un phénomène connu depuis fort longtemps, qui dans le cas du polyacrylamide et de l'oxyde de fer met en jeu des liaisons hydrogène entre les doublets libres des fonctions C=O des groupements amide du polymère et les hydrogènes des fonctions Fe-OH de surface.²¹ En ce qui concerne les particules de maghémite nanométriques, l'étude du gonflement des ferrogels (Thèse d'Alberto Galicia) a montré qu'il existe une interaction chimique entre un réseau de polyacrylamide réticulé et des nanoparticules de ferrofluide citaté, et que cette interaction est d'autant plus forte que la concentration en ions citrate dans le milieu est basse. Pour prouver que cet effet n'était pas lié au maillage du réseau mais uniquement à une compétition entre les équilibres d'adsorption du citrate et du polyacrylamide sur les nanoparticules, Delphine El kharrat a étudié en stage de DEA l'association de nanoparticules citatées et de chaînes linéaires de polyacrylamide (PAM) en solution.

¹⁸ N. Fauconnier, A. Bée, J. Roger, J. N. Pons, *J. Mol. Liq.* (1999) **83**, 233-242

¹⁹ O. Spalla and B. Cabane, *Colloid and Polymer Science* (1993) **271**, 357-371

²⁰ S. Desset, O. Spalla, P. Lixon and B. Cabane, *Langmuir* (2001) **17**, 6408-6418

²¹ Jean-Pierre Jolivet, *De la solution à l'oxyde*, CNRS Editions (1994) page 373

A cet effet, de longues chaînes linéaires de polyacrylamide commercial (PAM 5–6x10⁶g/mol, Polysciences) ont d'abord été dissoutes en dessous de C* (estimé à 2.4 g/L) dans des solutions de citrate de sodium, donnant des chaînes isolées dont le rayon de giration est estimé autour de 100nm. Puis les chaînes ont été mélangées avec des nanoparticules citratées S1S-Na₃Cit dans un rapport volumique de 2 entre le polymère et les particules, qui a été maintenu constant tout au long des dilutions successives nécessaires pour mesurer la viscosité intrinsèque du polymère en présence des particules, pour différentes salinités composées de citrate Na₃Cit et chlorure de sodium NaCl.

	50mM Na ₃ Cit	8mM Na ₃ Cit	2mM Na ₃ Cit	1mM Na ₃ Cit +24mM NaCl
PAM uniquement	535	595	591	481
PAM + particules	495, d _H =107nm	435, d _H =78nm	672, d _H =74nm	408, d _H =71nm

Tableau 1. Viscosité intrinsèque $[\eta]$ (g⁻¹.mL) mesurée par viscosimétrie capillaire et diamètre hydrodynamique pour des solutions de PAM mélangé avec 2 volumes de ferrofluide citaté S1S-Na₃Cit, à différentes salinités.

Les données ci-dessus montrent que la conformation des chaînes n'est pratiquement pas modifiée par les nanoparticules à forte concentration de citrate (la viscosité intrinsèque $[\eta]$ baisse seulement de 8% en présence de particules à 50mM de citrate en solution), alors que la présence des nanoparticules provoque une nette diminution du gonflement des chaînes lorsque la concentration citrate est plus basse (diminution de $[\eta]$ de 27% à 8mM de citrate). On peut suivre le même effet de **collapse des chaînes produit par les nanoparticules** en milieu peu concentré en citrate par diffusion de la lumière : le diamètre hydrodynamique des complexes polymère–ferrofluide qui vaut d_H=107 nm à 50mM de citrate libre diminue jusqu'à la valeur d_H=78 nm pour 8mM. La valeur plus élevée de la viscosité intrinsèque à 2 mM peut s'expliquer par un gonflement électrostatique en milieu faiblement salin. A l'inverse l'ajout de NaCl a pour effet de compenser la diminution de force ionique pour la valeur la plus basse en citrate (1mM), ce qui s'accompagne par un collapse des agrégats chaînes / nanoparticules. Ayant montré que les complexes polymère–particules gonflent et rétrécissent d'une manière analogue aux ferrogels réticulés, on s'est intéressé à leur morphologie par DNPA (Fig. 8a) et par AFM (Fig. 8b).

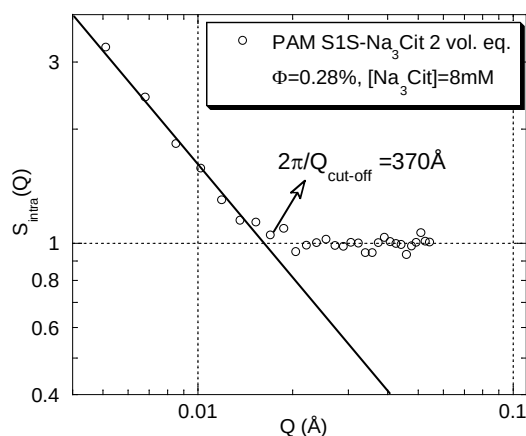


Fig. 8a): Mesures de DNPA pour une solution de PAM 0.6 g/L mélangée à 2 vol. de ferrofluide S1S-Na₃Cit à faible concentration de citrate (8mM).

La loi d'échelle de pente -1 donne le facteur de forme de bâtonnets rigides, de diamètre estimé à 37 nm d'après le cut-off.

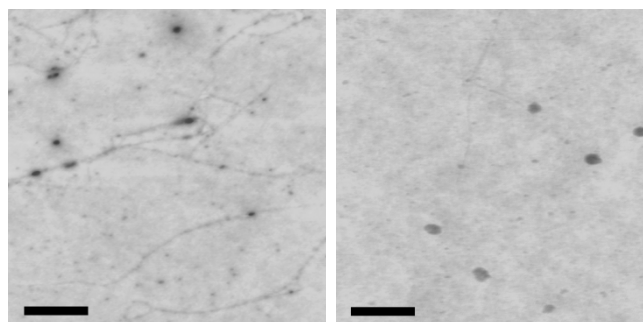


Fig. 8b): Images AFM des complexes PAM – S1S-Na₃Cit déposés sur mica à 8mM (à gauche) et 50mM (à droite) de citrate de sodium. La barre mesure 500nm. On observe des **colliers de perles** constitués de pelotes quasi-sphériques d'environ 80nm de diamètre reliées à des segments rigides pouvant mesurer jusqu'à 1μm de long.

Le contraste et l'épaisseur apparente des bâtonnets étant plus élevés à 8mM qu'à 50mM, on les interprète comme étant des chaînes polymères enrobées par une gaine inorganique constituée de nanoparticules adsorbées plus efficacement à 8 qu'à 50 mM.

On peut déduire de cette étude que les nanoparticules interagissent fortement avec les longues chaînes linéaires de polyacrylamide, et que ce couplage devient d'autant plus fort que la concentration en citrate de sodium libre en équilibre avec les ligands citrates est faible. Une **adsorption des particules sur les pelotes de polymère** est donc très vraisemblable, car elle permet d'expliquer l'effet spécifique de la concentration du citrate de sodium par la compétition entre les ligands citrates et les groupements polaires amides du polymère pour accéder à la surface d'oxyde de fer. Dans l'hypothèse d'une adsorption totale, on peut calculer qu'en moyenne 10^2 particules décorent une seule chaîne.

Hydrogels à la fois magnétiques et thermosensibles

L'objectif de la thèse de Delphine El kharrat, soutenue le 20 septembre 2007, était la réalisation d'hydrogels nanocomposites « multi-responsifs », capables d'absorber une grande quantité d'eau mais aussi sensibles à un champ magnétique, et éventuellement à la température. Pour élaborer ces « ferrogels », nous avons incorporé des nanoparticules magnétiques de maghémite ($\gamma\text{-Fe}_2\text{O}_3$) dans un réseau polymère hydrophile.

La synthèse des hydrogels (non dopés) et des ferrogels a été réalisée avec différents polymères hydrophiles dont le plus utilisé a été le PAM (polyacrylamide), soit à l'état d'homopolymère, soit comme bloc neutre d'un copolymère di-bloc double hydrophile (DHBC) PAM-PTEA (voir chapitre IV). A cette matrice neutre il était question d'associer le PNIPAM (poly-N-isopropylacrylamide) qui présente une température de transition LCST de 32°C afin de conférer au gel un caractère thermosensible en plus des propriétés magnétiques. Concernant les nanoparticules magnétiques, nous avons utilisé un ferrofluide stable autour de pH 7, obtenu par adsorption de citrate de sodium (Na_3Cit) à la surface de l'oxyde de fer. Au préalable, nous avons utilisé le tri en tailles spécifique des ferrofluide ioniques (basé sur leur démixtion par un électrolyte), nous permettant de considérer des nanoparticules de taille et de comportement magnétique différents : fraction S1S autour de 6 nm (superparamagnétiques) et fraction C1C autour de 9 nm (majoritairement ferrimagnétiques).

Une étude préliminaire des mélanges polymère – nanoparticules magnétiques nous a semblé indispensable avant de caractériser les ferrogels. En étudiant les propriétés statiques et dynamiques du polymère et du ferrofluide pris séparément puis dans les mélanges, nous avons mis en évidence des interactions dans les solutions de différents polymères hydrophiles (PAM, PEG, alginate, PAM-PTEA, PAM-PAA...) de masse molaire constante 2×10^5 g/mol et de nanoparticules. Cette attraction entre la surface d'oxyde et les polymères se manifeste non pas par une floculation macroscopique mais par la formation de micro-agrégats dispersés, diffusant la lumière mais ne sédimentant pas.

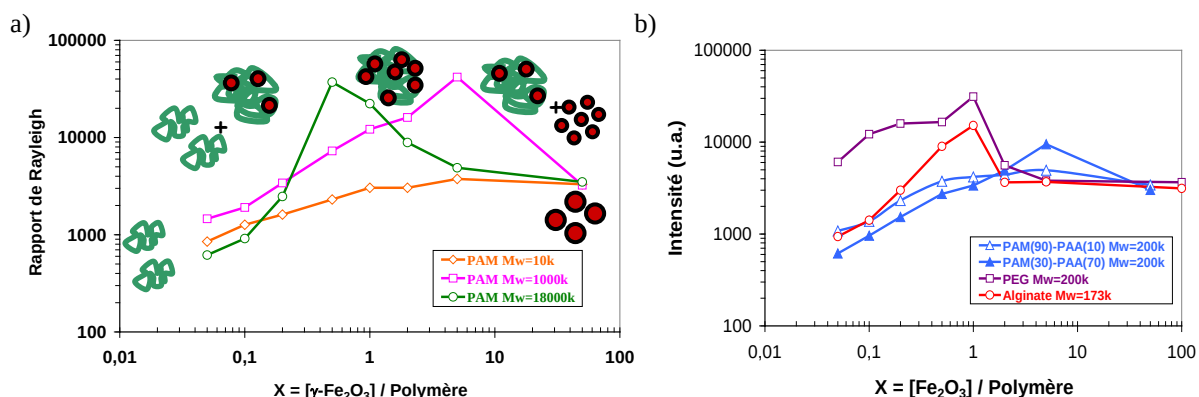


Fig. 9 : Étude de l'agrégation des nanoparticules magnétiques par diffusion statique de la lumière en fonction du ratio X (massique) des nanoparticules par rapport aux chaînes polymères de PAM de différentes masses molaires entre 10^4 à 18×10^6 g/mol (a) et, à masse molaire constante (2×10^5 g/mol) pour différents polymères.

Dans le cas du PAM de haut poids moléculaire, une diminution de la viscosité intrinsèque en présence des nanoparticules a été mesurée, résultant de l'augmentation de la compacité des chaînes par rapport à leur état gonflé en bon solvant. On a mis en évidence comme paramètre de contrôle la concentration $[Na_3Cit]$ qui présente une valeur optimale pour l'agrégation, différente selon la taille moyenne des nanoparticules (6 nm ou 9 nm). Le diagramme de phases – à l'intérieur duquel on se localise grâce au ratio X nanoparticules / polymère à concentration massique totale, pH et concentration $[Na_3Cit]$ constante – peut être balayé rapidement grâce à la diffusion de lumière statique et dynamique : la première nous renseigne sur la valeur de X optimale pour l'agrégation ainsi que sur la masse moléculaire des objets diffusants (« nombre d'agrégation »), tandis que la seconde nous donne leur taille hydrodynamique, de l'ordre de 60 à 100 nm.

Les observations à l'échelle moléculaire (AFM et TEM) complètent la description des agrégats de nanoparticules au sein du polymère en cernant leur forme qui va de la coque sphérique (cœur inorganique – couronne polymérique) pour les micro-coacervats électrostatiques avec les DHBC dont le bloc de PAM ne « pèse » que 30 kg/mol, à des objets filandreux rappelant des colliers de perle (Fig. 8b) obtenus pour les homopolymères de PAM de plus haute masse moléculaire (10^6 à 18×10^6 g/mol). Cette différence morphologique se retrouve par ailleurs sur les mesures de DNPA dont le comportement des courbes en fonction du vecteur de diffusion q confirme une structure en agrégats dense pour les coacervats avec les DHBC (Fig. 10) alors que les mélanges avec les homopolymères de PAM donnent des courbes plutôt en q^{-1} témoignant d'une décoration des chaînes polymères par les nanoparticules (Fig. 8a).

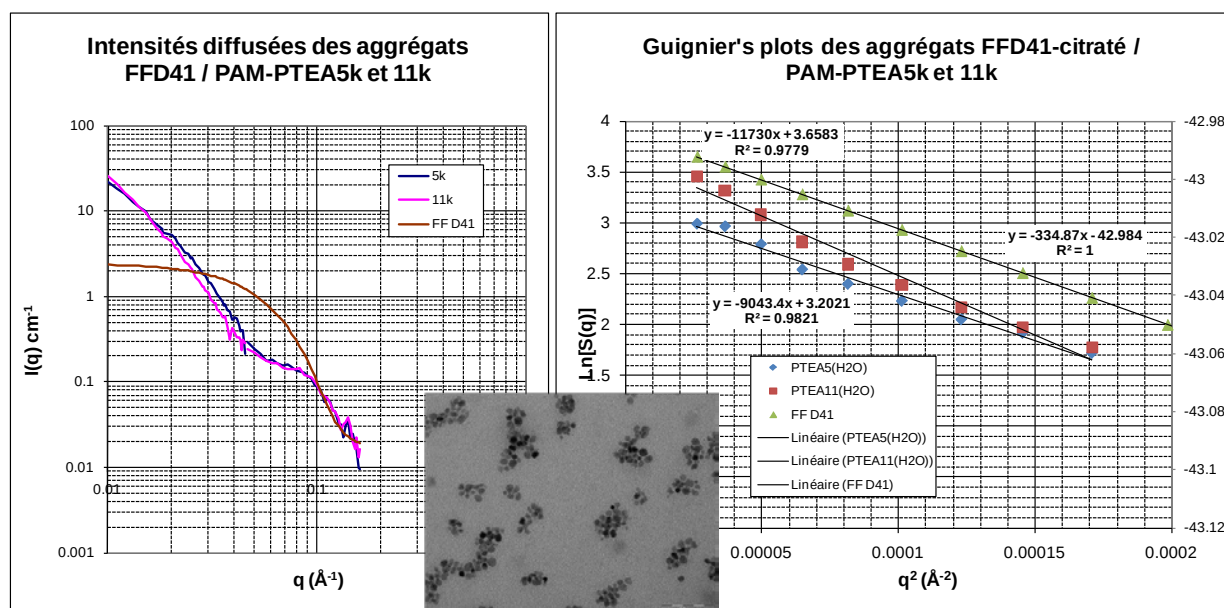


Fig. 10 : Signal de DNPA d'agrégats électrostatiques de petites nanoparticules magnétiques S1S (6 nm) avec le PAM-PTEA de masses 5k et 11k. Les rayons de giration déterminés dans le régime de Guinier mesurent respectivement $R_G=16.5$ nm et 18.8 nm, en accord avec l'image de ces agrégats en MET montrée en encart. Ces tailles sont du même ordre que les mailles caractéristiques dans un hydrogel,⁹ ce qui a permis de bloquer ces clusters magnétiques dans des gels de PNIPAM (Fig. 12).

On a utilisé enfin la biréfringence intrinsèque des nanoparticules d'oxyde de fer ferrimagnétiques (fraction C1C de diamètres environ 9 nm) pour étudier leur dynamique de rotation en présence ou non de chaîne de PAM, en fonction de la concentration $[Na_3Cit]$ qui a été variée entre 2 et 50 mM. L'analyse de la relaxation de biréfringence des mélanges et leur comparaison avec celle du ferrofluide seul a mis en évidence deux temps caractéristiques l'un court l'autre plus long qui ont été attribués respectivement aux nanoparticules isolées et aux nanoparticules à l'intérieur des agrégats, sans oublier une troisième population (environ 40% sauf à la plus haute concentration $[Na_3Cit]$) constituées de nanoparticules dont la rotation avec le champ magnétique est complètement bloquée

par le polymère. Concernant le mode de relaxation long, on a pu faire le parallèle de son évolution en fonction de $[\text{Na}_3\text{Cit}]$ avec celle des autres mesures hydrodynamiques (diffusion de lumière et viscosité intrinsèque).

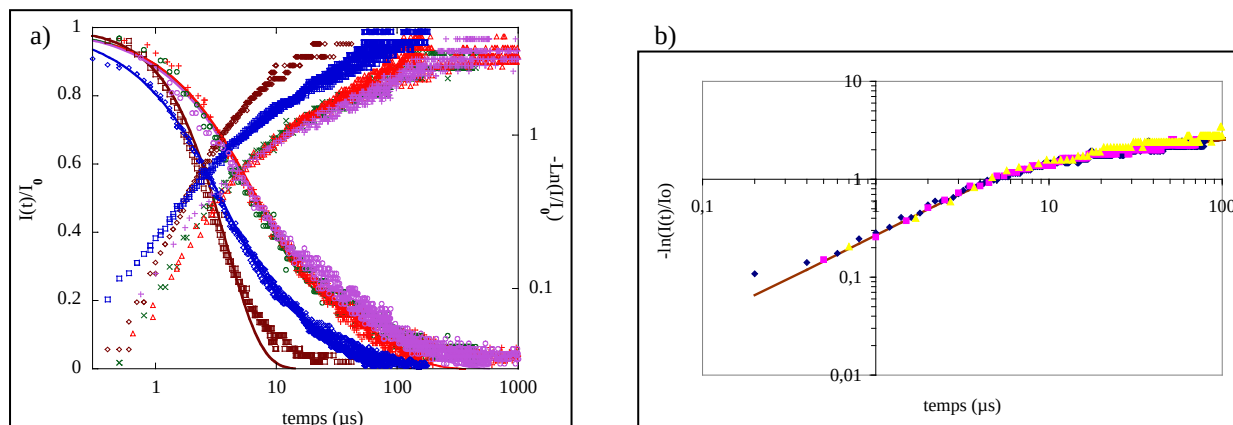


Fig. 11 : Exemple de mesure de relaxation de biréfringence dans un mélange en solution de PAM 6×10^6 g/mol et de nanoparticules C1C de tailles 9 nm (a) et dans un ferrogel de PAM réticulé à 1% (b) pour $[\text{Na}_3\text{Cit}] = 8 \text{ mM}$. On en tire un temps court ($3 \mu\text{s}$) correspondant à la relaxation des nanoparticules libres et un temps long ($50 \mu\text{s}$) témoignant d'agrégats d'un diamètre hydrodynamique de rotation de 70 nm.

Pour finir sur les solutions, on a conclu sur l'origine moléculaire des interactions nanoparticules – polymères qui sont respectivement :

i) de nature électrostatique pour les copolymères PAM-PTEA, entre les blocs cationiques de ces derniers et la surface négative des nanoparticules « citratées » ;

ii) de type liaison hydrogène pour les homopolymère de PAM, en référence à la bibliographie montrant de telles liaisons entre les atomes d'oxygène des fonctions amides primaires du PAM et les hydrogènes des fonctions carboxyles, soit directement celles de la surface d'oxyde de fer hydratée, soit celles des ligands citrates avec lesquels les chaînes de PAM sont en compétition pour l'équilibre d'adsorption.

A la lumière de cette étude sur les mélanges liquides, la structure nanocomposite des ferrogels à base de PAM réticulé par le N,N'-méthylène-bis-acrylamide (BAM) a été sondée, en s'appuyant sur les propriétés magnéto-optiques des nanoparticules à nouveau mises à profit pour mettre en évidence le ralentissement de la dynamique de rotation voire le blocage complet des nanoparticules par les chaînes de polymère (Fig. 11). Plus précisément, on a étudié l'effet d'une part du taux de réticulation du gel BAM/AM à la synthèse et d'autre part de la concentration $[\text{Na}_3\text{Cit}]$ de sorte que la fraction de nanoparticules bloquées totalement dans leur rotation varie entre à peine 5% et jusqu'à 88% en fonction de ces deux paramètres. Par ailleurs, c'est une spécificité des gels que d'avoir figé au moment de leur synthèse un état des conformations des chaînes polymères, en particulier la répartition spatiale des nœuds de réticulation. Ce figeage partiel des fluctuations thermiques (non ergodicité) rend les techniques de diffusion de la lumière très délicates pour étudier les ferrogels. Néanmoins, grâce à la relaxation de biréfringence, nous avons pu mesurer à l'intérieur des ferrogels des diamètres hydrodynamiques de rotation entre 30 et 100 nm proches des valeurs en solution, à partir de la distribution des temps de relaxation des agrégats de nanoparticules (temps longs).

Pour compléter la description microscopique des ferrogels, nous avons confronté les mesures dynamiques précédentes aux informations sur l'organisation des nanoparticules apportées par la DNPA. On bénéficie du contraste important des nanoparticules par rapport au réseau polymère dont le signal a été éteint dans un mélange adéquat d'eau légère et d'eau lourde. Ce type d'étude structurale avait déjà été conduit précédemment durant la thèse d'Alberto Galicia pour les ferrogels de PAM réticulés par le MBA. Nous avons utilisé la même approche consistant à éteindre les chaînes de polymère et à suivre le facteur de structure $S(q)$ des nanoparticules magnétiques pour détecter les hétérogénéités de la matrice dans le cas où celle-ci est constituée par du PNIPAM thermosensible.

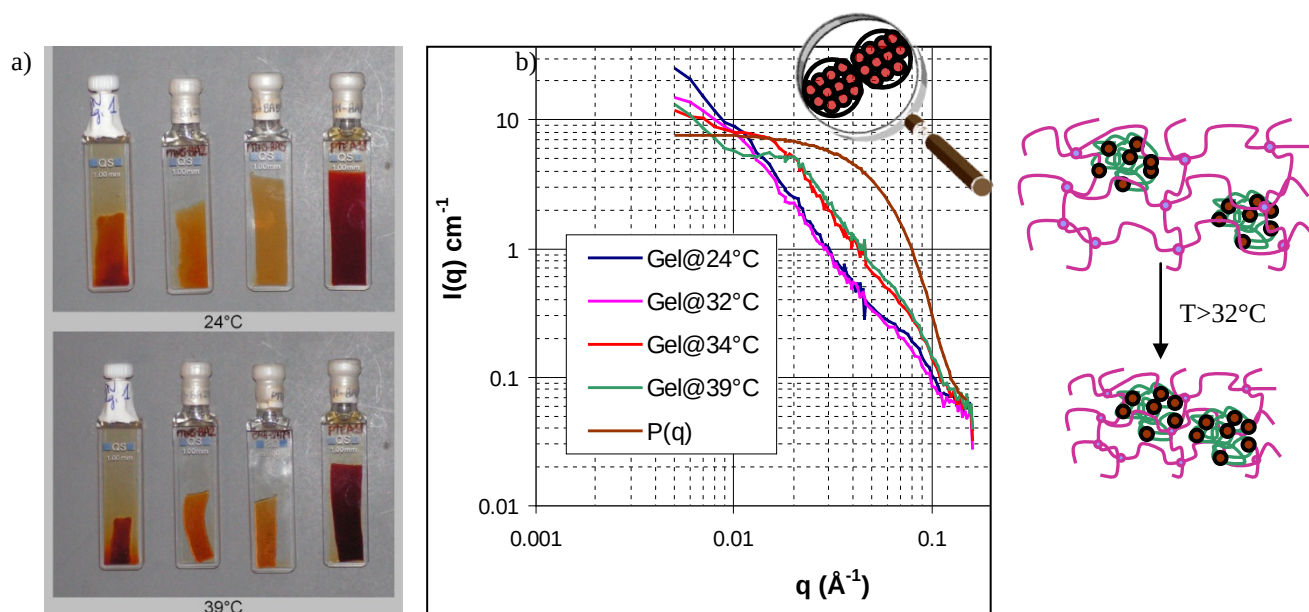


Fig. 12 : Réseau de PNIPAM semi-interpénétré par des agrégats de nanoparticules de 6 nm (S1S) avec le PAM_{30k}-PTEA_{11k}. La contraction de la matrice, visible sur les photos, entraîne un rapprochement des agrégats qui se traduit par l'apparition sur la courbe de DNPA d'un second pic de corrélation pour des valeurs de $q \approx 2 \times 10^{-2} \text{ \AA}^{-1}$ correspondant à une distance caractéristique entre les clusters $2\pi/q = 320 \text{ \AA} \approx 2R_G$.

L'obtention de gels à la fois chargés en nanoparticules magnétiques et présentant une forte variation du taux gonflement pour une température de transition marquée (32°C pour le PNIPAM pur) serait très intéressante pour le domaine du *drug delivery* dans lequel on recherche des matériaux répondant à divers stimuli. Plus précisément, on imagine qu'on puisse déclencher à distance la libération de médicaments par des vecteurs qui associeraient en leur sein à la fois du PNIPAM et des nanoparticules magnétiques: excitées par une onde électromagnétique radiofréquence, ces dernières provoqueraient un échauffement local qui déclencherait la transition du polymère, qui à son tour induirait une forte variation de perméabilité au produit. Cependant la difficulté réside dans le peu d'interaction entre le PNIPAM et l'oxyde de fer, qu'on attribue à un déficit de liaisons hydrogène quand on passe d'un polymère à groupements polaires amide primaire comme le PAM à un amide secondaire hydrophobe tel que le PNIPAM. L'augmentation du taux de réticulation, tout comme la réalisation de copolymères statistiques PAM-PNIPAM, se sont révélés inefficaces pour résoudre ce dilemme. Nous avons trouvé une solution originale qui limite le relargage des nanoparticules magnétiques tout en maintenant une transition marquée de la matrice de PNIPAM. Pour ce faire, on utilise bien des polymères hydrophiles interagissant fortement avec les nanoparticules, mais au lieu de copolymères statistiques avec le NIPAM on réalise plutôt des « copolymères topologiques », c'est-à-dire qu'on réalise un réseau de PNIPAM semi-interpénétré par des nanoparticules agrégées soit par du PAM de haut poids moléculaire, soit par des DHBC du type PAM-PTEA. Dans les deux cas, les expériences de DNPA ont montré que la matrice se contactait au-dessus le LCST et entraînait un rapprochement des pelotes mixtes nanoparticules – polymères (Fig. 12b) jusqu'à une distance de l'ordre de deux fois leur rayon de giration.

Préparation et étude de “microgels” et “minigels” magnétiques

Collaboration interne avec Christine Ménager

L'encapsulation homogène de particules inorganiques dans des microgels (billes de polymères réticulés) est un défi technologique, notamment pour les composites magnétiques où la nécessité d'une bonne répartition de la charge magnétique est indispensable afin d'avoir un comportement en gradient de champ magnétique quantifiable et reproductible. Pour des applications

de **tri magnétique de molécules biologiques**, on recherche aussi à obtenir des billes les plus magnétiques possibles et dans une gamme de tailles de 100 à 200 nm. La spécificité de notre approche est d'utiliser comme matrice des réseaux polymères hydrophiles (hydrogels), capables d'accueillir temporairement dans leur maillage des composés hydrosolubles, par opposition aux latex magnétiques commerciaux (billes Ademtech, Dynabeads, Estapor...), qui eux sont constitués d'un cœur hydrophobe, leur surface étant rendue hydrophile par greffage de molécules chargées^{22,23}.

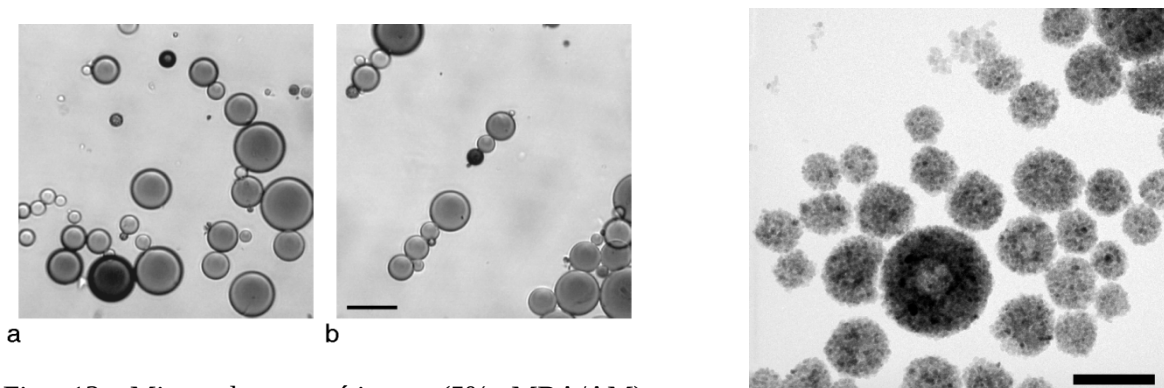


Fig. 13: Microgels magnétiques (5% MBA/AM) observés en microscopie optique pour un champ nul (a) et sous un champ appliqué $B=200$ Gauss (b), sous l'effet duquel ils forment des chaînes de manière réversible (Barre d'échelle = 20 μm).

c): Cliché MET de minigels (5% MBA/AM) sub-micrométriques, montrant une répartition homogène des nanoparticules (Barre d'échelle = 100 nm).

Ce travail sur les microgels s'inscrivait en parallèle de mes recherches sur les ferrogels macroscopiques. Lors du DEA de Jérôme Mangili encadré par Christine Ménager, on a mis à profit nos connaissances concernant l'état de dispersion des nanoparticules magnétiques citratées à l'intérieur des ferrogels de PAM réticulés par le MBA, ainsi que sur le taux de gonflement des gels et le relargage des particules. Les microgels et les minigels ont été synthétisés par **polymérisation radicalaire en émulsion inverse**. Selon la taille initiale des gouttelettes de l'émulsion, on obtient soit des **microgels de 10–50 μm** observables et manipulables par microscopie optique (figure 13a-b), soit des **minigels de 70–300 nm** que l'on étudie à l'état sec par microscope électronique (figure 13c).

On a pu mettre en évidence le gonflement dans l'eau de ces microgels magnétiques soit par observation directe en microscopie optique pour les plus gros, soit en diffusion de la lumière dynamique multi-angles pour les minigels. Comme on pouvait s'y attendre, leur diamètre est fonction de la nature du solvant et du taux de réticulation. On a ainsi montré que le diamètre hydrodynamique des minigels passe de 158 nm dans l'éthanol (mauvais solvant) à 195 nm dans l'eau, c'est à dire qu'ils doublent approximativement en volume²⁴. Par ailleurs, le taux d'encapsulation d'oxyde de fer magnétique a été quantifié par des mesures d'aimantation et des mesures gravimétriques, qui ont conduit à des pourcentages massiques d'oxyde par rapport au total de 64% et 34 % respectivement dans les microgels de taux de réticulation 5% et 2.5% (en poids MBA/AM). Les minigels sub-micrométriques possédaient quant à eux une charge magnétique de 69% en masse, ce qui les situait dans une gamme comparable aux latex magnétiques commerciaux.

Par rapport à ces premiers résultats publiés, la thématique a été développée dans deux voies : d'une part en vue d'améliorer la monodispersité en tailles des émulsions et donc des minigels ; d'autre part en visant l'objectif d'associer au caractère magnétique des billes une autre propriété, soit une réponse avec une variation du pH, soit avec une variation de la température, toujours en

²² J. Ugelstad, A. Berge, T. Ellingsen, R. Schmid, T.N. Nilsen, P. C.; Moerk, P. Stenstad, E. Hornes, O. Olsvik, *Progr. in Polym. Sci.* (1992) **17**(1), 87-161

²³ K. Landfester, *Adv. Mater.* (2001) **13**, 765; K. Landfester, *Macromol. Rapid. Commun.* (2001) **22**, 896

²⁴ C. Ménager, O. Sandre, J. Mangili, V. Cabuil, *Polymer* (2004), **45**, 2475-2481.

s'inspirant de ce que l'on développe sur des ferrogels macroscopiques, mais en l'adaptant pour les synthèses en milieu dispersé. Lors de son stage de Master 2 « Physico-Chimie Analytique » soutenu en 2005 sous ma direction, Tiphaine Châtelain a réalisé des microgels magnétiques de PAM plus monodisperse en cisillant les émulsions dans un rhéomètre de Couette, et a obtenu des microgels magnétiques thermo-sensibles, sur le modèle mésoscopique des réseaux semi-interpénétrés. Pour être injectés dans la circulation sanguine, de tels minigels devraient être suffisamment petits, idéalement moins de 200 nm. Pour ce faire, Tiphaine a effectué la polymérisation radicalaire du NIPAM soit en émulsion, soit en suspension, tout en confinant des nanoparticules enrobées de PAM. Nous pensons avoir démontré que même avec des interactions aussi peu spécifiques que des liaisons hydrogènes ou électrostatiques, nous pouvions jouer sur les paramètres de formulation (ratio X des nanoparticules par rapport aux chaînes complexantes, taux de réticulation de la matrice, concentration du citrate de sodium, etc...) et obtenir des matériaux déjà relativement évolués et pouvant facilement être mis en forme pour créer des dispositifs d'actuateurs « intelligents » ou des vecteurs pharmaceutiques.

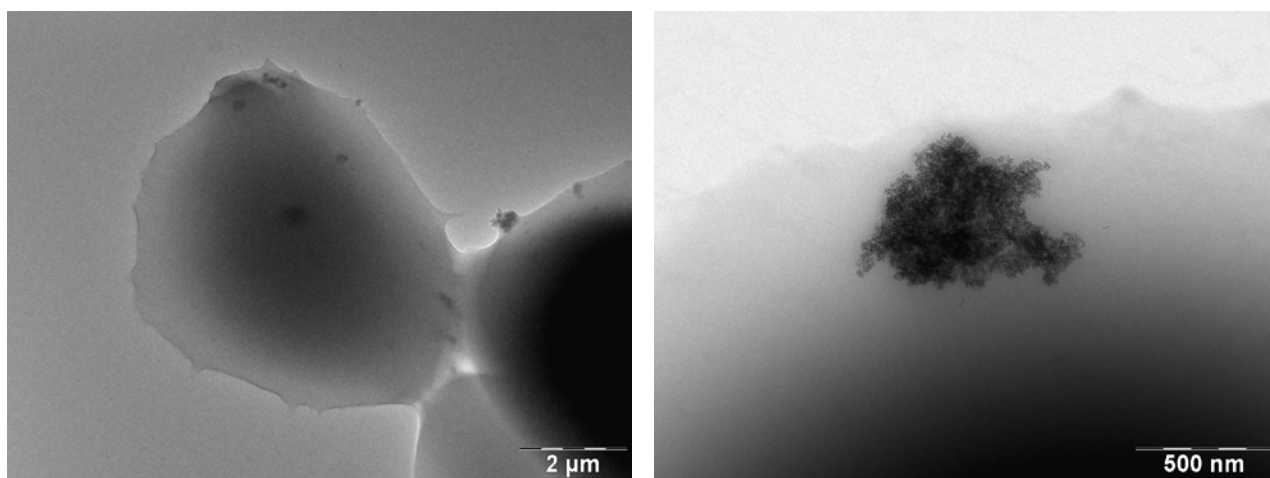


Fig. 14 a) Vue d'un microgel partiellement étalé sur la grille de microscope électronique contenant plusieurs agrégats de nanoparticules qui apparaissent comme des tâches sombres à l'intérieur du gel. b) Vue plus détaillée d'un agrégat situé près du bord du microgel, enrobé dans la matrice de PNIPAM (en gris sombre), contenant aussi 1.6% de MBA et 5 % de MAAC.

Pour étudier ces microgels à l'état hydraté, on utilise à la fois la diffusion dynamique de la lumière et la microscopie optique. En se basant sur une étude de Pelton par zétamétrie²⁵, Tiphaine a montré qu'on pouvait avantageusement incorporer 5% d'acide méthacrylique (MAAC) dans la matrice de PNIPAM, à la fois pour améliorer la rétention des nanoparticules (par liaison entre les carboxylates et l'oxyde de fer, en plus de l'enrobage par des chaînes de PAM) et pour assurer la stabilité colloïdale des microgels (en leur conférant une charge négative). Ce faisant, les microgels ont montré une réponse différente en fonction du pH : à pH 3, ils passent d'un diamètre 10 μm à l'état gonflé (20°C) à moins de 200 nm à l'état contracté (40°C) ; à pH 5, l'amplitude de la transition est moins élevée, tandis qu'à pH 7, elle est même supprimée, conduisant à des minigels très chargés et donc très hydrophiles, qui gonflent jusqu'au point de former ensemble un gel physique thixotrope.

Logiquement cette étude devait se poursuivre en provoquer la transition non par un chauffage macroscopique des minigels mais « à distance », par induction magnétique. Delphine El kharrat a fait un essai dans ce sens sur le montage d'hyperthermie radiofréquences de l'équipe de J.-C. Bacri et F. Gazeau au laboratoire MSC, en plaçant un ferrogel macroscopique dans un tube Ependorf. Mais pour mesurer une variation de diamètre hydrodynamique de minigels, il eût fallu monter une bobine directement sur un montage de diffusion de lumière, ce qui était un développement expérimental important pour lequel je n'ai pas pu obtenir de crédits. Je n'ai pas renoncé de le faire à Bordeaux avec d'autres objets, en mesurant leur coefficient de diffusion par "particle tracking" (voir projets).

²⁵ T. Hoare, R. Pelton, *Macromolecules* **37** (2004), 2544-2550.

Caoutchoucs magnétiques

Contacté en 2006 par un industriel (M Philippe Moureaux), j'ai proposé à celui-ci d'accueillir un stagiaire de Master 1 de l'UPMC, Jeoffroy Lefrère, dans son entreprise (CF GOMMA Barre Thomas à Rennes), pour tester son idée de réaliser des élastomères magnétiques pour des applications antivibratoires. Plus précisément, il s'agissait de pouvoir moduler leur module élastique avec un champ magnétique, afin de supprimer certaines résonances (dans les habitacles d'automobile ou bien les machines outils). Pour cela les charges inorganiques traditionnelles (noir de carbone et silice) dans des échantillons de caoutchouc naturel devaient être remplacées par des nanoparticules d'oxyde de fer. Devant la grande quantité de nanoparticules à synthétiser (2 kg de matière inorganique sèche pour remplir le « mélangeur pilote »), nous avons simplifié la synthèse de la magnétite Fe_3O_4 pour pouvoir être produite en telle quantité, avec une surface greffée par de l'acide oléique pour la dispersion dans le polymère. Après avoir résolu certains problèmes comme la teneur résiduelle en eau (qui provoquait des bulles dans le réacteur de vulcanisation à 160°C !), Jeoffrey a réalisé des éprouvettes de fractions volumiques croissantes (5%, 10% et 15%), ainsi qu'un échantillon à 30% bi-dispersé avec à la fois les nanoparticules d'oxyde de fer et des billes micrométriques de « fer carbonyle ».

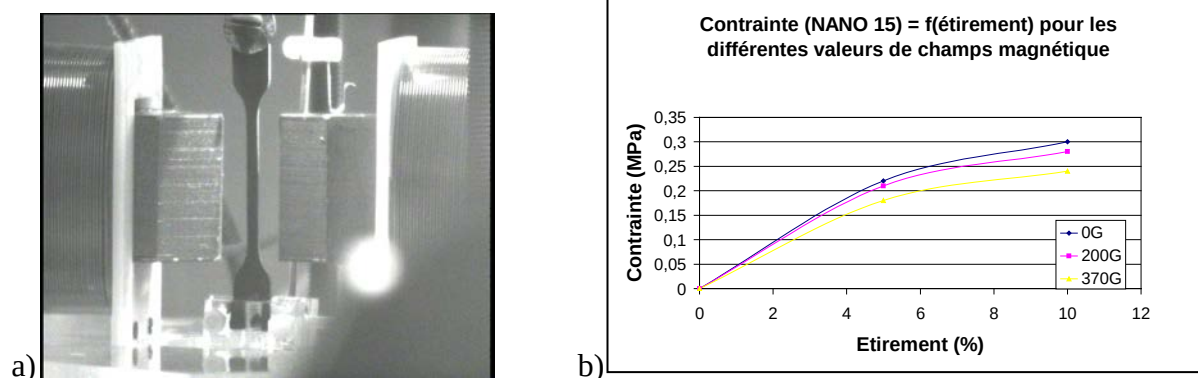
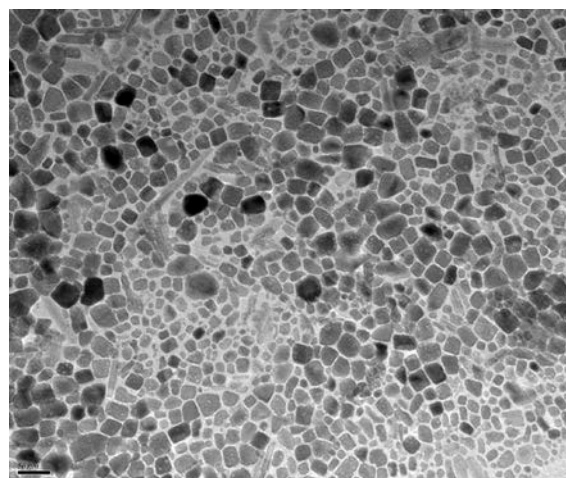


Fig. 15 : a) Eprouvette de caoutchouc magnétique dans une expérience de traction sous champ magnétique ; b) Courbe de traction pour l'échantillon chargé à 15% en magnétite : le module varie de 20% en champ B.

L'objectif était l'obtention d'élastomères dont la raideur puisse être ajustée par un champ magnétique appliqué. Même si l'effet magnéto-élastique mesuré sur nos échantillons n'était pas assez important pour les applications anti-vibratoires visées par l'industriel, je serais intéressé de reprendre cette étude notamment pour étudier la possibilité d'effectuer l'étape de vulcanisation sous champ magnétique, afin de renforcer l'anisotropie des élastomères (pré-alignement), comme indiqué récemment lors d'un congrès par Georges Bossis, qui étudie ces sujets à l'université de Nice.

L'effet du champ magnétiques pourrait se faire beaucoup plus sentir en employant des nanoparticules de grandes tailles ferrimagnétiques que l'on sait préparer (autour de 15 nm), avec de faibles distances inter-particulaires pour profiter des interactions dipolaires, ou bien même de la magnétique commerciale en poudre telle qu'imaginée ci-contre.

Fig. 16 : Nanoparticules de Fe_3O_4 très grandes tailles (de 30 à 50 nm) que l'on peut acheter chez Sigma-Aldrich, dont la dispersion dans une matrice polymère constituerait un challenge intéressant.



II. Vésicules et micelles magnétiques avec des copolymères diblocs amphiphiles "rod-coil"

Structures auto-assemblées de nanoparticules magnétiques et de copolymères diblocs amphiphiles

Collaboration depuis 2003 avec **Sébastien Lecommandoux** (Laboratoire de Chimie des Polymères Organiques (UMR5629 Université Bordeaux 1 / CNRS / ENSCPB), et **Annie Brûlet** du Laboratoire Léon Brillouin (UMR12 CNRS / CEA-Saclay) pour les études structurales.

Historique

Des vésicules dont la membrane est une bicouche de copolymères diblocs amphiphiles ont été introduites par Dan Hammer, Frank Bates et Denis Discher.²⁶ Par analogie avec les liposomes dont la membrane est composée d'une bicouche de lipides, ils furent baptisés polymersomes ou en français « polymérosomes ». Les challenges actuels concernent l'obtention de ces vésicules polymériques associant plusieurs fonctions et répondant à divers stimuli d'origines physiques (champs E ou B, lumière), chimiques (pH, salinité) ou biochimiques (molécules cibles).²⁷

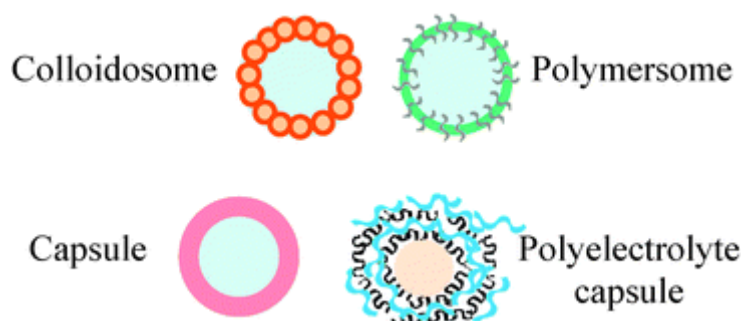


Fig.17 : Objets de géométrie coquille sphérique que l'on peut obtenir avec des polymères (d'après la revue de Yow et Routh²⁸). Parmi ceux-ci, seuls les polymérosomes composés d'une bicouche de copolymères diblocs amphiphiles ont une membrane véritablement fluide analogue à celles des liposomes au-dessus de la température de gel des lipides, mais néanmoins beaucoup plus visqueuse et moins perméable à l'eau.

L'étude a débuté après ma rencontre avec Sébastien Lecommandoux au [CERC3 Young Chemists Workshop 2002](#) "Water-soluble Polymers and their hydrogels", où nous avons été envoyés tous deux par le département Science Chimique du CNRS. Dès lors nous avons conçu des colloïdes hybrides oxyde de fer-polymère de structure soit micellaire, soit vésiculaire. Ces objets résultent de l'auto-assemblage des nanoparticules magnétiques avec des copolymères di-blocs de type "rod-coil". Ces copolymères amphiphiles comprenant un **bloc hydrophobe flexible** et un **bloc rigide peptidique hydrophile et pH-sensible** étaient synthétisés au LCPO, en combinant polymérisation anionique et par ouverture de cycle. Plus précisément il s'agissait de polybutadiène-*b*-poly(acide glutamique) (PB-*b*-PGA), dont l'indice de polymolécularité réduit ($I_p=1.1-1.2$) permettait d'obtenir des phases auto-assemblées bien définies, même si l'on suspecte depuis un travail d'Adi Eisenberg²⁹ que la polydispersité résiduelle des masses de chaînes soit plutôt un facteur bénéfique pour l'auto-assemblage en membranes. Ainsi le PB₄₀-*b*-PGA₁₀₀ formait-il dans l'eau des bicouches refermées sur elles-mêmes. Appartenant à cette nouvelle classe de vésicules polymériques introduites dans les années 90' par Frank Bates³⁰ et ses collègues de U Penn (*cf.* revues sur le sujet

²⁶ Bermudez, H.; Brannan, A. K.; Hammer, D. A.; Bates, F. S.; Discher, D. E., Molecular Weight Dependence of Polymersome Membrane Structure, Elasticity, and Stability. *Macromolecules* **2002**, 35, 8203-8208.

²⁷ Du, J.; O'Reilly, R. K., Advances and challenges in smart and functional polymer vesicles. *Soft Matter* **2009**, 5, 3544.

²⁸ Yow, H. N.; Routh, A. F., Formation of liquid core-polymer shell microcapsules. *Soft Matter* **2006**, 2, 940-949.

²⁹ O. Terreau, L. Luo, A. Eisenberg, *Langmuir* **19** (2003) 5601

³⁰ Y.-Y. Won, H. Davis, F. Bates, *Science* **283** (1999) 960

^{31,32}), elles présentaient par ailleurs la particularité de répondre à changement du pH extérieur par une variation importante de leur taille: leur diamètre hydrodynamique d_H passait ainsi de 200 nm en milieu acide à 300 nm en milieu basique (ou 250 nm en milieu très salin).³³ Cette transition est réversible et peu sensible à la salinité car elle repose non pas sur un simple gonflement par effet polyélectrolyte mais sur la nature peptidique du bloc PGA, transitant entre une conformation compacte en hélice α à pH acide et une autre plus étirée en pelote à pH basique.

L'un des objectifs de l'incorporation des nanoparticules de maghémite dans de tels objets supra-macromoléculaires était de s'en servir comme **sondes en diffusion des neutrons** pour étudier la transformation morphologique importante subie lors du changement de taille entre un pH acide et basique, en détectant peut-être un état transitoire avec une bicouche ouverte, ce qui ouvrirait bien des perspectives pour une libération contrôlée du contenu interne ! Pour cela on s'est servi du très bon contraste de l'oxyde de fer par rapport au polymère et au solvant, et on a mesuré leur facteur de structure à l'intérieur des agrégats $S_{intra}(q)$, qui nous renseigne d'une part sur la concentration locale de nanoparticules, d'autre part sur le facteur de forme de l'objet qu'elles décorent.³⁴ Un autre but était de conférer à l'objet tout entier une réponse à un champ magnétique, que ce soit une **déformation induite** (ellipsoïdale ou autre) ou une **libération d'une substance déclenchée par le champ**, sujet toujours d'actualité dans nos recherches (mais avec d'autres copolymères).

$\gamma\text{-Fe}_2\text{O}_3$ Nanoparticules	$\text{PB}_n\text{-}b\text{-PGA}_p$	$\text{PB}_{48}\text{-}b\text{-PGA}_{145}$ in H_2O	$\text{PB}_{48}\text{-}b\text{-PGA}_{114}$ in H_2O	$\text{PB}_{48}\text{-}b\text{-PGA}_{56}$ in H_2O	$\text{PB}_{48}\text{-}b\text{-PGA}_{20}$ in CH_2Cl_2
-surfacted in CH_2Cl_2 $d_0=6.8\text{nm}$ $\sigma=0.24$		A Magnetic Micelles		B Magnetic vesicles	
- acidic in HNO_3 (pH=1.2) $d_0=6.8\text{nm}$ $\sigma=0.24$					C Magnetic vesicles
		Objects are not properly dispersed (after 1 month)			
- citrated in Na_3Cit (pH=7) $d_0=6.6\text{nm}$ $\sigma=0.21$		not properly dispersed		D Magnetic vesicles	not properly dispersed

Tableau 2 : Objets colloïdaux hybrides obtenus par auto-assemblage de nanoparticules magnétiques et de copolymères amphiphiles “rod-coil”. Les lettres A, B, C et D représentent des objets que l'on a déjà réussi à préparer ; les cases restantes seront peut-être observées un jour avec de nouvelles « générations » de copolymères di-blocs synthétisés au LCPO.

³¹ D.E. Discher, A. Eisenberg, *Science* **297** (2002) 967

³² J.-F. Le Meins, O. Sandre, S. Lecommandoux, *Eur. Phys. J. E* **34** (2011) 14

³³ F. Chécot, S. Lecommandoux, Y. Gnanou, H. A. Klok, Water-soluble stimuli-responsive vesicles from peptide-based diblock copolymers. *Angew. Chem.-Int. Edit.* **2002**, 41 (8), 1339-1343

³⁴ J. Oberdisse, Y. Rharbi, F. Boué, *Computational and Theoretical Polymer Science* **10** (2000) 207–217

Ce travail a débuté par la vérification que les deux composants organique et inorganique pouvaient effectivement être combinés pour donner des objets bien définis et dispersés dans l'eau ou en solvant. Pour cela nous avons balayé une série de copolymères variant par la taille de leur bloc polypeptidique, le bloc hydrophobe étant maintenu constant, sa double-liaison offrant la possibilité d'être réticulée ultérieurement et ainsi de figer les structures déformées sous champ magnétique. Les possibilités d'association sont résumées par le Tableau 2 à double entrée, d'une part les copolymères:

- PB₄₈-b-PGA₁₁₄ et PB₄₈-b-PGA₁₄₅ qui s'auto-assemblent dans l'eau en micelles de diamètres hydrodynamiques respectivement 60 et 70 nm, le diamètre interne du cœur hydrophobe ayant été mesuré par DNPA à 14 nm pour les deux copolymères.³⁵

- PB₄₈-b-PGA₅₆ qui forme dans l'eau des vésicules analogues à celles de PB₄₀-b-PGA₁₀₀, mais de diamètre d_H égal à 100 nm et d'épaisseur de bicouche 14 nm.

- PB₄₈-b-PGA₂₀ qui se disperse non pas dans l'eau mais dans le dichlorométhane, sous forme de micelles inverses de d_H égal à 260 nm.

et d'autre part les ferrofluides, aqueux ou bien organiques, dont la polydispersité en tailles des grains magnétiques a été au préalable réduite par la méthode des démixtions fractionnées³⁶ :

- S2-HNO₃ qui est un ferrofluide aqueux acide (pH=1.2), dans lequel les particules présentent une dispersion de tailles caractérisée par un $d_0=6.8\text{nm}$ et $\sigma=0.24$, et dont la surface d'oxyde est recouverte simplement de protons H⁺, ce qui laisse un accès aisé pour une complexation par les groupements carboxylates des blocs PGA des copolymères.

- S2-CH₂Cl₂, provenant du ferrofluide précédent après greffage de tensioactif de type di-ester de l'acide phosphorique (Beycostat NE) pour une solubilisation dans le dichlorométhane.

- S1S-HNO₃, ferrofluide acide qui présente par ailleurs une distribution de tailles légèrement plus fine que le précédent, décrite par $d_0=6.6\text{nm}$ et $\sigma=0.21$.

- S1S-Na₃Cit, ferrofluide greffé par des ligands citrate de sodium pour une dispersion à pH≈7.

L'association de chaque copolymère mis en présence de 1 ou 2 équivalents de l'un des ferrofluides a été favorisée en éliminant d'abord le solvant, puis en re-dispersant les objets formés, après déprotonation des fonctions acides du copolymère avec NaOH. Cette technique est qualifiée de « **dissolution directe** » pour la différencier de la « nanopréciipitation » qui fut introduite par la suite avec des copolymères moins flexibles. Au bout de trois semaines d'agitation à température contrôlée, la structure des suspensions qui avaient atteint une forme d'équilibre et un état de dispersion satisfaisant des agrégats copolymères-nanoparticules a été analysée par DLS et DNPA.

Les échantillons PB₄₈-b-PGA₁₁₄ et PB₄₈-b-PGA₁₄₅ ont donné avec le ferrofluide surfacté S2-CH₂Cl₂ des **micelles magnétiques** de d_H compris entre 225 (1 équivalent) et 430 nm (2 équivalents). Leur cœur hydrophobe est rempli de nanoparticules très concentrées (Tableau 2, structures A), la fraction volumique locale étant d'environ 30% d'après la position du pic de structure en DNPA.

Les vésicules de PB₄₈-b-PGA₅₆ continuaient de se former dans l'eau en présence du ferrofluide S2-CH₂Cl₂, leur diamètre étant augmenté significativement (d_H égal à 620 et 210 nm pour 1 et 2 équivalents respectivement). Les nanoparticules surfactées étaient confinées à 2 dimensions au sein des membranes hydrophobes du copolymère, comme l'indiquaient conjointement les mesures de DNPA et les images de TEM et d'AFM. Il s'agissait alors du premier cas de **vésicules avec une membrane magnétique fluide**. Des préparations de coques magnétiques creuses avaient effectivement été déjà décrites, mais elles étaient basées sur la minéralisation d'une bille de polymère solide calcinée ultérieurement³⁷. Au contraire, les polymersomes auto-assemblés autour d'un cœur aqueux sont très mous et déformables, une configuration qui n'avait jusqu'alors été considérée que sur le plan théorique.³⁸

³⁵ F. Chécot, A. Brûlet, J. Oberdisse, Y. Gnanou, O. Mondain-Monval, S. Lecommandoux, *Langmuir* **21** (2005) 4308

³⁶ J.-C. Bacri, R. Perzynski, D. Salin, V. Cabuil, R. Massart, *J. Coll. Int. Sci.* **132** (1989), 43; R. Massart, E. Dubois, V. Cabuil, E. Hasmonay, *J. Magn. Magn. Mater.* **149** (1995) 1-5.

³⁷ E.L. Bizdoaca, M. Spasovaa, M. Farlea, M. Hilgendorff., F., Caruso, *J. Mag. Mag. Mat.* **240** (2002), 44

³⁸ Kern, N, Fourcade, B., *Europhys. Lett.* **38** (1997) 395

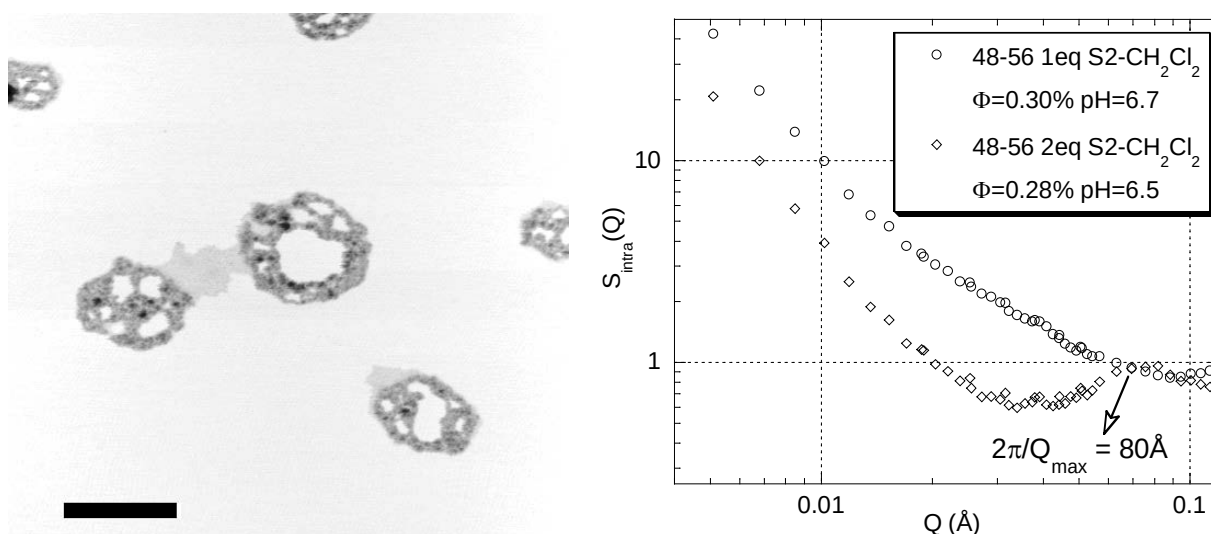


Fig. 18 : a) Image AFM de vésicules avec une membrane magnétique. Les bicouches de PB₄₈-b-PGA₅₆ ont pris en sandwich des nanoparticules hydrophobes (de diamètre moyen 6.6 nm) et présentent des trous qui pourraient soit être des déchirures lors du dépôt sur mica, soit pré-exister dans les membranes des vésicules encore dispersées dans la solution (barre = 500 nm, hauteur totale = 37 nm).

b) Facteur de structure de nanoparticules surfactées S2-CH₂Cl₂ mesuré par SANS (expérience n°6899 2003, PACE, LLB, CEA-Saclay), pour 1 ou 2 équivalents par rapport au PB₄₈-b-PGA₅₆.

A 1éq on observe à petit q une pente proche de -2 typique du facteur de forme d'un objet membranaire, tandis qu'à grand q l'absence du pic présent pour 2éq, indique que les particules ne sont plus au contact les unes des autres, et donc que les membranes sont vraisemblablement fluides.

Les vésicules qui s'auto-assemblent dans l'eau à partir de PB₄₈-b-PGA₅₆ ou bien PB₄₀-b-PGA₁₀₀ peuvent aussi être remplies dans leur compartiment aqueux interne avec un ferrofluide à pH7 tel que S1S-Na₃Cit. De la même manière que pour les liposomes magnétiques,³⁹ les nanoparticules non encapsulées ont été séparées des vésicules pleines de ferrofluide par un passage sur colonne chromatographique, ce que nous avons puis suivre clairement grâce au facteur de structure $S_{\text{intra}}(q)$.

Enfin pour le copolymère ayant le plus petit bloc hydrophile PB₄₈-b-PGA₂₀, nous avons trouvé qu'il s'auto-associait dans le dichlorométhane avec les nanoparticules dont la surface d'oxyde est simplement protonée partiellement, et qui peut donc interagir avec les groupements PGA. On obtient ainsi des **micelles magnétiques inverses** de d_H égal à 70 nm avec 2 équivalents de particules de S2-HNO₃ et 75 nm avec celles de S1S-HNO₃. Dans ce dernier cas on a noté aussi la présence d'objets bien plus gros ($d_H=470$ nm), qui pourraient être encore un exemple de vésicules fluides avec une membrane magnétique, mais dont cette fois l'espace entre les feuillettes de la bicouche est hydrophile et prend en sandwich des nanoparticules chargées.

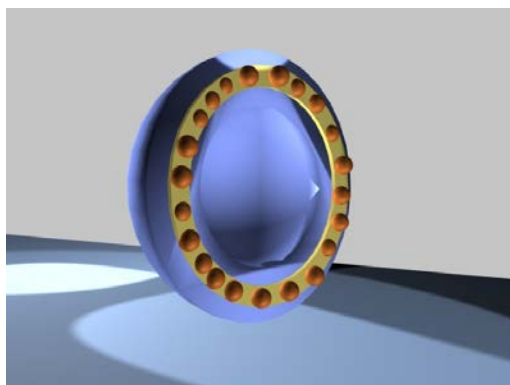
Maintenant que nous connaissons la structure de tels objets hybrides auto-assemblés, l'étape suivante de l'étude a consisté à **modifier leur forme grâce à un champ magnétique** et à le suivre par diffusion anisotrope des neutrons. Sous un champ magnétique statique on prévoyait d'obtenir une déformation ellipsoïdale simple. Avec un champ variable (notamment tournant), on pourrait bien produire des formes plus complexes, comme celles en étoiles de mer connues pour les gouttelettes de ferrofluide.⁴⁰ Ces formes originales constituent un défi pour le physico-chimiste qui veut les réaliser à l'échelle mésoscopique, alors que les micelles et polymersomes magnétiques seraient peut-être un moyen de les atteindre, et de les figer en réticulant ultérieurement les blocs PB insaturés.

³⁹ S. Lesieur, C. Grabielle-Madellmont, C. Menager, V. Cabuil, D. Dadhi, P. Pierrot, K. Edwards, *J. Am. Chem. Soc.* **125** (2003) 5266

⁴⁰ J.-C. Bacri, A. Cebers, R. Perzynski, *Phys. Rev. Lett.* **72** (1994) 2705

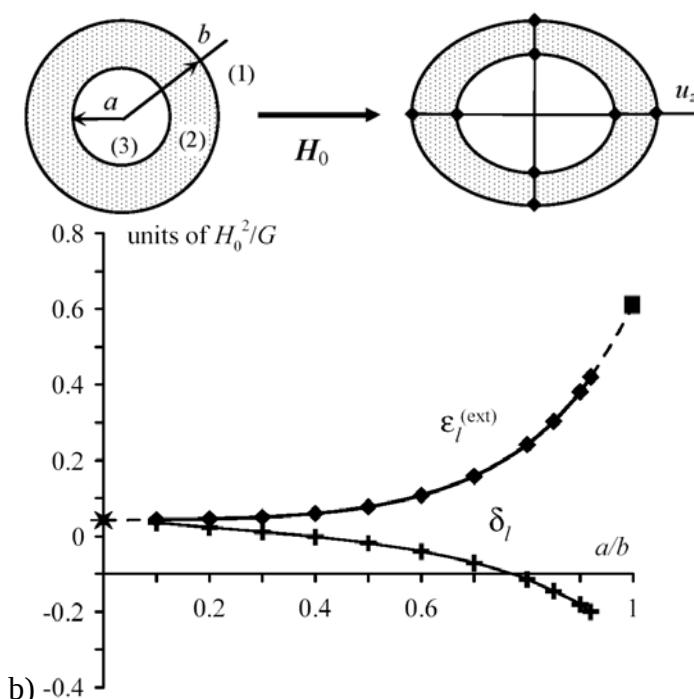
Polymersomes magnétiques sous champ statique

Connaissant la structure des objets hybrides copolymères-ferrofluides, la suite logique consistait à **modifier leur forme sous un champ magnétique** et à la suivre en diffusion anisotrope des neutrons. Les vésicules de PB₄₈-b-PGA₅₆ confinant des nanoparticules « surfactées » au sein de leur membrane hydrophobe, comme l'indiquent conjointement les mesures de DNPA (permettant en outre de mesurer l'épaisseur) et les images de TEM et AFM, constituaient le cas le plus original : en comparaison des coques magnétiques creuses décrites dans la littérature, basées sur le recouvrement d'un cœur sphérique par une couronne de polymère puis dissolution du template par attaque avec un acide⁴¹ ou un solvant,⁴² nos polymérosomes magnétiques constituaient le premier cas de vésicules avec une membrane à la fois magnétique et fluide, pouvant être réticulée. Le cas de la déformation sous champ statique d'une coque magnéto-élastique a été étudié théoriquement par Yuriy Raikher, de l'Institut de Mécanique des Milieux Continus à Perm en Russie qui possède une longue tradition de collaboration avec les expérimentateurs parisiens des ferrofluides (ECONET).⁴³ Il nous restait pour notre part à vérifier que les polymersomes étaient bien déformables par un champ de faible intensité, ce qui fut réalisé par des expériences de diffusion des neutrons aux petits angles sur le spectromètre PAXY du LLB au CEA-Saclay.^{44,45,46}



a)

Fig. 19 a) Vue d'artiste d'une vésicule confinant des nanoparticules magnétiques au sein de la membrane, subissant une déformation ellipsoïdale sous champ magnétique ; b) Déformation prévue par la théorie de coques magnétiques sous champ magnétique statique : la vésicule subit à la fois une elongation (ε) et une striction de sa membrane (δ), de module élastique G .



b)

L'effet attendu d'un champ B statique sur une coque magnétique molle est l'obtention d'une déformation ellipsoïdale. A l'avenir, on prévoit d'appliquer un champ B variant dans le temps (par exemple un champ tournant), afin de produire des déformations plus complexes, comme celles en étoiles de mer connues pour les gouttelettes de ferrofluide. Ces formes originales constituent un défi

⁴¹ Steadler, B.; Price, A. D.; Chandrawati, R.; Hosta-Rigau, L.; Zelikin, A. N.; Caruso, F., Polymer hydrogel capsules: en route toward synthetic cellular systems. *Nanoscale* **2009**, *1*, 68-73.

⁴² Liu, G.; Wang, H.; Yang, X., Synthesis of pH-sensitive hollow polymer microspheres with movable magnetic core. *Polymer* **2009**, *50*, 2578-2586.

⁴³ O.V. Stolbov, Yu.L. Raikher, Deformation of a ferrovesicle in a uniform magnetic field, *J. Magn. Magn. Mat.* **300** (2006) e199-e202

⁴⁴ S. Lecommandoux, O. Sandre, F. Chécot, J. Rodríguez-Hernández, R. Perzynski, Magnetic nano-composite micelles and vesicles, *Advanced Materials*, **17**(6), 712-718 (2005)

⁴⁵ S. Lecommandoux, O. Sandre, F. Chécot, R. Perzynski, Smart hybrid magnetic self-assembled micelles and hollow capsules, *Progress in Solid State Chemistry* **34**(2-4), 171-179 (2006)

⁴⁶ S. Lecommandoux, O. Sandre, F. Chécot, J. Rodríguez-Hernández, R. Perzynski, Self-assemblies of magnetic nanoparticles and di-block copolymers: Magnetic micelles and vesicles *J. Magn. Magn. Mat.* **300**(1), 71-74 (2006)

pour le physico-chimiste qui veut les réaliser à l'échelle mésoscopique, alors que nos agrégats copolymères diblocs-particules magnétiques et déformables sont de bons candidats pour les atteindre ainsi que pour les figer facilement en réticulant ultérieurement les blocs PB insaturés.

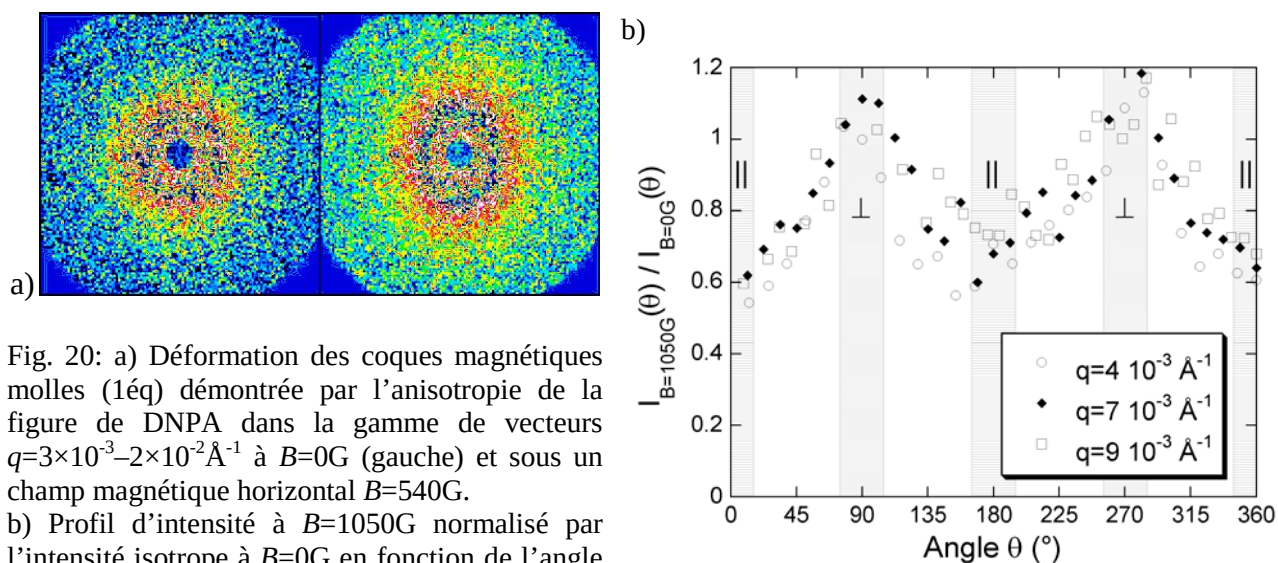


Fig. 20: a) Déformation des coques magnétiques molles (1éq) démontrée par l'anisotropie de la figure de DNPA dans la gamme de vecteurs $q=3 \times 10^{-3} - 2 \times 10^{-2} \text{ \AA}^{-1}$ à $B=0\text{G}$ (gauche) et sous un champ magnétique horizontal $B=540\text{G}$.

b) Profil d'intensité à $B=1050\text{G}$ normalisé par l'intensité isotrope à $B=0\text{G}$ en fonction de l'angle θ par rapport au champ B , pour différentes valeurs de q . Les zones hachurées représentent les directions $\parallel$ et $\perp$ par rapport à B .

En analysant les profils de diffusion (Kratky Porod plots), on a mesuré une magnéto-striction correspondant à une anisotropie d'épaisseur : $\delta_{\parallel}=9\text{nm}$ et $\delta_{\perp}=13\text{nm}$.

Polymersomes à la fois magnétiques et thermosensibles

Nous avons travaillé ensuite sur une 2^{ème} génération de polymérosomes que l'on voulait non seulement magnétiques mais aussi répondant à une élévation de la température. L'objectif à terme était d'utiliser le chauffage par induction radiofréquences des nanoparticules (« hyperthermie ») pour déclencher un changement de forme de la vésicule qui se traduise aussi par la libération d'un composé biologiquement actif. La thèse de Willy Agut encadrée par Sébastien Lecommandoux et Daniel Taton soutenue en janvier 2009 a permis de développer au LCPO une utilisation astucieuse de la « chimie clic » pour construire des copolymères à blocs, à partir de chaînes macromoléculaires de natures différentes synthétisées séparément puis attachées entre elles par cycloaddition 1,3-dipolaire de Huisgen entre un azoture et un alcynes.⁴⁷ Une différence notable avec la première génération de rod-coils réside dans la nature du bloc hydrophobe qui était cette fois-ci le bloc peptidique, à savoir le poly(L-glutamate de γ -benzyle) PBLG, tandis que le bloc hydrophile était le poly[méthacrylate de 2-(diméthyle amino)éthyle] PMADAME, qui démixe dans l'eau à une température supérieure à 39°C (LCST). Willy Agut a synthétisé d'autres copolymères dérivés de celui-ci en déprotégeant le PBLG pour donner le PGA hydrophile pH-sensible ou en quaternisant le PMADAME avec la propanesulfone pour donner un bloc zwitterionique avec une UCST au lieu de la LCST. Ils donnent des vésicules électrostatiques pH-sensibles (analogues aux capsules de polyélectrolytes représentées à la Fig. 1) qui peuvent être thermosensibles, mais elles n'ont pas encore été testées avec les nanoparticules magnétiques. Dans le cas du PMADAME₈₅-b-PBLG₄₁ qui donne des vésicules de 180 nm de diamètre avec une bicouche de 20 nm d'épaisseur, la tentative d'introduction de nanoparticules magnétiques surfactées de 6.3 nm a conduit à des objets sphériques plus petits (environ 60nm) mais surtout pleins, dans lesquels les nanoparticules sont agrégées de manière dense.

⁴⁷ Agut, W.; Taton, D.; Lecommandoux, S., A Versatile Synthetic Approach to Polypeptide Based Rod-Coil Block Copolymers by Click Chemistry. *Macromolecules* **2007**, *40*, 5653-5661.

Cette transition vésicule-micelle a été interprétée a posteriori comme provenant de la conformation hélicoïdale prononcée des blocs PBLG (mesurable par dichroïsme circulaire), qui pour un degré de polymérisation de 40 forment des cylindres rigides d'environ 6 nm de long. Leur rapport d'aspect prononcé (entre 2 et 3) confère à la membrane un caractère cristal liquide qui explique son incompatibilité avec des particules sphériques de taille comparable, par analogie avec la démixtion de particules sphériques et cylindriques décrite pour les cristaux liquides lyotropes par Henk Lekkerkerker⁴⁸, que l'on avait constaté aussi au LI2C/PECSA pour les thermotropes (cf. partie V).

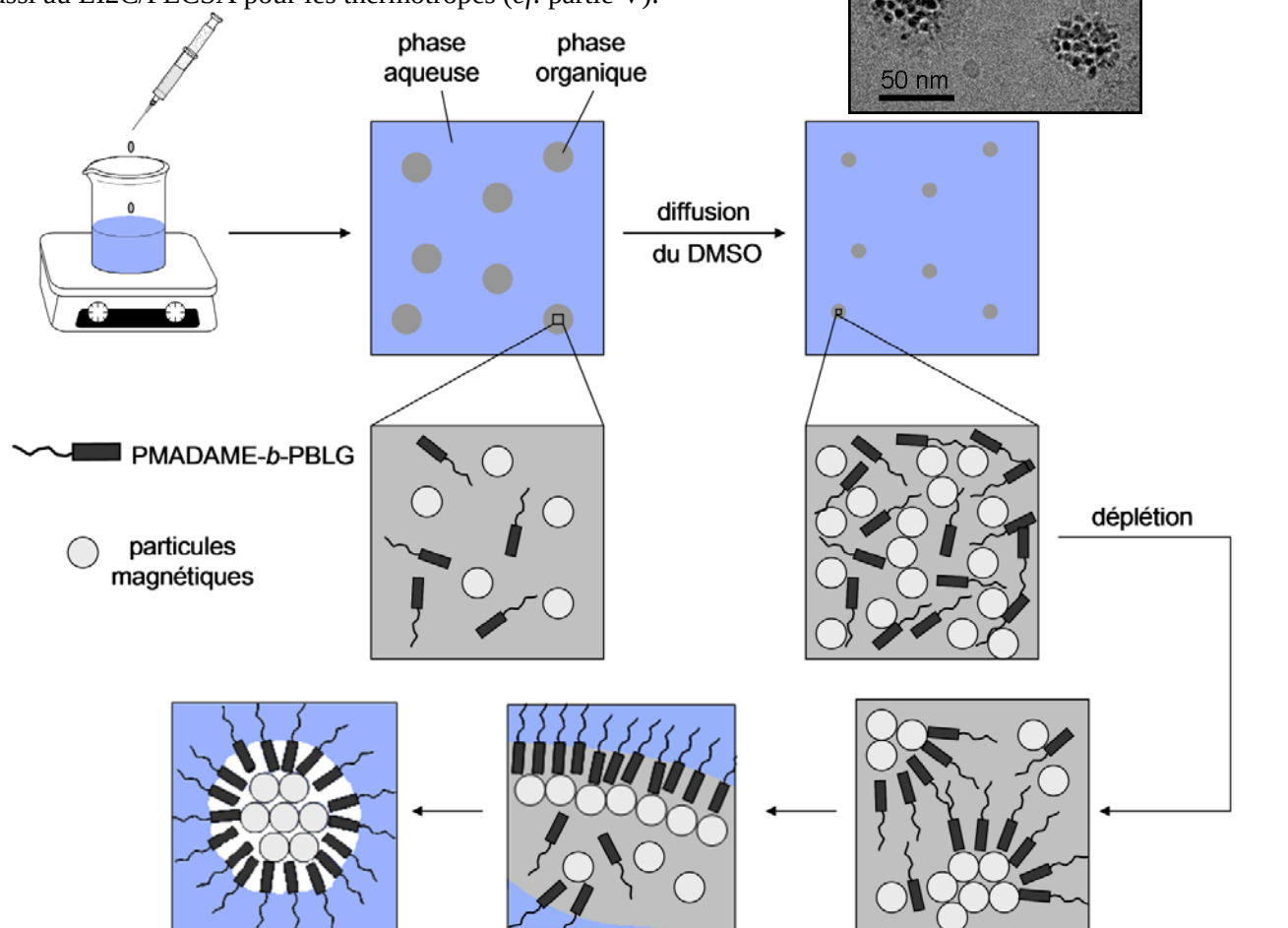


Fig. 21 : Schéma de la séparation de phase entre les nanoparticules magnétiques et les blocs rigides de PBLG (hélices α). L'interaction de déplétion entre deux nanoparticules au sein des bâtons a été évaluée à $3k_B T$.⁴⁹

Pour aller plus loin avec 2^{ème} génération de vésicules, nous pourrions décorer des vésicules cette fois électrostatiques PMADAME-*b*-PGA avec des nanoparticules d'oxyde de fer hydrophiles, un premier essai pour les coupler à des blocs d'acide polyglutamique (par précipitation-redispersion à partir d'un pH basique) s'étant révélé concluant. Des micelles magnétiques à cœur PBLG sont par ailleurs exploitées dans le cadre du projet [NanoTher](#) pour encapsuler des drogues hydrophobes.

⁴⁸ S.M Oversteegen, J.G.E.J Wijnhoven, C. Vonk, H.N.W. Lekkerkerker, Crystallization Kinetics and Morphology in Phase Separating and Sedimenting Mixtures of Colloidal Spheres and Rods, *J. Chem. Phys. B* **108** (2004), 18158-18163.

⁴⁹ W. Agut, D. Taton, Annie Brûlet, O. Sandre, S. Lecommandoux, Depletion Induced Vesicle-to-Micelle Transition from Self-Assembled Rod-Coil Diblock Copolymers with Spherical Magnetic Nanoparticles, *Soft Matter* **7** (2011), 9744.

Polymersomes magnétiques biocompatibles pour application en "drug delivery"

En attendant de poursuivre la voie des copolymères à LCST, une « troisième génération » de copolymères di-blocs formant des vésicules magnétiques a déjà été explorée par la thèse de Charles Sanson, soutenue en janvier 2010 au LCPO, puis dans le cadre du projet européen [NanoTher](#) : des blocs de faible masse molaire du polymère biodégradable poly(triméthylcarbonate) PTMC se sont révélés semi-cristallins, avec une température de fusion aux alentours de 35-40°C,⁵⁰ et peuvent être associés à un bloc PGA par une chimie de polymérisation par ouverture de cycle.⁵¹ Durant son stage de Master 2 à l'ENSCP, Odile Diou a effectué deux séjours d'expérimentation au laboratoire PECSA entrecoupés par une série d'expériences de diffusion des neutrons au LLB à Saclay. La dissolution directe employée avec les blocs polybutadiène flexibles ne donnant pas des objets nanométriques avec le PTMC beaucoup plus rigide, la technique dite de « nanoprécipitation » s'est révélée efficace pour préparer des vésicules de diamètres entre 120 et 200 nm jusqu'à un rapport massique élevé (70%) de nanoparticules magnétiques relativement au PTMC-*b*-PGA. Elles ont été caractérisées par diverses techniques structurales (magnétométrie, magnétophorèse, biréfringence magnéto-induite, DNPA) mais aussi vis-à-vis de leurs propriétés pour leurs applications en imagerie IRM (relaxivité T2) et en délivrance de drogue déclenchée par hyperthermie.⁵² Actuellement elles constituent le principal type de nanoparticules étudié à une large échelle dans le projet européen [NanoTher](#) sur des aspects aussi variés que la toxicité, la biodistribution, la production à grande échelle ("scale-up"), le ciblage grâce au greffage d'anticorps, la régression tumorale...

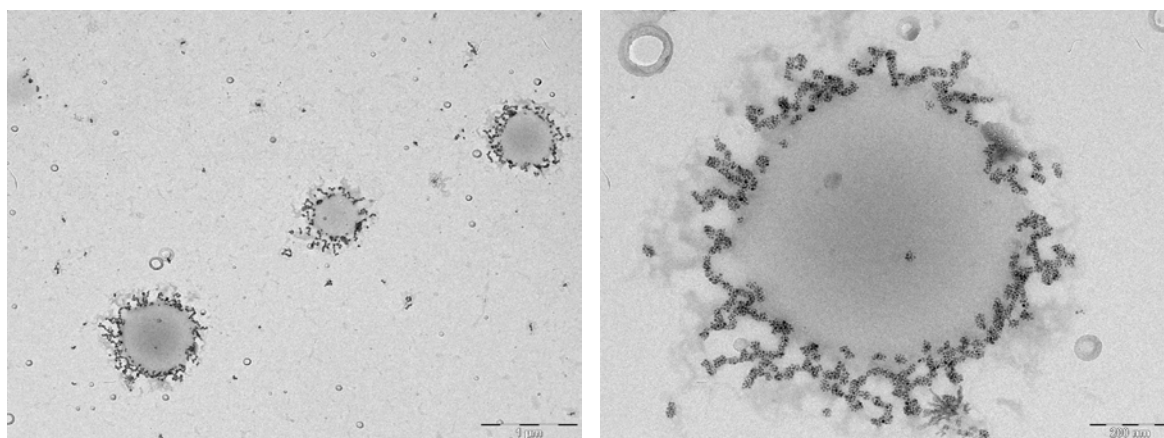


Fig. 22 : Clichés TEM de vésicules magnétiques WD15-50 formés par nano-précipitation en versant 10 volumes d'eau (en 15 min) dans une solution dans le DMSO de PTMC₂₄-*b*-PGA₁₉ mélangé à 50% en poids avec des nanoparticules de maghémite de diamètres 6-7 nm (recouvertes du surfactant Beycostat). Bien que les vésicules aient éclaté sur la grille, ces formes apparaissent nettement différentes de micelles pleines.

Pour ma part j'ai étudié tout particulièrement les propriétés physiques de ces vésicules. Avec Vincent Dupuis du PECSA nous avons détecté la fusion des chaînes de PTMC grâce à un pic de biréfringence dans une plage de températures 35-40°C (en accord avec les mesures calorimétriques). En analysant les trajectoires des vésicules dans un gradient de champ magnétique, on a pu découpler leur mouvement de diffusion Brownienne de leur migration magnétophorétique, décrit par la vitesse

de dérive :

$$\frac{v_{\text{mag}}}{\bar{V}_z(\frac{1}{2}B_0^2)} = \frac{\pi d_{\text{core}}^3}{(18)^2 \eta k_B T} \Phi_{\text{intra}} M_{\text{spe}}^2 d^2 \quad \text{Eq. (1)}$$

⁵⁰ C. Sanson, J-F. Le Meins, C. Schatz, A. Soum, S. Lecommandoux, Temperature responsive poly(trimethylene carbonate)-block-poly(L-glutamic acid) copolymer: polymersomes fusion and fission, *Soft Matter* **6** (2010) 1722–1730.

⁵¹ C. Sanson, C. Schatz, J-F. Le Meins, A. Brûlet, A. Soum, S. Lecommandoux, Biocompatible and Biodegradable Poly(trimethylene carbonate)-*b*-Poly(L-glutamic acid) Polymersomes: Size Control & Stability *Langmuir* **26**(2010), 2751

⁵² C. Sanson, O. Diou, E. Ibarboure, A. Soum, A. Brûlet, S. Miraux, E. Thiaudière, S. Tan, A. Brisson, V. Dupuis, O. Sandre, S. Lecommandoux, Doxorubicin Loaded Magnetic Polymersomes: a Multifunctional Nanocarrier for Theranostics, *ACS Nano* **5**(2) 1122-1140 (2011)

où d_{core} est le diamètre moyen des nanoparticules employées, M_{spe} leur aimantation spécifique, Φ_{intra} la fraction d'oxyde de fer dans l'objet de diamètre externe d . Bien que la vitesse de migration v_{mag} dans un gradient de l'ordre de 1 Tesla/cm soit de l'ordre de 1 $\mu\text{m/s}$, l'application d'un gradient localisé reste susceptible de provoquer une accumulation de vésicules magnétiques injectées dans la circulation sanguine si l'on tient compte de facteurs comme la perméabilité particulièrement élevée des tissus tumoraux.⁵³ Des expériences *in vitro* dans ce sens sont programmées (*cf.* projets).

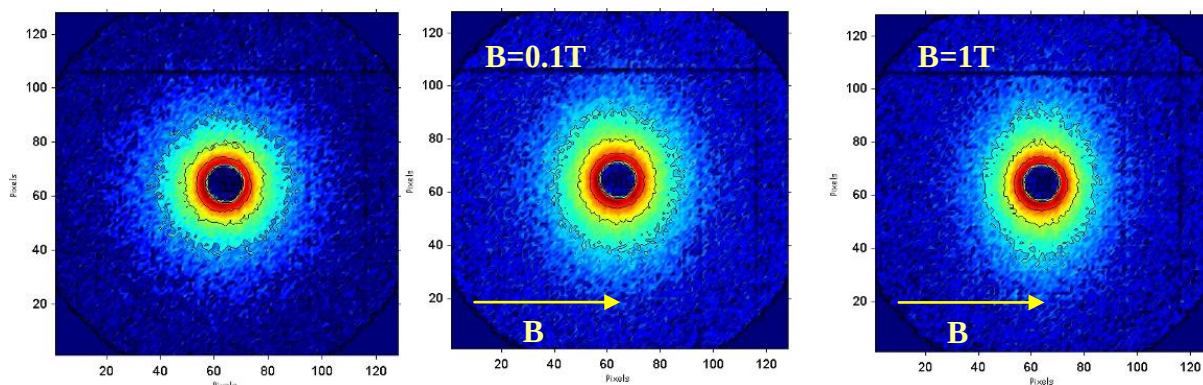


Fig. 23 : Figures de diffusion des neutrons aux petits angles des vésicules WD15-50, sous différentes valeurs de champ, pour une gamme de vecteurs de diffusion $q = 3 \times 10^{-3} - 3 \times 10^{-2} \text{ \AA}^{-1}$. Le code couleur correspond aux courbes iso-intensité.

Comme pour la première génération de vésicules, la DNPA a révélé le caractère vésiculaire de ces objets, ainsi que leur déformabilité sous champ magnétique. En plus l'AFM et la TEM réalisées au LCPO, la cryo-TEM réalisée dans le groupe d'Alain Brisson au CBMN a confirmé l'insertion des nanoparticules dans les membranes, donnant des images sans artefacts de séchage. Charles Sanson a optimisé par ailleurs la co-encapsulation d'une drogue anti-cancéreuse anthracycline, la doxorubicine (DOX) en même temps que les nanoparticules : des vésicules chargées à 50% en nanoparticules de 6-7 nm et 20% en DOX relativement au copolymère restent bien dispersées avec un $d_H=122 \text{ nm}$ (PDI=0.15).

La DOX est dosée par fluorescence dans une expérience de libération *in vitro* réalisée dans une membrane de dialyse. Ainsi son profil temporel de libération par les vésicules a-t-il pu être mesuré dans différentes conditions : en variant la température (5°C, 20°C, 37°C et 45°C), sous champ magnétique statique (sans effet), et à 23°C mais en présence d'un champ magnétique radio-fréquences (500 kHz 2.6mT) chauffant localement les nanoparticules magnétiques, et donc la membrane dans laquelle elles sont confinées. Cette expérience a prouvé le concept de libération de drogue par chauffage radio-fréquences, sans élévation macroscopique de la température.⁵²

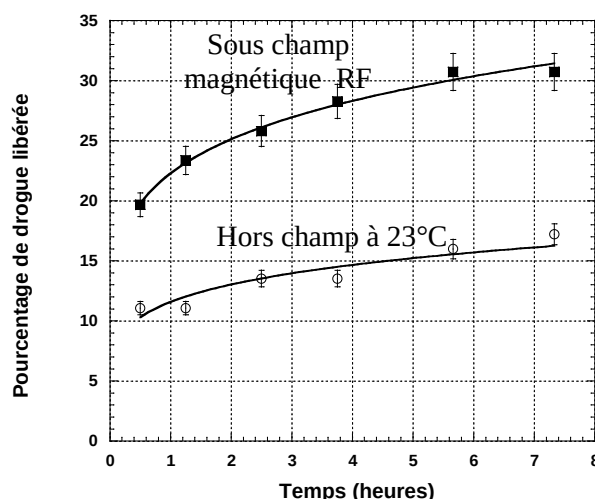


Fig. 24 : Profils de libération de la DOX.⁵²

Julie Thévenot, post-doctorante dans le cadre du projet NanoTher, a examiné le rôle de la taille des nanoparticules sur l'auto-assemblage. Pour cela j'avais préparé trois lots de nanoparticules de distributions de tailles indiquées par le Tableau 3, qui ont tous été recouverts de Beycostat pour être

⁵³ A. Nacev, C. Beni, O. Bruno, B. Shapiro, The behaviors of ferromagnetic nano-particles in and around blood vessels under applied magnetic fields, *J. Mag. Mag. Mat.* **323** (2011) 651-668

co-assemblés avec le PTMC-*b*-PGA à partir d'un mélange dans le DMSO. On a constaté que le pourcentage massique maximal d'oxyde de fer pouvant rentrer dans les membranes est de 70% pour les 6-7 nm, 50% pour les 8-10 nm et seulement 30% pour les 10-15 nm. L'influence respectivement de la taille des nanoparticules et du "feed weight ratio" (FWR) dans les membranes sur l'efficacité des vésicules en tant qu'agents de contraste négatif pour l'IRM est en cours d'étude avec des chercheurs italiens de l'INSTN, Paolo Arosio, Claudia Innocenti et Alessandro Lascialfari.⁵⁴ Une comparaison avec la loi d'échelle mise en évidence avec Yves Gossuin de l'université de Mons sera bientôt possible.⁵⁵ En ce qui concerne l'étude du ciblage « actif » des polymersomes vers les cellules tumorales, Hugo de Oliveira post-doctorant dans l'équipe au LCPO, a d'ores et déjà réussi le greffage d'herceptine, un anticorps monoclonal utilisé en thérapie qui est dirigé contre le récepteur HER2 des cellules métastatiques du cancer du sein. L'efficacité du ciblage est évalué à la fois *in vitro* par cytométrie de flux à l'institut CIC-bioGUNE de Bilbao et par bio-distribution *in vivo* par IRM au RMSB à Bordeaux et par tomographie par émission de positrons à l'université d'Athènes, tandis que l'efficacité thérapeutique pour faire régresser des tumeurs chez la souris est étudiée dans une entreprise pharmaceutique de Madrid... On se rend compte ainsi de l'effet de levier d'un réseau européen pour faire évaluer un système à visée thérapeutique en parallèle par différents partenaires!

Fraction du tri en tailles	Plage de tailles	d_H dans CH_2Cl_2 nm / PDI	Diamètres moyens par TEM	Diamètres moyens par VSM	Aimantation spécifique emu/g A/m	Relaxivité R_2 (1.5T) $s^{-1} mM^{-1}_{Fe}$
S1S2S3	6-7 nm	16 / 0.22	$d_n=4.9$ nm, $d_w=6.9$ nm ($N=600$)	$d_n=6.5$ nm, $d_w=7.5$ nm	55 ± 1 2.8×10^5	70
S1C2	8-10 nm	32 / 0.29	$d_n=8.5$ nm, $d_w=11.6$ nm ($N=3800$)	$d_n=8.2$ nm, $d_w=10.0$ nm	60 3.0×10^5	104
C1C2S3S4	10-15 nm	42 / 0.27	$d_n=13.8$ nm, $d_w=20$ nm ($N=1140$)	$d_n=10.4$ nm, $d_w=14.8$ nm	70 ± 1 3.5×10^5	293

Tableau 3 : Caractéristiques des diamètres (moyennes en nombre et en poids) de trois lots de nanoparticules, d'après la microscopie électronique et la magnétométrie. L'aimantation spécifique à saturation et la relaxivité transverse (efficacité comme agent de contraste négatif en IRM) sont aussi indiqués.

Que ce soit pour des micelles magnétiques de PEG-*b*-PBLG ou des vésicules de PTMC-*b*-PGA, la fraction volumique d'oxyde de fer à l'intérieur des objets sera $\phi_{intra} = N_{object}^{USPIO} \left(\frac{d_{USPIO}}{2R_G} \right)^3$ où le nombre de nanoparticules "ultra-small superparamagnetic iron oxide" est calculé géométriquement (Fig. 24) :

$$N_{vesicle}^{USPIO} = \pi \frac{d_H^2 \times \delta_{mb}}{V_{USPIO}} \times \frac{FWR}{\rho_{Fe_2O_3}} \left/ \left(\frac{FWR}{\rho_{Fe_2O_3}} + 1 - FWR \right) \right. \quad (\text{Eq. 2}) \quad \text{dans les vésicules}$$

$$\text{ou} \quad N_{micelle}^{USPIO} = \frac{\pi}{6} \frac{(0.775d_H)^3}{V_{USPIO}} \times \frac{FWR}{\rho_{Fe_2O_3}} \left/ \left(\frac{FWR}{\rho_{Fe_2O_3}} + 1 - FWR \right) \right. \quad (\text{Eq. 3}) \quad \text{dans les micelles.}$$

⁵⁴ P. Arosio, T. Orlando, F. Orsini, M. Corti, M. Mariani, C. Innocenti, J. Thévenot, H. de Oliveira, O. Sandre*, S. Lecommandoux, A. Lascialfari*, Hybrid Iron Oxide-Copolymer Nanoparticles as Improved Contrast Agents for Magnetic Resonance Imaging, *en preparation*.

⁵⁵ Y. Gossuin*, J-F. Berret, Q. L. Vuong, J. Fresnais, O. Sandre*, Universal Scaling Law to Predict the Efficiency of Magnetic Nanoparticles as MRI T2-Contrast Agents, *soumis*.

Ces formules sont compatibles avec les images de TEM, où elles conduisent par exemple pour un taux de chargement $FWR=10\%$ à une trentaine de nanoparticules de 6-7 nm dans une vésicule de PTMC-*b*-PGA et seulement 2 en moyenne pour les plus grosses tailles d'USPIO, 10-15 nm (Fig. 25).

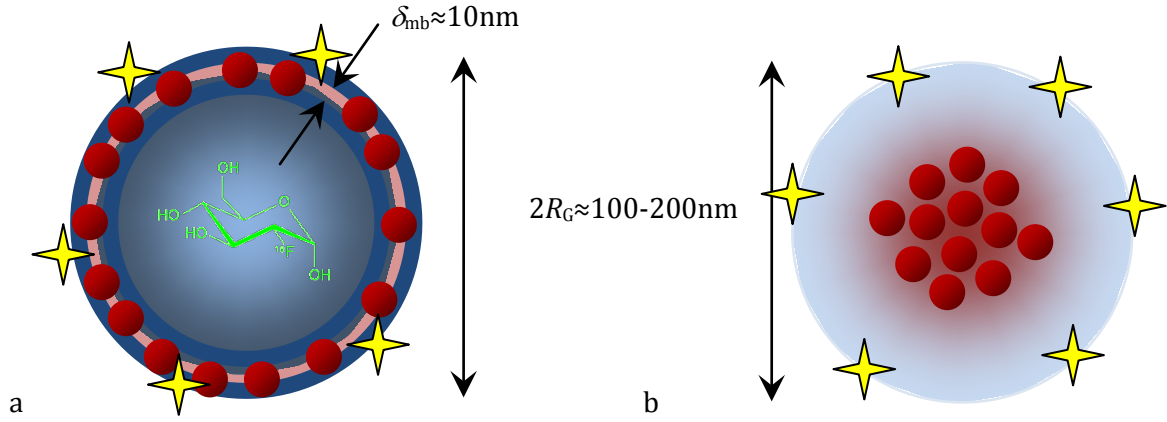


Fig. 24: Croquis permettant d'évaluer géométriquement le nombre de nanoparticules (USPIO) par objet dans le cas des vésicules (a) et des micelles (b). Le ratio théorique $2R_G/d_H$ est de 1 pour les vésicules et 0.775 pour les micelles. Les étoiles représentent des fluorophores pouvant aisément être greffés sur les blocs hydrophiles (se terminant par un groupement $-NH_2$). Une espèce hydrophile peut être encapsulée dans le compartiment aqueux des vésicules (ici du ^{18}F -désoxyglucose qui est un marqueur pour l'imagerie TEP).

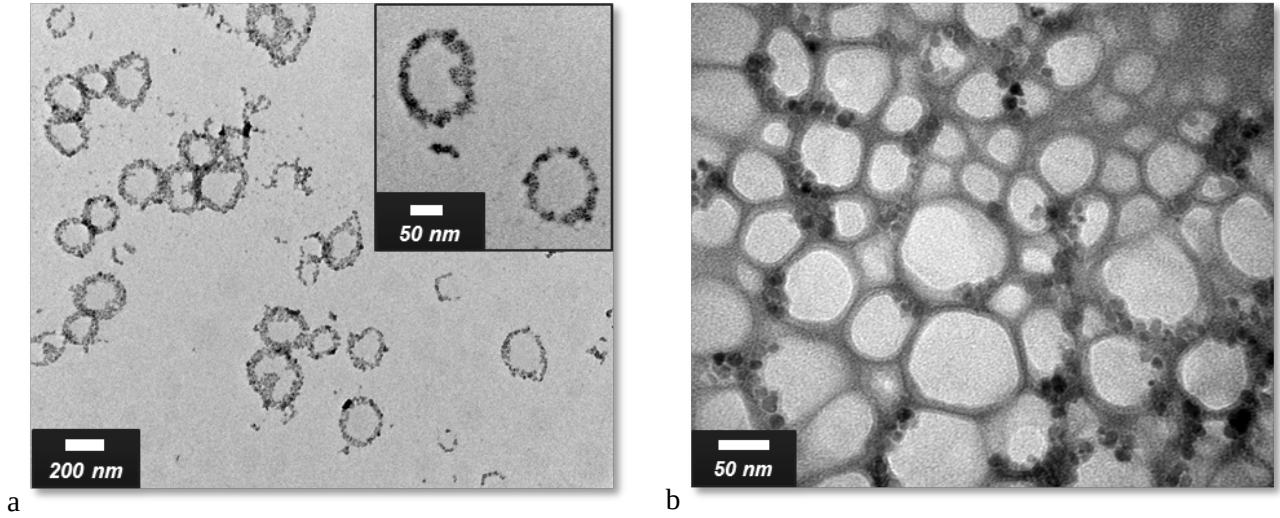


Fig. 25 : Vésicules de PTMC-*b*-PGA dopées avec un $FWR=10\%$ de nanoparticules 6-7 nm (a) et 10-15 nm (b). Clichés pris par Julie Thévenot sur un TEM à 80 kV Hitachi H7650 au Bordeaux Imaging Center.

En particulier on espère vérifier la loi d'échelle que l'on a mise en évidence sur une série d'agrégats contrôlés de nanoparticules préparés par coacervation et d'autres tirés de la littérature :⁵⁵

$$r_2 = \frac{R_2}{[Fe]} = \frac{2}{405} \frac{M_{Fe_2O_3}}{\rho_{Fe_2O_3}} \frac{\gamma^2 \mu_0^2}{D_{H_2O}} \Phi_{intra} M_{spe}^2 d^2 \quad (Eq. 4)$$

où l'on retrouve les mêmes dépendances que la vitesse magnétophorétique en fonction de M_{spe} , Φ_{intra} et d . En d'autres termes, **l'optimisation de vecteurs théranostiques peut se faire simultanément pour les propriétés de contraste IRM et de guidage par un gradient de champ magnétique.**

III. Films lamellaires avec des copolymères diblocs symétriques synthétisés par ATRP

Codirection avec **Valérie Cabuil** de la thèse de **Siham Douadi-Masrouki**, complétée par le stage IUT de **Romain Pintat** en collaboration avec **Maud Save** et **Bernadette Charleux** du Laboratoire Chimie des Polymères UMR7610 CNRS / UPMC et **Jean-Jaques Rousseau** du laboratoire DIOM de l'Université Jean Monnet à St Etienne.

Cette collaboration s'inscrivait initialement dans le cadre du réseau d'excellence européen FAME, tourné vers la recherche sur les matériaux fonctionnels, et devenu depuis un master international Erasmus Mundus. Elle avait pour objectif d'associer les propriétés d'auto-organisation des polymères synthétisés au LCP avec les propriétés d'orientation sous champ des nanoparticules magnétiques, afin de réaliser des éléments optiques contrôlables par un champ magnétique B appliqué de deux types :

(i) soit des films réfléchissants de type cristaux photoniques, avec un spectre de longueurs d'onde réfléchies large (visible, infrarouge, GHz...) et éventuellement sensible au champ B ;

(ii) soit des lames transparentes biréfringentes, pour moduler la polarisation de la lumière grâce à un champ B alternatif.

Les étapes importantes dans la création de ces matériaux étaient : la synthèse de la matrice polymère organisée en multi-couches réfléchissante (i) ou isotrope transparente (ii) ; le greffage ou l'adsorption de polymères à la surface des nanoparticules afin de les inclure facilement dans la matrice ; l'étude de l'organisation des nanoparticules au sein de la matrice. De tels systèmes lamellaires magnétiques avaient déjà été conçus une dizaine d'années plus tôt à l'occasion d'une collaboration entre Le Mans, Paris et Strasbourg.⁵⁶ L'obtention de nouveaux échantillons par des procédés de synthèse un peu différents était nécessaire pour reprendre et étendre ces travaux.

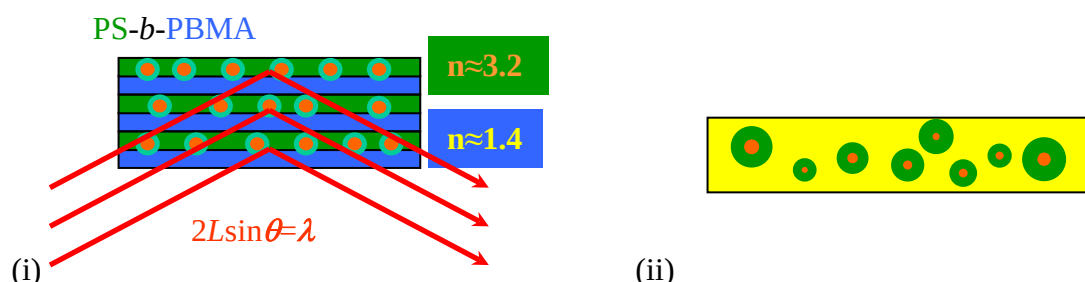


Fig. 26 : Schémas des deux systèmes de nanoparticules magnétiques (en orange) greffées par du polystyrène (en vert) et dispersées (i) soit dans une matrice lamellaire de copolymère polystyrène-*b*-polyméthacrylate de *n*-butyle (PS-*b*-PBMA) ; (ii) soit dans une matrice transparente, isotrope en champ nul et devenant biréfringente sous champ magnétique.

La réalisation de composants pour l'optique intégrée et les ondes Gigahertz utilisées en téléphonie mobile nécessite des matériaux en couche mince qui présentent une forte anisotropie. Les recherches actuelles se portent sur des matériaux de moindre coût et dont la mise en forme ne nécessite pas de traitement à haute température. Les copolymères di-blocs A-B conviennent car un simple recuit n'excédant pas 150 à 200°C suffit pour qu'ils s'auto-organisent en structures périodiques, de types lamellaires pour les copolymères symétriques (A et B de même poids moléculaire). Les films minces que l'on cherche à obtenir, de l'ordre du micron d'épaisseur, avec des couches multiples présentant une périodicité de plusieurs dizaines de nm (voire 100 nm) dopées fortement en nanoparticules magnétiques (de diamètre environ 6-7 nm) constitueraient peut-être un exemple de matériau à bandes interdites photoniques.⁵⁷ Les systèmes réfléchissants (i) trouveraient

⁵⁶ B. Hamdoun, D. Ausserre, S. Joly, Y. Gallot, V. Cabuil, C. Clinard, New Nanocomposite Materials, *J. Phys. II* (1996), **6**(4), 493-501 ; B. Hamdoun, D. Ausserre, V. Cabuil, S. Joly, Composites copolymères-nanoparticules : I. Période lamellaire dans le régime des "petites nanoparticules", *J. Phys. II* (1996), **6**(4), 503-510.

⁵⁷ Edrington, A. C., Urbas, A. M., DeRege, P., Chen, C. X., Swager, T. M., Hadjichristidis, N., Xenidou, M., Fetters, L. J., Joannopoulos, J. D., Fink, Y., Thomas, E. L., Polymer-Based Photonic Crystals, *Adv. Mater.* **2001**, *13*, 421-425.

ainsi des applications en tant que guides d'onde pour la lumière proche-infrarouge ($\lambda=1.55 \mu\text{m}$) à l'intérieur de fibres optiques polymères, présentant une bonne souplesse mécanique. Les structures auto-assemblées de copolymères di-blocs ont été proposées pour obtenir des BIP en raison de leurs différents types d'organisation (lamelles, cylindres en arrangement hexagonal, réseau cubique de sphères, réseaux bi-continus « gyroïdes »...), la périodicité de ces structures pouvant être ajustée par le choix du copolymère. De plus le fort contraste diélectrique entre les couches dopées par les nanoparticules inorganiques (indice $n\approx 3.2$) et les couches non dopées ($n\approx 1.4$) permettrait de réduire le nombre de couches périodiques nécessaire.

Pour les applications de type (ii) concernant les lames biréfringentes magnéto-induites, on recherchait au contraire une bonne transparence dans le domaine visible, c'est à dire l'absence d'inhomogénéités de taille supérieure à 50 nm. Dans ce cas les copolymères seraient utilisés uniquement pour recouvrir les nanoparticules, mais celles-ci seraient dispersées dans une matrice de polymère réticulé homogène. Pour concurrencer les modulateurs électro-optiques, relativement chers, il faudrait que les nanoparticules puissent se réorienter dans la matrice au moins jusqu'à des fréquences de 100 kHz, c'est à dire qu'elles devraient avoir une liberté de rotation aussi grande que dans un ferrofluide liquide. Les lames polarimétriques à base de matrices de silice obtenues par sol-gel ne répondent pas à cette exigence, car les nanoparticules magnétiques sont partiellement emprisonnées dans le réseau de silice, créant ainsi un phénomène de rémanence.⁵⁸ Dans ce projet déposé au BQR de l'UPMC, on proposait de conserver la liberté d'orientation des nanoparticules en les protégeant dans des cages fluides, par exemple avec une couronne de polymère au-dessus de sa T_g ou bien dans des gouttelettes de micro-émulsion, que l'on emprisonnait ensuite dans une matrice solide (matériau préparé en polymérisant l'émulsion d'une ferrofluide surfacté dans une phase continue contenant un monomère acrylique, que j'avais baptisé « émugel »). Mais le financement BQR n'ayant pas été obtenu, le projet a été malheureusement abandonné...

Au terme de l'étude, les échantillons devaient être envoyés au laboratoire Dispositifs et Instrumentation en Optoélectronique et Micro-ondes de l'Université Jean-Monnet pour y analyser leurs propriétés d'absorption et de réflexion dans le spectre visible et micro-ondes, mais au final seule une étude par ellipsométrie multi-longueurs d'ondes a pu être accomplie. Siham Douadi-Masrouki a néanmoins pu réaliser un travail avec le DIOM en parallèle de sa thèse sur des lames polarimétriques dopées en ferrofluides à base de silice sol-gel (mesures de la « rotation Faraday »).⁵⁹

Films lamellaires de copolymères di-blocs non dopés

On visait dans ce travail des phases lamellaires de copolymères di-blocs A-B. La première condition était donc de partir sur des degrés de polymérisation équivalents pour chaque bloc A et B ($N_A\approx N_B$). Alors la condition d'obtention d'un ordre lamellaire s'écrit $\chi N > 10.5$ pour un di-bloc symétrique, χ étant le paramètre de Flory-Huggins décrivant l'interaction entre les deux types de segments à la température de recuit.⁶⁰ On a choisis les blocs modérément incompatibles que sont le polystyrène (PS) et le poly(métacrylate de *n*-butyle) (PBMA),⁶¹ différant aussi fortement par leurs températures de transition vitreuse (respectivement 100°C et 30°C environ), ce qui n'aurait pas été le cas avec le copolymère plus courant PS-*b*-PMMA. On estimait $\chi_{140^\circ\text{C}}=0.045$ d'après une étude par DNPA de mélanges binaires d'homopolymères PS (deuté à 10%) et de PBMA, qui conduisait à $\chi/V_0=2.5\times 10^{-4} \text{ \AA}^{-3}$ (avec par ailleurs un volume moléculaire moyen $V_0=(V_{\text{PS}}V_{\text{PBMA}})^{1/2}\approx 180 \text{ \AA}^3$).⁶² On

⁵⁸ F. Chaput, J.-P. Boilot, M. Canva, A. Brun, R. Perzynski, D. Zins, *J. Non-Cryst. Solids* **160** (1993) 177-179.

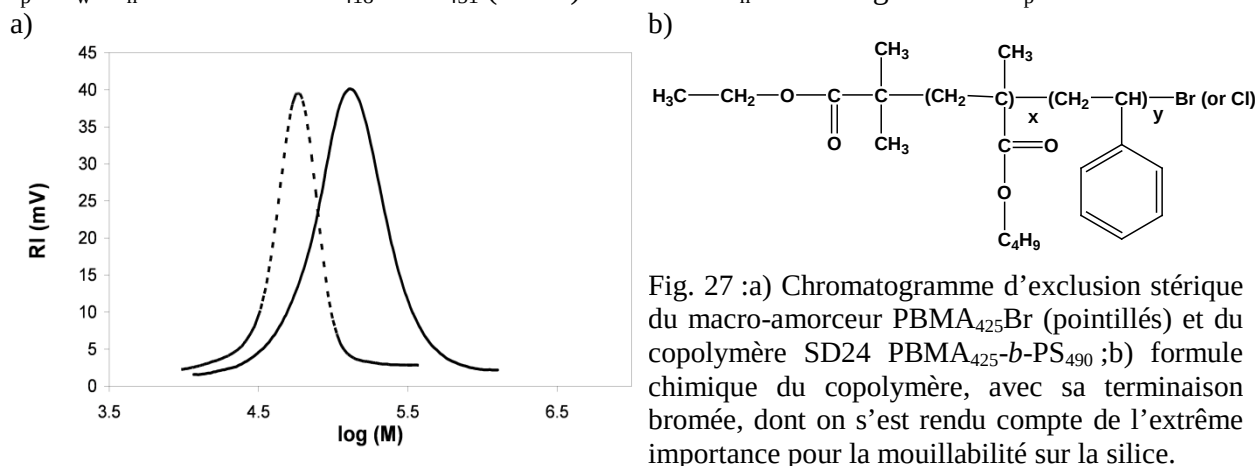
⁵⁹ F. Choueikani, F. Royer, S. Douadi, A. Skora, D. Jamon, D. Blanc, A. Siblini, *Eur. Phys. Low birefringent magneto-optical waveguides fabricated via organic-inorganic sol-gel process*, *J. Appl. Phys.* **47** (2009) 30401

⁶⁰ L. Leibler, *Theory of Microphase Separation in Block Copolymers*, *Macromolecules* **1980**, *13* (6), 1602-1617.

⁶¹ G. Vignaud, A. Gibaud, G. Grübel, S. Joly, D. Ausserré, J-F. Legrand, Y. Gallot, *Ordering of diblock PS-PBMA thin films: An X-ray reflectivity study* *Physica B: Condensed Matter*, **248** (1998) 250

⁶² Hammouda, B.; Bauer, B. J.; Russell, T. P., *Small-Angle Neutron Scattering from Deuterated Polystyrene/Poly(butyl methacrylate) Homopolymer Blend Mixtures*, *Macromolecules* **1994**, *27* (8), 2357-2359.

connaissait aussi $\chi_{160^\circ\text{C}} = 0.037$ pour le système d-PS / PMMA,⁵⁰ proche du nôtre. On en déduisait que le degré de polymérisation minimum N de chaque bloc devait être supérieur à 300. Considérant ce cahier des charges précis, on s'est orienté vers la synthèse de PS-*b*-PBMA de haute masse molaire par polymérisation radicalaire contrôlée par transfert d'atomes (ATRP), ce qui constituait en soit un challenge pour la chimie, le contrôle des réactions radicalaires n'étant aisé à garder jusqu'à des degrés de conversion de monomères élevés, nécessaires pour obtenir de fortes masses. Durant sa première année de thèse, Siham a réussi à préparer par ATRP deux lots de copolymères de tailles de blocs symétriques, de masses molaires importantes et disponibles en quantité suffisante pour les expériences suivantes : PBMA₄₂₅-*b*-PS₄₉₀ (échantillon SD24) de masse $M_n=105400$ g/mol et d'indice $I_p=M_w/M_n=1.38$ et PBMA₄₁₈-*b*-PS₄₅₁ (SD25) de masse $M_n=112000$ g/mol et d' $I_p=1.44$.⁶³



Des films de copolymères purs ont été déposés par spin-coating sur un substrat (lame de verre ou wafer de silicium pour l'optique, alumine pour sa faible absorption des micro-ondes). Tant qu'on cherchait à caractériser les structures, on utilisait des substrats atomiquement plans tels que le mica clivé ou le silicium pour les imager en microscopie à force atomique (AFM). La microscopie optique interférentielle était aussi utilisée donner une information qualitative rapide sur la qualité des films.

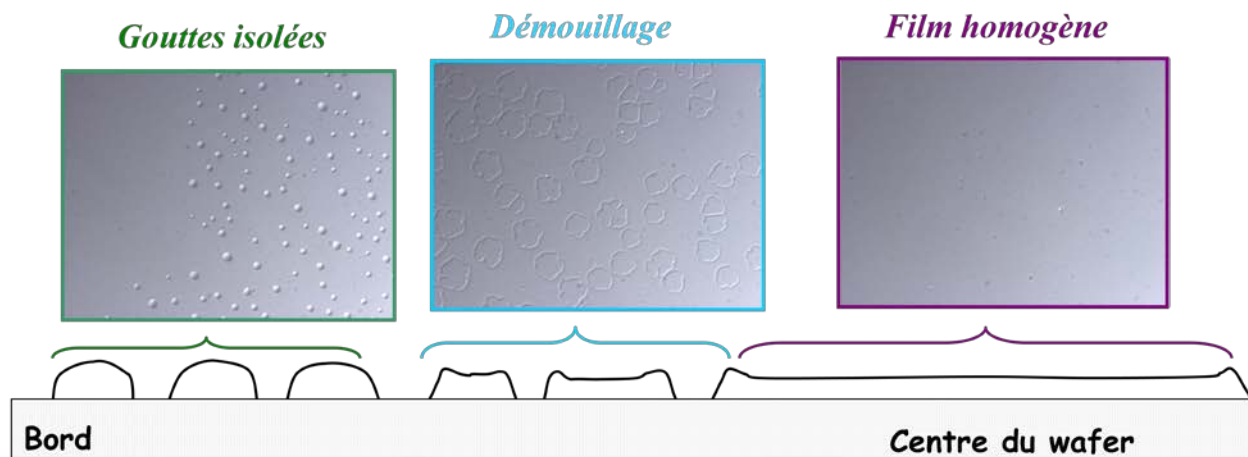


Fig. 28: a) Microscopie optique en réflexion avec un film de PBMA₄₁₈-*b*-PS₄₅₁ sur mica, observé avant recuit.

L'AFM est une méthode quantitative pour mesurer les dimensions latérales des structures, avec une résolution nanométrique en hauteur. Malgré les difficultés liées à la fragilité mécanique des échantillons de copolymères, on a pu avoir une idée de la période lamellaire à partir de la statistique des hauteurs mesurées des défauts de surface (Fig. 29), de types îlots ou trous selon la quantité de

⁶³ M. Save, B. Charleux, S. Douadi, O. Sandre, V. Cabuil, B. Hamdoun, "Synthesis of high molar mass poly(n-butyl methacrylate)-*b*-polystyrene diblock copolymers by ATRP: Formation of lamellar phases in thin films" *Polymer Preprints* (2005), 46(2), 440-441.

copolymère déposée.⁶¹ On a obtenu ainsi une estimation de la période $L_{AFM}=32.7\pm1.5$ nm, valeur vraisemblablement un peu sous-évaluée par l'enfoncement de la pointe d'AFM dans le copolymère.

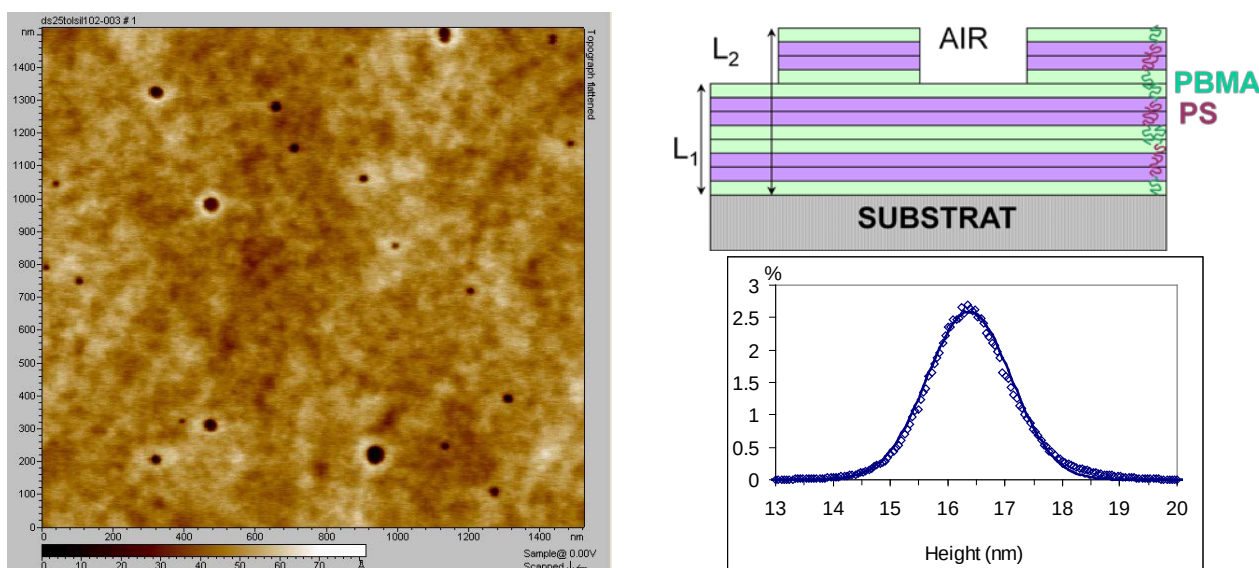


Fig. 29 : Film de PBMA₄₂₅-*b*-PS₄₉₀, d'épaisseur totale 60 nm d'après ellipsométrie (environ trois bicouches) observé par AFM. Sa surface présente des trous dont la statistique des hauteurs, $H_0=16.37\pm0.73$ nm, reflète indirectement la période lamellaire $L_2-L_1=L_{AFM}/2$.

On a mesuré en effet une valeur de période plus élevée $L_{NR}=38\pm1$ nm avec la réflectivité des neutrons au LLB-CEA Saclay (Figure 30).⁶⁴ Cette valeur était en total accord avec la loi d'échelle de la période moyenne en fonction de la masse molaire $L_{moy} \sim M_n^{0.69}$ qu'on a pu déduire de la littérature pour les diblocs PS-*b*-PBMA (Figure 31), avec un exposant proche de celui de la théorie de Helfand décrivant l'étirement des chaînes due à leur compaction latérale pour se ranger en lamelles.⁶⁵

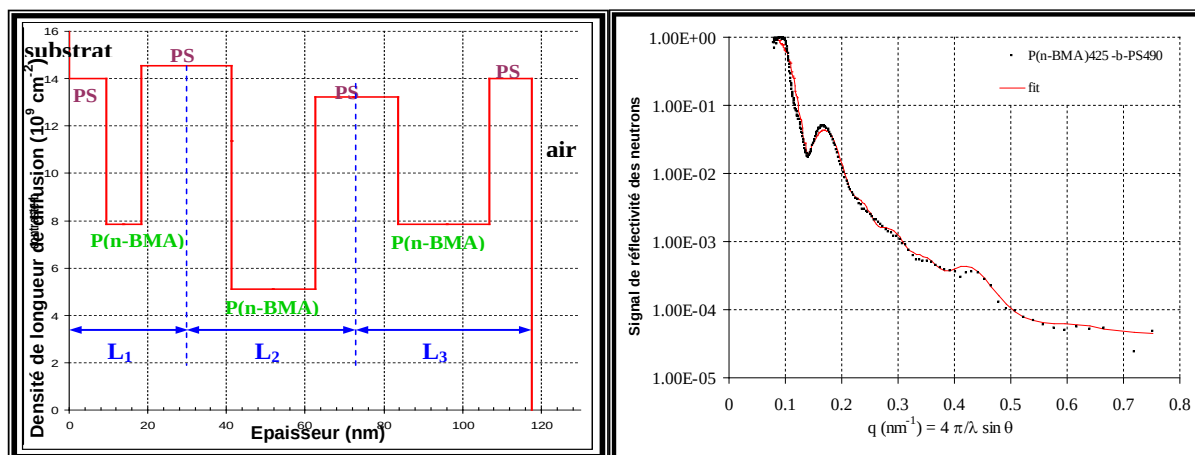


Fig. 30 : Courbe de réflectivité des neutrons d'un film mince de PBMA₄₂₅-*b*-PS₄₉₀ déposé sur silicium et son ajustement par un profil de densité de longueur de diffusion alternant entre des couches PS et PBMA. Contrairement aux travaux précédents utilisant un copolymère synthétisé par voie anionique, notre étude de réflectivité a conclu que le silicium était au contact de blocs PS et non pas PBMA, ce qu'on a attribué à une augmentation de la polarité des extrémités de chaînes terminées pas un atome de brome (Figure 27b).

⁶⁴ S. Douadi-Masrouki, B. Frka-Petesic, M. Save, B. Charleux, V. Cabuil, O. Sandre, Incorporation of magnetic nanoparticles into lamellar polystyrene-*b*-poly-(*n*-butyl methacrylate) diblock copolymer films: Influence of the chain end-groups on nanostructuration, *Polymer* **51** (2010) 4673-4685

⁶⁵ Helfand, E., *Macromolecules* **1975**, *8*, 552-556; Helfand, E.; Wasserman, Z. R., *Macromolecules* **1976**, *9*, 879-888.

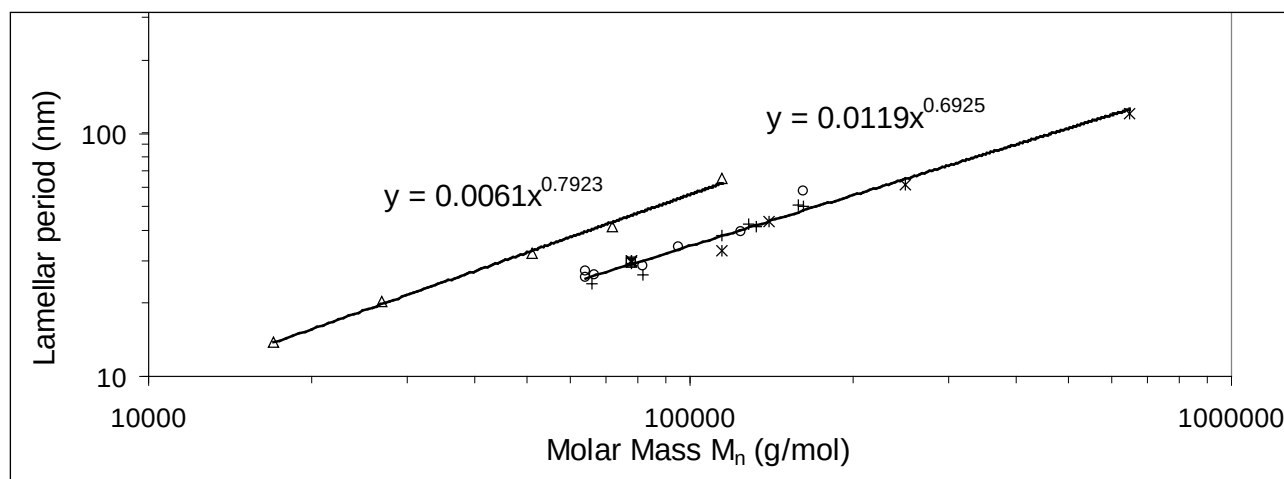


Fig. 31 : Lois d'échelle issues de l'étude bibliographique sur les valeurs publiées de période lamellaires L_{moy} en fonction de la masse molaires M_n . Triangles: mesures par SAXS pour PS-*b*-PI;⁶⁶ Cercles: mesures par SAXS pour PS-*b*-PBMA⁶⁷ ou PS-*b*-PBMA-MAH⁶⁸ et par DNPA pour dPS-*b*-PBMA;⁶⁹ Croix: mesures par réflectivité des neutrons pour (d)PS-*b*-PBMA^{70,71} ou PBMA-*b*-PS (ce travail); Astérisques: mesures d'après la hauteur des défauts à la surface des films, soit par microscopie optique interférentielle,⁷² soit par AFM^{71,73} pour (d)PS-*b*-PBMA ou PBMA-*b*-PS (ce travail); Carrés: mesures par réflectivité des rayons X pour (d)PS-*b*-PBMA.^{71,74} Les polymères ont toujours été recuits vers 150°C-165°C, excepté pour les masses molaires en-dessous de 70000 g.mol⁻¹ qu'il était nécessaire de recuire entre 200 et 260°C pour induire l'ordre lamellaire.

Polymérisation contrôlée du styrène à la surface des nanoparticules magnétiques

En vue des applications magnéto-optiques, on a réfléchi à optimiser le matériau magnétique afin qu'il présente un fort couplage des moments magnétiques et des axes d'anisotropie optique des grains, ainsi que de bonnes propriétés d'absorption en hyperfréquences. Le ferrite de cobalt CoO.Fe₂O₃ pouvait sembler optimal, cependant sa stabilité chimique est bien moins grande que la maghémite γ -Fe₂O₃, et par ailleurs on peut beaucoup moins bien contrôler les formes et les tailles des nanoparticules. Suivant une idée évoquée dans la littérature,⁷⁵ on a synthétisé spécialement pour cette étude des nanoparticules de ferrites mixtes Co_xFe_(1-x)O.Fe₂O₃, dont on a montré que leur aimantation spécifique M_{spe} était très élevée (4.0×10^5 et 4.6×10^5 A/m pour $x=3\%$ et 8% respectivement, soit 80 et 92 emu/g en unités CGS) alors que pour des nanoparticules de magnétite (ferrite de fer pur) ou de maghémite de taille équivalente, M_{spe} est d'ordinaire au mieux égale à 3.5×10^5 A/m (soit 70 emu/g).

⁶⁶ Hadziioannou, G.; Skoulios, A., *Macromolecules* **1982**, 15 (2), 258-262.

⁶⁷ Russell, T. P.; Karis, T. E.; Gallot, Y.; Mayes, A. M., *Nature* **1994**, 368, 729-731.

⁶⁸ Kim, J. K.; Kim, M. I.; Kim, H. J.; Lee, D. H.; Jeong, U.; Jinnai, H.; Suda, K., *Macromolecules* **2007**, 40 (21), 7590.

⁶⁹ Pollard, M.; Russell, T. P.; Ruzette, A. V.; Mayes, A. M.; Gallot, Y., *Macromolecules* **1998**, 31 (19), 6493-6498.

⁷⁰ Mansky, P.; Tsui, O. K. C.; Russell, T. P.; Gallot, Y., *Macromolecules* **1999**, 32, 4832-4837; Lauter-Pasyuk, V.; Lauter, H. J.; Gordeev, G. P.; Muller-Buschbaum, P.; Toperverg, B. P.; Jernenkova, M.; Petry, W., *Langmuir* **2003**, 19 (19), 7783-7788; Lauter-Pasyuk, V.; Lauter, H. J.; Ausserre, D.; Gallot, Y.; Cabuil, V.; Hamdoun, B.; Kornilov, E. I., *Physica B: Condensed Matter* **1998**, 248 (1-4), 243-245; Lefebure, S.; Cabuil, V.; Ausserre, D.; Paris, F.; Gallot, Y.; Lauter-Pasyuk, V., *Prog. Colloid Polym. Sci* **1998**, 110, 94-98; Lauter-Pasyuk, V.; Lauter, H. J.; Ausserre, D.; Gallot, Y.; Cabuil, V.; Kornilov, E. I.; Hamdoun, B., *Physica B: Condensed Matter* **1998**, 241-243, 1092-1094.

⁷¹ Joly, S.; Ausserre, D.; Brotons, G.; Gallot, Y., *European Physical Journal E* **2002**, 8, 355-363.

⁷² Coulon, G.; Collin, B.; Chatenay, D.; Gallot, Y., *J. Phys. II* **1993**, 3 (5), 697-717.

⁷³ Hamdoun, B.; Ausserre, D.; Joly, S.; Gallot, Y.; Cabuil, V.; Clinard, C., *J. Phys. II* **1996**, 6, 493-501; Fasolka, M. J.; Banerjee, P.; Mayes, A. M.; Pickett, G.; Balazs, A. C., *Macromolecules* **2000**, 33, 5702-5712.

⁷⁴ Vignaud, G.; Gibaud, A.; Grübel, G.; S. Joly; Ausserré, D.; Legrand, J.-F.; Gallot, Y., *Physica B* **1998**, 248, 250-257.

⁷⁵ S. Li, V. T. Johna, C. O'Connor, V. Harris, E. Carpenter *J. Appl. Phys.* (2000) **87**, 6223-6225; S Chakrabarti, S. K. Mandal, S. Chaudhuri, *Nanotechnology* **16** (2005) 506-511.

Une autre tâche importante de la thèse portait sur l'enrobage des nanoparticules inorganiques par des chaînes de polystyrène, afin de les rendre compatibles avec les blocs PS du copolymère. Plutôt qu'un procédé par adsorption d'un polymère fonctionnalisé en bout de chaîne par un groupe complexant de l'oxyde de fer ("grafting-on"), on s'est attaqué au problème ardu de faire croître des chaînes de PS à la surface des nanoparticules ("grafting-from"), tout en conservant un contrôle de la réaction (faible I_p) et surtout un état d'agrégation pas trop élevé pour pouvoir espérer ensuite les disperser au sein des lamelles. Un résultat satisfaisant a été obtenu en optimisant les conditions d'ATRP (synthèse en masse plutôt qu'en solvant) et en utilisant des nanoparticules triées en tailles.⁶⁴

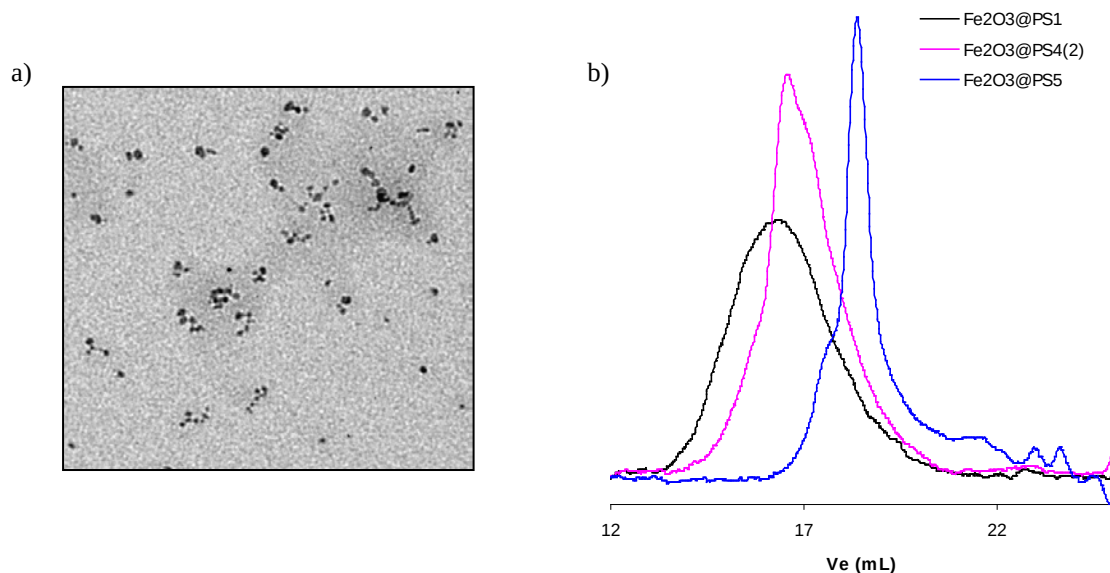


Fig. 32 : a) Nanoparticules magnétiques recouvertes d'une couche de PS observée par TEM par coloration avec RuO_4 ; b) Chromatographie d'exclusion stérique après greffage des chaînes de PS montrant un affinement de la distribution des chaînes ($I_p=1.4$) obtenu en modifiant les conditions de greffage par ATRP.

Films lamellaires dopés par les nanoparticules magnétiques

Après mélange de copolymère PBMA-*b*-PS avec des nanoparticules $\text{Fe}_2\text{O}_3@PS$ dans un co-solvant (toluène), dépôt sur silicium par spin-coating et recuit sous vide à 150°C , des films dopés en nanoparticules magnétiques d'épaisseur variées (mesurées par ellipsométrie) ont été obtenus.

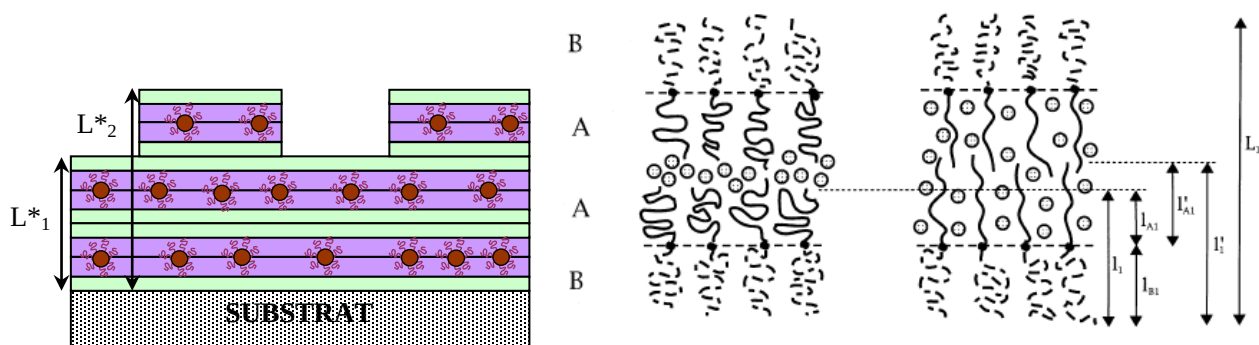


Fig. 33 : Schémas d'un film lamellaire PS-PBMA dopé par des nanoparticules recouvertes de chaînes de PS. Le croquis de droite tirée du travail théorique original de la thèse de B. Hamdoun⁵⁶ illustre les deux cas limites possibles selon que les nanoparticules sont expulsées entre les lamelles ou bien gonflent les chaînes de PS.

Grâce à la réflectivité des neutrons, on a montré un confinement des nanoparticules $\gamma\text{-Fe}_2\text{O}_3@PS$ dans les premières couches de PS en partant du substrat. Certes les fractions volumiques atteintes en nanoparticules magnétiques dans le film étaient plutôt faibles (Φ entre 0.025% et 0.15%), mais la période lamellaire a suivi la loi $L_{\text{moy}}=L_0(1+p\Phi)$ prédite par la théorie. Néanmoins même en tenant

compte du volume des chaînes de PS greffées, le paramètre p expérimental était aux alentours de 6 alors que sa valeur théorique est au maximum égale à 1 dans le cas où les nanoparticules gonflent les blocs PS.⁵⁶ Ce constat pourrait provenir de ce que nos échantillons présentaient une distorsion plus grande de l'ordre lamellaire : en observant plus attentivement les surfaces, on a constaté la présence de « craquelures » qui se sont révélées être des portions de lamelles orientées non plus parallèlement mais perpendiculairement à la surface (Figure 34). Au niveau des applications, le taux de gonflement des lamelles par les nanoparticules plus élevé qu'attendu par la théorie pourrait être avantageux : le maximum de réflectivité actuellement à une longueur d'onde $\lambda_{\max} = 4nL_{\text{moy}} \approx 290$ nm pourrait être décalé en plein domaine visible avec un PS-*b*-PBMA de $M_n = 200000$ g/mol, accessible par ATRP.

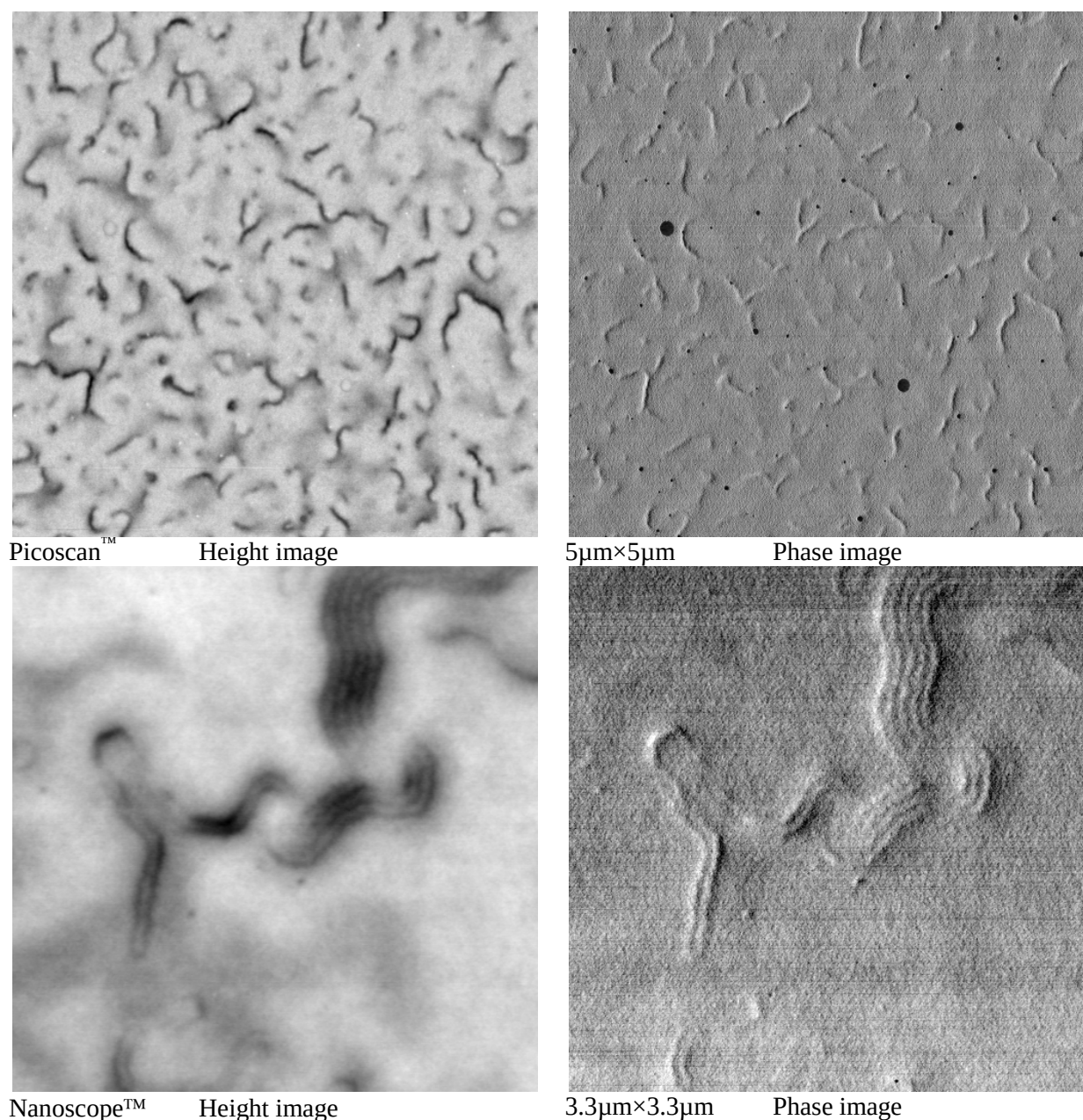


Fig 34: Visualisation par AFM de défaut d'orientation des lamelles à la surface du film de PBMA₄₂₅-*b*-PS₄₉₀ dopé avec les nanoparticules γ -Fe₂O₃@PS1 à une fraction volumique $\Phi = 0.025\%$ après recuit 72h à 150°C.

IV. Agrégats électrostatiques (coacervats) avec des copolymères diblocs neutres-chargés

Chaînes courtes de polyacrylate de sodium adsorbées en remplacement du citrate

Collaboration avec **Nicolas Biais** (Thèse de l'Université Paris Denis Diderot soutenue en 2004 au Laboratoire Matières et Systèmes Complexes, UMR 7057 CNRS)

Grâce à la technique de micro-injection adaptée pour les liposomes géants à laquelle il s'est formé par un séjour au laboratoire d'Owe Orwar de l'université technologique Chalmers en Suède,⁷⁶ Nicolas Biais que j'ai rencontré à l'ECIS 2002 a pu obtenir des vésicules magnétiques lipidiques encapsulant un ferrofluide citraté à des fractions volumiques élevées (quelques %) encore jamais atteintes avec les autres modes de préparation (réhydratation de film, émulsion triple...). Il nous est alors apparu que les ferrofluids citratés posaient un problème pour les manipulations sous une illumination intense avec microscope optique, en raison d'une instabilité photo-chimique du système fer-citrate-lumière,⁷⁷ dont on a montré avec Nicolas qu'elle s'accompagne d'une augmentation de la pression osmotique par dégradation photo-induite des molécules de citrate (Fig. 35a). Les vésicules très magnétiques sont instables sous le faisceau lumineux et présentent des oscillations de leur tension de membrane, à cause des variations de pression osmotique dues aux produits de photo-dégradation du citrate, causant aussi à plus long terme une déstabilisation du ferrofluide, voire une dissolution des nanoparticules. Afin qu'il puisse manipuler ses liposomes géants magnétiques micro-injectés pendant une longue durée, j'ai mis au point le **greffage de chaînes courtes de polyacrylate de sodium** (2,100) pour obtenir un ferrofluide dispersé à pH7 par des répulsions de nature à la fois électrostatique et stérique, qui présente par ailleurs la photo-stabilité nécessaire pour les micromanipulations sous microscope optique. Avec ce ferrofluide micro-injecté dans les vésicules, Nicolas a étudié durant son année d'ATER à Paris 7 des déformations originales dans des champs magnétiques oscillants, tournants, etc...

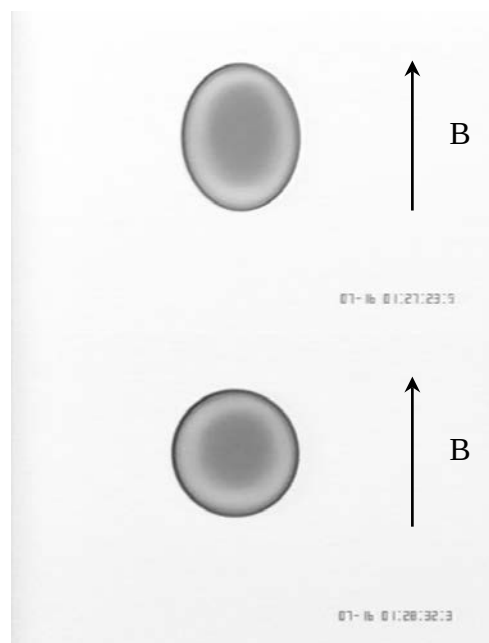
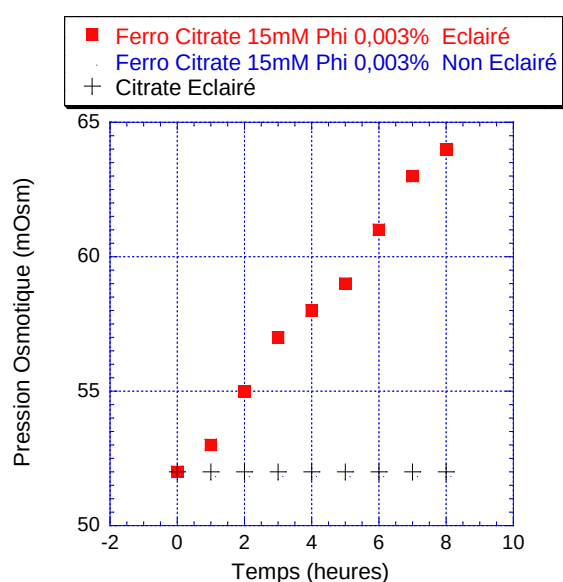


Fig. 35a) : En se dégradant sous l'effet combiné de la lumière et de la présence de fer, les molécules de citrate se dégradent en plusieurs molécules plus petites, ce qui augmente la pression osmotique.

b) après moins d'1 min d'éclairage, une vésicule lipidique remplie d'un ferrofluide citraté perd sa déformation ellipsoïdale sous champ magnétique (maintenu constant), car sa pression osmotique interne augmente, ce qui entraîne un afflux d'eau.

⁷⁶ A. Karlsson, R. Karlsson, M. Karlsson, A.S. Cans, A. Stromberg, F. Ryttsen, O. Orwar, *Nature* (2001)**409**,150

⁷⁷ T. D. Waite, F. M. Morel, *J. Coll. & Int. Sci.* (1984) **102**, 121-137

Aujourd'hui les ferrofluides couverts de polyacrylate de sodium sont toujours d'actualité : de par leur très grande stabilité due à des interactions à la fois stériques et électrostatiques, ils résistent à des salinités plus élevées que les ferrofluides citratés (jusqu'à 0.4 mol/L) y compris en milieu riche en protéines comme le plasma sanguin et ne présentent pas de toxicité cellulaire, comme démontré par Malak Safi qui soutiendra à la fin 2011 sa thèse au MSC⁷⁸ et par d'autres en Europe.⁷⁹ Dans ce mémoire je présenterai un autre domaine où ils peuvent remplacer avantageusement les ferrofluides citratés, en tant que nanoparticules négatives à pH7 pouvant servir de briques de base dans des structures auto-assemblées particulières avec des polymères de charge opposés, les « coacervats ».

Agrégats contrôlés de nanoparticules magnétiques enrobées par des copolymères di-blocs hydrophile neutre – hydrophile chargé pour des applications IRM

Collaboration avec Jean-François Berret (laboratoire MSC UMR7057 CNRS / Université Paris 7), Jérôme Fresnais, Vincent Dupuis et Régine Perzynski (PECSA)

Initiation du travail sur les coacervats

Les copolymères di-blocs hydrophile neutre – hydrophile chargé synthétisés par Rhodia, tel le poly(acrylamide)-bloc-poly(acrylate d'éthyl-triméthyl-ammonium) (PAM-*b*-PTEA) sont totalement solubles dans l'eau, tandis qu'ils s'auto-assemblent en présence d'autres colloïdes de charge opposée tels que des micelles de tensioactifs, pour former des agrégats mixtes très bien définis.⁸⁰ Ces copolymères s'étant avérés efficaces pour agréger aussi des nanoparticules inorganiques de CeO₂,⁸¹ il était naturel de les tester aussi avec les nanoparticules d'oxyde de fer magnétique.

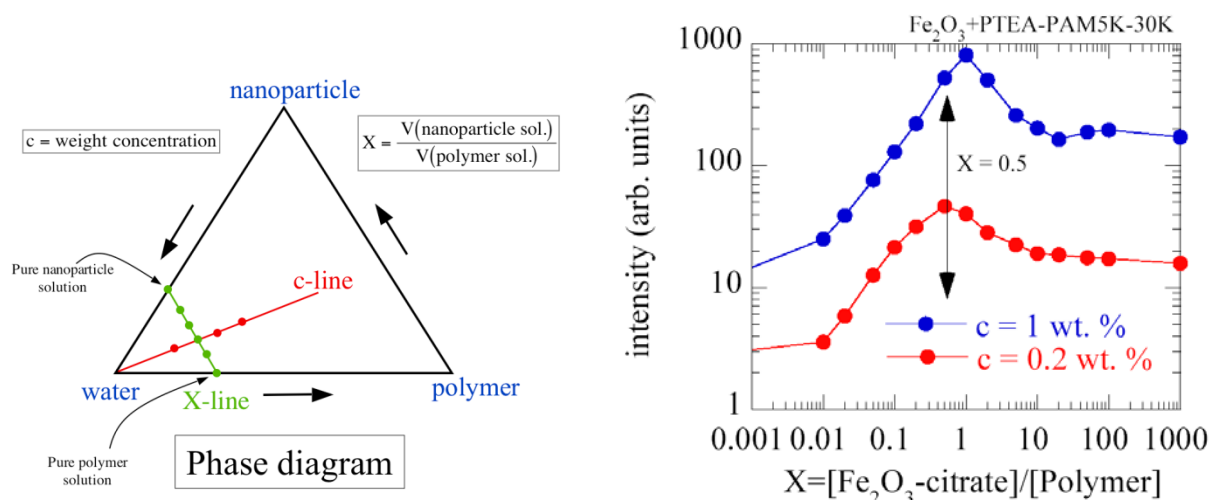


Fig. 36 a) Diagramme ternaire Nanoparticules / Copolymères / Eau. En pratique, on prépare deux solutions mères à la même concentration massique C, l'une de nanoparticules, l'autre de copolymère, puis on les mélange dans un rapport volumique X. L'association électrostatique entre les chaînes et les nanoparticules est quasi-instantanée.

b) Diffusion de lumière statique à 90°. Les intensités sont normalisées par la transmission (qui décroît avec X à cause de l'absorption par l'oxyde de fer). Par un ajustement de cette courbe avec un modèle théorique simple, on peut remonter au nombre d'agrégation N_{agg} (nombre moyen de nanoparticules par agrégat) et au ratio R de chaînes par nanoparticules. Ainsi on a trouvé $R=18$ et $N_{\text{agg}}=10$ à $C=0.2\%$, $R=10$ et $N_{\text{agg}}=9$ à $C=1 \%$.

⁷⁸ M. Safi, H. Sarrou, O. Sandre, N. Mignet, J-F. Berret, Interactions between sub-10-nm iron and cerium oxide nanoparticles and 3T3 fibroblasts: the role of the coating and aggregation state, *Nanotechnology* **21** (2010) 145103.

⁷⁹ M. Szekeres, E. Tombacz, Stability of ferocolloidal systems in biorelevant media, workshop "Structural aspects of biocompatible ferocolloids: stabilization, properties control & applications", Dubna, Moscow Region, 19-20 August 2011; A. Hajdu, I. Banyai, M. Babos, E. Tombacz, Carboxylated magnetic nanoparticles as MRI contrast agents, *Moscow International Symposium on Magnetism*, Moscow 21-25 August 2011.

⁸⁰ J.-F. Berret, B. Vigolo, R. Eng, P. Hervé, I. Grillot, L. Yang, *Macromolecules* **37** (2004), 4922-4930.

⁸¹ J.-F. Berret, K. Yokota, M. Morvan, Interactions Between Polymers and Nanoparticles: Formation of "Supermicellar" Hybrid Aggregates, *Soft Materials*, **2** (2004), 71-84.

Par ailleurs, la biodégradabilité des nanoparticules d'oxyde de fer les rend très utiles pour de nouvelles applications biomédicales. Ainsi servent-elles d'agents de contrastes négatifs en imagerie par résonance magnétique (IRM), par exemple en imagerie du cerveau pour marquer sous forme de zones noires les tumeurs cérébrales près desquelles la barrière hémato-encéphalique a été rompue. Basé sur la propriété de diminution du temps de relaxation transverse T_2 des spins nucléaires de l'eau dans l'environnement proche des nanoparticules superparamagnétiques,⁸² l'accélération de la relaxation des protons $R_2=1/T_2$ est d'autant plus prononcée que les nanoparticules sont denses et concentrées localement (à fraction volumique moyenne en agent de contraste égale). C'est pourquoi les agrégats électrostatiques de nanoparticules et de copolymères di-blocs neutre-chargés présentent un intérêt évident pour cette application. Le bloc chargé servant à la complexation de l'oxyde de fer, le bloc neutre peut être ajusté afin d'apporter la répulsion stérique nécessaire à stabiliser ces objets et les rendre furtifs dans la circulation sanguine (l'optimisation des paramètres pharmacocinétiques est cruciale, même pour les agents de contraste qui ne sont pas destinés à rester longtemps dans l'organisme, mais doivent au moins subsister dans le sang pendant une période de l'ordre de l'heure).

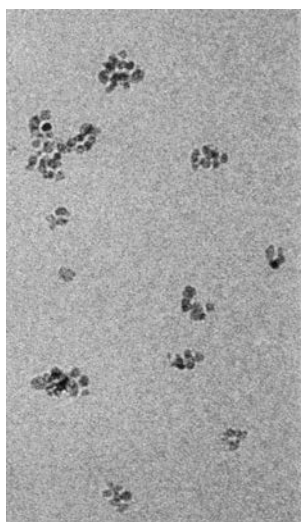


Fig. 37 : Image par Cryo-TEM prise au centre de recherches de Rhodia à Aubervilliers montrant des agrégats de $\gamma\text{Fe}_2\text{O}_3$ @citrate avec $\text{PAM}_{30\text{k}}\text{-PTEA}_{5\text{k}}$ ($C=0.2\%$ $X=0.5$). Le nombre de grains magnétiques par agrégat est compatible avec la valeur mesurée par diffusion de lumière $N_{\text{agg}}=10$.

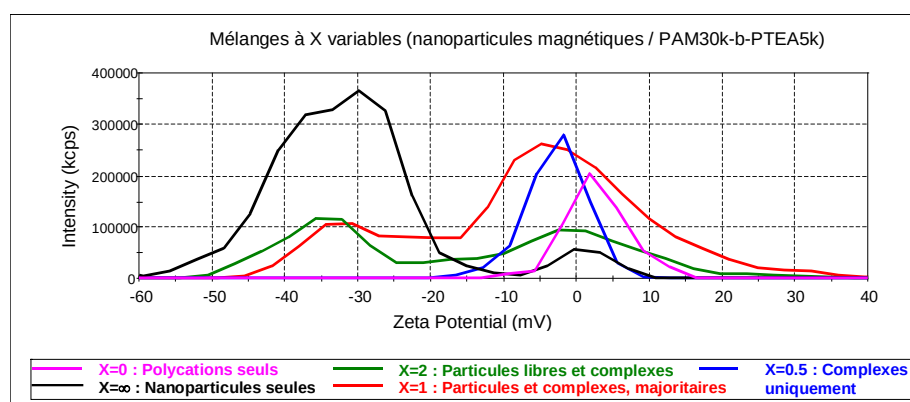


Fig. 38 : Mesures de zétamétrie par vélocimétrie laser sur un Malvern Nano ZS avec les cocervats préparés à $C=0.2\%$.

Le cas $X=\infty$ désigne le ferrofluide pur, stabilisé à pH 7 grâce à l'adsorption préalable de ligands citrates sur les nanoparticules. On mesure alors bien un potentiel zêta moyen négatif $\zeta = -32$ mV. Le copolymère pur $\text{PAM}_{30\text{k}}\text{-PTEA}_{5\text{k}}$ ($X=0$) apparaît quant à lui faiblement positif, à $\zeta = +3$ mV, en accord avec la charge nominale du bloc cationique. Pour les plus grandes quantités de nanoparticules inorganiques par rapport à celle de copolymère ($X=2$ et $X=1$), le mélange des deux systèmes colloïdaux se traduit par la coexistence de complexes neutres et de nanoparticules libres, au potentiel zêta négatif et inchangé par rapport au ferrofluide citraté pur. Ce n'est qu'en léger excès de polycations ($X=0.5$) que l'on détecte uniquement des complexes nanoparticules / copolymères, quasiment neutres.

Les mesures au laboratoire MSC sur un relaxomètre Bruker Minispec mq20 (0.47 Tesla) ont montré un effet très prononcé sur la relaxivité $r_2=R_2/[\text{Fe}]$ des agrégats, multipliée par 4 par rapport aux nanoparticules dispersées individuellement. Ce résultat a été publié à peu près simultanément avec celui obtenu par l'équipe de Jinming Gao aux USA avec aussi des clusters de nanoparticules magnétiques, confinées cette fois dans le cœur hydrophobe de micelles polymères de $\text{PCL-}b\text{-PEG}$.⁸³

⁸² C. Corot, P. Robert, J-M. Idée, M. Port, Recent advances in iron oxide nanocrystal technology for medical imaging, *Adv. Drug Deliv. Rev.* **58** (2006) 1471–1504; S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. Vander Elst, R. N. Muller, Magnetic Iron Oxide Nanoparticles: Synthesis, Stabilization, Vectorization, Physicochemical Characterizations, and Biological Applications, *Chem. Rev.* (2008) **107**, 2064-2110.

⁸³ H. Ai, C. Flask, B. Weinberg, X. Shuai, M. D. Pagel, D. Farrell, J. Duerk, J. Gao, Magnetite-Loaded Polymeric Micelles as Ultrasensitive Magnetic Resonance Probes, *Adv. Mat.* (2005) **17**, 1949-1952.

La « coacervation » qui consiste à associer deux types de colloïdes de charges électrostatiques opposées conduisant à l'apparition d'une phase mixte est en fait une technique de formulation très ancienne. On pense par exemple aux procédés ancestraux des vignerons qui ajoutent des polymères naturels (albumine d'œuf, gomme arabique...) afin de clarifier leur vin. Dans le cas de la « micro-coacervation », la phase obtenue n'est pas macroscopique (précipité, floc...) mais reste en suspension. C'est le cas par exemple des complexes formés entre l'ADN et les protéines histones.⁸⁴

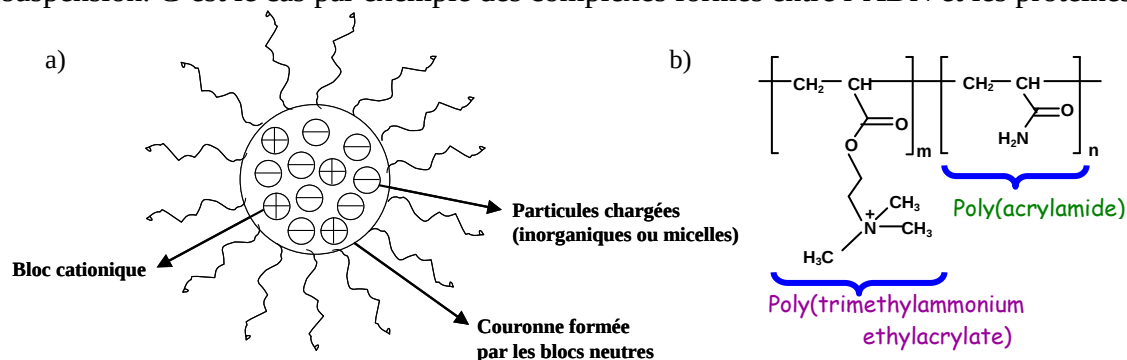


Fig. 39 : a) Schéma d'un agrégat *core-shell* obtenu par coacervation de colloïdes chargés par des copolymères comportant un bloc de chargé opposé et un bloc neutre ; b) Formule des copolymères PAM-*b*-PTEA synthétisés à Rhodia par une technique de polymérisation radicalaire contrôlée (MADIX, analogue de la RAFT avec agents de transfert xanthates). La présence d'une couronne neutre entourant un cœur dense est responsable de la limitation de taille des agrégats : la coacervation s'arrête à une taille mésoscopique (typiquement 100-200 nm) et ne s'étend pas sous la forme d'un précipité macroscopique.

La société Rhodia commercialise des copolymères diblocs neutres-chargés appelés par la suite DHBC (*double hydrophilic bloc copolymers*), qui dans un premier temps ont été utilisés pour effectuer la microcoacervation de micelles de tensioactifs chargées (DTAB).⁸⁵ Ayant appliqué aussi ce concept à l'association de nanoparticules inorganiques chargées (des terres rares à base de cérium ou d'yttrium), Jean-François Berret m'a proposé d'étudier ensemble la complexation électrostatique par les DHBC cationiques des nanoparticules d'oxyde de fer négatives, en premier lieu celles recouvertes de citrate.⁸⁶ Ayant montré les propriétés remarquables de ces clusters magnétiques en tant qu'agents de contraste pour l'IRM, on a aussi étudié leurs profils de sédimentation dans un fort gradient de champ magnétique, permettant de séparer des agrégats de tailles différentes qu'on peut obtenir en variant la masse du bloc cationique (PTEA_{5k} ou PTEA_{11k}).⁸⁷ De part la couronne neutre qui les entoure, les micro-coacervats avec le PAM-*b*-PTEA présentent une stabilité colloïdale accrue par rapport aux nanoparticules nues, pouvant résister à une force ionique élevée : ce n'est qu'au-dessus de 0.4–0.5 mol/L de sel (NaCl ou NH₄Cl) que les espèces se dissocient. Leur caractère répulsif (*anti-fouling*) voire furtif (*stealth effect*) en fait de bons candidats pour l'introduction en milieu biologique. Notons que si elle fut informelle pendant deux ans, la collaboration entre le MSC, le LI2C/PECSA et le LCPO a ensuite bénéficié d'un soutien financier par l'ANR dans le cadre des appels à projets blancs de 2007. Le projet intitulé "IsoThermal Calorimetry as a Probe of Interactions between Magnetic Nanoparticles, Plasma Protein and Cells" a permis d'acquérir cette technique très

⁸⁴ Wilhelm, F. X.; Wilhelm, M. L.; Erard, M.; Daune, M. P. *Nucleic Acids Res.* (1978) **5**, 505; Burton, D. R.; Butler, M. J.; Hyde, J. E.; Phillips, D.; Skidmore, C. J.; Walker, I. O. *Nucleic Acids Res.* (1978) **5**, 3643.

⁸⁵ Colloidal complexes obtained from charged block copolymers and surfactants: a comparison between small-angle neutron scattering, cryo-TEM, and simulations. Berret, J.-F., Hervé, P., Aguerre-Chariol, O., Oberdisse, J., *J. Phys. Chem. B* **107**, 8111-8118 (2003)

⁸⁶ Berret J-F, Schonbeck N, Gazeau F, El Kharrat D, Sandre O, Airiau M, Vacher A, Controlled Clustering of Superparamagnetic Nanoparticles Using Block Copolymers: Design of New Contrast Agents for Magnetic Resonance Imaging *JACS* (2006) **128**(5) 1755–1761; Stable oxide nanoparticle clusters obtained by complexation, Berret J-F, Sehgal A, Morvan M, Sandre O, Vacher A, Airiau M, *J. Coll. and Inter. Sci.* (2006) **303** 315–318

⁸⁷ Berret J-F, Sandre O, Mauger, A Size Distribution of Superparamagnetic Particles Determined by Magnetic Sedimentation, *Langmuir* (2007) **23**(6) 2993–2999

sensible qu'est le titrage par calorimétrie isotherme (ITC) pour mesurer les interactions mises en jeu par la complexation électrostatique des colloïdes. Une autre partie, réalisée par les collègues, a concerné la quantification du degré de furtivité (*stealthness*) des coacervats envers des protéines plasmiques, modélisées dans un premier temps par la BSA, en parallèle à MSC sur les nanoparticules d'oxyde de fer greffées citrate ou polyacrylate,⁸⁸ et des nanoparticules de silice avec le Pys-*b*-PEG au LCPO⁸⁹ : ces deux études, alliant l'ITC et la DLS, ont toutes deux montré l'effet de densité de la brosse polymère sur celle de la couronne de protéines plasmiques adsorbées. Pour ma part j'ai réalisé l'étude structurale des agrégats,⁹⁰ et ceux-ci m'ont servi par ailleurs pour piéger les nanoparticules magnétiques dans les hydrogels de PNIPAM (*cf.* Partie I).

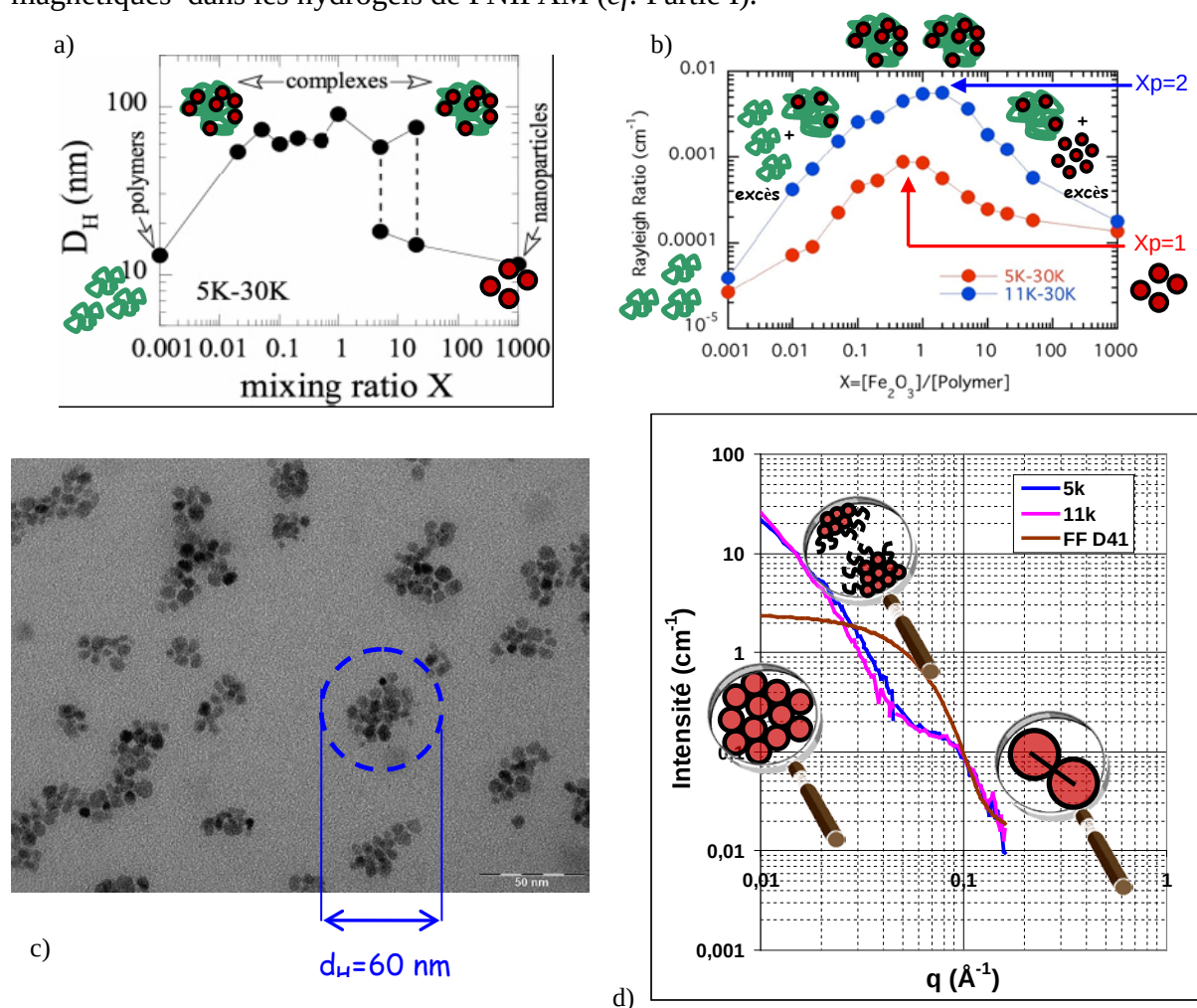


Fig. 40 : Etude structurale de la complexation de nanoparticules magnétiques citrées de diamètres 6 nm par des copolymères diblocs PAM_{30k}-PTEA_{5k} et PAM_{30k}-PTEA_{11k} par diffusion de la lumière dynamique (a) et statique (b). L'observation au TEM montre des agrégats denses (c). La DNPA renseigne sur le rayon de giration et les distances au contact (d).

Que ce soit avec les nanoparticules citrées ou bien greffées avec le polyacrylate, les facteurs de forme des coacervats (d'après les facteurs de structure intra-agrégats), présentent systématiquement un « creux de corrélation », qui est la signature en diffusion de neutrons des distances statistiquement moins probables entre les centres de nanoparticules du fait de la couronne neutre de polyacrylamide.

⁸⁸ M. Safi, J. Courtois, M. Seigneuret, H. Conjeaud, J.-F. Berret, The effects of aggregation and protein corona on the cellular internalization of iron oxide nanoparticles, *Biomaterials* xxx (2011) 11 pages.

⁸⁹ S. Louguet, A. Kumar, G. Sigaud, E. Duguet, S. Lecommandoux, C. Schatz, A physico-chemical investigation of PEG-block-poly(L-lysine) copolymer adsorption onto silica nanoparticles. *J. Coll. and Inter. Sci.* (2011) **359**, 413-422.

⁹⁰ J. Fresnais, J.-F. Berret, L. Qi, J.-P. Chapel, J.-C. Castaing, O. Sandre, B. Frka-Petesic, R. Perzynski, J. Oberdisse, F. Cousin, Universal scattering behavior of co-assembled nanoparticle-polymer clusters, *Phys. Rev. E* **78**, 040401 R (2008)

Coassemblage magnétique de forme contrôlée par la cinétique de dessalement hors champ (sphères) ou sous champ magnétique (aiguilles cylindriques)

De même que les répulsions électrostatiques entre colloïdes de charges de même signe dans un électrolyte sont écrantées par la force ionique (théorie de Debye-Hückel), le phénomène opposé de coacervation entre des nanoparticules magnétiques et des polymères DHBC de charges opposées est aussi sensible à la salinité. Durant son post-doctorat financé par le projet ANR "ITC-Nanoprobe" d'abord six mois à MSC avec J.-F. Berret puis douze mois avec moi au laboratoire PECSA, Jérôme Fresnais a montré qu'un tel écrantage de l'interaction électrostatique par la salinité suivi d'un dessalement dans des conditions contrôlées permettait de contrôler la géométrie des agrégats mixtes (sphères parfaites ou cylindres) et la taille des agrégats mixtes sur une gamme de 50 nm à 50 µm.

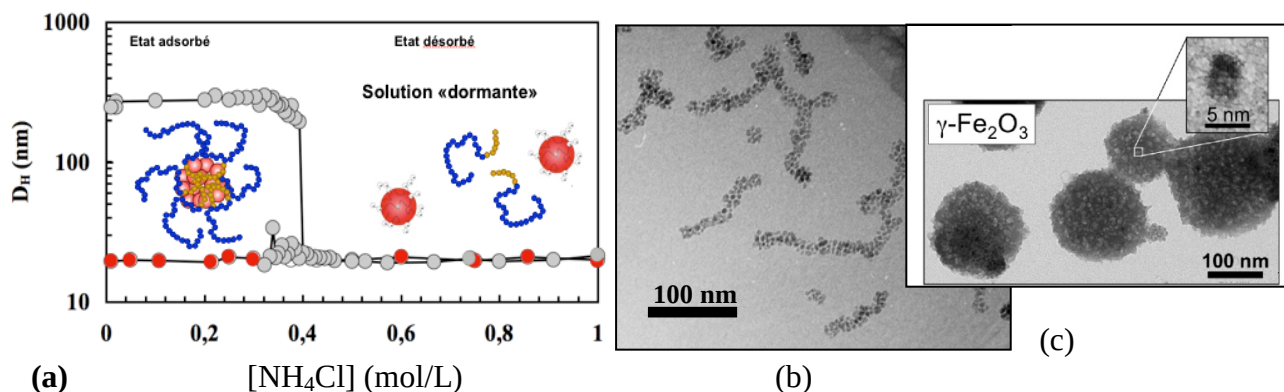


Fig. 41 : a) Taille hydrodynamique mesurée en fonction de la salinité; b) agrégats par mélange direct ("one-shot"); c) agrégats préparés par mélange au dessus de $C_s^* = 0.4$ mol/L puis dialyse (hors champ magnétique).

Comme indiqué sur la Fig. 41, les nanoparticules magnétiques chargées négativement par l'acide polyacrylique PAA_{2k} et les copolymères di-blocs neutres-cationiques (PAM_{30k}-b-PTEA_{11k}) mélangés à une concentration de sel NH₄Cl supérieure à $C_s^* = 0.4$ mol/L restent sans interaction entre eux (« solutions dormantes »). Ce n'est que par abaissement de la salinité en deçà d'un seuil C_s^* , par simple dilution ou bien par dialyse, que les colloïdes s'associent entre eux. Si les agrégats formés par mélange direct étaient bien denses en nanoparticules comme mesuré par DNPA,⁹⁰ leur forme globale pouvait être anisotrope voire vermiculaire (Figure 41b). Au contraire ceux obtenus par contrôle de la salinité présentent une géométrie parfaitement sphérique (Figure 41c), avec un diamètre déterminé par la cinétique de dessalement (plus petit avec une dilution brusque, plus gros par dialyse lente).

Une idée très astucieuse de Jérôme a été d'appliquer un champ magnétique durant toute l'étape de dialyse, ce qui provoque une agrégation orientée sous forme de cylindres denses en nanoparticules magnétiques,⁹¹ pouvant être dopés avec des colloïdes organiques fluorescents.⁹² En outre la longueur moyenne de ces aiguilles magnétiques augmente avec l'intensité du champ magnétique appliqué pendant l'assemblage. Une fois formées, les aiguilles peuvent être aisément manipulées sous microscope optique par des champs magnétiques : durant sa thèse au PECSA dirigée par Régine Perzynski, Bruno Frka-Petesic a étudié leur dynamique de réorientation lorsqu'on bascule la direction du champ de 90° et de rotation dans un champ magnétique tournant.⁹³ Sans rentrer dans les calculs, on peut comprendre qualitativement la Fig. 42b donnant l'inverse du temps caractéristique de basculement τ_{90° mesuré aussi bien par microscopie optique que par relaxation de biréfringence,

⁹¹ J. Fresnais, J.-F. Berret, B. Frka-Petesic, O. Sandre, R. Perzynski, Controlled co-assembly of iron oxide nanoparticles and polymers: towards the generation of highly persistent super-paramagnetic nanorods, *Adv. Mat.* **20** (2008) 3877-3881

⁹² J. Fresnais, E. Ishow, O. Sandre, J.-F. Berret, Electrostatic Co-assembly of Magnetic Nanoparticles and Fluorescent Nanospheres: A Versatile Approach Towards Bimodal Nanorods, *Small* **5** (22), 2533-2536 (2009)

⁹³ J. Fresnais, J.-F. Berret, B. Frka-Petesic, O. Sandre, R. Perzynski, Reorientation kinetics of superparamagnetic nanostructured rods, *J. Phys.: Cond. Mat.* **20**(49), 494216/1-6 (2008); B. Frka-Petesic, K. Erglis, J.-F. Berret, A. Cebers, V. Dupuis, J. Fresnais, O. Sandre, R. Perzynski, Dynamics of organic/inorganic magnetic nanostructured rods under rotating field, *J. Magn. Magn. Mat.* **323**(10) 1309-1313 (2011)

pour des aiguilles de longueur calibrées de 5 et 10 μm . Le couple magnétique variant comme le volume physique de l'aiguille D^2L tandis que le couple de friction augmente avec le volume hydrodynamique L^3 , d'où la loi d'échelle $1/\tau_{90} \propto (D/L)^2$. De même Bruno a observé $1/\tau_{90} \propto H^2$ pour les aiguilles composées de nanoparticules superparamagnétiques de $\gamma\text{-Fe}_2\text{O}_3$, et $1/\tau_{90} \propto H$ pour des aiguilles dures à base de CoFe_2O_4 ferrimagnétique, conformément au couple magnétique attendu (produit vectoriel du champ et du moment, induit dans le premier cas et permanent dans le second).

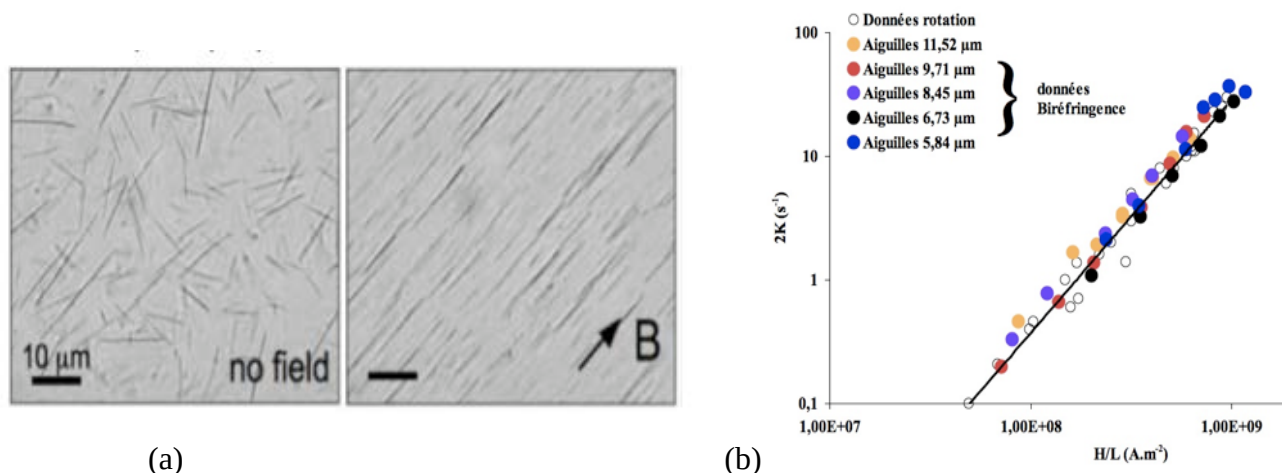


Fig. 42 : a) Aiguilles magnétiques préparées par dessalement sous champ ; b) Constante cinétique de basculement à 90° mesurée soit par microscopie optique, soit par relaxation de biréfringence, en fonction du ratio du champ appliqué sur la longueur moyenne des aiguilles.⁹⁴

Jérôme Fresnais qui a été recruté comme chargé de recherche CNRS au PECSA en octobre 2009 a bâti son projet de recherche sur l'utilisation des aiguilles magnétiques et leur dispersion dans le PDMS pour former des surfaces structurées avec des micro-plots orientables pour un contrôle actif de la mouillabilité et le déplacement de gouttes, avec des perspectives en micro-fluidique de surface.

Des agrégats contrôlés pour mieux comprendre les agents de contraste T_2 en IRM

Depuis début 2009, je collabore avec Jean-François Berret, Jérôme Fresnais et Yves Gossuin du groupe de biophysique à l'Université de Mons (Belgique) sur l'influence de l'état d'agrégation (taille et géométrie) sur la relaxivité transverses r_2 des particules magnétiques qu'on a défini plus haut, en vue de leur utilisation comme agents de contraste en IRM. La communication soumise récemment est jointe en annexe, mais je tiens ici à présenter l'étude bibliographique⁹⁵ que nous avons effectuée pour démontrer la loi d'échelle universelle entre la valeur de r_2 à haut champ ($\geq 1\text{T}$ pour que les moments magnétiques des particules soient saturés) et trois paramètres simples définissant les objets: le diamètre (d'après TEM ou DLS), la fraction volumique en matériau magnétique et l'aimantation.

⁹⁴ J. Fresnais, B. Frka-Petecic, O. Sandre, J-F. Berret, R. Perzynski, V. Dupuis, Orientational behavior of an assembly of superparamagnetic rods, *Physics Procedia* (2010) **9**, 15-19

⁹⁵ Forge, D.; Gossuin, Y.; Roch, A.; Laurent, S.; Vander Elst, L.; Muller, R. N. *Contrast Med. Mol. Imaging* **2010**, 5, 126; Koenig, S. H.; Kellar, K. E.; Fujii, D. K.; Gunther, W. H. H.; Briley-Saebø, K.; Spiller, M. *Acad. Radiol.* **2002**, 9, S5; Pinho, S. L.; Pereira, G. A.; Voisin, P.; Kassem, J.; Bouchaud, V. Etienne, L.; Peters, J. A.; Carlos, L.; Mornet, S.; Galdes, C. F.; Rocha, J.; Delville, M.-H. *ACS Nano* **2010**, 4, 5339; Jung, C. W.; Jacobs, P. *Magn. Reson. Imaging* **1995**, 13, 661; Paquet, C.; de Haan, H. W.; Leek, D. M.; Lin, H.-Y.; Xiang, B.; Tian, G.; Kell, A.; Simard, B. *ACS Nano* **2011**, 5, 3104; Xie, X.; Zhang, C. *J. Nanomaterials* **2011**, 152524; Ai, H.; Flask, C.; Weinberg, B.; Shuai, X.; Pagel, M. D.; Farrell, D.; Duerk, J.; Gao, J. *Adv. Mater.* **2005**, 17, 1949; Martina, M.-S.; Fortin, J.-P.; Ménager, C.; Clément, O.; Barratt, G.; Grabielle-Madellmont, C.; Gazeau, F.; Cabuil, V.; Lesieur, S. *J. A. C. S.* **2005**, 127, 10676; Beaune, G.; Levy, M.; Neveu, S.; Gazeau, F.; Wilhelm, C.; Ménager, C. *Soft Matter* **2011**, 7, 6248; Cheong, S. *et al.*, *Angew. Chem. Int. Ed.* **2011**, 50, 4206; Yang, J. *et al.*, *Angew. Chem. Int. Ed.* **2007**, 46, 8836.

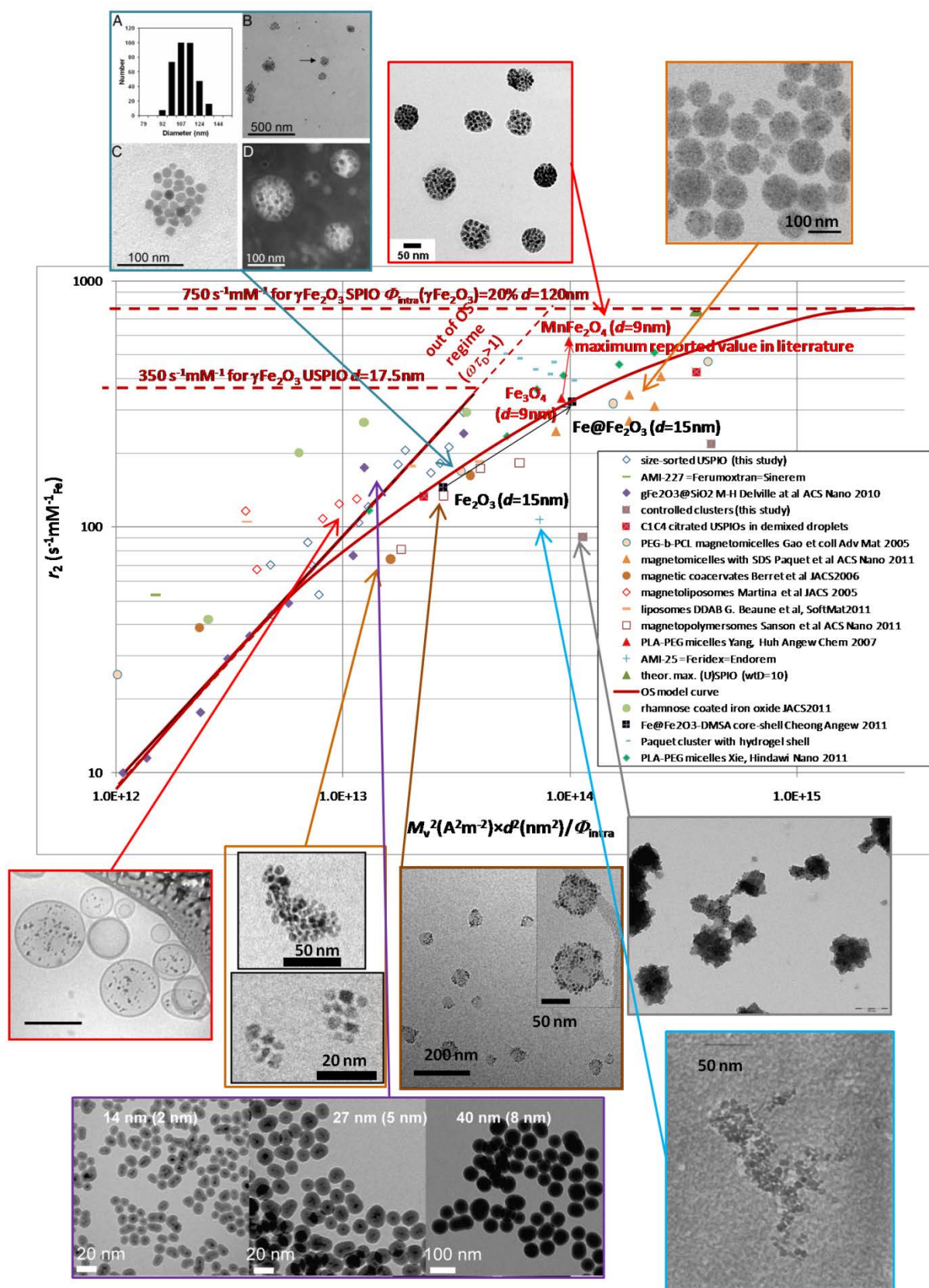


Fig. 43 : Relaxivité transverse à haut champ ($\geq 1T$) apparaissant linéaire avec le carré de l'aimantation et du diamètre divisé par la fraction volumique intra-agrégat, pour des nanoparticules individuelles ($\phi_{intra}=100\%$) et des clusters (petits ou dilués) dans le régime « sphère externe » : $r_2 = 1.1 \times 10^{-11} m_s^2 \times d^2 \times \phi_{intra}$ dans ce régime.

V. Dispersions de nanoparticules magnétiques dans des cristaux liquides (ferronématiques)

Cristaux liquides thermotropes ferronématiques

avec Cristina da Cruz (ATER 2002/2003) et Valérie Cabuil, suivi du projet de 3ème année ESPCI de Julien Deseigne en 2006

Si l'idée de disperser des particules colloïdales dans un cristal liquide thermotrope remonte à un article théorique de F. Brochard et P.-G. de Gennes d'il y a une quarantaine d'années,⁹⁶ le dopage de cristaux liquides thermotropes par des charges inorganiques en vue d'améliorer leurs caractéristiques tant électriques⁹⁷ que magnétiques⁹⁸ n'a pu être réalisé qu'à des fractions volumiques très faibles, de l'ordre de 10^{-5} . Lors de son année d'ATER au LI2C, Cristina da Cruz avait pour projet d'orienter par un champ magnétique les domaines du 4-cyano-4'-pentylbiphenyl (5CB), cristal liquide nématique thermotrope couramment utilisé, grâce à un dopage convenable par des nanoparticules de maghémite. Elle a utilisé pour cela le tensioactif Beycostat (BNE), adapté aux dispersions dans les huiles organiques. L'étude du comportement de phases du système ternaire (matrice nématique 5CB – nanoparticules magnétiques – surfactant BNE) a mis en évidence une séparation de phase en micro-gouttelettes de phase magnétiques mais isotropes dans la phase nématique non magnétique.⁹⁹

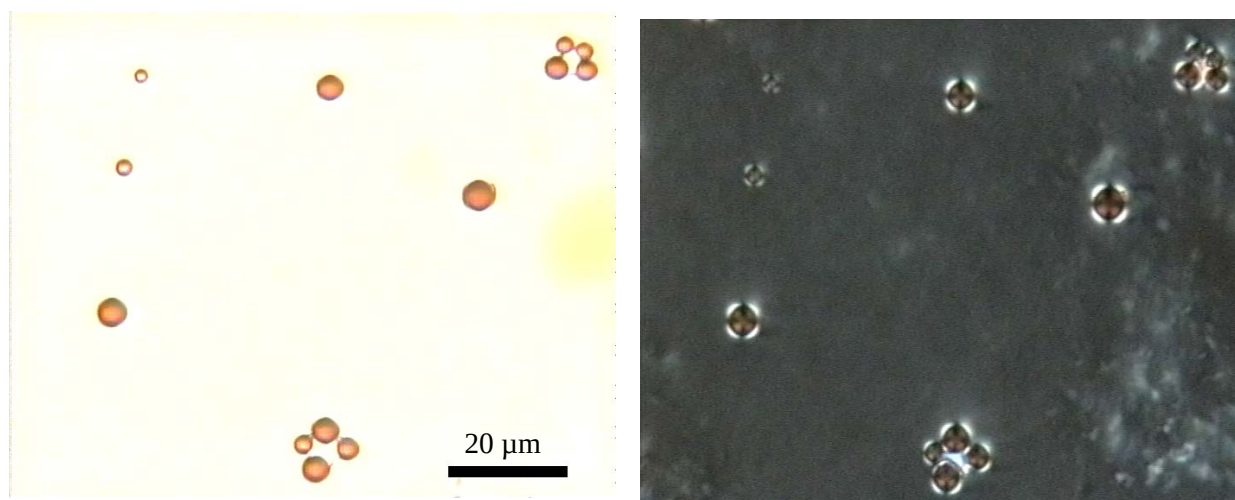


Fig. 44 : a) Ferronématique démixtée en gouttes magnétiques dispersées dans la phase nématique du 5CB non magnétique, entre polariseurs parallèles ; b) idem entre polariseurs croisés: la croix noire à l'intérieur de chaque goutte correspond à un défaut ponctuel du directeur n à l'extérieur des gouttes.

Au dessus de la température de clarification $T_{(N-I)}$, on n'observe qu'une seule phase, isotrope et magnétique, véritable ferrofluide pour lequel le 5CB joue le rôle de solvant dispersant parfaitement les nanoparticules. Mais lorsque la température descend en dessous de $T_{(N-I)}$, le système démixte entre des gouttelettes magnétiques concentrées (malheureusement de phase isotrope) dispersées dans une phase continue nématique (mais non magnétique). La calorimétrie différentielle (DSC) a permis d'attribuer la variation de $T_{(N-I)}$ uniquement au tensioactif (Beycostat) désorbé des nanoparticules et dissout à l'état d'impureté dans le 5CB. La diffusion des rayons X aux petits angles (SAXS) a permis quant à elle de mesurer la fraction volumique des nanoparticules à l'intérieur des gouttes $\Phi_{loc}=18\%$.

⁹⁶ F. Brochard, P.-G. de Gennes, Theory of Magnetic Suspensions in Liquid Crystals, *J. Phys. (France)* **31** 691 (1970)

⁹⁷ A. Glushchenko, C. Il Cheon, J. West, F. Li, E. Büyüktanir, Y. Reznikov, A. Buchnev, Ferroelectric Particles in Liquid Crystals: Recent Frontiers, *Mol. Cryst. & Liq. Cryst.*, **453**, 227-237 (2006)

⁹⁸ M. Konerackf, V. Kellnerova, P. Kopcansky, T. Kuczynski, Study of magnetic Fredericksz transition in ferronematic, *J. Magn. Magn. Mat.* **140-144** (1995) 1455-1456

⁹⁹ C. da Cruz, O. Sandre, V. Cabuil, Phase Behavior of Nanoparticles in a Thermotropic Liquid Crystal. *J. Chem. Phys. B* **109** (2005), 14292-14299

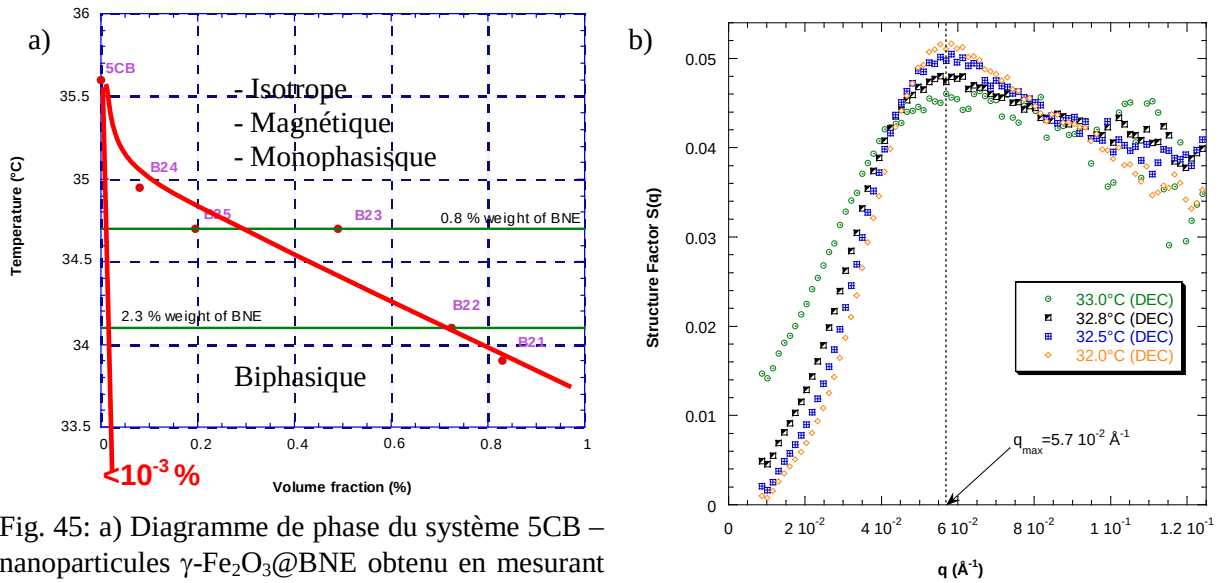


Fig. 45: a) Diagramme de phase du système 5CB – nanoparticules $\gamma\text{-Fe}_2\text{O}_3\text{@BNE}$ obtenu en mesurant la température $T_{(N-I)}$ par DSC en fonction de Φ , fraction volumique en nanoparticules. Le domaine nématique est biphasique (gouttes magnétiques dans une phase continue non magnétique). BNE désigne le surfactant greffé sur les nanoparticules.

b) Facteur de structure des nanoparticules mesuré par SAXS (sur la ligne D24 au LURE en 2003) au-dessous de la température d'apparition des gouttelettes $T_c=32.8^\circ\text{C}$ pour $\Phi=0.725\%$ (B22).

Nous avons par la suite étudié la déformation de ces gouttelettes en champ magnétique (Fig. 46), permettant de mesurer la tension de surface entre les deux phases ($\sigma \approx 5 \times 10^{-2}$ mN/m).

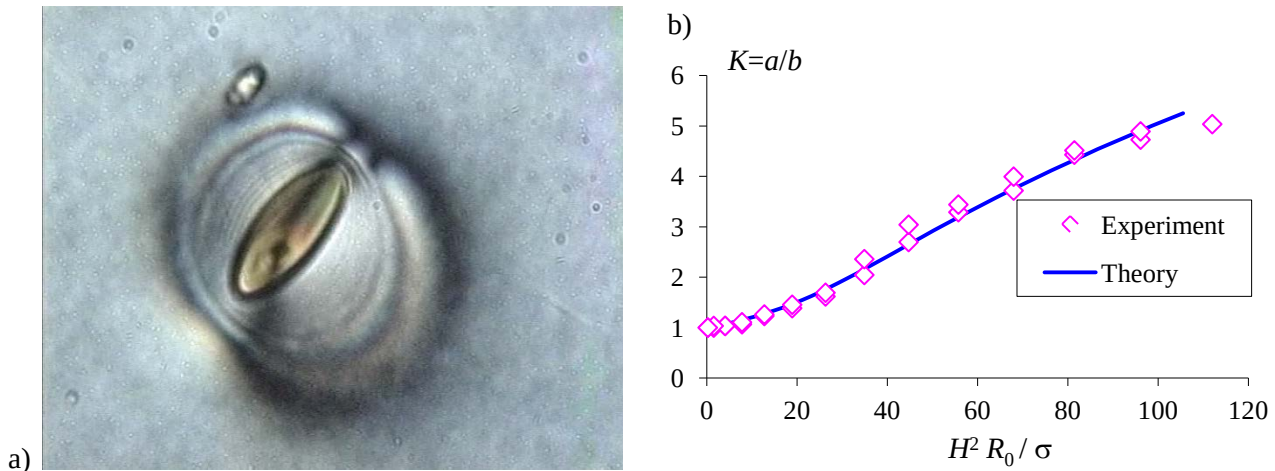


Fig. 46 : Goutte magnétique dans le 5CB démixté, de rayon initial $R_0=5.4 \mu\text{m}$, déformée par un champ magnétique d'amplitude H_0 croissante de 0 à 100 Oe ($R_0 \approx 5 \times 10^{-4}$ cm, $\sigma \approx 5 \times 10^{-2}$ dyn/cm en CGS).

Si des ferronématiques lyotropes ont été obtenus dès les années 90', aucun expérimentateur n'a encore obtenu de véritable système ferronématique thermotrope tel que celui décrit par F. Brochard et P.-G. de Gennes, en raison de la perturbation de la matrice cristal liquide dans laquelle on tente de disperser des nanoparticules, trop importante pour qu'elle conserve son ordre nématique. Alors que de nombreuses équipes travaillent activement sur des inclusions micellaires ou inorganiques dans les cristaux liquides (T. Bellini en Italie, C. Blanc à Montpellier, Y. Reznikov en Ukraine, P. Kopčanský en Slovaquie, I. Muševic en Slovénie...), j'ai souhaité étudier pour ma part une autre piste, liée à la forme des nanoparticules qui n'est pas favorable au mélange dans le cas de sphères et de bâtons.¹⁰⁰

¹⁰⁰ M. Adams, Z. Dogic, S. L. Keller, and S. Fraden, Entropically driven microphase transitions in mixtures of colloidal rods and spheres, *Nature* **393**, 349-352 (1998).

Ainsi dans une dispersion à 0.1% v/v de particules sphériques au milieu de mésogènes calamitiques (molécules allongées), qui dans le cas du 5CB s'associent en doublets modélisés par des cylindres de 2.57 nm de long et 0.3 nm de diamètre, on évalue l'interaction par déplétion entre des particules sphériques de 7 nm de diamètre à environ $40k_B T$, quand bien même la compatibilité chimique serait parfaite (comme avec le greffage de surfactants mésogènes sur les particules, tels que ceux synthétisés par G. Toquer pendant sa thèse avec C. Blanc en 2006). Le même calcul montre que l'interaction de déplétion entre sphères dispersées dans des bâtons de 5CB devient égale à $k_B T$ uniquement pour un rayon $R=0.45$ nm, ce qui est compatible avec le résultat d'auteurs chinois qui ont dopé du 5CB par des complexes organométalliques paramagnétiques de taille sub-nanométrique, orientant les domaines avec des champs magnétiques de l'ordre de 0.3 Telsa.¹⁰¹ Dès lors, je me suis proposé de remplacer les nanoparticules quasi-sphériques par des nano-bâtonnets.

Orientation en champ magnétique de nanoparticules antiferromagnétiques et son usage pour tenter d'abaisser le champ seuil de la transition de Fredericksz de cristaux liquides avec Vincent Dupuis, Habib Hayeb et Emmanuelle Lacaze (INSP-UPMC Paris 6), après les stage de TEP de L3 de chimie de Jean-Baptiste Delsuc et Kathy Simoes en 2007, de 3^{ème} année ESPCI de Christelle Vatry en 2008 (bourse de stagiaire ingénieur CNRS) et les projets de L3 de physique de Jérémie Guedj et Michaël Gonzalez Diaz en 2009.

Grâce au dispositif utilisé d'ordinaire pour mesurer la biréfringence magnéto-induite des ferrofluides,¹⁰² nous avons observé la transition de Fredericksz du 5CB soumis à des orientations antagonistes entre la surface de la cuve (ancrage soit homéotrope soit planaire) et le volume (orientation perpendiculaire à la précédente induite par un champ magnétique). La mesure de biréfringence sous champ H avait déjà été décrite dans la littérature pour mesurer l'anisotropie de susceptibilité magnétique $\Delta\chi=\chi_{||}-\chi_{\perp}\approx 2\times 10^{-6}$ du 5CB pur en phase nématique,¹⁰³ mais aussi pour suivre l'orientation magnétique de suspensions aqueuses de particules inorganiques anisotropes de goethite α -FeOOH. Si de tels nano-bâtonnets à une fraction volumique supérieure à un seuil de 8% présentent une phase nématique lyotrope, en régime dilué aussi leur paramètre d'ordre S_2 devient non nul sous champ magnétique H et qui plus est change de signe pour une valeur critique H_c mise en évidence à Orsay par B Lemaire.¹⁰⁴ Cette transition d'orientation due à la compétition entre l'énergie d'un moment magnétique permanent (parallèle aux bâtonnets pour la goethite) et celle d'un moment induit (du fait d'un $\Delta\chi$), minimale dans une direction perpendiculaire, peut être généralisée à d'autres nanoparticules antiferromagnétiques présentant un moment magnétique résiduel du fait de lacunes ou de défauts des spins de surface.¹⁰⁵ Nous nous étions d'abord intéressés à des particules plaquettaires de $Ni(OH)_2$ pour lesquelles nous n'avons pas trouvé de phase nématique discotique (à haute fraction volumique elles gélifient mais restent isotropes) mais qui présentent néanmoins une orientation perpendiculaire à un champ magnétique appliqué.¹⁰⁶ Nous sommes ensuite passés à l'étude de nano-bâtons : la goethite m'a semblé pour cela un matériau aisé à synthétiser, avec des caractéristiques de taille et de forme pouvant être optimisées dans le but de doper efficacement des nématiques

¹⁰¹ Y. Xiang, T. Li, L. Zi-Yang, L. Ji, An intermolecular coupling between guest and host in the doped liquid crystal system, *J. Appl. Phys.* (2007), **101**, 036109

¹⁰² E. Hasmonay, E. Dubois, J.-C. Bacri, R. Perzynski, Yu. L. Raikher, V. I. Stepanov, Static magneto-optical birefringence of size-sorted γ -Fe₂O₃ nanoparticles, *Eur. Phys. J. B* **5**, 859 (1998)

¹⁰³ T. Moses, B. Durall, G. Frankowiak, Magnetic birefringence in a liquid crystal: An experiment for the advanced undergraduate laboratory, *Am. J. Phys.* **68** (3), March 2000

¹⁰⁴ B.J. Lemaire, P. Davidson, J. Ferré, J.P. Jamet, D. Petermann, P. Panine, I. Dozov, J.-P. Jolivet, Physical properties of aqueous suspensions of goethite (α -FeOOH) nanorods Part I: In the isotropic phase, *Eur. Phys. J. E* **13**, 291-308 (2004)

¹⁰⁵ Yu. L. Raiker, V. I. Stepanov, Magneto-orientational behavior of a suspension of antiferromagnetic particles, *J. Phys. Cond. Mat.* **20** (2008) 204120

¹⁰⁶ M. Meyer, Y.L. Raikher, O. Sandre, A. Bee, V. Cabuil, V. Dupuis, P. Licinio, R. Perzynski, Magneto-orientational properties of ionically stabilized aqueous dispersions of $Ni(OH)_2$ nanoplatelets. *Eur. Phys. J. E*, 2008. 26(4): 355-360

thermotropes. Ce type de bâtonnets antiferromagnétiques a par ailleurs été combiné récemment avec un lamellaire lyotrope.¹⁰⁷

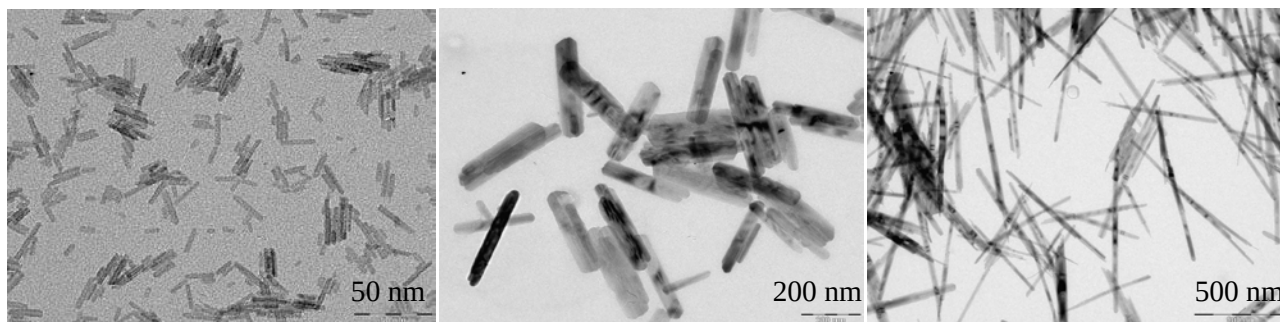


Figure 47 : Clichés MET de nano-bâtonnets antiferromagnétiques de tailles très différentes: a) akaganéite $\text{Fe}_8\text{O}_8(\text{OH})_8\text{Cl}_{1.35}$ de longueur moyenne 20 nm ; b) goethite $\alpha\text{-FeOOH}$ de 250nm ou c) de 500 nm.

Ainsi en variant les conditions de synthèse (température, choix de la base et des sels de Fe^{III}) en nous inspirant de la littérature,¹⁰⁸ nous avons obtenu des échantillons de longueurs très différentes de 20 à 500 nm (Fig. 47). Par ailleurs, le dopage par des ions paramagnétiques Co^{2+} ou Ni^{2+} a permis d'agir sur les valeurs du moment permanent et de l'anisotropie de susceptibilité antiferromagnétique, comme l'ont montré les mesures de SQUID réalisées à l'INSP. Nous avons pu ainsi relier les différentes mesures du champ H_c de la transition d'orientation (magnétométrie, biréfringence sous champ, diffusion anisotrope des neutrons) pour toute une série d'échantillons (Fig. 48 et tableau 4).

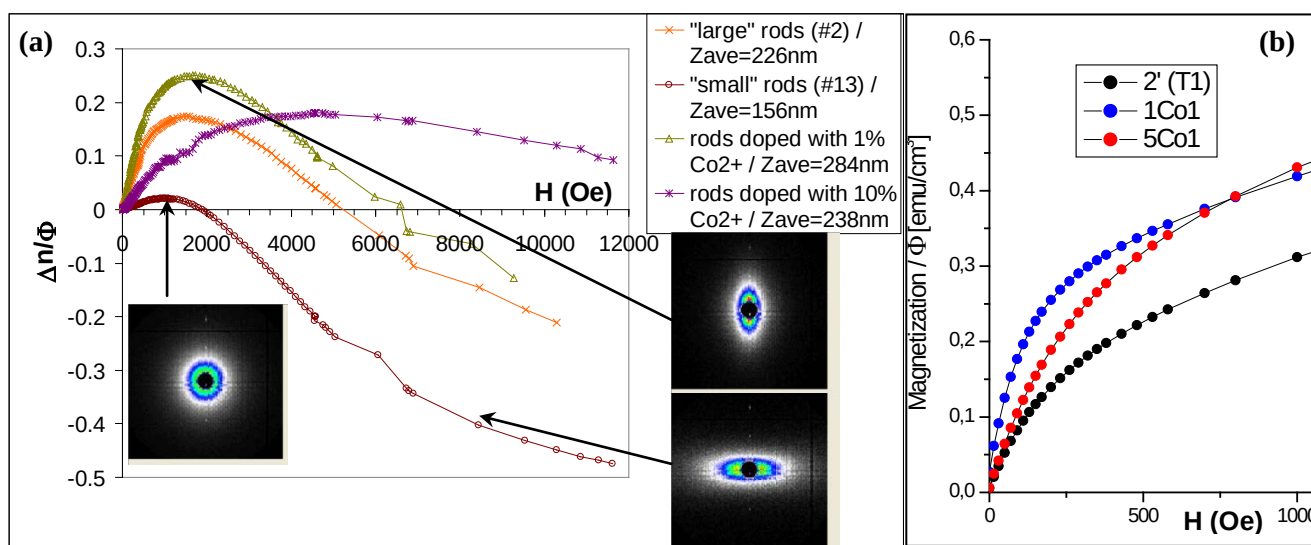


Figure 48 : a) Courbes de biréfringence en fonction du champ magnétique appliqué pour des suspensions de bâtonnets de tailles et de compositions variées (goethite pure et dopée à 1% ou 10% de cobalt). Les figures de diffusion anisotrope des neutrons (PAXY CEA-Saclay octobre 2008) illustrent l'orientation moyenne d'abord parallèle au champ pour $H < H_c$ puis perpendiculaire pour $H > H_c$. L ; b) Courbes de magnétométrie SQUID.

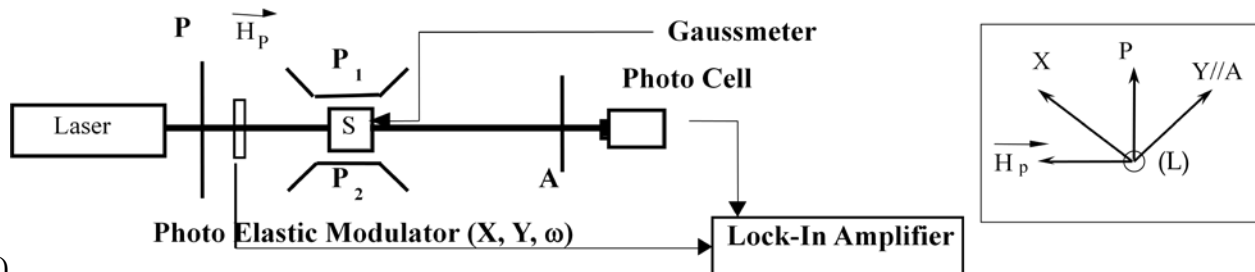
Echantillon	Ratio Co/Fe réel	Longueur moyenne	Anisotropie χ_a/Φ	Moment résiduel
Goethite pure	0	205 nm	1.44×10^{-3}	$58400 \mu_{\text{Bohr}}$
Dopée 1% Co	1%	284 nm	1.53×10^{-3}	$112500 \mu_{\text{Bohr}}$
Dopée 5% Co	2.6%	169 nm	1.59×10^{-3}	$43800 \mu_{\text{Bohr}}$

Tableau 4 : Mesures magnétiques (SQUID) sur des échantillons de goethite pure ou dopée au cobalt.

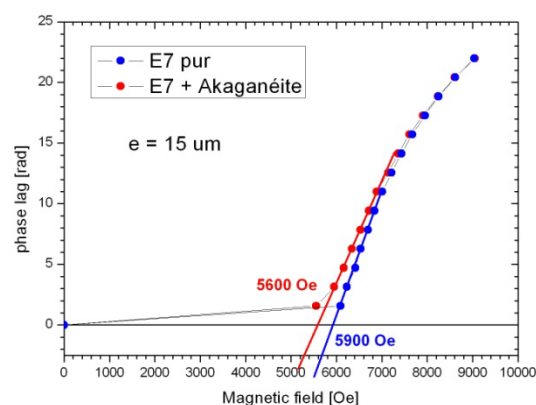
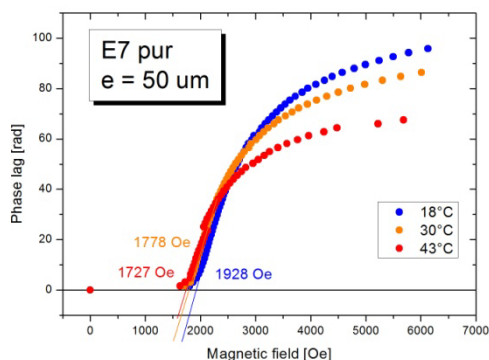
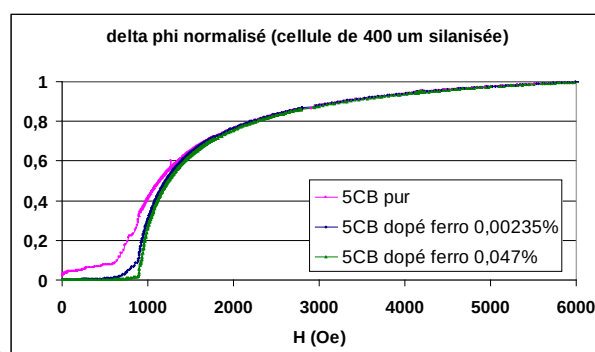
¹⁰⁷ D. Constantin, P. Davidson, C. Chanéac, Lyotropic lamellar phase doped with a nematic phase of magnetic nanorods, *Langmuir* **26**, 7 (2010) 4586-4589.

¹⁰⁸ Krehula, S.; Popovic, S.; Music, S., Synthesis of acicular $\alpha\text{-FeOOH}$ particles at a very high pH. *Materials Letters* **54**, (2002) 108-113.

Parallèlement à cette étude de synthèse, nous avons étudié la possibilité de doper un cristal liquide thermotrope (5CB pur ou mélange eutectique – dit mélange E7 – de mésogènes cyano-paraphénylènes) par ces nanoparticules allongées afin d'abaisser le champ seuil de Fredericksz, partant de l'idée intuitive que des particules allongées se disperseront mieux car elles perturberont moins le champ directeur du nématique, ce qui a été démontré pour des cristaux liquides lyotropes.¹⁰⁹ De premiers résultats encourageants ont été obtenus avec des nanoparticules d'akaganéite dont la taille n'est que de huit fois environ la taille des mésogènes : dispersées dans le mélange E7, elles provoquent un abaissement du champ seuil de la transition de Fredericksz de 590 mT à 560 mT et de 99 mT à 55 mT pour des épaisseurs respectives de 15 μm et 500 μm . Pour mener à bien cette étude, nous avons porté le plus grand soin à la qualité des ancrages des molécules organiques aux interfaces avec les parois de la cuve, en bénéficiant de l'expérience d'Emmanuelle Lacaze et d'Habib Hayeb de l'INSP. Il faudrait maintenant faire de même pour réaliser un ancrage planaire des molécules du nématique la surface des nanoparticules inorganiques, afin d'améliorer leur état de dispersion à une fraction volumique plus importante et démontrer l'obtention d'un véritable ferronématique.



a) Figure 49 : a) Dispositif expérimental pour la mesure de la biréfringence sous champ magnétique ; b) Résultat pour un cristal liquide 5CB pur et dopé par des nanoparticules superparamagnétiques quasi-sphériques. L'ancrage planaire sur les parois de la cellule explique un niveau initial de biréfringence quasi-nul, tandis que le signal optique augmente fortement quand le champ passe la valeur seuil de la transition de Fredericksz H_F ; c) Détermination du seuil H_F du mélange E7 pur à plusieurs températures au dessous de $T_{(N-I)}=65^\circ\text{C}$; d) Léger abaissement du seuil H_F mesuré entre le E7 pur et le E7 dopé par des nano-bâtonnets d'akaganéite de 20.6 ± 8.6 nm de long (expériences réalisées par H. Hayeb et V. Dupuis).



¹⁰⁹ Mondiot, F.; Chandran, S. P.; Mondain-Monval, O.; Loudet, J. C., Shape-Induced Dispersion of Colloids in Anisotropic Fluids. *Phys. Rev. Lett.* **103** (2009), 4.

VI. Synthèses assistées par l'outil microfluidique : des nanoparticules aux vésicules magnétiques

Miniaturiser la synthèse des ferrofluides en canal micro-fluidique

Collaboration entamée par Valérie Cabuil avec Caroline Derec (MSC UMR 7057 CNRS Univ. Denis Diderot Paris 7), Hervé Willaime et Patrick Tabeling du Laboratoire de Microfluidique, MEMS et Nanostructures UMR 7083 Gulliver de l'ESPCI

En organisant à Paris en 2006 un « atelier du savoir » du CNRS sur les potentialités de la microfluidique pour l'étude de la réactivité chimique, Valérie Cabuil avait dans l'idée de rapprocher les chimistes de ce domaine en pleine expansion. Dès lors je me suis intéressé à l'adaptation de la synthèse des nanoparticules magnétiques en microfluidique. L'idée sous-jacente était de contrôler la cinétique de nucléation et de croissance des nanoparticules en utilisant l'équivalence « temps – distance » des flux laminaires dans des micro-canaux (des positions différentes dans le canal correspondent à des temps de réaction différents). Bien qu'il dût comporter une part technologique non négligeable, nous souhaitions poursuivre ce travail avec une approche de physico-chimistes.

Les prémices : PIR Microfluidique et projet de 3A ESPCI de Virginie Watel en 2006

Parallèlement à cette idée, nous avons reçu un soutien financier du CNRS dans le cadre de son « programme intégré de recherche » sur la microfluidique pour notre participation à un projet porté par Caroline Derec, sur l'utilisation des nanoparticules magnétiques pour accélérer la diffusion et le mélange entre deux liquides miscibles, l'un magnétique, l'autre pas. L'équipe des physiciens au MSC avait réalisé une miniaturisation astucieuse pour appliquer un champ magnétique très localement (Fig. 50), et désirait ainsi mesurer l'efficacité du mélange des deux fluides en fonction du champ magnétique appliqué et des paramètres de l'écoulement (nombre de Péclet).¹¹⁰ Une miniaturisation des mesures de biréfringence était aussi envisagée dans le cadre du projet PIR.

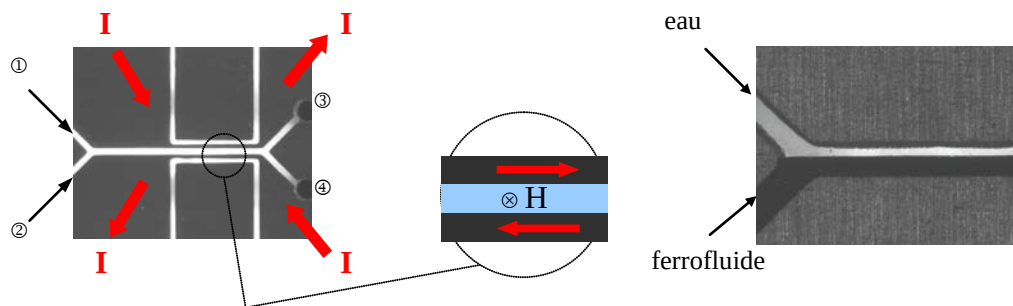


Fig. 50 : Croquis (vu de haut) du micro-canal et des fils conducteurs permettant d'appliquer un champ magnétique local de géométrie contrôlée, dans le plan de l'interface entre les deux fluides (Caroline Derec).

Même si notre contribution à ce projet est restée faible, il marqua profondément nos choix scientifiques et technologiques : entre la microfluidique digitale basée sur les écoulements bi-phasiques de gouttes régulières en taille et la microfluidique continue utilisant des fluides miscibles, nous avons choisi de nous focaliser sur la seconde pour aborder la question de la synthèse des nanoparticules magnétiques. Dans les deux cas les faibles dimensions des canaux induisent un contrôle de la cinétique de réaction par la diffusion des espèces réactives, soit entre une goutte et une phase continue pour l'émulsion, soit dans un front de mélange diffusif entre des liquides miscibles. Ce détail est intéressant à souligner car d'autres collègues, pour les synthèses de nanoparticules de silice, d'or ou de semi-conducteurs fluorescents, avaient choisi eux des méthodes en émulsion.¹¹¹

¹¹⁰ C. Derec, M. Smerlak, J. Servais, J.-C. Bacri, Anomalous diffusion in microchannel under magnetic field, *Physics Procedia* **9** (2010) 109–112 C; Derec, C. Wilhelm, J. Servais, J.-C. Bacri, *Microfluid. Nanofluid.* **8**, 123 (2010)

¹¹¹ L. Frenz, A. El Harrak, M. Pauly, S. Bégin-Colin, A. D. Griffiths, J.-C. Baret *Angew. Chem. Int. Ed.* 2008, **47**, 6817; A. Abou-Hassan, O. Sandre, V. Cabuil, Microfluidics in Inorganic Chemistry, *Angew. Chem. Int. Ed.* 2010, **49**, 6268.

Les tous premiers essais de synthèse de nanoparticules magnétiques en microfluidique furent réalisés par des étudiantes de 3^{ème} année de l'ESPCI, Lorraine Raboin en 2004 encadrée par Patrick Tabeling et Valérie Cabuil, puis Virginie Watel en 2006 avec moi. Les réactifs étaient simplifiés à l'extrême, avec d'un côté un mélange de sels de fers en milieu acide (pour éviter la précipitation d'hydroxydes), de l'autre une solution de base forte « non flocculante », l'hydroxyde de tétraméthylammonium, afin de réaliser la réaction de coprécipitation de sels de fer ferreux et ferrique suivante :



Les puces étaient constituées d'un canal à double-entrée « en Y », photographié ci-dessous.

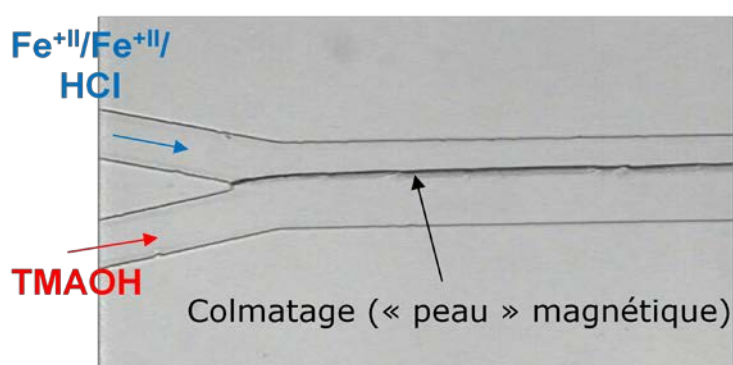


Fig. 51 : Premiers essais de synthèse de ferrofluide dans un canal en Y (projets de L. Raboin et V. Watel).

La magnétite colloïdale apparaissait bien à l'interface des deux solutions, mais malgré les contre-ions de la base TMA^+ encombrés et non flocculants, les nanoparticules ne se dispersaient pas et formaient une peau solide entre les deux écoulements. A ce problème attribué à un collage des nanoparticules sur les parois de verre de la cellule on trouva une solution, tous deux schématisés à la figure suivante.

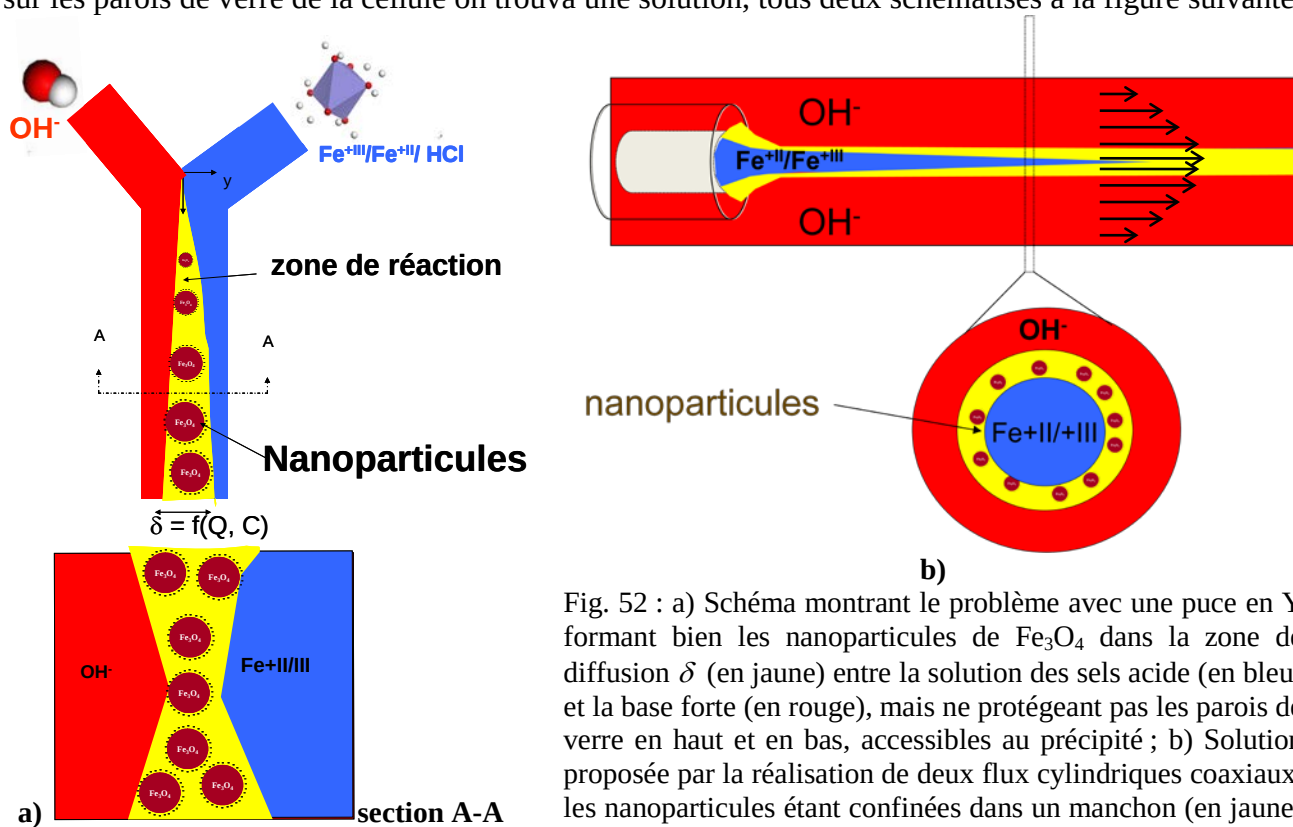


Fig. 52 : a) Schéma montrant le problème avec une puce en Y formant bien les nanoparticules de Fe_3O_4 dans la zone de diffusion δ (en jaune) entre la solution des sels acide (en bleu) et la base forte (en rouge), mais ne protégeant pas les parois de verre en haut et en bas, accessibles au précipité ; b) Solution proposée par la réalisation de deux flux cylindriques coaxiaux, les nanoparticules étant confinées dans un manchon (en jaune) qui n'a pas de risque d'entrer en contact avec les parois (si le parallélisme des deux capillaires est ajusté). Cette géométrie dite "co-flows" ou "flow focusing" a été popularisée par les travaux de David Weitz en microfluidique digitale.

Obtention des premières puces efficaces : Thèse d'Ali Abou Hassan de 2007 à 2009

Dès le début de sa thèse, Ali Abou Hassan a validé le concept grâce à une puce intermédiaire entre la milli-fluidique et la micro-fluidique fabriquée avec des capillaires et du PDMS, ne nécessitant aucun recours à la photolithographie.¹¹² Plus tard nous avons exploré la possibilité d'utiliser des puces plus complexes *via* une collaboration avec Toru Maekawa au centre d'excellence bio-nano électronique de l'Université de Toyo (<http://bionano.toyo.ac.jp>), qui a permis à Ali Abou Hassan de séjourner un mois au Japon pour y construire des dispositifs en quartz. Malheureusement ceux-ci se sont révélés trop compliqués à utiliser en pratique, en raison d'un problème de mouillage induit par un traitement avec un gaz fluoré. On est alors revenu à une technologie plus simple à base de PDMS en collaboration avec Hervé Willaime à l'ESPCI pour la réalisation des masques.

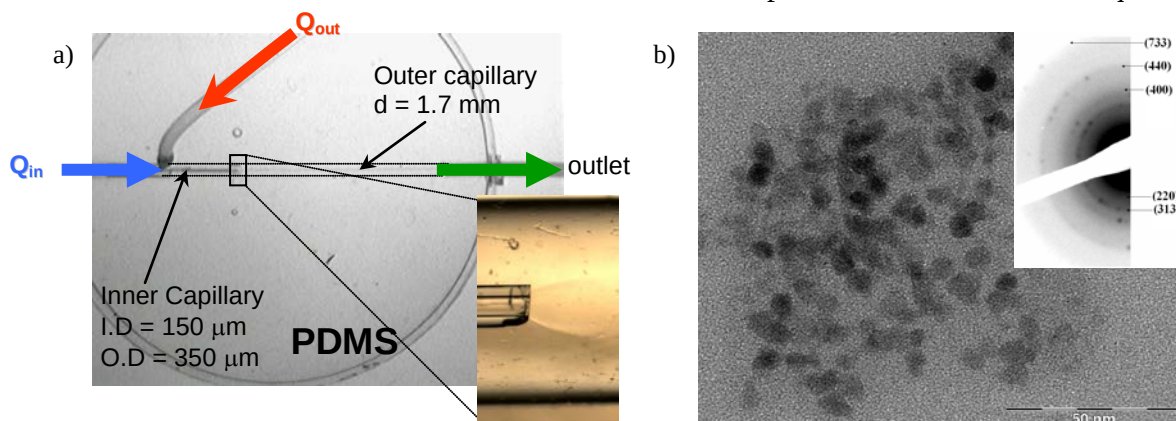


Fig. 53 : a) Dispositif microfluidique réalisant le mélange de deux flux laminaires coaxiaux. L'image agrandie montre le bout du capillaire interne d'où sort la solution acide de Fe^{+II} et de Fe^{+III} débouchant dans le flux de solution alcaline de TAMOH. En isolant la solution ferreuse et ferrique des parois, on évite le phénomène de colmatage du capillaire par la précipitation des nanoparticules, qui sont colloïdalement stables dans TAMOH ; b) Images TEM et cliché de microdiffraction des premières nanoparticules synthétisées en microfluidique.

Suivi *in-situ* de la synthèse de nanoparticules en canal micro-fluidique par CLSM et SAXS

Thèse d'Ali Abou-Hassan dirigée par Valérie Cabuil, avec Jean-François Dufrêche et Guillaume Mériguet du PECSA pour la modélisation des flux et des phénomènes de transport, Vincent Dupuis et Olivier Spalla (CEA-LIONS) pour le suivi en SAXS.

La géométrie de canal microfluidique a été choisie pour réaliser deux flux laminaires coaxiaux, le flux interne contenant les sels précurseurs ferreux et ferrique acides, le flux externe la base forte TAMOH. En plus de la taille des capillaires, les paramètres de contrôle sont les concentrations respectives des solutions et les débits Q_{interne} et Q_{externe} qui sont réglés avec des pousse-seringues. Le profil de l'écoulement schématisé sur la figure 52b a ensuite été étudié beaucoup plus en détail par un modèle numérique associant l'hydrodynamique (Navier-Stokes) et les équations de transport pour les espèces majoritaires à savoir H^+ et OH^- , sans considérer les espèces du fer composées non seulement de Fe^{2+} et Fe^{3+} mais aussi de tous les complexes polymétalliques intermédiaires vers l'état de nanoparticules, dont les coefficients de diffusion sont inconnus.¹¹³ Par contre nous avons ajouté à la fois dans les simulations et dans les expériences une molécule sonde, la fluorescéine, dont l'intensité d'émission est un bon traceur du pH. Nous en avons tiré une méthode de cartographie du pH à l'intérieur du canal par comparaison des simulations par éléments finis (COMSOL) et d'images de microscopie confocale à balayage laser (CLSM), qui par référence au diagramme de spéciation du

¹¹² A. Abou Hassan, O. Sandre, V. Cabuil, P. Tabeling, Synthesis of iron oxide nanoparticles in a microfluidic device: preliminary results in a coaxial flow millichannel, *Chem. Comm.* **2008**(15): 1783-1785, [lien](#).

¹¹³ A. Abou-Hassan, J-F. Dufreche, O. Sandre, G. Meriguet, O. Bernard, V. Cabuil, Fluorescence Confocal Laser Scanning Microscopy for pH Mapping in a Coaxial Flow Microreactor: Application in the Synthesis of Superparamagnetic Nanoparticles. *J. Phys. Chem. C*, 2009, 113(42): 18097-18105, [lien](#).

fer dans l'eau nous a permis de prévoir les zones d'apparition de la magnétite colloïdale Fe_3O_4 (grosso modo les zones près du saut de pH de la réaction acide fort-base forte).

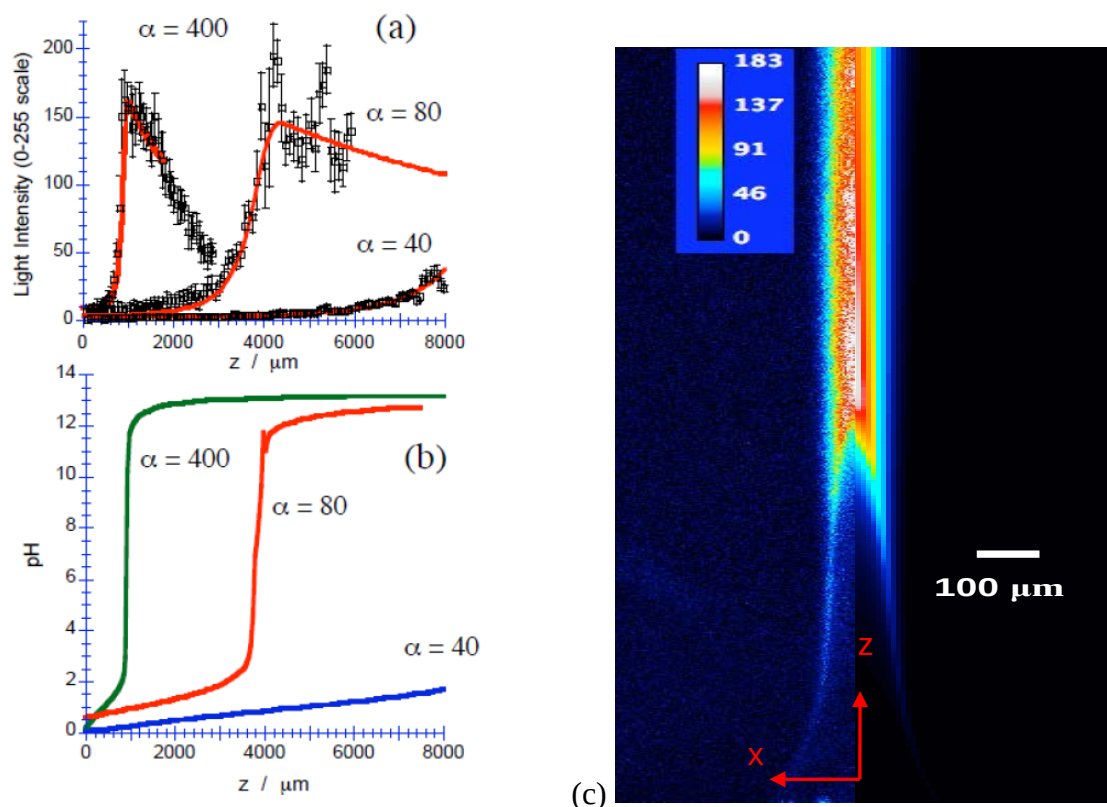


Fig. 54 : (a) Profils expérimentaux de l'intensité de fluorescence mesurée par microscopie confocale et courbes ajustées par le modèle couplant l'équation de Navier-Stokes et les équations de transport, pour différentes valeur du rapport des débits $\alpha = Q_{\text{ext}}(\text{OH}^-)/Q_{\text{int}}(\text{H}^+)$; (b) Profil du saut de pH dans le plan médian du canal ($x=0$) en fonction de la coordonnée longitudinale z ; (c) Image composite formée à gauche de l'intensité de fluorescence expérimentale, à droite de la concentration simulée de la forme déprotonée de la fluorescéine.

Les simulations montrent en particulier que la « longueur de mélange » des réactifs dans le canal, définie comme la distance z nécessaire pour que le pH atteigne 7, vaut par exemple 800 μm pour $\alpha=400$ et 3600 μm pour $\alpha=80$. Cette distance est importante à connaître pour définir l'instant $t=0$ dans l'optique de mesures cinétiques de la réaction de coprécipitation du Fe^{2+} et du Fe^{3+} , dans l'esprit des travaux publiés par l'équipe d'Olivier Spalla au LIONS sur la synthèse des sols d'or.¹¹⁴ L'étude cinétique de la nucléation/croissance des nanoparticules de magnétite présentant encore un certain nombre de difficultés expérimentales à surmonter (vitesse de réaction beaucoup plus grande qu'avec l'or, densité de longueur de diffusion des rayons X plus faible...), nous avons commencé par étudier le cas de nanoparticules de Fe^{III} uniquement, qui bien qu'antiferromagnétiques présentent néanmoins un intérêt du fait de leur forme anisotrope (voir la partie suivante sur les cristaux liquides). Un dispositif micro-fluidique composé de deux réacteurs successifs l'un pour la nucléation, l'autre pour la croissance de nano-bâtonnets de goethite $\alpha\text{-FeOOH}$ à température contrôlée (60°C) a montré que celle-ci suit vraisemblablement un mécanisme dit « agrégation orientée » à partir de premiers germes de ferrihydrite mal cristallisés.¹¹⁵

Nous avons en outre effectué une première expérience de diffusion des rayons X aux petits angles sur la ligne SWING du synchrotron SOLEIL en canal microfluidique, qui n'était pas dans des

¹¹⁴ B. Abécassis, F. Testard, O. Spalla, P. Barboux, Probing in situ the Nucleation and Growth of Gold Nanoparticles by Small-Angle X-ray Scattering, *Nano Letters* 7(2007), 1723-1727

¹¹⁵ A. Abou-Hassan, O. Sandre, S. Neveu, V. Cabuil, Synthesis of Goethite by Separation of the Nucleation and Growth Processes of Ferrihydrite Nanoparticles Using Microfluidics, *Angew. Chem.-Int. Ed.* 48 (2009), 2342-2345, [lien](#).

conditions optimales de flux, mais a permis néanmoins de suivre les toutes premières étapes de la réaction de synthèse de magnétite, à savoir la précipitation de l'hydroxyde de Fe^{III} , plus insoluble que celui de Fe^{II} . Les objets formés étaient de type plaquettaire, comme l'indiquent les courbes d'intensité modélisables par des facteurs de forme de disques plats de rayon R et d'épaisseur $2H$, que l'on a attribué à la forme cristalline bernalite de $\text{Fe}(\text{OH})_3$.¹¹⁶

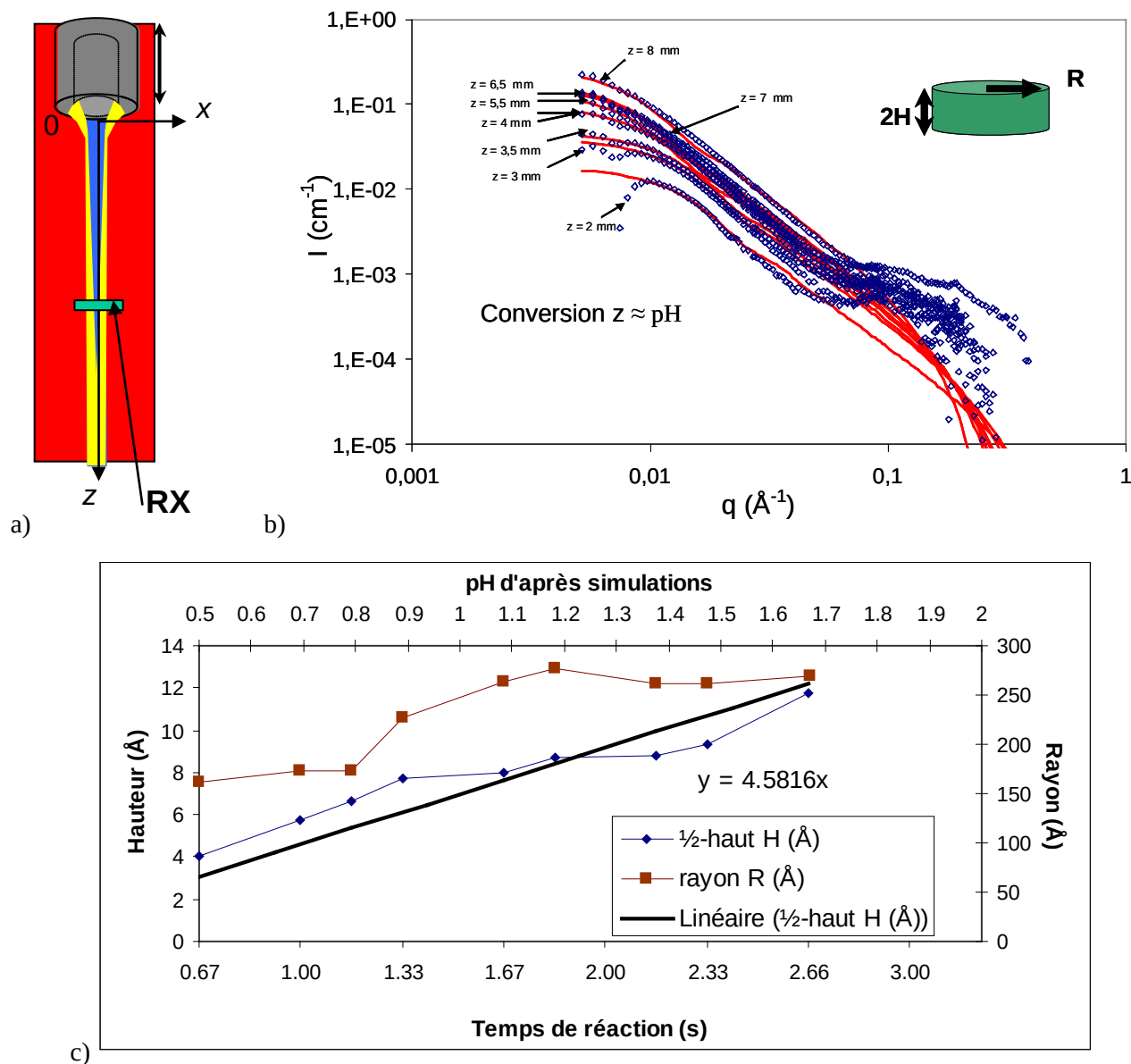


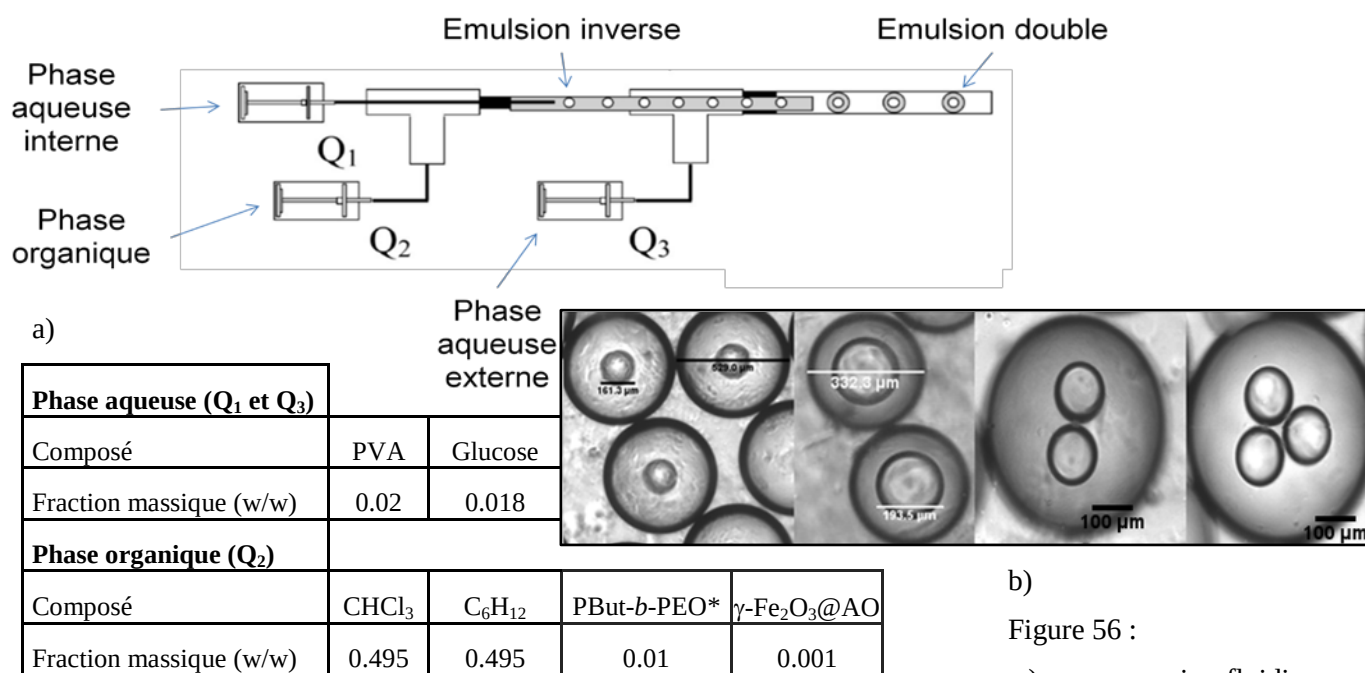
Fig. 55 : Première expérience de suivi de la réaction par SAXS sur la ligne SWING du synchrotron SOLEIL. a) Le faisceau de rayons X étant à une position fixe dans le capillaire, l'embouchure du jet interne était translatée afin de varier la position longitudinale z . Pour une question de détection non optimale de l'intensité diffusée, nous avons dû baisser le ratio des débits à une valeur $\alpha=40$ qui ne permettait pas un mélange rapide des réactifs (cf. Fig. 54b) ; b) dans ces conditions, les courbes d'intensité correspondaient à des pH croissants et non pas à des temps croissants à partir d'instant initial ; c) néanmoins on a pu modéliser l'évolution des courbes par la croissance de plaquettes d'hydroxyde $\text{Fe}(\text{OH})_3$ qui représentent les tous premiers germes.

¹¹⁶ C.A. McCammon, A. Pring, H. Keppler, T. Sharp, A study of Bernalite, $\text{Fe}(\text{OH})_3$, using Mössbauer spectroscopy, optical spectroscopy and transmission electron microscopy, *Phys Chem Minerals* (1995) 22,11-20

Préparation microfluidique de vésicules hybrides magnétiques

Stage de Master 2 de Damien Habault (élève de 3A à l'ENSCBP avril-septembre 2011)

L'équipe « polymer nanotechnologies for life science » du LCPO utilise diverses méthodes pour formuler des polymersomes à visée pharmaceutique encapsulant un principe actif (doxorubicine, Paclitaxel, siRNA..) dans une gamme de tailles 50 – 200 nm pour être injectés dans le sang : dissolution directe pour les copolymères fluides, nanopréciipitation pour les diblocs plus rigides à bloc hydrophobe vitreux ou semi-cristallin.¹¹⁷ Pour former des vésicules dites « géantes » c'est-à-dire de diamètres 5 à 150 μm avec des objectifs plus tournés vers la biophysique et le biomimétisme (« cellules artificielles »), on a le choix entre l'électroformation,¹¹⁸ l'émulsion-centrifugation¹¹⁹ mais aussi l'émulsion double en canal microfluidique.¹²⁰ L'objectif du stage de master était l'utilisation de la puce microfluidique mise au point au LCPO en collaboration avec le LOF (Fig. 56) pour formuler des vésicules géantes hybrides, avec des nanoparticules magnétiques insérées dans la membrane du polymersome (plutôt que dans leur cavité hydrophile) de la même manière que les polymersomes magnétiques sub-micrométriques préparés par nano-précipitation (cf. partie II). L'un des objectifs était de pouvoir observer en temps réel au microscope optique le comportement de telles vésicules soumises à un chauffage magnétique radio-fréquences (cf. projet), sous la forme de la libération d'une espèce fluorescente (simple colorant ou bien molécule thérapeutique de doxorubicine). Il s'agissait donc d'établir les conditions expérimentales (choix des solvants, traitements de surface, concentrations du copolymère et des nanoparticules, débits respectifs...) permettant d'obtenir des polymersomes de diamètres de l'ordre de 100 μm , et de vérifier leur stabilité sur plusieurs jours.



b)
Figure 56 :

a) puce microfluidique constituée de trois capillaires emboîtés (diamètres respectifs : 40 μm , 150 μm et 540 μm) permettant de produire des émulsions doubles de tailles contrôlée;¹²⁰ b) exemples de gouttes d'émulsions obtenues avec des jeux de débits respectifs $Q_1/Q_2/Q_3$: 0.5/49.5/300, 0.5/0.5/1000, 0.5/49.5/200 et 0.5/49.5/150 ($\mu\text{L} \cdot \text{min}^{-1}$) et pour les compositions indiquées. *masse molaires $M_n=2500/1300$ ou 6500/3900 g/mol (Polymer Source)

¹¹⁷ C. Sanson, J.-F. Le Meins, C. Schatz, A. Soum, S. Lecommandoux, Temperature responsive poly(trimethylene carbonate)-block-poly(L-glutamic acid) copolymer: polymersomes fusion and fission, *Soft Matter*, **6** (2010) 1722–1730

¹¹⁸ A. Carlsen, N. Glaser, J.-F. Le Meins, S. Lecommandoux, Block copolymer vesicle permeability measured by osmotic swelling and shrinking, *Langmuir*, **27** (2011), 4884–4890.

¹¹⁹ M. Marguet, O. Sandre, S. Lecommandoux, “Gelly” polymersomes: towards cell structural cell mimicry, *Langmuir*

¹²⁰ A. Perro, C. Nicolet, J. Angly, S. Lecommandoux, J.-F. Le Meins, A. Colin, Mastering a double emulsion in a simple co-flow microfluidic to generate complex polymersomes, *Langmuir*, **27** (2011), 9034–42.

On sait que microfluidique permet d'obtenir des émulsions extrêmement calibrées, et donc idéalement des polymersomes aussi bien contrôlés une fois que le solvant organique s'est évaporé et a laissé à la place à une membrane polymère. Néanmoins les publications de l'équipe de David Weitz mentionnent des difficultés pour maintenir l'émulsion double stable durant tout le processus de séchage, avec en particulier un effet de changement de la qualité du solvant durant l'évaporation.¹²¹ Ce problème de stabilité de l'émulsion ayant été surmonté pour les compositions choisies (tableau Fig. 56), il subsistait celui de l'excès de copolymère créant une excroissance de la membrane une fois le solvant évaporé (image ci-contre). L'introduction des nanoparticules magnétiques stabilisées par une monocouche d'acide oléique à hauteur de 0.1 wt.% d'oxyde de fer dans le solvant organique (soit $\Phi=2\times 10^{-4}$ v/v) a offert une solution originale, basée sur une compétition entre une force magnétique appliquée et la force de trainée visqueuse, pour extraire les vésicules magnétiques débarrassées de leur polymère en trop (Fig 57). Une puce de séparation et d'évaporation (à travers une fine épaisseur de PDMS perméable à l'air et aux solvants) a été pour cela construite, en collaboration avec Jaques Leng du LOF (UMR 5258 CNRS/Rhodia).

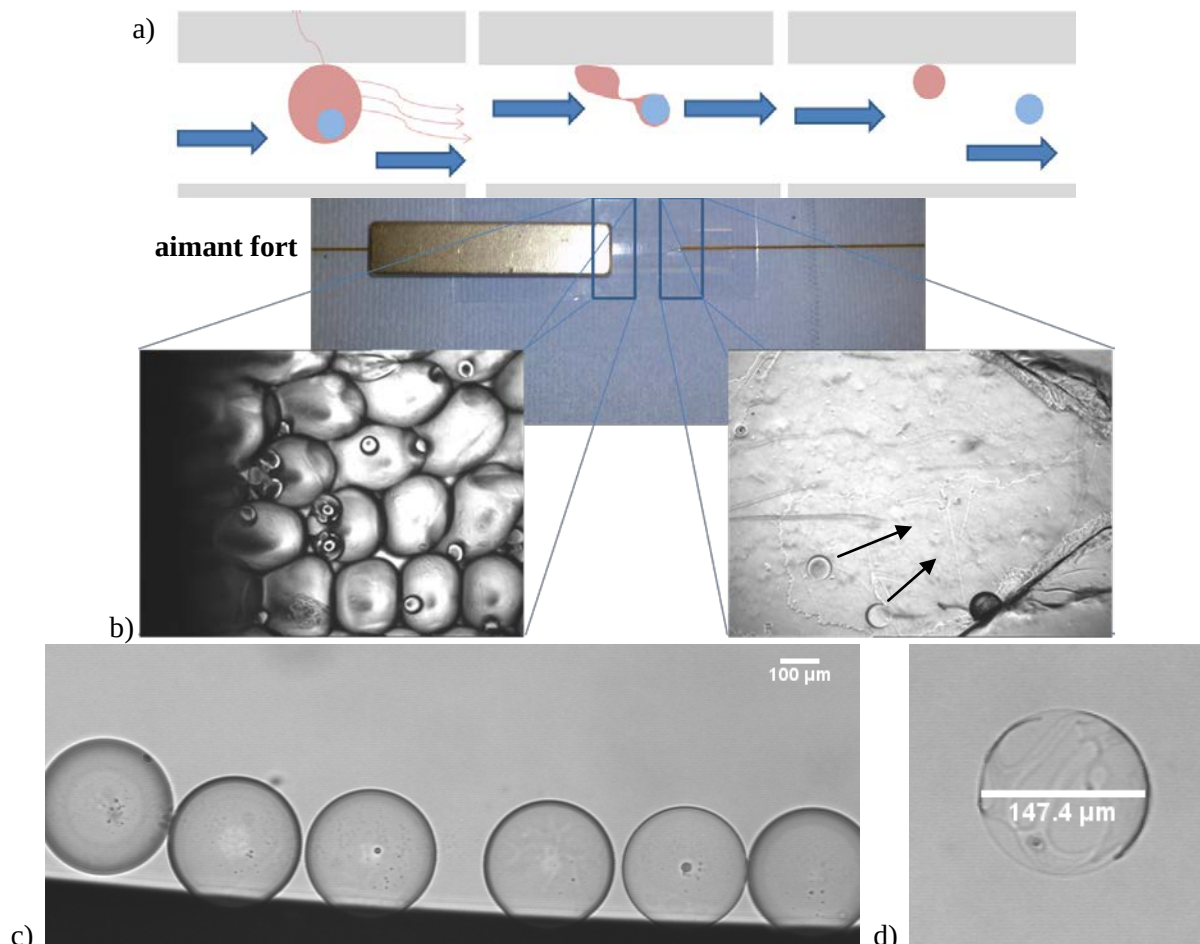
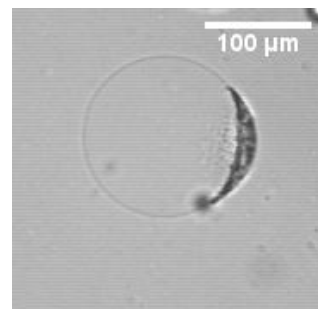


Fig. 57 : a) schéma de l'extraction des cœurs aqueux des gouttes d'émulsions double par compétition entre les forces magnétiques et de cisaillement; b) puce de récupération construite avec le LOF (les flèches indiquent le déplacement des vésicules vers la sortie ; c) vésicules magnétiques contenant encore un peu de solvant ; d) idem après séchage. La membrane des vésicules possède un caractère à la fois magnétique et caoutchoutique.

¹²¹ Hayward, R. C., Utada, A. S., Dan, N., & Weitz, D. A, Dewetting instability during the formation of polymersomes from block-copolymer-stabilized double emulsions, *Langmuir*, **22** (2006), 4457-61; Shum, H. C., Santanach-Carreras, E., Kim, J.-W., Ehrlicher, A., Bibette, J., & Weitz, D., Dewetting-induced membrane formation by adhesion of amphiphile-laden interfaces, *J. A. C. S.*, **133** (2011), 4420-4426

I. Projet : nouveaux vecteurs « théragnostiques » avec les polymersomes magnétiques

De nos études précédentes sont ressortis plusieurs enseignements sur la faisabilité de l'obtention de nano-vecteurs magnétiques polymères pour la « théragnostique », combinaison du diagnostique rendu possible par la visualisation par IRM d'une surconcentration de particules magnétiques près d'une tumeur et de la thérapie par destruction des cellules cancéreuses. Plutôt qu'un échauffement direct des cellules, nous visons un mécanisme reposant sur l'action biochimique d'une drogue libérée par la vésicule grâce à la réponse de sa membrane suite à l'élévation très locale de température autour des nanoparticules magnétiques (à l'échelle de la dizaine de nm), avant que la chaleur n'ait pu diffuser sur une plus grande distance. Or il n'existe aucun thermomètre à une échelle de 10-100 nm (même un thermomètre basé sur le changement avec la température du pas de l'hélice, donc de couleur, d'un cristal liquide cholestérique, a une taille minimale de l'ordre de la longueur d'onde de la lumière). Nous proposons donc de mesurer d'abord la réponse en taille des vésicules composites avec une élévation macroscopique de température. Puis nous utiliserons cet abaque pour déterminer, à partir des changements structuraux constatés, la température locale induite par les radiofréquences appliquées. D'ores et déjà nous avons constaté une réponse de l'augmentation de la vitesse de libération de l'espèce Dox par des vésicules de PTMC₂₄-b-PGA₁₉ qui est égale à 2/3 de celle obtenue avec un échauffement macroscopique à 37°C,⁵² ce qui est un résultat très encourageant compte tenu du fait que les nanoparticules de 6-7 nm utilisées pour ces premiers essais n'étaient pas optimisées pour l'hyperthermie : le pouvoir spécifique de chauffage augmentant drastiquement avec la taille, il est 20 fois plus élevé pour des nanoparticules de diamètre 10-12 nm.¹²² Une de mes premières tâches à mon arrivée à Bordeaux a été d'effectuer de nouvelles synthèses avec les compétences acquises durant ces années au laboratoire PECSA et de trier les nanoparticules selon leurs tailles.

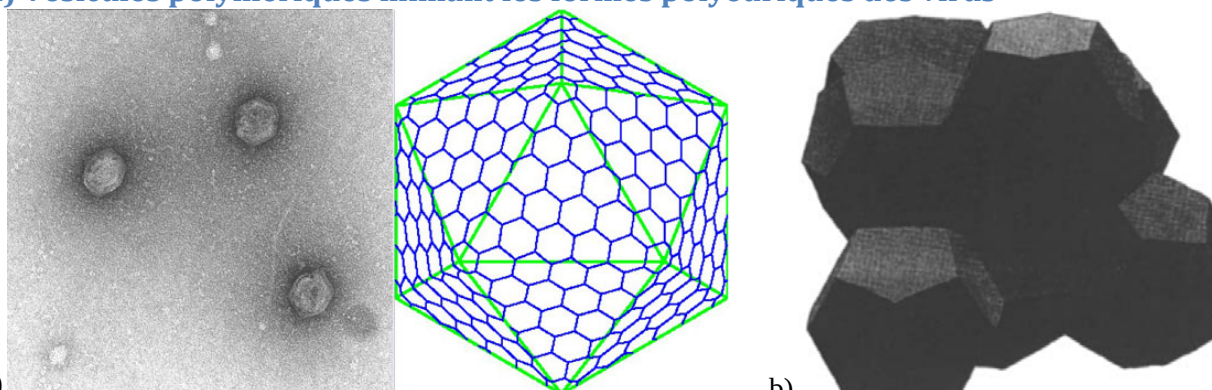
Depuis mon arrivée au LCPO en avril 2010, j'ai repris ma collaboration avec Sébastien Lecommandoux et son équipe. Etant sur place, j'ai pu apporter plus efficacement mon aide sur les aspects physicochimiques (obtention de polymérosomes géants – 10 à 150 µm – pour les études en microscopie optique et la micromanipulation ; couplage des nanoparticules et des copolymères, en les mettant en contact à différentes étapes (soit au moment de la formation des vésicules par nanoprécipitation ou émulsion double en canal microfluidique, soit ultérieurement). J'ai pu discuter avec les chimistes de la conception de nouveaux copolymères à blocs, et je vais moi-même me lancer dans la synthèse en dirigeant une thèse en cotutelle avec l'université de Waterloo). A plus long terme je souhaite explorer aussi les deux voies suivantes : l'une est l'obtention de polymérosomes non plus sphériques mais facettés comme le sont un certain nombre de capsides protéiques de virus, avec l'idée sans doute audacieuse de tester le pouvoir immunisant de telles structures artificielles ; l'autre est l'encapsulation d'acides nucléiques, soit d'ADN double brins relativement robuste pour étudier sa cinétique de libération (translocation) à travers un pore membranaire et tester un modèle biophysique,¹²³ soit d'ARN monobrin et en particulier de petites séquences d'ARN interférent (siRNA) réputées très fragiles qui sont très utiles pour des thérapies géniques anti-cancéreuses.¹²⁴

¹²² Fortin, J.-P.; Wilhelm, C.; Servais, J.; Ménager, C.; Bacri, J.-C.; Gazeau, F., Size-Sorted Anionic Iron Oxide Nanomagnets as Colloidal Mediators for Magnetic Hyperthermia. *J.A.C.S.* **2007**, *129*, 2628-2635; Ahrentorp, F.; Astalan, A.; Jonasson, C.; Blomgren, J.; Bin, Q.; Thompson Mefford, O.; Yan, M.; Courtois, J.; Berret, J.-F.; Fresnais, J.; Sandre, O.; Dutz, S.; Müller, R.; Johansson, C., Sensitive High Frequency AC Susceptometry in Magnetic Nanoparticle Applications. In *The 8th International Conference on the Scientific and Clinical Applications of Magnetic Carriers*, Häfeli, U.; Schütt, W.; Zborowski, M., Eds. AIP Conference Proceedings Series: Rostock, Germany, **2010**, *1311*, 213.

¹²³ P.-G de Gennes, Passive entry of a DNA molecule into a small pore, *Proc. Natl. Acad. Sci. USA* **96** (1999) 7262.

¹²⁴ Crombez, L.; Morris, M. C.; Dufort, S.; Aldrian-Herrada, G.; Nguyen, Q.; Master, G. M.; Coll, J.-L.; Heitz, F.; Divita, G., Targeting cyclin B1 through peptide-based delivery of siRNA prevents tumour growth, *Nucleic Acids Research*, **37** (2009), 4559-4569.

a) Vésicules polymériques mimant les formes polyédriques des virus



a) Virus à capsid icosaédrique. A gauche, image TEM de Sulfolobus Icosahedral Turreted Virus (STIV) découvert dans le parc de Yellowstone ([George Rice](#), Montana State Univ.). A droite, reconstruction 3d à partir de cryoTEM du réseau de protéines « capsomères » à la surface du STIV (Liand Tang, Scripps Research Institute). De nombreux autres virus ont une capsid ainsi composée de 20 faces triangulaires (phages, HIV..)

b) Cellule de Weaire et Phelan dans une mousse à 3d.¹²⁵ Cette maille de 2 dodécaèdres et 6 tétradécaèdres permettant de paver l'espace et de minimiser la surface pour un volume donné a été utilisée en architecture ("Watercube" des JO 2008 à Pékin).

Afin de mimer la géométrie non sphérique des capsides protéiques de certains virus (Fig. 58a), je compte m'appuyer sur la réticulation photochimique de polymérosomes (par exemple *via* la double liaison du bloc polybutadiène) soumis à certaines contraintes physiques. En particulier le figeage des déformations sous champ magnétique suivi de leur visualisation par TEM devrait nous permettre de corroborer nos études jusque là par diffusion des neutrons. Mais pour atteindre des formes facettées, je vais procéder à une compression osmotique des suspensions de polymérosomes (pour les empiler de manière dense) suivie d'une augmentation de leur volume, selon les blocs par effet d'une augmentation du pH (PGA) ou d'une diminution de la température (PMADAME ou PNIPAM). La surface totale de membrane étant fixée par la quantité totale de copolymère présente, on s'attend à obtenir une structure facettée comme une mousse, minimisant le rapport surface/volume (Fig. 58b).

b) Encapsulation de brins d'ADN dans des polymérosomes et étude de leur translocation à travers des pores membranaires

Je souhaite m'investir par ailleurs dans l'encapsulation d'acides nucléiques à l'intérieur des polymérosomes, bénéficiant au LCPO de l'interaction avec Elisabeth Garanger qui possède un grand savoir faire biochimique. L'encapsulation par des vésicules de copolymères amphiphiles hybrides bloc peptidique-bloc synthétique (l'un des deux étant thermosensible) est différente de la stratégie développée par Gillies Divita à Montpellier utilisant des peptides amphiphiles qui eux ne forment des micelles qu'en présence de siRNA par complexation électrostatique.¹²⁶ En plus des applications théragnostiques, je souhaite résoudre un problème biophysique introduit par P-G. de Gennes il y a 10 ans qui est la vitesse de libération d'un filament d'ADN à travers un pore dans une membrane. J'ai une idée pratique pour tester ce modèle datant de mon post-doc avec Deborah Fygenson à San Barbara, qui consiste à mesurer l'évolution du diamètre hydrodynamique du duo vésicules-ADN au cours du temps. L'interaction à Bordeaux avec le biologiste Olivier Lambert sera très profitable puisqu'il a étudié par cryo-TEM le mécanisme de libération de l'ADN par les phages T5 et la complexation de siRNA.

¹²⁵ Weaire, D.; Phelan, R., A counter-example to Kelvin's conjecture on minimal surfaces. *Philosophical Magazine Letters* **1994**, 69 (2), 107-110.

¹²⁶ M. C. Morris, E. Gros, G. Aldrian-Herrada, M. Choob, J. Archdeacon, F. Heitz, G. Divita, *Nucleic Acids Research*, **2007**, Vol. 35, No. 7 e49

c) *In vitro* assays of local heating and drug release with magnetic polymersomes

Our first measurements of *in vitro* DOX accelerate release by PTMC-*b*-PGA vesicles heated either by macroscopic or induction heating correlate with the endothermic peak at 34–40°C observed in microcalorimetry measurements attributed to the melting point of PTMC nanocrystalline domains in the membranes. The melting of (liquid)-crystalline domains has been indeed recognized as a strong factor for the increased permeability of vesicles made of mixtures of phospholipids around the “main chain transition temperature”.¹²⁷ On the practical side, the electronics necessary for Radio-Frequency induction heating was purchased by LCPO to one of the companies selling such devices for soldering purposes in jewelry artwork or bonding applications in dental prosthesis industry, including a power generator in the frequency range 750–1000 kHz with two low inductance coils for different geometries of the implemented RF heating:

- (i) a small inducting coil (1 turn, $\varnothing=15$ mm) that can be positioned precisely on a microscope stage to visualize giant polymersomes (5 μ m or larger) possibly deformed by the heating or biological cells during the hyperthermia treatment; coupling this coil to the microfluidic chip, we will evidence the minute temperature raise in sample droplets' trains in a mainstream of oil with lowest thermal conductivity and transparency in the RF range; the purpose in that case is to follow the variation of the diffusion coefficient D_v of thermo-sensitive polymersomes triggered by the absorption of a radio frequency wave as a direct proof of the modification of their hydrodynamic size or of the local temperature in their close vicinity (and hence of their membrane permeability).
- (ii) a larger inducting coil (4 turns, $\varnothing=50$ mm) to fit Petri dishes for incubation of cells or even a small animal (mouse) placed inside a home-made plastic restrainer for the therapeutic evaluations.

The setup is sketched on Figure 59. In all cases the experimentalist will be protected from the strong RF-field by a shield of μ -metal to fulfill the upper dose of RF irradiation $f_{RF} \times H_0 < 4.85 \times 10^8 \text{ Am}^{-1}\text{s}^{-1}$ recommended by Article 16(1) of European Directive 89/391/EEC, pp. 1–29 (2004) 347 dealing with “the minimum health and safety requirements regarding the exposure of workers to the risks arising from electromagnetic fields”.

Prior to those RF-wave induced shape and size modifications of nanocomposite vesicles, the specific loss powers (SLP in W/g) of our synthesized magnetic nanoparticles will be assessed by measuring the macroscopic temperature increment as a function of time for a concentrated suspension of MNPs. We know that USPIOs of diameters sharply centered around 10 nm exhibit a $SLP \approx 275 \text{ W/g}$ for a RF magnetic field intensity around 14 mT at 410 kHz.¹²² A vesicle WD15-50 (*i.e.* obtained by nanoprecipitation from DMSO into water in 15 min and loaded with 50 wt% USPIOs) having a radius $R_v=190$ nm contains about 2300 USPIOs in its membrane and encapsulate a water volume around $3 \times 10^{-14} \text{ cm}^3$. The iron oxide content per vesicle being $3 \times 10^{-15} \text{ g}$, the vesicle irradiates a heating power $P \approx 8 \times 10^{-13} \text{ W}$. If this power was dissipated only in the internal core of the vesicle filled with water (heat capacity $C_p \approx 4 \text{ J/K/g}$), the time necessary to heat up the vesicle by 1°C would be $(4 \times 3 \times 10^{-14} \text{ J}) / (8 \times 10^{-13} \text{ W}) = 0.15 \text{ s}$. However, this “adiabatic” estimate is unrealistic: it does not consider the thermal diffusivity in the surrounding medium ($k_m \approx 0.6 \text{ W/m/K}$ for water). A rough estimate leads to an equilibrium temperature increase $\Delta T \approx P / (k_m a)$ where P is the total power dissipated by all the vesicles submitted to magnetic induction and a is the size (radius) of the container (either vessel *in vitro* or tissue *in vivo*). Contrary to a common naïve idea, one cannot use intra-cellular hyperthermia to obtain a quantitative raise of temperature of tissues doped with MNPs if their size is smaller than 1 mm (a typical size of a solid tumor).¹²⁸ A recently reported experiment of magneto-chemotherapy (doxorubicin release *in cellulo*) needed to use a large quantity of magnetic polymer beads (~100 mg) separated from the cells by a short distance (~10 μ m).¹²⁹ In our project, we intend to reach a reasonable value of RF magnetic field application (*e.g.* 10 min) by using droplets (~200 μ m in diameter) in a microfluidic channel (~600 μ m) both to mimic the environment of blood capillaries alimentering the tumors and to minimize the thermalization effect of the aqueous buffer. Using standard flow focusing microfluidics well handled at LCPO after a collaboration with LOF (UMR 5258 Rhodia),¹²⁰ we have encapsulated the nanovesicles inside droplets separated by a continuous phase of an oil thermostated at 37°C, with a low dielectric constant (to be transparent to RF fields) and the lowest possible thermal conductivity to minimize heat dissipation outside the droplets. The temperature of the continuous oil phase will be assessed by an infra-red camera in a wavelength range 8–14 μ m with a spatial resolution of 60 μ m. The challenge of our project consists in measuring precisely the local temperature increase in the close vicinity of the vesicles at a nanoscale (10–100 nm) and comparing it with the following theoretical estimate: each droplet of radius $a=100 \mu\text{m}$ and volume $\approx 4 \text{ nL}$ ($4 \times 10^{-6} \text{ cm}^3$) will contain vesicles at a concentration 30

¹²⁷ Xiang, T.-X.; Anderson, B. D., Phase structures of binary lipid bilayers as revealed by permeability of small molecules. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1998**, 1370, 64-76.

¹²⁸ Rabin, Y., Is intracellular hyperthermia superior to extracellular hyperthermia in the thermal sense? *Int. J. Hyperthermia* **2002**, 18, 194-202.

¹²⁹ Brulé, S.; Levy, M.; Wilhelm, C.; Letourneur, D.; Gazeau, F.; Ménager, C.; Le Visage, C., Doxorubicin Release Triggered by Alginate Embedded Magnetic Nanoheaters: A Combined Therapy. *Advanced Materials* **2011**,

mg/mL thus containing 60 ng of iron oxide in $\sim 2 \times 10^7$ vesicles. The total power being $P \approx 16 \mu\text{W}$, the time necessary to heat up the droplet by 1°C (neglecting heat conduction into oil) should be $(4 \times 4 \times 10^{-6} \text{ J}) / (16 \times 10^{-6} \text{ W}) = 1 \text{ s}$. We will design a microfluidic chip with a long serpentine channel ($\sim 4 \text{ cm}$) corresponding to a residence time of at least 1 min inside the coil of the magnetic inductor with the used flow-rates ($\sim 10 \mu\text{L}/\text{mn}$). The experiment will use at least one of the following methods to detect a local temperature increase:

- (i) diffusion coefficient D_v of the vesicles measured by particle tracking compared to an abscissa curve vs. T obtained by dynamic light scattering;
- (ii) spectrum shift (at least $1 \text{ nm}/^\circ\text{C}$) of a thermochromic dye located inside the membranes as a reported betain compound confined in Pluronic micelles;¹³⁰
- (iii) IR thermography at the scale of the whole droplets (with a resolution of $60 \mu\text{m}$ better than their size).

By using a fluorinated oil such as Fluorinert FC-84 (3M) with $k_m = 0.059 \text{ W/m/K}$ at 37°C as thermal insulating liquid, we expect that the droplets have their temperature elevated by $\Delta T = P / (k_m a) \approx 6^\circ\text{C}$ in less than a minute along their way in the channel.

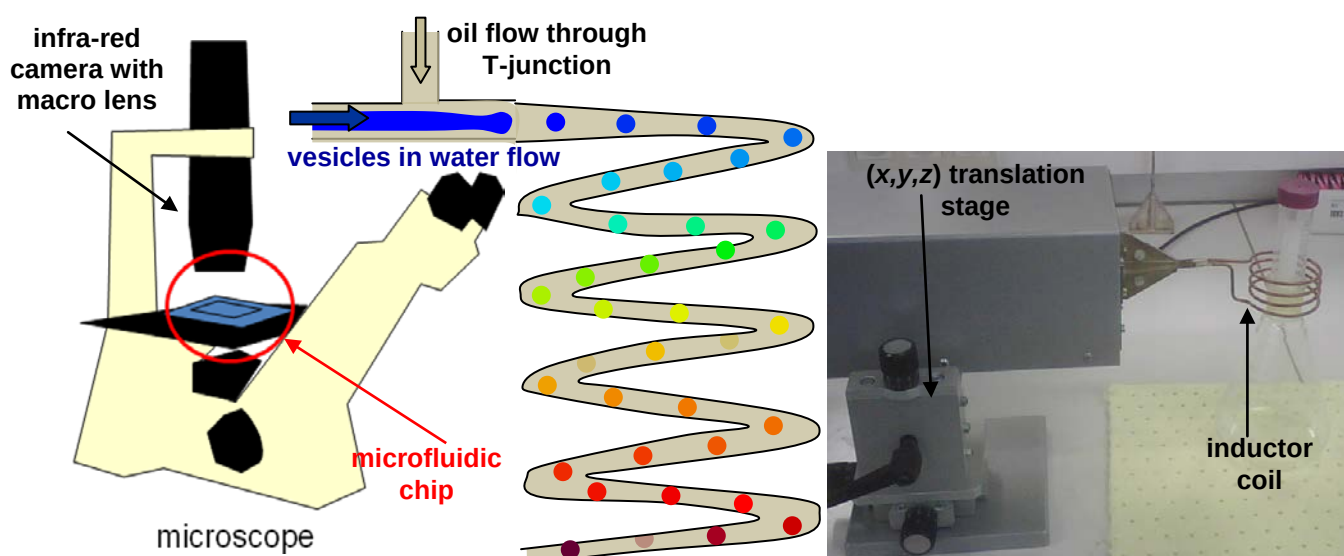


Fig. 59: RF magnetic induction set-up on the stage of an inverted microscope to be built at LCPO. The vesicles will be suspended in a series of droplets circulating in a fluorinated oil initially at 37°C . The IR camera will represent temperature on a color scale. The fluorescence images will give access to the local temperature close to the membranes either by particle tracking or with a T-reporter dye.

d) Migration of magnetic vesicles under a gradient of DC magnetic field

When the magnetic field intensity is not homogeneous but undergoes a spatial gradient, one observes a migration of magnetic polymersomes towards the direction of increasing field intensity called “magnetophoresis”. As checked in a test experiment, this drift superposes to the random walk of the polymersomes, which are Brownian colloids at a low Péclet's number (ratio of convective to diffusive velocities).⁵² Thus we will analyze carefully the trajectories with a dedicated particle tracking plugin of the ImageJ software to measure independently their diffusion coefficient D_v and migration velocity V_{drift} (Fig. 60). By equilibrating Stokes' drag force with the magneto-phoretic force, we see that the drift velocity is proportional to the magnetic dipole per vesicle m_v (product of m_p by the number of encapsulated USPIOs N_{nano}) and inversely proportional to the hydrodynamic radius:

$$6\pi\eta \times R_H \times V_{\text{drift}} = m_v \times L(B_0) \times \frac{dB}{dz}$$

where the degree of orientation of the magnetic dipoles is given by Langevin's function $L(B_0)$.

In preliminary experiments, we found a number of USPIOs $N_{\text{nano}} \approx 2300$ and a hydrodynamic radius $R_H = 190 \text{ nm}$ for vesicles with a 50 wt% payload of USPIOs, which exhibited a drift velocity $\approx 1 \mu\text{m/s}$ under a magnetic field gradient $dB/dz \approx 0.185 \text{ T/cm}$ created by a strong permanent magnet.⁵²

¹³⁰ Aliaga, C.; Briones, L.; Rezende, M. C.; Tirapegui, C., The thermochromism of the E_T(30) betaine in a micro-heterogeneous medium: A spectral and dynamics simulation study. *J. Coll. Inter. Sci.* **2010**, 349, 565–570.

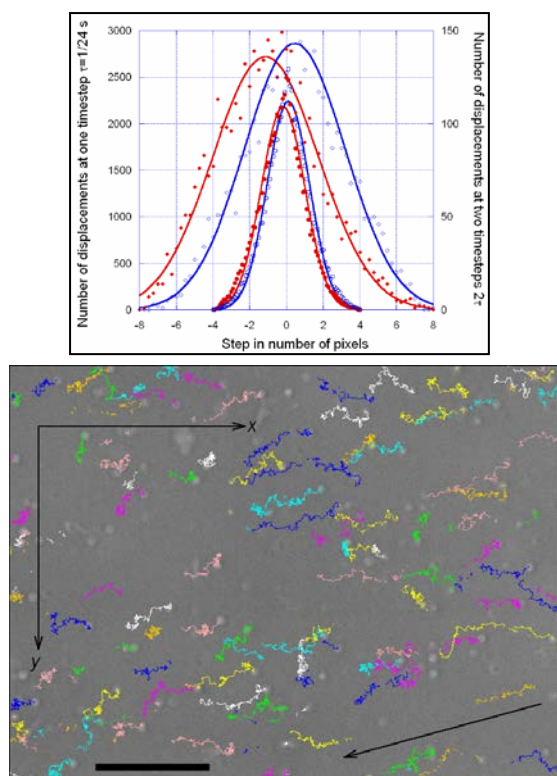


Fig. 60: Colored trajectories of WD15-50 vesicles under magnetic field gradient of direction indicated by the arrow. Bar is 20 μm . The histograms contain ~ 64000 individual displacements every $\tau=42$ ms time-step.

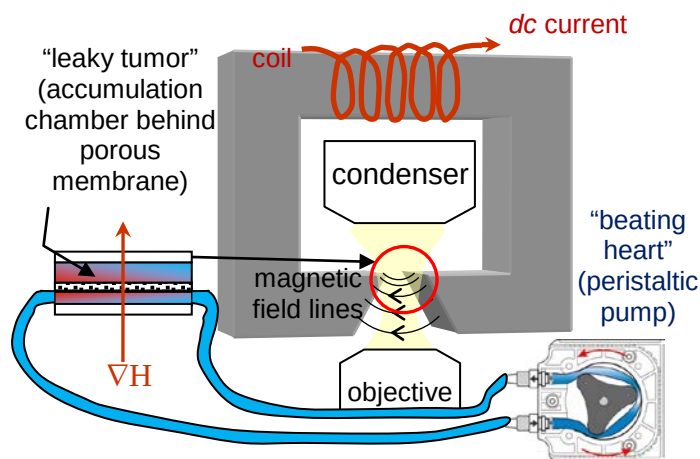


Fig. 61: Sketch of a hydrodynamic circuit to build for *in vitro* modeling of the magnetic guiding experiment to be performed later *in vivo*. The field lines created by an electromagnet are guided by a sharply edged polar piece, reaching a field gradient up to 30 T/cm.¹³¹ The solenoid is deported apart from the optical axis of the microscope by the use of a ferrite open ring.

According to literature, the effectiveness of *in vitro* migration of vesicles in a magnetic field gradient by sticking a strong magnet near the tumor for several hours (before removing it for the biodistribution analysis e.g. by IRM) is not straightforward and must rely on some favorable conditions: the magnetophoretic velocities ranging between 1-3 $\mu\text{m/s}$ for individual vesicles ~ 200 nm in diameter¹³² and 10-20 $\mu\text{m/s}$ for aggregates¹³³ are indeed at least one order of magnitude lower than the slowest velocity V_{stream} in the small capillaries (≈ 100 $\mu\text{m/s}$ in rats and ≈ 500 $\mu\text{m/s}$ in humans). Recently, a hydrodynamic numerical simulation showed that in addition to the ratio $V_{\text{drift}}/V_{\text{stream}}$ and to Péclet's number, the regime effectively observed between accumulation near the magnet or washing out by the blood stream was governed by a third parameter: this is Renkin's reduced diffusion coefficient, which is the ratio of diffusion constants of the magnetic NPs respectively in the center of the blood vessel (taking into account the collisions not only with the solvent molecules but also the red blood cells) and near the epithelium membrane, when the dimensions of the interstices between the cells (*fenestrae*) become comparable to the size of the NPs.⁵³ We propose to build a close-loop microfluidic circuit schematizing the blood circulation (Fig. 61) and to measure independently the diffusion coefficients D_V and the migration velocities V_{drift} of the vesicles in various conditions by particle tracking (Fig. 60) in order to prepare *in vivo* experiments of "tumor homing". In particular, the discrepancy in the inter-cellular space between a normal capillary (~ 60 nm) and one near a tumor (>600 nm) much more leaky will be simulated by coupling a filtration membrane of controlled pores' size to the circuit (Fig. 61).

e) Magnetic Micelles of Arborescent Polymers as Therapy and Diagnostic Agents

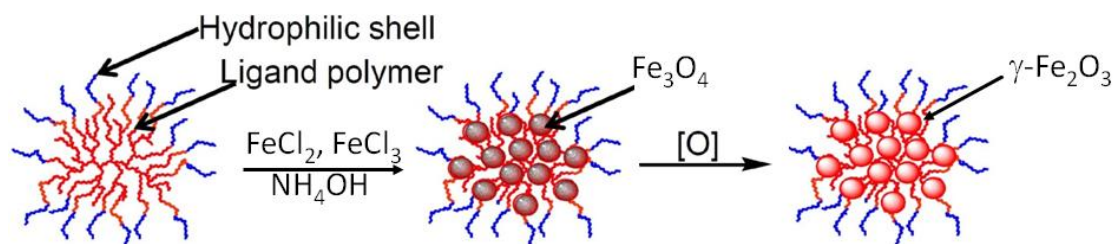
Thèse en cotutelle IDS-FunMat avec le Pr Mario Gauthier de l'université de Waterloo

¹³¹ Lim, J.; Lanni, C.; Evarts, E. R.; Lanni, F.; Tilton, R. D.; Majetich, S. A., Magnetophoresis of Nanoparticles. *ACS Nano*, 5 (2011) 217-226.

¹³² Fortin-Ripoche, J.-P.; Martina, M. S.; Gazeau, F.; Ménager, C.; Wilhelm, C.; Bacri, J.-C.; Lesieur, S.; Clément, O., Magnetic Targeting of Magnetoliposomes to Solid Tumors with MR Imaging Monitoring in Mice: Feasibility, *Radiology*, 239 (2006) 415-424.

¹³³ Sinha, A.; Ganguly, R.; Puri, I. K., Magnetic separation from superparamagnetic particle suspensions. *J. Mag. Mat.*, 321 (2009), 2251-2256.

Polymeric micelles loaded with magnetic iron oxides (*e.g.* Fe_3O_4 , magnetite or $\gamma\text{-Fe}_2\text{O}_3$, maghemite) are being investigated in a number of biomedical applications including Magnetic Resonance Imaging (MRI) contrast agents and for the radio frequency wave-induced destruction of cancer cells.¹³⁴ These can be obtained by the adsorption of amphiphilic block copolymers onto preformed nanoparticles,^{44,49,86} or through the self-assembly of block copolymers into micelles and complexation with iron salts.¹³⁵ Both approaches have their limitations. For example spherical micelles are only formed over a narrow block copolymer composition range, and they can dissociate if the solvency conditions (solvent type, pH, temperature, etc.) change; their size is also difficult to vary over a wide range.



We now propose a new strategy for the preparation of polymer-stabilized magnetic nanoparticles with stealth properties (invisible to the immune system) in biological media, potentially useful as MRI contrast agents and for cancer treatment by magnetic hyperthermia. We will use amphiphilic branched copolymers with a dendritic architecture (arborescent polymers) both as template for the iron cores' synthesis and to provide a repulsive (stabilizing) shell around the nanoparticles. The challenge for MRI and hyperthermia applications consists in maintaining large (*e.g.* 15–25 nm) nanoparticles dispersed in blood serum, *i.e.* in a high ionic strength and protein content environment. These macromolecules are derived from successive grafting reactions of linear polymer segments.¹³⁶ Unimolecular water-soluble micelles in a size range from 5–200 nm can be derived from these materials by adding polar polymer segments in the last grafting cycle to form a hydrophilic shell.¹³⁷ This unique approach will provide stable micelles independently of the solvency conditions used, and whose characteristics (size, flexibility) are predetermined in their synthesis. We will explore the use of amphiphilic arborescent copolymers (synthesized in Waterloo) to generate magnetic nanoparticles of uniform yet controllable size, as shown schematically below. These copolymers will incorporate side-chains acting as ligands for iron salts such as poly(2-vinylpyridine), poly(acrylic acid), and poly(glutamic acid), and a shell of biocompatible poly(ethylene oxide), polyglycidol, or polyoxazoline chains to provide steric stabilization. The magnetic properties of the nanoparticles will be investigated in Bordeaux with TEM, XRD, and magnetometry measurements to evaluate their potential in MRI diagnostic applications. The hyperthermic properties in radio-frequency fields will be studied at LCPO, which owns the equipment and knowledge to characterize the energy absorption rate of magnetic nanoparticles subjected to *ac* magnetic fields. Collaboration with a pharmaceutical company will be developed to explore all the possible outcomes of the magnetic unimacromolecular micelles in the fields of medical imaging and therapy.

The project will require alternating 6–12 months stays of the PhD student in Waterloo and in Bordeaux for the synthetic and the characterization portions of the work, respectively. After a selection process involving 75 candidates, we selected Mrs An Vo-Thu Nguyen who graduated in organic chemistry at Ho Chi Minh City university in Vietnam and Roskilde university in Denmark.

¹³⁴ a) A. Aqil, S. Vasseur, E. Duguet, C. Passiranic, J-P. Benoît, A. Roch, R. N. Muller, R. Jérôme, C. Jérôme *Eur. Polym. J.* **2008**, *44*, 3191–3199.; b) J-P. Fortin, F. Gazeau, C. Wilhelm *Eur. Biophys. J.* **2008**, *37*, 223–228

¹³⁵ J. I. Abes, R. E. Cohen, C. A. Ross *Mater. Sci. Eng.* **2003**, *C23*, 641.

¹³⁶ S. J. Teertstra, M. Gauthier *Prog. Polym. Sci.* **2004**, *29*, 277.

¹³⁷ a) M. Gauthier, L. Tichagwa, J.S. Downey, S. Gao *Macro* **1996**, *29*, 519; b) Gauthier *et al. Macro* **2003**, *36*, 2642

J. Collaborations nationales et internationales

a) au LCPO

Titulaire d'une chaire IUF junior et de la médaille de bronze du CNRS, Professeur ENSCPB, Sébastien Lecommandoux est l'un des chercheurs à avoir introduit en France l'auto-assemblage supramacromoléculaire à son retour de post-doc chez Samuel Stupp à Northwest University. Il est coordinateur de l'un des 4 thèmes du projet du LCPO intitulé "Polymer Nanotechnology for Life Science", où interviennent plusieurs acteurs parmi lesquels, sans être exhaustif : Christophe Schatz, Maître de conférences, qui synthétise et étudie des copolymères pour les applications biologiques¹³⁸ ; Jean-François Le Meins,¹³⁹ Maître de conférences, qui s'intéresse à une nouvelle méthode microfluidique de préparation des vésicules et à leur micromanipulation ; Elisabeth Garanger, Post-doctorante biochimiste ayant passé une étape de pré-sélection de l'IECB devant un jury international, qui maîtrise la synthèse peptidique et la culture cellulaire ; Daniel Taton, Professeur, chimiste qui a été en détachement dans la société Rhodia où il a contribué au développement de la polymérisation radicalaire contrôlée MADIX pour synthétiser des copolymères à blocs doubles hydrophiles (DHBC).¹⁴⁰ Son expertise lui ayant permis de synthétiser des polymères aux structures des plus complexes (dendrimères et nanogels¹⁴¹), il développe actuellement des procédés de synthèse novateurs qui permettront d'obtenir les copolymères « hybrides ».

b) sur le campus de Bordeaux

- ICMCB (UPR 9048) : Etienne Duguet, Professeur, et Stéphane Mornet, Chargé de Recherche, qui travaillent tous deux depuis longtemps sur le développement de nanoparticules magnétiques pour les applications d'hyperthermie, dans le cadre du projet européen FAME sur les hybrides organiques-inorganiques multifonctionnels et du GIS Matériaux Aquitaine. Ils sont les auteurs d'une revue très complète sur les applications biomédicales des nanoparticules magnétiques¹⁴² et de nombreux travaux sur les colloïdes Janus ou plus complexes.¹⁴³ Ensemble ils ont développé les nanoparticules VUSPIO (pour "versatile ultrasmall supermagnetic iron oxides") greffées par un aminopropylsilane et couplées à des molécules de Dextran partiellement oxydées, qui constituent une plateforme très intéressante pour être injectées en milieux biologiques.¹⁴⁴

- CBMN (UMR 5248) : Olivier Lambert, Directeur de recherche, spécialiste de cryo-microscopie électronique et de ses développements les plus pointus (reconstruction 3d par cryotomographie) pour imager des nano-structures biologiques réputées peu contrastées et fragiles. Il a étudié le rôle de la protéine FhuA dans le mécanisme de pénétration de l'ADN des bactériophages (virus qui s'attaquent aux bactéries)¹⁴⁵ et des liposomes multi-lamellaires chargés en nanoparticules magnétiques.¹⁴⁶

¹³⁸ Schatz, C.; Louguet, S.; Le Meins, J.-F.; Lecommandoux, S., Polysaccharide-block-polypeptide Copolymer Vesicles: Towards Synthetic Viral Capsids. *Angew. Chem.-Int. Edit.* **2009**, *48*, 2572–2575.

¹³⁹ Le Meins, J.-F.; Houg, C.; Borsali, R.; Taton, D.; Gnanou, Y., Morphological Changes Induced by Addition of Polystyrene to Dextran-Polystyrene Block Copolymer Solutions. *Macromol. Symp.* **2009**, *281*, 113-118.

¹⁴⁰ Taton, D.; Wilczewska, A.-Z.; Destarac, M., Direct Synthesis of Double Hydrophilic Statistical DiandTriblock Copolymers Comprised of Acrylamide and Acrylic Acid Units via the MADIX Process. *Macromol. Rapid Commun.* **2001**, *22*, 1497-1503.

¹⁴¹ Poly, J.; Wilson, D. J.; Destarac, M.; Taton, D., Synthesis of Poly(vinyl acetate) Nanogels by Xanthate-Mediated Radical Crosslinking Copolymerization. *Macromol. Rapid Commun.* **2008**, *29*, 1965-1972.

¹⁴² Mornet, S.; Vasseur, S.; Grasset, F.; Duguet, E., Magnetic nanoparticle design for medical diagnosis and therapy. *J. Mater. Chem.* **2004**, *14* (2161-2175).

¹⁴³ Perro, A.; Reculusa, S.; Pereira, F.; Delville, M.-H.; Mingotaud, C.; Duguet, E.; Bourgeat-Lami, E.; Ravaine, S., Towards large amounts of Janus nanoparticles through a protection-deprotection route. *Chem. Commun.* **2005**, 5542-5543; Taveau, J.-C.; Nguyen, D.; Perro, A.; Ravaine, S.; Duguet, E.; Lambert, O., New insights into the nucleation and growth of PS nodules on silica nanoparticles by 3D cryo-electron tomography. *Soft Matter* **2008**, *4*, 311-315.

¹⁴⁴ S. Mornet, J. Portier, E. Duguet, A method for synthesis and functionalization of ultrasmall superparamagnetic covalent carriers based on maghemite and dextran, *J. Mag. Mat* **293** (2005) 127–134.

¹⁴⁵ Bohm, J.; Lambert, O.; Frangakis, A. S.; Letellier, L.; Baumeister, W.; Rigaud, J. L., FhuA-mediated phage genome transfer into liposomes: A cryo-electron tomography study. *Current Biology* **2001**, *11* (15), 1168-1175.

- LOF (UMR 5258) : Jaques Leng, Chargé de Recherche CNRS, qui a développé une méthode de séchage contrôlé en puce microfluidique en profitant de la perméabilité naturelle du PDMS aux gaz, permettant d'étudier des diagrammes de phase des colloïdes concentrés.¹⁴⁷

c) au niveau national

- UPMC : Vincent Dupuis (PECSA) et Emmanuelle Lacaze (INSP) sur les ferronématiques
- MSC-Paris 7 : Jean-François Berret, malgré la fin « administrative » de nos projets communs, l'ANR blanche ITC-Nanoprobe et le projet européen Nano-3T (7^{ème} PCRD n°CE 214137).
- CEA-Saclay : LLB (Annie Brûlet, Fabrice Cousin) pour les études de SANS et LIONS (Olivier Spalla) pour le SAXS, en partenariat avec Ravier Perez et Florian Meneau du synchrotron SOLEIL.

d) et international

- Yuryi Raikher de l'institut de mécanique des milieux continus de Perm (Russie) qui développe des modèles théoriques en particulier sur les ferronématiques, les ferrogels,...
- Yves Gossuin de l'UMons (Belgique), avec qui j'ai soumis une communication sur l'optimisation des propriétés des particules magnétiques comme agents de contraste IRM.⁵⁵
- Amithaba Sarkar du département de chimie organique de l'Indian Association for the Cultivation of Science à Calcutta en Inde, sur le greffage d'un antibactérien sur les nanoparticules et la réalisation de particules à cœur magnétique et coque d'or par greffage de carbènes de Fisher.

K. Conclusion générale

Ce mémoire aura présenté, j'espère d'une manière suffisamment cohérente, le fil de mes recherches durant les dix dernières années. Plusieurs sujets ne sont pas si disjoints qu'on pourrait croire à première vue, possédant en fait des « correspondances » au sens baudelairien du terme ! Par exemple la synthèse de nano-bâtonnets de goethite intervient à la fois dans la thématique sur les cristaux liquides et celle sur la microfluidique, le concept d'interaction entre nanoparticules par effet de déplétion à la fois dans le sujet sur les ferronématiques et la transition vésicule-micelle de copolymères diblocs, le dopage de la maille de goethite ou de maghémite par des ions nickel ou cobalt à la fois sur les propriétés magnétiques, optiques, mais aussi d'agents de contraste en IRM et le guidage magnétique (les lois d'échelles ayant montré que ces deux dernières propriétés pouvaient être optimisées simultanément)... J'ai choisi délibérément d'écrire le manuscrit dans un style vivant, parfois anecdotique, présentant les résultats dans leur contexte humain ou historique. Cela correspond en effet à ma vision de la science, qui est très compétitive, mais doit rester à une échelle humaine, l'unité de taille étant l'équipe de recherche, au sein d'un laboratoire cohérent mais surtout ouvert sur le monde (comme en témoignent les vingt nationalités différentes présentes au LCPO !), à une époque où les scientifiques du monde entier et en particulier des pays émergents apportent désormais une contribution excellente, et non plus seulement le trio des « vieilles nations » que sont l'Europe, les USA et le Japon.

¹⁴⁶ Faure, C.; Meyre, M.-E.; Trépout, S.; Lambert, O.; Lebraud, E., Magnetic Multilamellar Liposomes Produced by In Situ Synthesis of Iron Oxide Nanoparticles: "Magnetoliposomes". *J. Phys. Chem. B*, **113** (2009), 8552-8559.

¹⁴⁷ H. Bodiguel, J. Leng, Imaging the drying of a colloidal suspension, *Soft Matter*, **6** (2010) 5451-5460; J. Leng, *Phys. Rev. E*, **82** (2010), 021405.

L. Annexe : production scientifique exhaustive (septembre 2011)

1. Peer-reviewed articles in international journals:

N°	Authors	Reference	Title	Times cited
1*	Letellier, D.; Sandre, O.; Menager, C.; Cabuil, V.; Lavergne, M.	<i>Materials Science & Engineering, C: Biomimetic Materials, Sensors and Systems</i> (1997), C5 (2), 153-162 http://dx.doi.org/10.1016/S0928-4931(97)00035-0	Magnetic tubules	21
2	Sandre, O.; Browaeys, J.; Perzynski, R.; Bacri, J.-C.; Cabuil, V.; Rosensweig, R. E.	<i>Physical Review E: Statistical Physics, Plasmas, Fluids, and Related Interdisciplinary Topics</i> (1999), 59 (2-A), 1736-1746 http://link.aps.org/doi/10.1103/PhysRevE.59.1736	Assembly of microscopic highly magnetic droplets: Magnetic alignment versus viscous drag	25
3	Sandre, O.; Gorre-Talini, L.; Ajdari, A.; Prost, J.; Silberzan, P.	<i>Physical Review E: Statistical Physics, Plasmas, Fluids, and Related Interdisciplinary Topics</i> (1999), 60 (3), 2964-2972 http://link.aps.org/doi/10.1103/PhysRevE.60.2964	Moving droplets on asymmetrically structured surfaces	46
4	Sandre O; Moreaux L; Brochard-Wyart, F.	<i>Proceedings of the National Academy Of Sciences USA</i> (1999 Sep 14), 96 (19), 10591-10596 http://dx.doi.org/10.1073/pnas.96.19.10591	Dynamics of transient pores in stretched vesicles	135
5	Brochard-Wyart, F.; de Gennes, P. G.; Sandre, O.	<i>Physica A: Statistical Mechanics and Its Applications</i> (Amsterdam) (2000), 278 (1-2), 32-51 http://dx.doi.org/10.1016/S0378-4371(99)00559-2	Transient pores in stretched vesicles: role of leak-out	69
6	Brochard-Wyart, F.; Buguin, A.; Martin, P.; Martin, A.; Sandre, O.	<i>Journal of Physics: Condensed Matter</i> (2000), 12 (8A), A239-A244 http://iopscience.iop.org/0953-8984/12/8A/330/	Adhesion of soft objects on wet substrates	3
7	Bernard, A.-L.; Guedeau-Boudeville, M.-A.; Sandre, O.; Palacin, S.; di Meglio, J.-M.; Jullien, L.	<i>Langmuir</i> (2000), 16 (17), 6801-6808 http://pubs.acs.org/doi/abs/10.1021/la991342p	Permeation through Lipid Bilayers by Adhesion of Giant Vesicles on Decorated Surfaces	23
8*	Sandre, O.; Menager, C.; Prost, J.; Cabuil, V.; Bacri, J.-C.; Cebers, A.	<i>Physical Review E: Statistical Physics, Plasmas, Fluids, and Related Interdisciplinary Topics</i> (2000), 62 (3-B), 3865-3870 http://link.aps.org/doi/10.1103/PhysRevE.62.3865	Shape transitions of giant liposomes induced by an anisotropic spontaneous curvature	22
9	Moreaux, L. ; Sandre, O.; Mertz, J.	<i>Optics Letters</i> (2000), 25 , 320-322 http://dx.doi.org/10.1364/OL.25.000320 et errata 25 , 678-678 http://dx.doi.org/10.1364/OL.25.000678	Membrane imaging by simultaneous second-harmonic generation and two-photon microscopy	112
10	Moreaux, L.; Sandre, O.; Blanchard-Desce, M.; Mertz, J	<i>Molecular Crystals and Liquid Crystals Science and Technology Section B Nonlinear Optics</i> (2000), 25 (1-4), 183-188	Simultaneous second-harmonic generation and two-photon excited fluorescence microscopy	0
11	Moreaux, L.; Sandre, O.; Mertz, J.	<i>Journal of the Optical Society of America B: Optical Physics</i> (2000), 17 (10), 1685-1694 http://dx.doi.org/10.1364/JOSAB.17.001685 republié dans <i>SPIE milestone series</i> (2003), 175 , 59- 68	Membrane imaging by second-harmonic generation microscopy	166

12	Moreaux, L.; Sandre, O.; Charpak, S.; Blanchard-Desce, M.; Mertz, J.	<i>Biophysical Journal</i> (2001 Mar), 80 (3), 1568-74 http://dx.doi.org/10.1016/S0006-3495(01)76129-2	Coherent scattering in multi-harmonic light microscopy	142
13	Karatekin, E.; Sandre, O.; Brochard, F.	<i>Polymer International</i> (2003) 52 , 486-493 http://dx.doi.org/10.1002/pi.1007	Transition pores in vesicles	15
14	Karatekin, E.; Sandre, O.; Guitouni, H.; Borghi, N.; Puech, P.H.; Brochard, F.	<i>Biophysical Journal</i> (2003), 84 , 1734-1749 http://dx.doi.org/10.1016/S0006-3495(03)74981-9	Cascades of transient pores in giant vesicles: Line tension and transport	92
15*	Galicia, J.A.; Sandre, O.; Cousin, F.; Guemghar, D.; Ménager, C.; Cabuil, V.	<i>Journal of Physics: Condensed Matter</i> (2003), 15 , S1379-S1402 http://iopscience.iop.org/0953-8984/15/15/306/	Designing magnetic composite materials using aqueous magnetic fluids	11
16*	Ménager, C.; Sandre, O.; Mangili, J.; Cabuil, V.	<i>Polymer</i> (2004), 45 , 2475-2481 http://dx.doi.org/10.1016/j.polymer.2004.02.018	Preparation and swelling of hydrophilic magnetic microgels	34
17*	Lecommandoux, S.; Sandre, O.; Chécot, F.; Rodriguez-Hernandez, J.; Perzynski, R.	<i>Advanced Materials</i> (2005), 17 , 712-718 http://dx.doi.org/10.1002/adma.200400599	Magnetic Nano-composite Micelles and Vesicles	59
18*	Da Cruz, C.; Sandre, O.; Cabuil, V.	<i>Journal of Physical Chemistry B</i> (2005), 109 (30), 14292-14299 http://pubs.acs.org/doi/abs/10.1021/jp0455024	Phase behavior of nanoparticles in a thermotropic liquid crystal	16
19*	J.-F. Berret, N. Schonbeck, F. Gazeau, D. El Kharrat, O. Sandre, A. Vacher, M. Airiau	<i>Journal of the American Chemical Society</i> (2006), 128 (5), 1755 – 1761 http://dx.doi.org/10.1021/ja0562999	Controlled Clustering of Superparamagnetic Nanoparticles Using Block Copolymers: Design of New Contrast Agents for Magnetic Resonance Imaging	138
20*	S. Lecommandoux, O. Sandre, F. Chécot, J. Rodriguez-Hernandez, R. Perzynski.	<i>Journal of Magnetism and Magnetic Materials</i> 300 (2006), 71-74 http://dx.doi.org/10.1016/j.jmmm.2005.10.035	Self-assemblies of magnetic nanoparticles and di-block copolymers : Magnetic micelles and vesicles.	12
21*	S. Lecommandoux, O. Sandre, F. Chécot, R. Perzynski.	<i>Progress in Solid State Chemistry</i> 34 (2006) 171-179 http://dx.doi.org/10.1016/j.progsolidstchem.2005.11.050	Smart hybrid magnetic self-assembled micelles and hollow capsules	21
22*	J.-F. Berret, A. Sehgal, M. Morvan, O. Sandre, A. Vacher, M. Airiau	<i>Journal of Colloid and Interface Science</i> 303 (2006) 315–318 http://dx.doi.org/10.1016/j.jcis.2006.07.025	Stable oxide nanoparticle clusters obtained by complexation	25
23*	J.-F. Berret, O. Sandre, A. Mauger	<i>Langmuir</i> 23 (2007) 2993–2999 http://dx.doi.org/10.1021/la061958w	Size Distribution of Superparamagnetic Particles Determined by Magnetic Sedimentation	22
24*	A. Abou Hassan, O. Sandre, V. Cabuil, P. Tabeling	<i>Chemical Communications</i> (2008), Issue 15, 1783 - 1785 http://dx.doi.org/10.1039/b719550h	Synthesis of iron oxide nanoparticles in a microfluidic device: preliminary results in a coaxial flow millichannel	13

25*	M. Meyer, Yu. L. Raikher, O. Sandre, A. Bée, V. Cabuil, V. Dupuis, P. Licinio, and R. Perzynski	<i>Eur. Phys. J. E</i> 26 , 355–360 (2008) http://dx.doi.org/10.1140/epje/i2007-10338-5	Magneto-orientational properties of ionically stabilized aqueous dispersions of Ni(OH) ₂ nanoplatelets	2
26*	J. Fresnais, J.-F. Berret, B. Frka-Petesic, O. Sandre, R. Perzynski	<i>Advanced Materials</i> 20 , 3877–3881 (2008) http://dx.doi.org/10.1002/adma.200800846	Controlled co-assembly of iron oxide nanoparticles and polymers: towards the generation of highly persistent super-paramagnetic nanorods	27
27*	J. Fresnais, J.-F. Berret, L. Qi, J.-P. Chapel, J.-C. Castaing, O. Sandre, B. Frka-Petesic, R. Perzynski, J. Oberdisse, and F. Cousin	<i>Physical Review E</i> 78 , 040401R (2008) http://dx.doi.org/10.1103/PhysRevE.78.040401	Universal scattering behavior of coassembled nanoparticle-polymer clusters	16
28*	Ishow E*, Brosseau A, Clavier G, Nakatani K, Tauc P, Fiorini-Debuisschert C, Neveu S, Sandre O, Leautic A	<i>Chemistry of Materials</i> 20 (21), 6597-6599 (2008) http://dx.doi.org/10.1021/cm802264c	Multicolor Emission of Small Molecule-Based Amorphous Thin Films and Nanoparticles with a Single Excitation Wavelength	12
29*	Fresnais, J.; Berret, J.-F.; Frka-Petesic, B.; Sandre, O.; Perzynski, R.*	<i>Journal of Physics: Condensed Matter</i> 20 (49), 494216/1-6 (2008) http://dx.doi.org/10.1088/0953-8984/20/49/494216	Reorientation kinetics of superparamagnetic nanostructured rods	5
30*	A. Abou-Hassan*, O. Sandre, S. Neveu, V. Cabuil	<i>Angew. Chem. Int. Ed.</i> 48 , 2342-2345 (2009) http://dx.doi.org/10.1002/anie.200805933	Synthesis of Goethite by Separation of the Nucleation and Growth of Ferrihydrite Nanoparticles by Microfluidics	11
31*	A. Galicia, F. Cousin, E. Dubois, O. Sandre, V. Cabuil, R. Perzynski*	<i>Soft Matter</i> 5 , 2614-2624 (2009) http://dx.doi.org/10.1039/b819189a	Static and dynamic structural probing of swollen Polyacrylamide ferrogels	8
32	J. Fresnais, E. Ishow*, O. Sandre, J.-F. Berret*	<i>Small</i> 5 (22), 2533-2536 (2009) http://dx.doi.org/10.1002/sml.200900703	Electrostatic Co-assembly of Magnetic Nanoparticles and Fluorescent Nanospheres: A Versatile Approach Towards Bimodal Nanorods	2
33*	A. Abou-Hassan*, J.-F. Dufrêche, O. Sandre, G. Mériguet, O. Bernard, V. Cabuil	<i>J. Chem. Phys. C</i> 113 (42) 18097-18105 (2009) http://dx.doi.org/10.1021/jp9069459	Fluorescence confocal scanning microscopy for pH mapping in a coaxial flow microreactor: application in the synthesis of superparamagnetic nanoparticles	3
34*	A. Abou-Hassan*, O. Sandre, V. Cabuil*	<i>Angew. Chem. Int. Ed.</i> 49 (36) 6268-6286 <i>Angew. Chem.</i> 122 (36) 6408-6428 (2010) http://dx.doi.org/10.1002/anie.200904285 http://dx.doi.org/10.1002/ange.200904285	Microfluidics for Inorganic Chemistry (Review) Mikrofluidik in der anorganischen Chemie (Aufsatz)	5
35*	M. Safi, H. Sarrou, O. Sandre, N. Mignet, J.-F. Berret*	<i>Nanotechnology</i> 21 (2010) 145103 (10pp) http://dx.doi.org/10.1088/0957-4484/21/14/145103	Interactions between sub-10-nm iron and cerium oxide nanoparticles and 3T3 fibroblasts: the role of the coating and aggregation state	3

36	A-S. Robbes, J. Jestin, F. Meneau, F. Dalmas, O. Sandre, J. Perez, F. Boué, F. Cousin*	<i>Macromolecules</i> 43 (13) (2010) 5785-5796 http://dx.doi.org/10.1021/ma100713h	Homogeneous Dispersion of Magnetic Nanoparticles Aggregates in a PS Nanocomposite: Highly Reproducible Hierarchical Structure Tuned by the Nanoparticles' Size	2
37*	S. Douadi-Masrouki, B. Frka-Petescic, M. Save, B. Charleux, V. Cabuil, O. Sandre*	<i>Polymer</i> 51 (21) (2010) 4673-4685 http://dx.doi.org/10.1016/j.polymer.2010.08.043	Incorporation of magnetic nanoparticles into lamellar polystyrene- <i>b</i> -poly(n-butyl methacrylate) diblock copolymer films: influence of the chain end-groups on nanostructuration	0
38*	C. Sanson, O. Diou, E. Ibarboure, A. Soum, A. Brûlet, S. Miraux, E. Thiaudière, S. Tan, A. Brisson, V. Dupuis, O. Sandre*, S. Lecommandoux*	<i>ACS Nano</i> 5 (2) 1122-1140 (2011) http://pubs.acs.org/doi/abs/10.1021/nn102762f	Doxorubicin Loaded Magnetic Polymersomes: a Multifunctional Nanocarrier for Theranostics	4
39*	J-F. Le Meins*, O. Sandre and S. Lecommandoux*	<i>Eur. Phys.J.E</i> 34 (2) 14 (2011) http://dx.doi.org/10.1140/epje/i2011-11014-y	Recent trends in the tuning of polymersomes' membrane properties (colloquium paper)	1
40*	W. Agut, D. Taton, Annie Brûlet, O. Sandre* and S. Lecommandoux*	<i>Soft Matter</i> 7 9744-9750 (2011) http://xlink.rsc.org/?doi=C1SM05638G	Depletion Induced Vesicle-to-Micelle Transition from Self-Assembled Rod-Coil Diblock Copolymers with Spherical Magnetic Nanoparticles	0
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2. Invited talks in international conferences:

N°	Date and place	Name of conference	Title of conference
1*	Institute of Physics of University of Latvia, Riga, Lettonie 16-18 septembre 2004	"Magnetic forces nano- and biotechnologies"	Magnetic vesicles
2	Moscow, Russia, 21-25 th August 2011	Moscow International Symposium on Magnetism (MISM 2011) – Section 6 "Soft Magnetic Matter"	Combining polymers with magnetic nanoparticles
3	São Paulo, Brazil, 13-17 February 2012	Summer School on Complex Fluids	Magnetic nanoparticles: synthesis, properties and biomedical applications

3. Peer-reviewed proceedings papers:

N°	Authors	Reference	Title
1	Sandre, O.; Rosensweig, R. E.; Bacri, J.-C.; Cabuil, V.; Perzynski, R.	ERMIR'97 International Conference on Electro-Rheological Fluids, Magneto-Rheological Suspensions and their Applications (Yonezawa, Japon, 22-25 juillet 1997)	New ferrofluid composites: Magnetism and optics experiments
2	Moreaux, L.; Sandre, O.; Blanchard-Desce, M.; Mertz, J	ICONO'5 (Davos, 12-16 mars 2000) publié dans <i>Molecular Crystals and Liquid Crystals Science and Technology Section B Nonlinear Optics</i> (2000), 25 (1-4), 183-188	Simultaneous second-harmonic generation and two-photon excited fluorescence microscopy

3*	El kharrat, D.; Sandre, O.; Perzynski, R.; Chécot, F.; Lecommandoux, S.	Proceedings of the 9 th Japanese-French seminar on Magnetic Fluids (Sendai, Japon, 6 novembre 2003) p.104-107 http://hal.archives-ouvertes.fr/hal-00174174/	Self-assembly of polymers or copolymers and ferrofluids leading to either 1-d, 2-d or 3-d aggregates decorated with magnetic nanoparticles
4*	El kharrat, D.; Sandre, O.; Licinio, P.; Perzynski, R.	The 3 rd International Symposium on Slow Dynamics in Complex Systems (Sendai, Japon 3-8 novembre 2003) <i>American Institute of Physics Conference Proceedings</i> (2004) 708 , 122-123 http://link.aip.org/link/?APCPCS/708/122/1	Adsorption of Magnetic Nanoparticles onto Polyacrylamide Chains in Dilute Polymer Solutions and "Ferrogel" Networks
5	Cabuil, Valerie; Menager, Christine; Guemghar, Dihya; Sandre, Olivier; Perzynski, Regine.	Abstracts of Papers, 229th ACS National Meeting, San Diego, CA, United States, March 13-17, 2005 229: U722-U722 471-COLL Part 1 http://oasys2.confex.com/acs/229nm/techprogram/	Magnetic vesicles
6	S. Lecommandoux, O. Sandre, F. Chécot, R. Perzynski	EMRS 2005 - SPRING MEETING - SYMPOSIUM I "Advanced Functional Nanomaterials"	Smart Hybrid Magnetic Self-Assembled Micelles and Hollow Capsules
7	Save, Maud; Charleux, Bernadette; Douadi, Siham; Sandre, Olivier; Cabuil, Valerie; Hamdoun, Bassam.	Abstracts of Papers, 230th ACS National Meeting, Washington, DC, United States, Aug. 28-Sept. 1, 2005 (2005), POLY-504. <i>Polymer Preprints</i> (2005), 46 (2), 440-441. http://www.polyacs.org/503.html	Synthesis of high molar mass poly(n-butyl methacrylate)-b-polystyrene diblock copolymers by ATRP: Formation of lamellar phases in thin films.
8	S. Lecommandoux, O. Sandre, F. Chécot, J. Rodriguez-Hernandez, R. Perzynski.	The 2 nd Moscow International Symposium on Magnetism (MISM, June 2005) <i>J. Mag. Mag. Mat.</i> 300 (2006), 71-74 http://dx.doi.org/10.1016/j.jmmm.2005.10.035	Self-assemblies of magnetic nanoparticles and di-block copolymers: Magnetic micelles and vesicles.
9	S. Lecommandoux, O. Sandre, F. Chécot, R. Perzynski.	<i>Progress in Solid State Chemistry</i> 34 (2006) 171-179 http://dx.doi.org/10.1016/j.progsolidstchem.2005.11.050	Smart hybrid magnetic self-assembled micelles and hollow capsules
10*	B. Frka-Petesic, J. Fresnais, J-F. Berret, V. Dupuis, R. Perzynski and O. Sandre	The 3 rd Moscow International Symposium on Magnetism (MISM, 20-25 th June 2008) <i>J. Mag. Mag. Mat.</i> 321 (2009) 667-670 http://dx.doi.org/10.1016/j.jmmm.2008.11.021	Stabilization and controlled association of superparamagnetic nanoparticles using block copolymers
11	S. Douadi-Masrouki, B. Frka-Petesic, O. Sandre, F.Cousin, V.Dupuis, R. Perzynski, V.Cabuil	The 3 rd Moscow International Symposium on Magnetism (MISM Moscow, Russia, June 20-25, 2008) <i>Solid State Phenomena</i> 152-153 , 194-197 (2009) http://dx.doi.org/10.4028/www.scientific.net/SSP.152-153.194	Neutron reflectivity on polymer multilayers doped with magnetic nanoparticles
12	J. Fresnais, J.-F. Berret, B. Frka-Petesic, O. Sandre, R. Perzynski R	Nanotechnology Conference and Trade Show (Nanotech 2008) Boston, MA, June 01-05, 2008 NSTI NANOTECH 2008, Vol 2, Technical Proceedings - Life Sciences, Medicine, And Bio Materials Pages 689-692	Elaboration of superparamagnetic nanorods using iron oxide nanoparticles and polymers
13	F. Ahrentorp, C. Jonasson, J. Blomgren, A. P. Astalan, J. Blomgren, Q. Bin, O. Thompson Mefford, M. Yan, J. Courtois, J-F. Berret, J. Fresnais, O. Sandre, R. Müller, C. Johansson	The 8 th International Conference on the Scientific and Clinical Applications of Magnetic Carriers, May 25-29, 2010, Rostock, Germany, in <i>American Institute of Physics Conference Proceedings Series</i> , Vol 1311, pp 213-223 http://scitation.aip.org/dbt/dbt.jsp?KEY=APCPCS&Volume=1311&Issue=1	Sensitive High Frequency AC Susceptometry in Magnetic Nanoparticle Applications

14	B. Frka-Petecic, K. Erglis, J.-F. Berret, A. Cebers, V. Dupuis, J. Fresnais, O. Sandre, R. Perzynski,	12 th International Conference on Magnetic Fluids (ICMF12) August 1 - 5, 2010 Sendai, Japan <i>J. Magn. Magn. Mat.</i> 323 (10) 1309-1313 (2011) http://dx.doi.org/10.1016/j.jmmm.2010.11.036	Dynamics of organic/inorganic magnetic nanostructured rods under rotating field
15	J. Fresnais, B. Frka-Petecic, O. Sandre, J.-F. Berret, R. Perzynski, V. Dupuis	12 th International Conference on Magnetic Fluids (ICMF12) August 1 - 5, 2010 Sendai, Japan <i>Physics Procedia</i> (2010) 9 , 15-19 http://dx.doi.org/10.1016/j.phpro.2010.11.005	Orientational behavior of an assembly of superparamagnetic rods
16	J.A. Galicia, F. Cousin, E. Dubois, O. Sandre, V. Cabuil, R. Perzynski	12 th International Conference on Magnetic Fluids (ICMF12) August 1 - 5, 2010 Sendai, Japan <i>J. Magn. Magn. Mat.</i> 323 (10) 1211-1215 (2011) http://dx.doi.org/10.1016/j.jmmm.2010.11.008	Local structure of polymeric ferrogels

4. Publications without peer-reviewing: (Scientific press and other media)

N°	Auteurs	Référence	Titre
1	I. Peterson	Science News Vol.156 No. 12:	Taking the pop out of cell-like balloons.
2	Trisha Gura	Science Now 20 septembre 1999:	Puncturing a Cellular Riddle
3	Hervé This	Pour La Science No. 270 avril 2000	«Cellules» entrouvertes. Quand elles adhèrent à un support, des cellules artificielles s'ouvrent et se dégonflent progressivement
4	Réalisation : Christine Dory	Archimède (Arte), émission du 18 avril 2000	Vésicules artificielles

5. Communications at international congresses or symposia:

N°	Date and place	Name of congress	Type and title of communication
1	Sendai, Japon 3-8 novembre 2003	The 3 rd International Symposium on Slow Dynamics in Complex Systems	Poster "Adsorption of Magnetic Nanoparticles onto Polyacrylamide Chains in Dilute Polymer Solutions and "Ferrogel" Networks" D. El kharrat, <u>O. Sandre</u> , P. Licinio, V. Cabuil
2	4-9 juillet 2004, Paris	IUPAC World Polymer Congress MACRO 2004: 40th International Symposium on Macromolecules	Poster "Magnetic Nano-composite Micelles and Vesicles" <u>S. Lecommandoux</u> , <u>O. Sandre</u> , F. Chécot, J. Rodriguez-Hernandez, R. Perzynski
3	4-9 juillet 2004, Paris	IUPAC World Polymer Congress MACRO 2004: 40th International Symposium on Macromolecules	Poster "Interactions between polymer chains and magnetic nanoparticles in dilute solutions and ferrogel networks" D. El kharrat, J. A. Galicia, E. Dubois, F. Cousin, <u>O. Sandre</u> , P. Licinio, R. Perzynski, V. Cabuil
4	Guarujá, São Paulo, Brazil - August 02 - 06 - 2004	10th International Conference on Magnetic Fluids	Oral "Tuning of the interactions in magnetic fluids and consequences on their properties" <u>E. Dubois</u> , A. Galicia, V. Cabuil, O. Sandre, F. Cousin, F. Boué, A. Cebers, R. Perzynski

5*	Kottayam, Kerala - Inde 21-23 mars 2005	International Conference on Advances in Polymer Blends, Composites, IPNS and Gels: Macro to Nano Scales	Oral "Synthesis of block copolymers by ATRP for the combination with magnetic nanoparticles to obtain nano-composite films" <u>S. Douadi</u> , O. Sandre, M. Save, J.J Rousseau, B. Hamdoun, B. Charleux, V. Cabuil
6*	Strasbourg 31 mai – 3 juin 2005	EMRS 2005 - SPRING MEETING - SYMPOSIUM I "Advanced Functional Nanomaterials"	Oral "Smart Hybrid Magnetic Self-Assembled Micelles and Hollow Capsules." S. Lecommandoux, O. Sandre, F. Chécot, R. Perzynski
7*	Moscou, Russie 25-30 juin 2005	"Moscow International Symposium on Magnetism" (MISM)	Oral "Magnetic Nano-composite Micelles and Vesicles" S. Lecommandoux, O. Sandre, F. Chécot, J. Rodriguez-Hernandez, <u>R. Perzynski</u>
8*	Riga Jurmala, Lettonie 27 juin - 1er juillet 2005	Joint 15th Riga and 6th PAMIR International Conference on Fundamental and Applied MHD	Oral "Supermagnetic nanoparticles and oppositely charged polymers: design of new contrast agents for Magnetic Resonance Imaging" <u>J.-F. Berret</u> , F. Gazeau, D. El kharrat and O. Sandre
9	Washington DC, USA 28 août-1er septembre 2005	2005 Fall 230 th NATIONAL ACS MEETING – POLY division	Poster "Synthesis of high molar masses Poly(N- Butyl Methacrylate)-B-Poly(Styrene) diblock copolymers by ATRP. Formation of lamellar phases in thin films". S. Douadi, O. Sandre, V. Cabuil, <u>M. Save</u> , B. Charleux, B. Hamdoun
10*	San Diego, CA, USA March 13-17, 2005	229th ACS National Meeting	Oral "Magnetic vesicles" <u>V. Cabuil</u> , C. Ménager, D. Guemghar, O. Sandre, R. Perzynski
11	Budapest, Hungary 13-16 September 2005	8th International Symposium Polymers for Advanced Technologies	Poster "Magnetic Nano-composite Micelles and Vesicles" <u>S. Lecommandoux</u> , O. Sandre, F. Chécot, J. Rodriguez-Hernandez, R. Perzynski
12	Miyazaki, Japon 24-25 novembre 2005	The 3rd International Symposium on Bioscience and Nanotechnology	Poster "Coupling of magnetic nanoparticles with polymer chains and networks" D. El kharrat, O. Sandre, <u>V. Dupuis</u> , R. Perzynski, V. Cabuil
13*	13-16 May 2006, Orlando, Florida USA	Particles 2006 conference: Medical/Biochemical Diagnostic, Pharmaceutical, and Drug Delivery Applications of Particle Technology	Oral "Controlled Clustering Of Superparamagnetic Nanoparticles Using Block Copolymers : Design Of New Contrast Agents For Magnetic Resonance Imaging" J.-F. Berret & O. Sandre
14*	Beijing, CHINA May 18th – 20th	International Conference on Polymer Processing ICPP 2007	Oral "The Lamellar Structure In Magnetic Nanocomposite Thin Films" <u>S. Douadi</u> , O. Sandre, M. Save, B. Charleux, V. Cabuil
15*	Kawagoe, Japon 4-5 décembre 2007	The 5th International Symposium on Bioscience and Nanotechnology	Poster "Probing the internal structure of magnetic nanocomposites – thermo-sensitive gels and lamellar films – respectively by small angle neutron scattering and neutron reflectivity" S. Douadi-Masrouki, B. Frka-Petesic, D. El kharrat, <u>O. Sandre</u> , M. Save, B. Charleux, V. Cabuil
16	Kawagoe, Japon 4-5 décembre 2007	The 5th International Symposium on Bioscience and Nanotechnology	Oral "Self-assembled clusters of magnetic nanoparticles and di-block copolymers" B. Frka-Petesic, J. Fresnais, J.-F. Berret, V. Dupuis, <u>R. Perzynski</u> and O. Sandre

17	Kawagoe, Japon 4-5 décembre 2007	The 5th International Symposium on Bioscience and Nanotechnology	Poster "Synthesis of colloidal iron oxide in microchannel: preliminary results in a coaxial flows millichannel" <u>A. Abou-Hassan</u> , O. Sandre, V. Cabuil
18	27 June – 1 July 2008, Lund, Sweden	7th Liquid Matter Conference	Oral: "Controlled co-assembly of iron oxide nanoparticles and polymers: towards the generation of highly persistent superparamagnetic nanorods" J. Fresnais, J.-F. Berret, B. Frka-Petesic, O. Sandre, R. Perzynski
19	Ecole Polytechnique, Palaiseau, 17-18 janvier 2008	SOLEIL Users's Meeting 2008	Poster "Probing the internal structure of magnetic nanocomposites – thermo-sensitive gels and lamellar films – respectively by small angle neutron scattering and neutron reflectivity" S. Douadi-Masrouki, D. El kharrat, B. Frka-Petesic, R. Perzynski, <u>O. Sandre</u> , V. Cabuil Poster "Synthesis of colloidal iron oxide in microchannel and objective of SAXS study <i>in situ</i> " <u>A. Abou Hassan</u> , O. Sandre, V. Cabuil
20*	26-29 août 2008, Strasbourg, France	11èmes Journées de la Matière Condensée (JMC11) de la Société Française de Physique	Oral « Etude de l'effet d'un champ magnétique sur l'orientation d'un cristal liquide nématique thermotrope (5CB) ou lyotrope (goethite) » C. Vatry, <u>O. Sandre</u> , V. Dupuis Poster « Coacervats core-shell de copolymères et de nanoparticules magnétiques » <u>B. Frka-Petesic</u> , V. Dupuis, R. Perzynski, O. Sandre, J. Fresnais et J.-F. Berret
21	13-16 octobre 2009, Arcachon, France	Aquitaine Conferences 2009 on Polymers	Poster "Probing with neutrons the internal structure of magnetic nanocomposites: thermo-sensitive hydrogels, multi-lamellar diblock copolymer films" S. Douadi, B. Frka-Petesic, <u>O. Sandre</u> , M. Save, B. Charleux, V. Cabuil Poster "Electrostatic complexation to synthesise superparamagnetic fluorescent rods" J. Fresnais, J.F. Berret, E. Ishow, V. Dupuis, R. Perzynski, O. Sandre
22	20-21 janvier 2010, Palaiseau, France	5 th SOLEIL Users' Meeting	Poster "Magnetite nanoparticles synthesis probed in situ by SAXS in a continuous flow micro-reactor: detection and measurement of the first Fe(OH) ₃ precipitates" A. Abou-Hassan, V. Dupuis, <u>O. Sandre</u> , O. Spalla Poster "Tuning the Dispersion of Magnetic Fillers in Nanocomposites" <u>A.-S. Robbes</u> , F. Meneau, F. Cousin, J. Jestin, O. Sandre, D. Gigmes, F. Boué
23	San Francisco, USA March 21-25, 2010	2010 SPRING NATIONAL ACS MEETING – POLY division	Oral "Magnetic polymersomes for combined imaging and therapy" C. Sanson, O. Diou, A. Soum, O. Sandre, <u>S. Lecommandoux</u>
24	April 27- 29 th 2010, Kottayam, India	International Conference on Nanomaterials: Synthesis, Characterization and Applications (ICN 2010)	Oral "Microfluidic synthesis of magnetic nanoparticles" A. Abou-Hassan, V. Cabuil, <u>S. Neveu</u> , O. Sandre
25	1 ^{er} juillet 2010, LAAS, Toulouse	Les nanotechnologies en cancérologie: projets, applications cliniques et enjeux au sein du Cancéropôle Grand Sud-Ouest	Poster « Polymersomes Magnétiques pour la Théranostique Anti-Cancéreuse (Imagerie et Thérapie combinées) » C. Sanson, W. Agut, O. Diou, A. Soum, E. Thiaudière, P. Voisin, S. Miraux, <u>O. Sandre</u> , S. Lecommandoux

26	24 août 2010, UTT, Troyes	12èmes Journées de la Matière Condensée (JMC12) de la SFP Minicolloque n°10 : Nanoparticules magnétiques: synthèse et propriétés	Oral « Tri en tailles de nanoparticules de maghémite synthétisées par coprécipitation alcaline et sélection des plus grands diamètres pour les applications physiques et médicales » M. Yan M., J-F. Berret, J. Courtois, J. Fresnais, <u>O. Sandre</u>
27	25 août 2010, UTT, Troyes	12èmes Journées de la Matière Condensée (JMC12) de la SFP Minicolloque n°7 : Nanoparticules et nanocomposites : de l'élaboration aux applications biomédicales	Oral « Polymersomes Magnétiques pour la Théranostique Anti-Cancéreuse (Imagerie et Thérapie combinées) » C.Sanson, W. Aut, O. Diou, A. Soum, <u>O.Sandre</u> , S. commandoux Oral « Particules d'oxyde de fer pour l'IRM, influence du type d'oxyde et de l'agglomération » <u>Y. Gossuin</u> , V. Dupuis, J-F. Berret, O. Sandre
28	11-14 avril 2011, Bilbao, Espagne	NanoSpain and NanoBio&Med 2011 conferences at the ImagineNano event	Oral “Doxorubicin Loaded Magnetic Polymersomes: Theranostic Nanocarriers for MR Imaging and Magneto-Chemotherapy” <u>O. Sandre</u> , C. Sanson, O. Diou, J. Thévenot, E.Ibarboure, A. Soum, A. Brûlet, S. Miraux, E. Thiaudière, S. Tan, A. Brisson, S. Lecommandoux
29	26 th June-July 1 st , Grenada, Spain	European Polymer Federation Congress (EPF2011)	Poster “Phospholipid/Block Copolymer Hybrid Vesicles: a new approach to modulate membrane properties for drug delivery” M. Chemin, P-M. Brun, S. Lecommandoux, O. Sandre, <u>J-F. Le Meins</u>
30			

6. Seminars, workshops:

N°	Date and place	Name of seminar or workshop	Type and title of communication
1	Institut Curie, Paris avril 1997	Rencontres Physique-Biologie-Chimie de la montagne Ste Geneviève	Oral « Manipulation de tubules avec un champ magnétique »
2	Institut Curie, Paris 5 novembre 1997	Séminaire du Laboratoire Physico-Chimie Curie	Oral « Deux histoires de ferrofluides : gouttes tournantes et liposomes carrés »
3	Jussieu, Paris novembre 1997	4 th French-Japanese seminar on Magnetic Fluids	Oral “Assembly of microscopic magnetic droplets”
4	Ascona, Suisse, 21-25 juin 1998	Workshop “Giant Vesicles”	Oral “Oblate–Prolate Transition of Ellipsoidal Giant Magnetoliposomes due to an anisotropic spontaneous curvature”
5	Ecole Normale Supérieure, Paris, octobre 1998	Séminaire de clôture de la chaire B. Pascal attribuée à R. E. Rosensweig “Les liquides magnétiques : vers des applications géophysiques – quelques travaux en collaboration avec de jeunes chercheurs de la région d’Île de France”	Oral « Dynamique de rotation de microgouttes de ferrofluide »
6	Laboratoire de Spectrométrie Physique, Université Joseph Fourier, Grenoble, 19 octobre 1999	Séminaires Matière molle - Matière grise (séminaires communs au Grephe et au groupe Matière molle et Biologie de l'équipe Phase)	Oral « Mises sous tension de liposomes géants : des modèles de perméation et de fusion membranaires »
7	Jussieu, Paris, 14 janvier 2000	Séminaire Matière Molle et Biologie	Oral « Mises sous tension de liposomes géants : des modèles de perméation et de fusion membranaires »

8	Collège de France, Paris 10 mars 2000	Séminaire du laboratoire Physique de la Matière Condensée	Oral « Adhésion et rupture des vésicules : Pores de fusion et pores de fuite »
9	Obernai, 27-30 juin 2000	GDR "Films Moléculaires Bidimensionnels"	Oral « Imagerie de membranes par microscopie de fluorescence et de seconde harmonique »
10	Jussieu, Paris 2 avril 2001	Séminaire du Laboratoire de Physico-Chimie Biomoléculaire et Cellulaire	Oral « Mesure de l'ordre des molécules dans une membrane: microscopie de second harmonique et imagerie de l'anisotropie de fluorescence »
11*	European Centre La Foresta, Vaalbeek, Belgique, 28 février-2 mars 2002	CERC3 Young Chemists Workshop 2002 "Water-soluble Polymers and their hydrogels"	Oral "Preparation and properties of magnetic hydrogels or ferrogels"
12*	Florence, Italie (2003)	17th Annual meeting of the European Colloid and Interface Society (ECIS)	Oral "Nanosized nickel hydroxide dispersion" <u>M. Meyer</u> , A. Bee, O. Sandre, P. Licinio, R. Perzynski, R. Garrigos, V. Cabuil
13*	Tohoku University - Sendai - Japon 6/7 novembre 2003	9 th Japanese-French seminar on Magnetic Fluids	Oral "Self-assembly of polymers or copolymers and ferrofluids leading to either 1-d, 2-d or 3-d aggregates decorated with magnetic nanoparticles" D. El kharrat, <u>O. Sandre</u> , R. Perzynski, F. Chécot, S. Lecommandoux
14	Paris, 24-25 mai 2004	EPF Workshop on Polymer Nanoparticles in Life Science	Poster "Magnetic pH-sensitive Polymersomes" S.Lecommandoux, <u>O. Sandre</u> , F. Chécot, J. Rodriguez-Hernandez, R. Perzynski
15*	Paris, 17 juin 2004	Séminaire Externe du Collège de France	Oral « Systèmes complexes à base de ferrofluides et de polymères : ferrogels, microgels et polymérosomes magnétiques »
16*	Paris, 29 juillet 2004	10 th Japanese-French seminar on Magnetic Fluids	Oral "Magnetic Microgels and Minigels: Preparation, Swelling and Instabilities" C. Ménager, <u>O. Sandre</u> , J. Mangili, V. Cabuil
17	Nancy, 30 août-3 septembre 2004	9èmes Journées de la Matière Condensée - Minicolloque : « Nouveaux défis en matière molle »	Poster « Interaction entre des nanoparticules magnétiques et des polymères » D. El Kharrat, O. Sandre, V. Dupuis, R. Perzynski, V. Cabuil
18	Paris 11 mars 2005	"Club Nanomatériaux pour les Sciences du Vivant"	Poster "Stabilisation and Controlled Association of Fe ₂ O ₃ -Magnetic Nanoparticles using Block Copolymers" <u>J.-F. Berret</u> , <u>O. Sandre</u> , F. Gazeau J.-C. Bacri
19*	Autrans (Vercors) 6 au 10 juin 2005	Colloque Nano-Hybrides 2	Oral "Supermagnetic nanoparticles and oppositely charged polymers: design of new contrast agents for Magnetic Resonance Imaging" <u>J.-F. Berret</u> , F. Gazeau, D. El kharrat and O. Sandre
20*	Bordeaux 19-20 octobre 2005	Colloque Imageries & Polymères (IMAPOL)	Oral "γ-Fe ₂ O ₃ Magnetic nanoparticles and oppositely charged polymers: design of new contrast agents for Magnetic Resonance Imaging", J.-F. Berret, F. Gazeau and <u>O. Sandre</u>
21	Bordeaux 19-20 octobre 2005	Colloque Imageries & Polymères (IMAPOL)	Poster "The lamellar structure in symmetric diblock copolymer thin films studied by imagery techniques" <u>S. Douadi</u> , <u>O. Sandre</u> , M. Save, J.J Rousseau, B. Hamdoun, B. Charleux, V. Cabuil

22	Université de Technologie de Belfort-Montbéliard – site de Sévenans, 7-8 décembre 2005	8 ^{ème} Colloque industriel CIMATS Nano matériaux, nano composites, nano structures : un point sur la réalité industrielle	Poster « Films polymères magnétiques nano-composites pour le guidage optique et micro-ondes » S. Douadi, <u>O. Sandre</u> , M. Save, J.J Rousseau, B. Hamdoun, B. Charleux, V. Cabuil
23*	Montpellier 13 janvier 2006	Séminaire du Laboratoire des Colloïdes, Verres et Nanomatériaux (LCVN UMR5587 CNRS / Université de Montpellier 2)	Oral « Auto-assemblage de nanoparticules magnétiques et de copolymères: Gels, Minigels, Vésicules et Coacervats Magnétiques »
24	Tours 1-2 février 2006	Journées scientifiques du CEA HYPERMAG « La rencontre du magnétisme et des hyperfréquences »	Poster « Films lamellaires de copolymères à blocs dopés par des couches magnétiques pour des applications de guidage optique visible et hyperfréquences » <u>S. Douadi</u> , O. Sandre, M. Save, J.J Rousseau, B. Charleux, V. Cabuil
25*	Montpellier 6-7 avril 2006	Montpellier Nanocomposites Workshop 2006 (réseau européen SOFTCOMP)	Oral “Trapping magnetic nanoparticles in a semi-interpenetrated PNIPAM hydrogel to yield a soft magnetic and thermo-sensitive nanocomposite”
26*	Bristol PA USA 1 ^{er} juin 2006	Complex Fluid Laboratory Rhodia Research Center (UMR166 CNRS / Rhodia)	Oral “Self-assembly of magnetic nanoparticles & copolymers: Gels, Minigels, Vesicles, Coacervates, Lamellar Films”
27*	Paris 21-22 septembre 2006	Toyo-Paris International Seminar of the Bio-Nano Center of Excellence (Toyo University, Japan)	Oral “Dispersion of magnetic nanoparticles in a nematic liquid crystal host: Phase diagram, Fredericks transition and deformation of droplets” J. Deseigne, C. Da Cruz, V. Cabuil and <u>O. Sandre</u> Oral “Efficient doping of polymer gels by maghemite nanoparticles to obtain both magnetic and thermo-sensitive materials” <u>D. El kharrat</u> , V. Cabuil, O. Sandre Oral “Synthesis of block copolymer by ATRP for the combination with magnetic nanoparticles to obtain nano-composite films” <u>S. Douadi</u> , O. Sandre, M. Save, B. Charleux, V. Cabuil
28*	Utrecht, 10-12 octobre 2007	Nancy-Paris-Utrecht Meeting on liquid crystals at the Van't Hoff Laboratory for Physical and Colloidal Chemistry	Oral “Dispersion of magnetic nanoparticles in a nematic liquid crystal host: Phase behavior, Fredericks transition and deformation of droplets” J. Deseigne, C. Da Cruz, V. Cabuil and <u>O. Sandre</u>
29*	Perm, Russie, 26 novembre 2007	Séminaire à l'Institut de Mécanique des Milieux Continus de l'Académie des Sciences de Russie – division de l'Oural	Oral “Dispersion of magnetic nanoparticles in a nematic liquid crystal host: Phase behavior, Fredericks transition and deformation of droplets” J. Deseigne, C. Da Cruz, V. Cabuil and <u>O. Sandre</u>
30	Paris, 11 janvier 2008	Journée « Jeunes Chercheurs – Nanochimie » du pôle CNano-Ile de France	Oral “La microfluidique: un outil pour la synthèse de nanoparticules inorganiques? Influence des paramètres hydrodynamiques sur la synthèse de nanoparticules d'oxyde de Fe ^{II} et Fe ^{III} dans un canal millifluidique” A. Abou Hassan, <u>O. Sandre</u> , V. Cabuil

31	20 novembre 2008, Pessac (France)	Séminaire externe du Laboratory Of Future (LOF) UMR 5258 CNRS/Rhodia/Bordeaux-1	Oral "Orientation par un champ magnétique d'un cristal liquide nématique et de colloïdes allongés (nano-bâtonnets de goethite) : vers l'obtention d'un véritable cristal liquide ferromagnétique" Christelle Vatry, Ali Abou Hassan, Vincent Dupuis et <u>Olivier Sandre</u>
32	27-28 novembre 2008, Leuven (Belgique)	"Kick-off meeting" du réseau européen Nano-3T	Oral "Ferrofluids at LI2C and their applications"
33	27-28 novembre 2008, Montpellier (France)	European Workshop on 'Nanocomposites & Polymer Dynamics' (réseau SoftComp)	Oral "Controlled co-assembly of iron oxide nanoparticles and polymers: towards the generation of highly persistent super-paramagnetic nanorods" <u>J. Fresnais</u> , J.-F. Berret, B. Frka-Petescic, O. Sandre, R. Perzynski
34*	8-11 septembre 2009, Orsay (France)	14 ^{ème} Colloque Francophone des Cristaux Liquides (CFCL2009) – Systèmes anisotropes auto-organisés	Oral « Orientation par un champ magnétique d'un cristal liquide nématique et de nano-bâtonnets de goethite : vers l'obtention d'un véritable cristal liquide ferromagnétique », C. Vatry, A. Abou Hassan, V. Dupuis, <u>O. Sandre</u>
35	8 octobre 2010, Institut Curie, Paris Cours donné aussi en juin 2008	2h de cours dans le stage de formation continue UPMC/CNRS intitulé « Colloïdes et Nanoparticules » organisé par Françoise Brochard	Les ferrofluides: un exemple de suspension colloïdale stable avec des grains magnétiques, et une source d'inspiration pour créer de nouveaux "matériaux intelligents"
36	12 octobre 2009, Pessac (France)	Séminaire externe du LCPO (UMR5629 CNRS/IPB/ENSCP)	Oral « Elaboration et étude de matériaux et d'objets colloïdaux nano-composites à base de nanoparticules magnétiques et de copolymères »
37	18 janvier 2010, Paris (France)	Séminaire externe du laboratoire Gulliver (UMR7093 CNRS/ESPCI)	Oral « Quelques apports des nanoparticules magnétiques en matière molle: cristaux liquides, polymersomes et microfluidique »
38	9 février 2010, Mons (Belgique)	Séminaire au Service de Physique Expérimentale et Biologique de l'Université de Mons-Hainaut	Oral « Matériaux et colloïdes à base de nanoparticules magnétiques et de (co)polymères »
39	4-6 octobre 2010, Pessac	NanoTher M24 Meeting	Workshop of European network
40	14-15 octobre 2010, Pessac	Recent Advances on Supramolecular Systems involving Biological Molecules or Bioinspired Compounds (SUPRABIO)	Poster "Doxorubicin loaded magnetic polymersomes: a multifunctional nanocarrier for theranostics" C. Sanson, W. Agut, O. Diou, A. Soum, E. Thiaudière, P. Voisin, S. Miraux, O. Sandre, S. Lecommandoux
41	14-15 avril 2011, Bilbao, Espagne	BioInspired Nanotechnology COST Action TD1003 Kick-Off Meeting	Oral "Polymersomes: a route to design Theranostic Nanocarriers" (WG3) C. Schatz, <u>O. Sandre</u> , J.-F. Le Meins, E. Garanger, S. Lecommandoux
42	28-29 th June 2011, Aarhus, Denmark	"Bimodal PET-MRI molecular imaging technologies and applications for in vivo monitoring of disease and biological processes" COST Action TD1007 Kick-Off Meeting	Oral "Polymersomes: a route to design Theranostic Nanocarriers" (WG3) C. Schatz, <u>O. Sandre</u> , J.-F. Le Meins, E. Garanger, S. Lecommandoux
43	12 juillet 2011, Plateforme Génomique Fonctionnelle de Bordeaux	1ères rencontres du Réseau Santé Industrie Recherche Aquitain (2ADI) « Thérapies Guidées par l'imagerie: théranostique et transport ciblé »	Poster "Nanocarriers from Polymers & Size-sorted Magnetic Nanoparticles" <u>O. Sandre</u> , S. Lecommandoux

44	19-20 August, 2011, Dubna, Moscow Reg., Russia Joint Institute of Nuclear Research – Frank Laboratory of Neutron Physics	Workshop “Structural aspects of biocompatible ferrocolloids: stabilization, properties control and application”	Oral “Depletion Induced Vesicle-to-Micelle Transition from Self-Assembled Rod-Coil Diblock Copolymers with Spherical Magnetic Nanoparticles” W. Agut, D. Taton, A. Brûlet, S. Lecommandoux, <u>O. Sandre</u>

7. Book Chapters:

N°	Authors	Reference	Title of chapter
1	Sandre, O.; Ménager, C.; Prost, J.; Cabuil, V.; Bacri, J.-C.; Cebers, A.	<u>Giant Vesicles</u> , Perspectives in Supramolecular Chemistry (Vol. 6) P.-L. Luisi and P. Walde Eds. (2000), John Wiley & Sons, Inc., Chichester (UK), pages 169-180 http://dx.doi.org/10.1002/9780470511534.ch11	Chapter 11: Oblate–Prolate Transition Of Ellipsoidal Giant Magnetoliposomes: Experiments showing an Anisotropic Spontaneous Curvature
2	A. Abou-Hassan, O. Sandre, V. Cabuil	<u>Microfluidic Devices in Nanotechnology: Applications</u> , June 2010 edited by Challa S. S. R. Kumar, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ ISBN: 978-0-470-59069-0 Hardcover (407 pages) http://eu.wiley.com/WileyCDA/WileyTitle/productCd-0470590696.html	Chapter 9: Microfluidics for the synthesis of iron oxide nanoparticles Pages 323-360

8. Patents:

N°	Inventor(s)	Owner(s)	Reference/Date	Title
1	Sandre, O.; Lecommandoux, S.	IPB, CNRS	FR10-04789 8/12/2010	Vésicules formées à partir d'un copolymère à blocs amphiphile, comprenant des nanoparticules magnétiques et au moins une molécule thérapeutique.

M. Annexe 2 : tirés à part d'articles (un par chapitre)

Static and dynamic structural probing of swollen Polyacrylamide ferrogels

J.A. Galicia^{1,2}, F. Cousin³, E. Dubois¹, O. Sandre¹, V. Cabuil¹, R. Perzynski^{1,*}

¹ Université Pierre et Marie Curie – PECSA – UMR 7195 CNRS – UPMC – ESPCI, case 51, 4 Place Jussieu, 75252 Paris cedex 05 – France

² Fac. de Ing. Química, Benemerita Universidad Autónoma de Puebla, Edif 147/102 Ciudad Universitaria, 18 sur y San Claudio, Puebla, Pue. Mexico

³ Laboratoire Léon Brillouin, UMR 12 CNRS-CEA, CE-Saclay, 91191 Gif-sur-Yvette - France

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* corresponding author, e-mail : regine.perzynski@upmc.fr

The local structure of hybrid ferrogels resulting from the incorporation of γ -Fe₂O₃ nanoparticles in a polyacrylamide polymeric network is probed from a static and a dynamic point of view, in various synthesis conditions and at swelling equilibrium. Relaxation of magneto-optical birefringence and Small Angle Neutron Scattering measurements show an adsorption of the nanoparticles onto the polymer ascribed to H-bonds. These measurements allow sorting out the conditions leading either - to a homogeneous scaffold reinforced by the nanoparticles or - to a nano-structured composite with a 2D nanoparticle decoration on the walls of percolating pockets, initially filled by the ferrofluid during the synthesis and leaking out during the swelling. Two parameters rule the structure and the properties of these ferrogels : - the osmotic pressure ratio R_{Π} (ratio of the osmotic pressure of the initial ferrofluid to that of the hydrogel) and the steric parameter R_S (ratio of the nanoparticles volume to the mesh volume of the polymeric hydrogel).

Introduction

Ferrogels are hybrid materials resulting from the mixing of either micrometric or nanometric magnetic particles with polymeric chains. Such composites are usually developed for their magneto-elastic properties.^{1,2} The particles, frequently aggregated, reinforce the polymeric matrix mechanically.^{3,4,5} More generally, nanocomposites successfully combining polymers and magnetic nanoparticles result from the synthesis of multifunctional 3D architectures rationally assembled at the nanoscale. We disperse here magnetic nanoparticles of controlled size and coating inside polyacrylamide (PAM) hydrogels. PAM is well known for its swelling properties in aqueous media, it is often used as super-absorbing microbeads and in biochemistry.⁶ Our aim here is to monitor the local structure of our ferrogel by using the nanoparticles both as fillers and as probes, an aspect quite rarely reported in literature⁷ even though polymers are widely used to template different complex nanoparticles assemblies.⁸ By tuning the physico-chemical parameters of the system, we are able to control for example - the volume fraction of internalized nanoparticles and their release, - the swelling of the ferrogel, - the local homogeneity of the hybrid material, - the proportion of internalized nanoparticles free to rotate and those blocked in rotation. The control of the fraction of the internalized particles at equilibrium after swelling is specifically important for the mechanical properties, as it has been shown that the Young modulus of the ferrogel strongly depends on this fraction.^{2,9}

The synthesis⁹ is performed by polymerizing acrylamide (AM) monomers in an aqueous suspension of maghemite (γ -Fe₂O₃) nanoparticles in the presence of the cross-linking agent (BAM). The ratio $CL = BAM/AM$ allows a tuning of the mesh-size of the polymeric network. The ferrogels are then let to reach their equilibrium inside a swelling bath because they are out-of-equilibrium materials in the synthesis conditions.

To probe our ferrogels from both a static and a dynamic point of view, we take advantage of the physical properties of the dispersed nanoparticles. We study their local organization inside the medium by Small Angle Neutron Scattering (SANS)^{10,11}, taking benefit from the possibility to match the scattering length density of PAM in mixture of H₂O and D₂O. Being magnetic monodomains and also optically uniaxial, the nanoparticles give optical responses to magnetic solicitations. We perform a nano-mechanical probing of the hybrid system by a magneto-optical birefringence method, which is specific to ferrofluids.^{12,13,14} We are thus able to obtain a local, structural and rheological information on the hybrid system at the length scale of the nanoparticles.

In the following, we first discuss the parameters of the initial hydrogels and ferrofluids that can influence the local structure of ferrogels. After exposing our choices for the current study, we present the synthesis and the physico-chemical properties of the ferrogels. Their structural and magneto-relaxational properties are then presented and discussed in terms of homogeneity/inhomogeneity of the complex magnetic medium.

1. Choice of components for designing ferrogels with versatile structures

If one wants to define a reproducible procedure for synthesizing ferrogels, versatile enough to tune the structure of the ferrogel from very homogenous to heterogeneous, the first step is to find physico-chemical conditions for the synthesis at which the individual components of the system are homogenous prior to mixing.

This implies to choose dispersions of magnetic nanoparticles with an interparticle potential dominated by repulsion in order to avoid any kind of aggregation of the nanoparticles before ferrogel synthesis. This has led us to work with ionic

dispersions of magnetic nanoparticles based on maghemite ($\gamma\text{-Fe}_2\text{O}_3$), of typical size 10 nm, coated by citrate species that provide them with a negative charge in water at neutral pH.¹⁵ For a citrate salt concentration $[\text{Na}_3\text{Cit}]$ lying between 2.10^{-3} mol.L⁻¹ and 10^{-2} mol.L⁻¹, the electrostatic repulsion dominates the interparticle attraction and the phase behavior is well described by the usual fluid-solid phase diagram of a repulsive system, even when a magnetic field is applied.¹⁶ *We have decided to fix the pH to 7.2 for all the experiments described in the paper and the citrate salt concentration $[\text{Na}_3\text{Cit}]$ to 8.10^{-3} mol.L⁻¹.*

For the hydrogel, it means to work in a range of cross-linking CL where single hydrogels have both (i) good mechanical performances and (ii) an homogeneous structure. *We have thus decided to work in a range of CL lying between 0.5% and 2% because the gels are too soft to be manipulated below CL = 0.5% and PAM hydrogels are known to present local heterogeneities of cross-linkers^{17,18} above CL = 2%.* For these cross-linking ratios $0.5\% \leq \text{CL} \leq 2\%$, the mesh-size ξ_{synth} of the polymeric hydrogel after the synthesis ranges between 11 and 17 nm (see Table SI.1 of Supporting Informations (SI)).

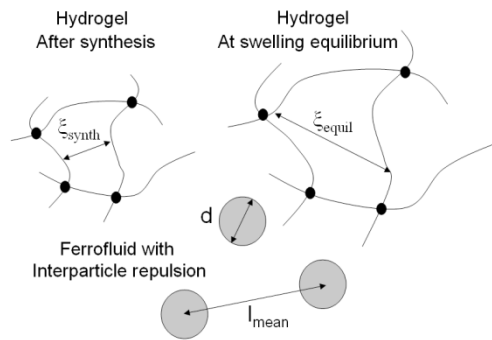


Figure 1 – **Characteristic sizes of the individual components which are mixed together to form the ferrogel** – **Hydrogel** : mesh size after synthesis ξ_{synth} , (upper left) and at swelling equilibrium ξ_{equil} (upper right). **Ferrofluid** : characteristic diameter d of the magnetic nanoparticle and interparticle distance l_{mean} (down).

Two parameters may play a decisive role on the structure and on the properties of the ferrogel when the components are mixed:

- the osmotic parameter R_{Π} , ratio of the osmotic pressure of the suspension of magnetic nanoparticles and that of the hydrogel,
- the steric parameter R_S , ratio of the characteristic volumes of these components.

Both R_{Π} and R_S depend on the nanoparticle characteristics and on the choice of cross-linking ratio, R_{Π} being also dependent on the volume fraction Φ_{synth} of the initial ferrofluid (which fixes the initial interparticle distance l_{mean}).

The osmotic parameter $R_{\Pi} = \Pi^{FF} / \Pi_{\text{swl}}^{HG}$ is the ratio of the osmotic pressures that characterizes the two components of the mixture in the initial conditions (see SI). If the osmotic pressure Π^{FF} of mixing of the nanoparticles with the solvent in the pure ferrofluid is much larger than the initial swelling pressure Π_{swl}^{HG} of the pure hydrogel, it may be impossible to incorporate nanoparticles in the gels.

The steric parameter $R_S = d^3 / \xi_{\text{synth}}^3$ is the ratio between the nanoparticle characteristic volume and the mesh volume ξ_{synth}^3 of the polymeric hydrogel, supposed homogeneous, immediately after synthesis. If R_S is too large, the presence of the nanoparticles may prevent to form a homogeneous gel.

Prior to mix any components, it is thus important to choose carefully the mesh size ξ_{synth} of the hydrogel after synthesis as

well as its swelling pressure Π_{swl}^{HG} , together with the nanoparticle size and the osmotic pressure Π^{FF} of the ferrofluid dispersion. The way in which all these details are obtained, is given in SI.

2. Ferrogels: synthesis and characterizations

a) Synthesis

The polymerization of the ferrogels is performed by a free radical polymerization at a given CL, in the aqueous dispersion of magnetic nanoparticles, at a given Φ_{synth} tuned from 1% to 7% and at $[\text{Na}_3\text{Cit}] = 8.10^{-3}$ mol.L⁻¹. All chemicals, including the monomer AM and the cross-linker BAM are purchased from Sigma-Aldrich. The reactional mixture of global volume 3mL, contains acrylamide monomers AM (concentration 0.5 mol.L⁻¹), cross-linking agent N,N'-methylene-bis-acrylamide (BAM) and the initiator (Ammonium persulfate at a concentration of 4.10^{-4} mol.L⁻¹). Solutions are degassed under nitrogen for 5 minutes to eliminate oxygen, which would hamper the polymerization. The solutions are maintained at 70°C during two hours to achieve the completion of the reaction. The ferrogel appears as a red/orange material because of the trapped $\gamma\text{-Fe}_2\text{O}_3$ particles.

At the end of the synthesis, the ferrogels are molden as cylinders of polymeric concentration $\varphi_{\text{AM}}^{\text{synth}} \sim 3\%$. They are carefully removed from their vessel and then swollen in an aqueous solution at $[\text{Na}_3\text{Cit}] = 0.008$ mol.L⁻¹. The swelling equilibrium of the ferrogels is reached in three weeks during which the swelling bath is changed several times. The particles, which are not trapped, are released in the swelling bath.⁹ Both phenomena of solvent swelling and particle release change the volume fraction of particles Φ_{equil} inside the gel which is then smaller than Φ_{synth} .

b) Conditions chosen for the synthesis of ferrogels

The determination of the characteristic spatial scales of the individual components (table 1 and 2 of SI) enable us to choose conditions for synthesis of ferrogels where R_S is higher or lower than 1 and R_{Π} is varied on a large range. Two different batches of ferrofluids with slightly different typical size of nanoparticles (FFA with $d = 12$ nm and FFB with $d = 11$ nm) are used here. Ferrogels prepared with nanoparticles FFA (resp. FFB) are denoted FGA (resp. FGB).

For CL = 0.5% and 1%, R_S is less than 1 for both kinds of nanoparticles FFA and FFB. The case of FFA with CL = 1% has been already considered in ⁹. In the following we thus limit ourselves to the synthesis of ferrogels at CL = 0.5% with FFA to picture ferrogels with $R_S < 1$ and we compare the results with the ones from ⁹. To picture ferrogels with $R_S \geq 1$ we consider both FFA and FFB for CL = 2% as $R_S > 1$ for FFA and $R_S \sim 1$ for FFB. Table 1 summarizes the values of R_S in the various conditions of ferrogel synthesis.

FGA CL = 0.5 %	FGA CL = 1 %	FGB CL = 2 %	FGA CL = 2 %
$R_S = 0.35$	$R_S = 0.7$	$R_S = 1$	$R_S = 1.3$

Table 1 – **Steric parameter R_S values for the various ferrogels**

Figure 2 compares the swelling pressure Π_{swl}^{HG} of the hydrogels to the equation of state $\Pi^{FF}(\Phi)$ of the ferrofluid dispersions at $[\text{Na}_3\text{Cit}] = 8.10^{-3}$ mol.L⁻¹ as given by the Carnahan-Starling formalism – equ. (SI.7). It allows us, by using osmotic stress, to

adjust precisely the osmotic pressure Π^{FF} inside the ferrofluid used for preparing ferrogels and thus also the volume fraction of particles Φ_{synth} . We prepare ferrogels in a large range of known osmotic parameter R_{Π} varying from $R_{\Pi} \ll 1$ to $R_{\Pi} \sim 1$. Let us point out that it has not been possible here to synthesize macroscopic homogeneous ferrogels FGA at CL = 2%, that would correspond to $R_{\Pi} \geq 1$.

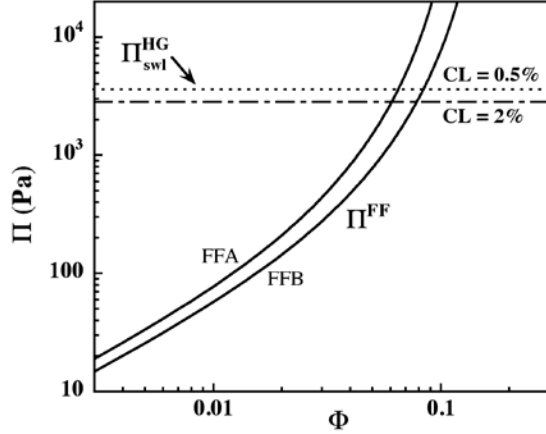


Figure 2 – **Hydrogels** : Initial swelling pressure $\Pi_{\text{swI}}^{\text{HG}}$ at CL=0.5% (horizontal dotted line) and at CL = 2% (horizontal dashed-dotted line) (Table SI.1 of SI). **Ferrofluids** : Carnahan Starling adjustments of the osmotic pressure $\Pi^{FF}(\Phi)$ of nanoparticles FFA and FFB following equ. (SI.7) – full lines (parameters : see table SI.2).

c) Structural and rotational probing of the ferrogels

We probe the structural organization of nanoparticles in the ferrogels on a local scale by SANS. The experiments are performed in LLB (Saclay, France) on the PAXY spectrometer. We play on the neutronic contrast of the solvent to match the polymer scattering. The solvent is a mixture $\text{H}_2\text{O}/\text{D}_2\text{O}$ (44/56 vol%). Its density of neutron scattering length is equal to that of the polymer, taking into account the exchange between hydrogen and deuterium of the two labile protons of the PAM repetition unit. In these conditions, the scattering coming from the nanoparticles is mainly of nuclear origin, with a contrast $\Delta\rho^2$ equal to $1.33 \cdot 10^{21} \text{ cm}^{-4}$, the magnetic contribution being less than 7% of the nuclear one.¹⁹ The scattered intensity is :

$$\frac{I(q, \Phi)}{\Phi} = \Delta\rho^2 F(q) S(q, \Phi) \quad (1)$$

where $F(q)$ is the form factor of the nanoparticles and $S(q, \Phi)$ the structure factor of the dispersed nanoparticles. The structure factor in swollen ferrogels at various volume fractions of nanoparticles are obtained by dividing the scattering of ferrogels by the form factor of the nanoparticles measured with pure and dilute ferrofluids (shown for FFB in Fig SI.2 of SI). The shape of the structure factor reflects the homogeneity/inhomogeneity of the nanoparticles dispersion and allows measuring the most probable value of the interparticle distance inside the complex medium.

A complementary probing of the rotational degree of freedom of the nanoparticles at the nanoscale, inside the medium where they are dispersed, is also realized. Each nanoparticle of maghemite bears a magnetic moment $\vec{\mu}$ and an optical anisotropy axis $\vec{u}$, which are linked together by the anisotropy energy. Under an applied field $\vec{H}$, the magnetic moment $\vec{\mu}$ of each nanoparticle orientates along the field direction. If the nanoparticle is free to rotate in its carrier medium, this also rotates mechanically the core of the

nanoparticle together with its optical axis. The alignment (even partial) of the optical axis $\vec{u}$ of the nanoparticles imparts the system with a macroscopic optical birefringence Δn . Comparing the birefringence induced in a ferrogel to that induced in a ferrofluid based on the same nanoparticles allows quantifying the proportion of nanoparticles free to rotate in the ferrogel and to characterize dynamically this rotation (see part 3 of SI). Two magneto-optical experiments are here performed to probe the rotation :

- A static measurement of the birefringence up to high fields $\vec{H}$ (of the order of 10^3 kA/m)²⁰; In high fields, the birefringence Δn saturates and is then proportional to the number of nanoparticles that can rotate within the sample (see SI - part 2).
- A dynamic measurement of the optical birefringence relaxation after a pulse of magnetic field with a small amplitude; this relaxation can be written as a stretched exponential decay

$$I(t) = I_0 \exp(-(\tau/\tau_0)^\alpha) \quad (2)$$

where τ_0 is a characteristic time of rotation and the exponent α is related to the width of the distribution of relaxation times in the dispersion. In a pure and dilute ferrofluid, this relaxation reduces to the rotational diffusion of the nanoparticles.

More details on these experiments are given in part 2 of SI. They provide us with a powerful structural and rheological probing of the ferrogels on the nanoscale.

d) Swelling ratio and particle release of the ferrogels

These ferrogels present remarkable swelling properties in particular because they are out-of-equilibrium in the synthesis conditions. During the swelling process, some internalized nanoparticles may escape from the gel and are released in the swelling bath.

The swelling ratio of the ferrogels G is here defined as :

$$G = \frac{m_{\text{water}}}{m_{\text{polymer}}} = \frac{m_{\text{FG}} - m_{\text{dry}}}{m_{\text{dry}} - m_{\text{part}}} \quad (3)$$

It is measured as follows. The ferrogel is weighed (m_{FG}) and dried at 70°C in order to obtain the mass of the dry ferrogel (m_{dry}). The mass of $\gamma\text{-Fe}_2\text{O}_3$ (m_{part}) in the ferrogel is deduced from iron concentration determination by using either chemical titration or Atomic Absorption Spectroscopy.

Note that following expression (3), the swelling ratio of the ferrogel G_{synth} at the end of the synthesis (thus before swelling) is already of the order of a few tens in our conditions. Hereafter we denote by G_{equil} the swelling ratio of the ferrogel at swelling equilibrium.

In the pure hydrogels, the corresponding swelling ratios $G_{\text{synth}}^{\text{HG}}$ and $G_{\text{equil}}^{\text{HG}}$ are obtained by using exp. (3) with $m_{\text{part}} = 0$.

At swelling equilibrium, the ratio of nanoparticle release is calculated with:

$$R = \frac{m_{\text{part}}^{\text{synth}} - m_{\text{part}}^{\text{equil}}}{m_{\text{part}}^{\text{synth}}} \quad (4)$$

where $m_{\text{part}}^{\text{synth}}$ is the initial mass of $\gamma\text{-Fe}_2\text{O}_3$ introduced at the ferrogel synthesis. The release ratio R is a characteristic parameter of the swollen ferrogels. It quantifies the nanoparticles ability to escape from the gel during swelling. The ferrogel always reaches an equilibrium state for which both R and G_{equil} remain constant.

Figure 3 presents the swelling ratio G_{equil} of the ferrogels as a function of Φ_{synth} . For a comparison, the values obtained in ref.⁹ are also plotted. The full line corresponds to the value G_{synth} at the end of the synthesis before swelling. It is independent on the nanoparticle size distribution and varies from 25 to 27 in our range of Φ_{synth} . Whatever Φ_{synth} and CL (except for $\Phi_{\text{synth}} = 9\%$ and $\text{CL}=1\%$) the value G_{equil} at the end of the swelling process is larger than G_{synth} . Moreover, whatever the volume fraction Φ_{synth} introduced, G_{equil} is lower than the swelling ratio of the hydrogel $G_{\text{equil}}^{\text{HG}}$ at the same cross-linking ratio CL (see Fig. 3). The introduction of nanoparticles within the polymeric network strongly modifies the affinity of solvent for the polymer, expressing that the swelling pressure $\Pi_{\text{swell}}^{\text{HG}}$ of the ferrogel is weaker than the one of the corresponding hydrogel.

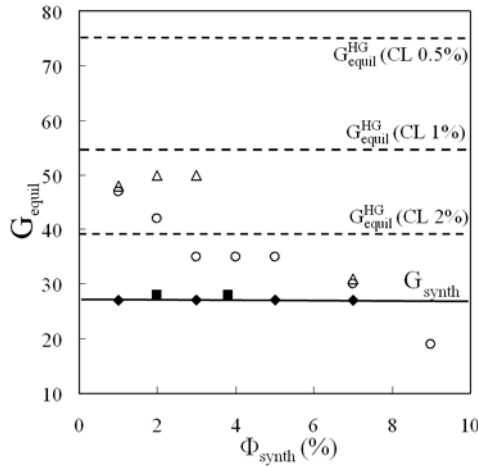


Figure 3 – **Swelling ratio of swollen ferrogels** G_{equil} as a function of the initial volume fraction of nanoparticles Φ_{synth} : FGA with $\text{CL}=0.5\%$ (open triangles), $\text{CL}=1\%$ (open circles – data from ref.⁹) and $\text{CL}=2\%$ (solid squares); FGB – $\text{CL}=2\%$ (solid diamonds); The horizontal lines correspond to the swelling ratio G_{synth} at the end of synthesis (full line) and to the swelling ratio of hydrogels at various CL (dashed lines).

As observed for the hydrogels, the swelling ratio G_{equil} of ferrogels at constant Φ_{synth} decreases with increasing the cross-linking ratio. If Φ_{synth} increases at constant CL, G_{equil} decreases except for $\text{CL}=2\%$, ferrogels then do not swell ($G_{\text{equil}} \sim G_{\text{synth}}$ for FGA and FGB).

Figure 4 shows the evolution of the release ratio R as a function of the initial volume fraction Φ_{synth} in the ferrogels.

For FGA ferrogels, the release ratio R increases with Φ_{synth} more or less linearly up to $\Phi_{\text{synth}} = 4\%$ ($R_{\text{II}} \leq 0.2$) and is independent on CL. For higher Φ_{synth} ($R_{\text{II}} > 0.2$), it stops at a maximal value of the order of 45% for $\text{CL} = 0.5\%$ as it was observed in ref.⁹ with FGA samples at $\text{CL} = 1\%$.

For FGB ferrogels at $\text{CL} = 2\%$, there is no release at low Φ_{synth} ($R_{\text{II}} < 0.2$) and a very large release ratio (about 85%) at larger Φ_{synth} ($R_{\text{II}} > 0.2$). The difference of release ratios R between FGA and FGB samples at cross-linking ratio of 2% at low Φ_{synth} (both associated with $R_{\text{II}} \leq 0.2$) points out the important role of R_s . Both systems do not swell. But samples made with FFA ($R_s > 1$) release nanoparticles while samples made with FFB ($R_s \sim 1$) do not.

The mass conservation implies:

$$\Phi_{\text{equil}} = \Phi_{\text{synth}} (1 - R) \frac{G_{\text{synth}}}{G_{\text{equil}}} \quad (5)$$

This relation is well verified experimentally. The evolution of the volume fraction Φ_{equil} obtained at the end of the swelling process as a function of Φ_{synth} is presented in figure 5 for the different ferrogels.

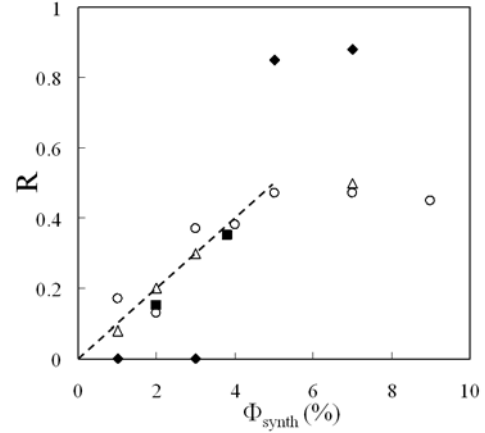


Figure 4 – **Release ratio R versus Φ_{synth}** . Same symbols as in Figure 3. The dashed line corresponds to R proportional to Φ_{synth} at $R_{\text{II}} \leq 0.2$ for FGA.

At low cross-linking ratios ($\text{CL} = 0.5\%$ and 1% - FGA – open symbols) the results in Fig. 5 are roughly equivalent up to $\Phi_{\text{synth}} = 7\%$: Φ_{equil} is an increasing function of Φ_{synth} with Φ_{equil} always smaller than Φ_{synth} . As the samples swell, the mesh size of the polymeric network initially larger than particles still increases allowing the nanoparticles release. On the contrary for $\text{CL} = 2\%$ - FGB (solid diamonds), we have to distinguish two situations: $\Phi_{\text{synth}} \leq 3\%$ ($R_{\text{II}} \leq 0.2$) and $\Phi_{\text{synth}} > 3\%$ ($R_{\text{II}} > 0.2$). At low Φ_{synth} we obtain $R=0$ and $G_{\text{equil}} = G_{\text{synth}}$ and thus $\Phi_{\text{equil}} = \Phi_{\text{synth}}$ (see Fig. 5). As the samples do not swell, the mesh size of the polymeric network remains always constant and close to the particle diameter. This indeed limits the translational diffusion of particles within the network and prevents release. For $\Phi_{\text{synth}} > 3\%$ the behavior drastically changes: R is large (Fig. 4) and Φ_{synth} decreases to a value of the order of 1% (Fig. 5).

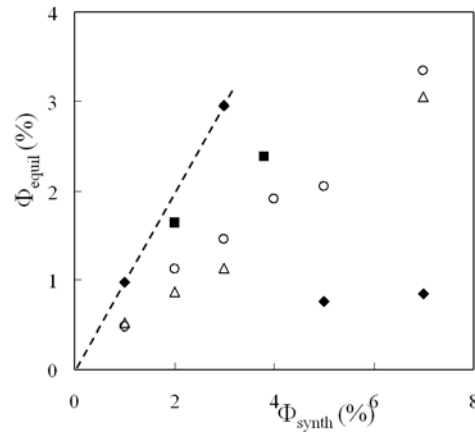


Figure 5 – **Swollen ferrogels** – Volume fraction of nanoparticles Φ_{equil} at swelling equilibrium versus the initial volume fraction Φ_{synth} for various ferrogels. Same symbols as in Figure 3. The dashed line corresponds to $\Phi_{\text{equil}} = \Phi_{\text{synth}}$.

3. Ferrogels at $R_s < 1$

We study here the structure and properties of ferrogels before and after swelling in the limiting case where $R_s < 1$. We choose thus to work with FGA samples at $\text{CL} = 0.5\%$ (see table 3) and we vary R_{II} in a large range.

a) Rheological probing before swelling

The nanoparticles able to rotate in the samples before swelling are probed dynamically by relaxation of birefringence for three different Φ_{synth} (1%, 3% and 7%). The experiments are performed on ferrogels instantaneously after the synthesis before they are placed in their swelling bath. The measurement of the relaxation time τ_0 and of the initial intensity I_0 enable to obtain the volume fraction of rotating nanoparticles in ferrogel $\Phi_{\text{synth}}^{\text{rot}}$ by comparison with the values of the pure ferrofluid (see principle in part 3 of SI). The values of $\Phi_{\text{synth}}^{\text{rot}}/\Phi_{\text{synth}}$, $\Phi_{\text{synth}}^{\text{rot}}$ and of $\Phi_{\text{synth}}^{\text{block}}$ ($= \Phi_{\text{synth}} - \Phi_{\text{synth}}^{\text{rot}}$) are collected in table 2. Although the average mesh size of the polymeric network is superior to the particle diameter ($R_s = 0.35$), there is a large amount of nanoparticles which are not allowed to rotate within the ferrogel. This suggests an interaction with the polymeric network. However the proportion of rotating particles increases from 15% at $\Phi_{\text{synth}} = 1\%$ up to 64% at $\Phi_{\text{synth}} = 7\%$.

Φ_{synth} (%)	τ_0 (μs)	$\Phi_{\text{synth}}^{\text{rot}}/\Phi_{\text{synth}}$	$\Phi_{\text{synth}}^{\text{rot}}$ (%)	$\Phi_{\text{synth}}^{\text{block}}$ (%)
1	3.2	0.15	0.15	0.85
3	2.7	0.34	1.0	2
7	1.9	0.64	4.5	2.5

Table 2 - **Non swollen ferrogels. FGA-CL=0.5%** - Low field magneto-optical birefringence. Φ_{synth} volume fraction of nanoparticles in the ferrogel; τ_0 characteristic relaxation time; $\Phi_{\text{synth}}^{\text{rot}}$ and $\Phi_{\text{synth}}^{\text{block}}$ volume fractions of respectively rotating and blocked nanoparticles. For FFA dilute ferrofluids, we obtain $\tau_0^{\text{FF}} = 5.4 \mu\text{s}$ (see Table SI.2 of SI).

The decay of intensity can be adjusted by a stretched exponential of relaxation time τ_0 which stretched exponent α is always close to 0.75, smaller than that of the pure ferrofluid which is close to ~ 1 . An advanced data treatment, presented in SI, enables to compare quantitatively the time distribution of rotating nanoparticles within ferrogels to the ones in the ferrofluid (see figure SI.4 of SI). It shows that in these unswollen ferrogels only short times are remaining. Nanoparticles of longer relaxation time seem to be blocked by the polymer within the ferrogels.

At low volume fractions Φ_{synth} of nanoparticles ($\Phi_{\text{synth}} = 1\%$ and 3%, $R_{\text{II}} < 0.1$), a large amount of nanoparticles is blocked by the presence of the polymeric chains. Let us note however that, at these Φ_{synth} , the proportion of nanoparticles free to rotate $\Phi_{\text{synth}}^{\text{rot}}/\Phi_{\text{synth}}$ in the ferrogels at the end of synthesis is roughly equal to R the release ratio of nanoparticles during the swelling process (see table 2 and figure 4). When R_{II} becomes large, this is no more true, namely at $\Phi_{\text{synth}} = 7\%$ ($R_{\text{II}} \sim 0.5$), $\Phi_{\text{synth}}^{\text{rot}}/\Phi_{\text{synth}}$ is found significantly larger than R .

b) Rheological probing at swelling equilibrium

We study here the rheological properties of ferrogels after swelling when the synthesis is at thermodynamic equilibrium. We perform here both static magneto-optical experiments in high magnetic fields and dynamic magneto-optical experiments in low magnetic fields. If both experiments can provide the amount of particles blocked in the ferrogels, one has to keep in mind that magnetic energy applied to a nanoparticle to produce its mechanical rotation are very different. In high fields experiments, it is of the order of its magnetic anisotropy energy, namely here $\sim 2kT_{\text{amb}}$.²¹ The blocked nanoparticles in high field experiments should thus be strongly coupled to the polymeric network, at least through an energy $8 \cdot 10^{-21}$ J.

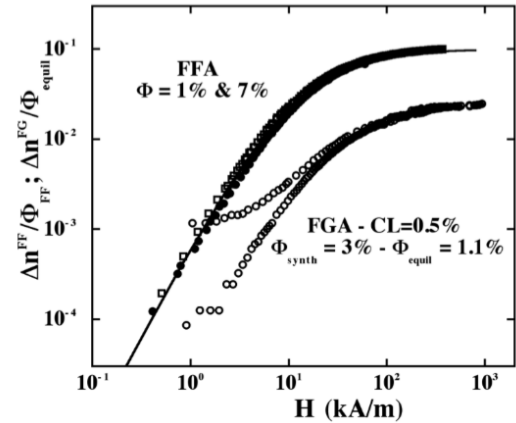


Figure 6 - **Reduced birefringence** $\Delta n^{\text{FF}}/\Phi^{\text{FF}}$ of ferrofluids FFA at $\Phi = 1\%$ (solid circles) and 7% (open squares) – full line best fit by Langevin formalism (see part 2 of SI); $\Delta n^{\text{FG}}/\Phi^{\text{FG}}$ of ferrogel FGA at swelling equilibrium (at CL = 0.5, $\Phi_{\text{synth}} = 3\%$, $\Phi_{\text{nequil}} = 1.1\%$) as a function of applied field H .

Figure 6 plots the birefringence Δn^{FG} as a function of magnetic field for a ferrogel at $\Phi_{\text{equil}} = 1.1\%$ ($\Phi_{\text{synth}} = 3\%$) and compares it to that of pure ferrofluid Δn^{FF} , normalized by the volume fraction inside the medium. Even though, both curves saturate in high fields, however they saturate at different values $\Delta n_s/\Phi$. In the ferrogel it is equal to 0.024 to be compared to the corresponding value 0.1 in ferrofluid FFA (see table SI.2 in SI). This difference comes from the fact that inside the ferrogel a significant amount of nanoparticles is unable to rotate mechanically within ferrogel under the action of a high magnetic field. In the ferrogel, 76% of the nanoparticles are tightly blocked by the polymeric network at 10^3 kA/m.

Moreover, a hysteresis is observed when the field is decreased back to zero. At the time scale of the experiment (about 15 mn for the field decrease), an important proportion of nanoparticles keeps their mechanical orientation in zero field. This hysteresis disappears within a few hours denoting the existence of slow relaxations in the swollen system. The mechanical rotation of the nanoparticles are thus hindered by the polymers inside these ferrogels, even in fields as high as 10^3 kA/m.

Φ_{synth} (%)	Φ_{equil} (%)	$\Phi_{\text{equil}}^{\text{rot}}/\Phi_{\text{synth}}$		$\Phi_{\text{equil}}^{\text{rot}}$ (%)		$\Phi_{\text{equil}}^{\text{block}}$ (%)		τ_0 (μs)
		HF	LF	HF	LF	HF	LF	
1	0.5	0.34	0.05	0.17	0.02	0.33	0.48	4.4
3	1.1	0.24	0.10	0.26	0.11	0.84	0.99	3.5
7	3.0	0.14	0.21	0.42	0.63	2.58	2.37	2.5

Table 3 - **Ferrogels at swelling equilibrium. FGA-CL=0.5%** - Magneto-optical birefringence. Φ_{synth} initial volume fraction; Φ_{equil} volume fraction at swelling equilibrium; τ_0 characteristic relaxation time; $\Phi_{\text{equil}}^{\text{rot}}$ and $\Phi_{\text{equil}}^{\text{block}}$ volume fractions of respectively rotating and blocked nanoparticles. HF refers to High-Field experiments and LF to Low-Field experiments.

Table 3 reports the respective volume fractions of blocked particles and of particles free to rotate within the ferrogel for $\Phi_{\text{equil}} = 0.5\%$, 1.1% and 3% . After swelling, the volume fraction $\Phi_{\text{equil}}^{\text{rot}}$ of blocked nanoparticles in high field is close to Φ_{equil} . The volume fractions of rotating nanoparticles obtained in low fields in dynamic magneto-optical experiments are very

similar to the ones deduced from high fields measurements (table 3). It shows that the ratio of blocked nanoparticles in low field experiments corresponds to the particles coupled with the polymeric network. The characteristic times τ_0 of rotation remains short compared to the one of pure ferrofluid (see the distribution times in SI).

c) Comparison of ratios of nanoparticles blocked in ferrogel before and after swelling

If the nanoparticles, blocked before swelling, remain blocked at swelling equilibrium, it means that their number can be obtained with:

$$\Phi_{\text{equil}}^{\text{block}} = \Phi_{\text{synth}}^{\text{block}} G_{\text{synth}}/G_{\text{equil}} \quad (6)$$

The comparison of table 2 and 3 enables to check this assumption. We compare in table 4 the experimental values $\Phi_{\text{equil}}^{\text{block}}$ to the calculated ones (using equation 6). This is in very good accordance. *The particles blocked just after synthesis remain blocked during swelling.* It also means, because the volume fraction of polymer φ_{AM} is proportionnal to $1/G$ (see SI), that the ratio $\Phi_{\text{equil}}^{\text{block}}/\varphi_{\text{AM}}$ (either equal to $\Phi_{\text{synth}}^{\text{block}}/\varphi_{\text{AM}}^{\text{synth}}$ or to $\Phi_{\text{equil}}^{\text{block}}/\varphi_{\text{AM}}^{\text{equil}}$) remains constant during the swelling process (see table 4).

Φ_{synth} (%)	$\Phi_{\text{synth}}^{\text{block}}$ meas. (%)	$\Phi_{\text{equil}}^{\text{block}}$ meas. (%)	$\Phi_{\text{equil}}^{\text{block}}$ calc. (%)	$\Phi_{\text{synth}}^{\text{block}}/\varphi_{\text{AM}}^{\text{synth}}$ meas.	$\Phi_{\text{equil}}^{\text{block}}/\varphi_{\text{AM}}^{\text{equil}}$ meas.
1	0.85	0.48	0.48	0.30	0.31
3	2	0.99	1.08	0.72	0.66
7	2.5	2.37	2.17	0.90	1.00

Table 4 – **FGA - CL=0.5%** - Volume fraction of blocked nanoparticles in ferrogels at swelling equilibrium ($\Phi_{\text{equil}}^{\text{block}}$) and in synthesis conditions ($\Phi_{\text{synth}}^{\text{block}}$). Comparison between measurements of tables 2 and 3, and calculation with equation 6; $\Phi_{\text{synth}}^{\text{block}}/\varphi_{\text{AM}}^{\text{synth}}$: volume ratio of nanoparticles with respect to polymer in the synthesis conditions; $\Phi_{\text{equil}}^{\text{block}}/\varphi_{\text{AM}}^{\text{equil}}$: volume ratio of nanoparticles with respect to polymer at swelling equilibrium.

d) SANS experiments and structure of ferrogels

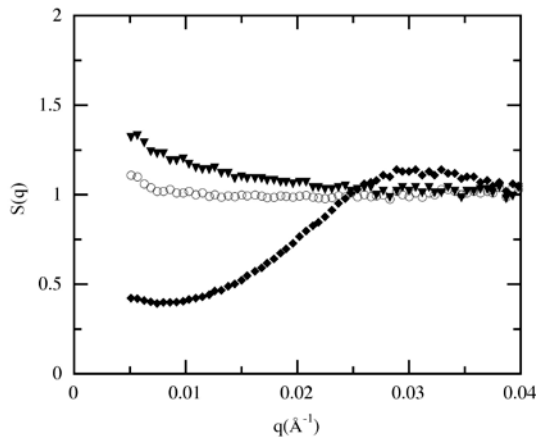


Figure 7 – **Ferrogels at swelling equilibrium FGA - CL = 0.5 %** - Structure factor of the nanoparticles $S(q)$ versus q at $\Phi_{\text{equil}} = 0.5\%$ (solid triangles), 1.1% (open circles) and 3% (solid diamonds).

The structure factor $S(q, \Phi)$ of swollen ferrogels at various volume fractions are presented in figure 7. They are obtained by dividing the scattering of ferrogels by the form factor of the ferrogels measured on pure ferrofluids. As almost all the

particles remaining in the ferrogels are coupled to the polymeric network, the structure is not at all similar to that of a pure ferrofluid solution. It is the *structure of a matched polymeric network decorated with nanoparticles*. For $\Phi_{\text{equil}} = 0.5\%$ and 1% , the structure factor is roughly equal to 1, except at low q where there is a slight upturn. The nanoparticles behave like a dilute system where nanoparticles are randomly organized with a low effective attraction.

At $\Phi_{\text{equil}} = 3\%$, which corresponds to a sample synthesized in concentrated regime ($\Phi_{\text{synth}} = 7\%$), there is still the slight upturn at low q and a correlation peak at $0.032 \text{ \AA}^{-1}$ ($\sim 200 \text{ \AA}$ in real space). This peak corresponds to the most probable interparticle distance inside the polymeric network. Let us assume that the polymeric network is homogeneous with a mesh size ξ . In the framework of this hypothesis, the initial mesh size before swelling would be here $170 \text{ \AA}$ (see table 1 of SI) and would remain of the order of $170 \text{ \AA}$ after swelling (see Fig 3). ξ is of the same order of magnitude as the most probable interparticle distances. It is thus a rather homogeneous polymeric network that the nanoparticles are ‘decorating’.

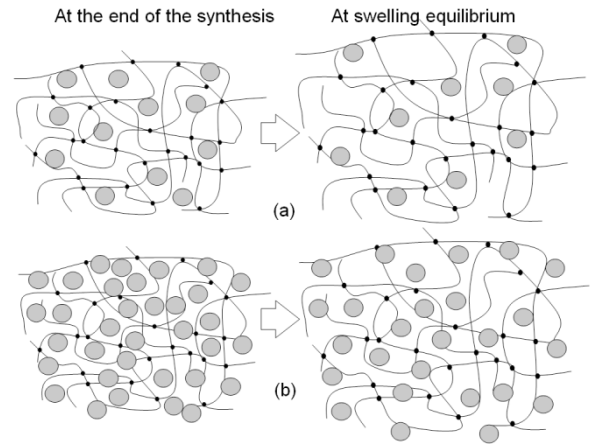


Figure 8 – **Route 1 : $R_s < 1$ - FFA CL = 0.5%** - Pictures of gels at the end of the synthesis (left) and at swelling equilibrium (right), (a) low Φ_{synth} (1% or 3%); (b) high Φ_{synth} (7%).

Moreover the correlation peak corresponds also to the mean distance between nanoparticles in a pure ferrofluid solution at 3% in repulsive regime (see Fig SI.2). The particles fill thus all the available space in the swollen ferrogel and each mesh contains a nanoparticle. The covering of the polymeric network by nanoparticles is thus close to saturation. Note that if in this structure 80% of the nanoparticles are blocked in rotation, 20% still rotates as if in a liquid environment, without polymer hindrance, with a size of the order of the mesh of the network.

The structures of the ferrogels made with CL = 0.5% with FFA (at the end of the synthesis and at swelling equilibrium) are pictured in figure 8 for the whole range of Φ_{synth} .

4. Ferrogels at $R_s \sim 1$

We study here the structure and properties of ferrogels after swelling when $R_s \sim 1$. We choose thus to work with FGB samples at CL = 2 % (see table 1) and we vary R_{II} in a large range. As seen in Fig 3, there is no swelling at all for such samples: G_{equil} remains very close to G_{synth} . However, there are two different releasing behaviors depending on Φ_{synth} . At low Φ_{synth} (1% and 3%), there is no release. Oppositely above $\Phi_{\text{synth}} = 3\%$, there is a very large release, as Φ_{equil} decreases to a value of the order of 1%. The following measurements enlighten these two very different behaviors

a) Rheological probing at swelling equilibrium

We have deduced from low-field dynamical birefringence the volume fractions $\Phi_{\text{equil}}^{\text{rot}}$ of rotating nanoparticles and $\Phi_{\text{equil}}^{\text{block}}$ of blocked nanoparticles in the swollen ferrogels (see table 5). A large proportion of the nanoparticles is blocked whatever Φ_{synth} . Table 5 gives also the ratio $\Phi_{\text{equil}}^{\text{block}} / \Phi_{\text{AM}}^{\text{equil}}$ for each Φ_{synth} .

Φ_{synth} (%)	Φ_{equil} (%)	τ_o (μs)	$\frac{\Phi_{\text{equil}}^{\text{rot}}}{\Phi_{\text{equil}}}$	$\Phi_{\text{equil}}^{\text{rot}}$ (%)	$\Phi_{\text{equil}}^{\text{block}}$ (%)	$\frac{\Phi_{\text{equil}}^{\text{block}}}{\Phi_{\text{AM}}^{\text{equil}}}$ meas.
1	0.97	4.4	0.05	0.05	0.92	0.33
3	2.95	2.24	0.26	0.76	2.19	0.79
5	0.75	3.6	0.08	0.06	0.69	0.25
7	0.82	3.43	0.26	0.22	0.60	0.22

Table 5 - **Ferrogels at swelling equilibrium. FGB-CL=2% - low field magneto-optical birefringence.** - Same notations as in table 3

There is a majority of blocked nanoparticles at any Φ_{synth} . However in the samples at $\Phi_{\text{synth}} = 3\%$ and 7% , about $\sim 25\%$ of the amount of nanoparticles entrapped in the swollen system are still rotating. This is clearly different from the case where $R_s < 1$ where most of the particles free to rotate at the end of the synthesis are released during the swelling process since almost all of the particles remaining in ferrogels are blocked at swelling equilibrium. This is indeed related here to the tight mesh size of the network when $R_s \sim 1$. Moreover the characteristic time τ_o of the rotating nanoparticles is here still of the same order of magnitude as τ_o^{FF} (the relaxation time in the pure ferrofluid – see Table SI.2) and the stretched exponent has a constant value of ~ 0.9 , also close to the one of the pure ferrofluid. It shows that the movement of the rotating nanoparticles is not strongly hindered by the polymeric network nor related to the existence of large nanoparticle agglomerates. These rotating nanoparticles should be located in a liquid environment within the sample. It enables us to conclude that the network is not homogeneous. Indeed, since $R_s \sim 1$ and since the ferrogel does not swell, an homogeneous network is not compatible with particles in a liquid environment.

At $\Phi_{\text{synth}} > 3\%$, the very strong release of nanoparticles from ferrogels that do not swell implies the existence of large inhomogeneities within the synthesized samples. The volume fraction of blocked nanoparticles being quite constant in these swollen samples, it also suggests a saturation of the fraction of the polymeric network accessible to the nanoparticles.

b) SANS experiments and structure of ferrogels

The SANS structure factors of the four ferrogels are presented in Figure 9. At low Φ_{synth} (1 %) $S(q)$ is rather flat, while at larger Φ_{synth} , $S(q)$ presents a slight upturn at low q together with a maximum at a wave-vector $q_{\text{max}} = 2\pi/l_{\text{mp}}$ associated with the most probable interparticle distance l_{mp} .

For sake of reasoning, let us first assume that the polymeric network is here homogeneous. It would lead to a mesh size $\xi_{\text{hom}} \sim 110 \text{ \AA}$ (see table SI.1) very close to the size of the nanoparticles. A network decorated with one nanoparticle per mesh (as in part 3) would present a maximum of $S(q)$ at $2\pi/\xi_{\text{hom}} \sim 0.057 \text{ \AA}^{-1}$ (see inset of figure 9 – dashed line) much larger than the experimental $q_{\text{max}} = 2\pi/l_{\text{mp}}$ (open circles). This is

in any case not possible because it would imply much larger volume fractions of nanoparticles than the present ones.

Let us now assume that the spatial distribution of nanoparticles in the ferrogel is homogeneous with a mean interparticle distance $l_{\text{mean}}(\Phi) = d_o \sqrt[3]{\frac{\pi}{6\Phi}}$. The maximum of $S(q)$

will then be located at $q_{\text{mean}}(\Phi) = 2\pi/l_{\text{mean}} = \frac{2\pi}{d_o} \sqrt[3]{\frac{6\Phi}{\pi}}$, which is

plotted as a full line in inset of Fig. 9. Surprisingly, in these ferrogels, q_{max} is experimentally very close to $2\pi/l_{\text{mean}}(\Phi_{\text{synth}})$ (and for $\Phi_{\text{synth}} > 3\%$ sensibly larger than what would be expected for $2\pi/l_{\text{mean}}(\Phi_{\text{equil}})$). It allows to conclude that for:

- the spatial distribution of nanoparticles is clearly heterogeneous in the ferrogel.

- $l_{\text{mean}}(\Phi_{\text{synth}})$ is close to l_{mp} , even if slightly larger. The swollen ferrogel has a kind of “memory” of the initial large volume fraction of nanoparticles in the synthesis bath.

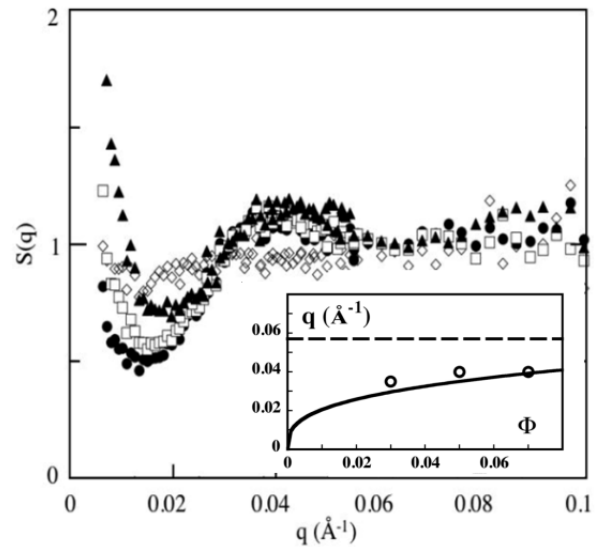


Figure 9 - **Ferrogels at swelling equilibrium. FGB CL = 2%** Structure factor $S(q)$ versus q at $\Phi_{\text{synth}} = 1\%$ (Γ), $\Phi_{\text{synth}} = 3\%$ (λ); $\Phi_{\text{synth}} = 5\%$ (open squares); $\Phi_{\text{synth}} = 7\%$ (σ). **Inset** – q versus Φ (Dashed line : $q_{\text{max}} = 2\pi/\xi_{\text{hom}}$; Full line : q_{mean} versus Φ ; Open circles : experimental $q_{\text{max}} = 2\pi/l_{\text{mp}}$ from fig.9a versus Φ_{synth})

The increase of the structure factor at low q observed for $\Phi_{\text{synth}} \geq 3\%$ also shows that the nanoparticles are here organized in a very heterogeneous way within the ferrogels. There are areas of the ferrogel in which there is a high density of nanoparticles and areas empty of nanoparticles. In the areas of the ferrogel rich in nanoparticles, two situations can be considered: (i) the nanoparticles are locally concentrated or (ii) the nanoparticles form large aggregates. This second situation is not possible because we do not observe a correlation peak corresponding to the contact of two particles at $2\pi/d_o \sim 0.08 \text{ \AA}^{-1}$ which should be obtained in case of the presence of dense aggregates.²² There are thus spatial regions of the ferrogels where nanoparticles have been concentrated.

Since a large amount of the nanoparticles is coupled with the polymeric network inside the swollen ferrogel, the heterogeneous spatial distribution of nanoparticles shows that the polymeric network is also heterogeneous with regions of high polymer density coexisting with regions of low polymer density. The mass conservation of the polymer implies then that there are some regions of the ferrogel where the mesh size

of the network is inferior to the diameter of the nanoparticles. These parts are thus not accessible to the nanoparticles. The structure of the samples is thus composed of: (i) some regions with a high density of polymer and a small mesh size without nanoparticles, (ii) some regions where the nanoparticles are linked to the polymeric network and (iii) some pockets where there are no polymers and eventually some free nanoparticles in a fluid environment.

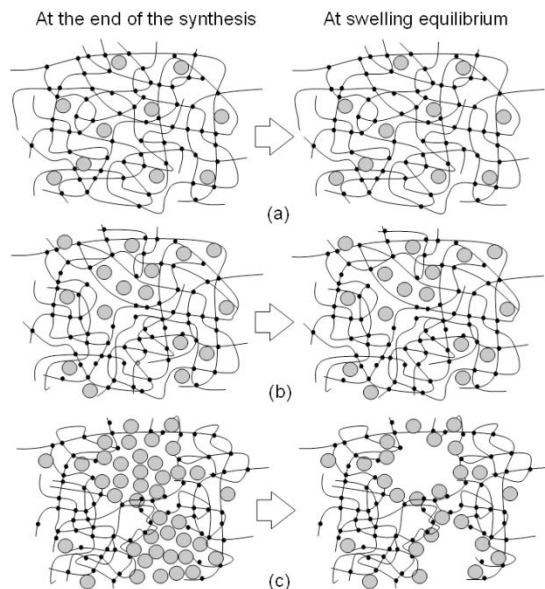


Figure 10 – **Route 2 : $R_S \sim 1$ - FFB CL = 2%** - Pictures of gels at the end of the synthesis (left) and at swelling equilibrium (right), (a) low Φ_{synth} (1%); (b) intermediate Φ_{synth} (3%); (c) high Φ_{synth} (5% or 7%).

At $\Phi_{\text{synth}} \geq 3\%$, the presence of liquid pockets containing nanoparticles may explain why we observe a correlation peak. The situation of the nanoparticles in the pockets is close to the one observed with pure ferrofluid solutions with a concentration of the order of $\sim 7\text{--}8\%$ (at $\Phi_{\text{synth}} > 3\%$). It leads to a correlation peak at the same wavevector (see the scattering spectra of the pure FFB ferrofluid at $\Phi = 7\%$ in figure SI.2), but with an interparticle distance less defined. However in these systems, the total amount of rotating nanoparticles is at most 25% (of Φ_{equil}), the majority of the nanoparticles is blocked in rotation and thus linked to the polymeric network. Taking into account the observed intensity of the correlation peak, these blocked nanoparticles must also contribute to the peak and they thus should decorate the polymeric network with the same most probable distance. In this context, we can easily imagine that, during the swelling process, the pockets of the already very heterogeneous networks can percolate favoring the release of nanoparticles. This process then leaves behind a decoration of (blocked) nanoparticles on the surface of the empty pockets with the same interparticle distance as the ferrofluid previously contained in these pockets. This explains in the swollen ferrogels (prepared with $\Phi_{\text{synth}} = 5\%$ and $\Phi_{\text{synth}} = 7\%$): (i) their large release ratio (see Fig. 4), (ii) their low value of Φ_{equil} because the polymeric network outside the pockets is very tight, (iii) their large proportion of blocked nanoparticles and (iv) the potential remaining of some isolated fluid pockets.

If we assume that at $R_S \sim 1$, the particles blocked after the synthesis remain blocked after the swelling process as it is observed at $R_S < 1$, it would also explain the rather low proportion of blocked nanoparticles at the end of the synthesis. The blocked nanoparticles are mainly decorating the pockets and are not introduced in the regions with a tight polymeric

network. Indeed at $\Phi_{\text{synth}} = 3\%$, decorated pockets full of nanoparticles in a liquid environment also exist but remain isolated. The nanoparticles cannot be released because they cannot cross the regions where the polymeric mesh size is lower than their diameter. The pocket decoration confirms the existence of a specific interaction between the nanoparticles.

The structures of FGB ferrogels at CL = 2% (at the end of the synthesis and after swelling) are pictured in figure 10 for the whole investigated range of Φ_{synth} .

5 - Discussion

The different local structures of ferrogels obtained in parts 3 and 4 shows that the *homogeneity of ferrogels is controlled by the synthesis conditions* and is tuned by both R_S and R_{II} . Indeed as the polymeric network is cross-linked by covalent bonds its homogeneity cannot change during swelling.

R_S is the main parameter that influences the homogeneity of the ferrogel in the synthesis conditions. If $R_S < 1$, the nanoparticles can easily enter in the meshes of the hydrogel without any distortion. Oppositely, for $R_S > 1$ an entrapped nanoparticle will necessarily forces the mesh size in its immediate surrounding to be larger than the average mesh. The presence of the nanoparticles will thus bring inhomogeneities within the whole ferrogel. The difference of release ratios between FGA and FGB samples, at CL = 2% and low Φ_{synth} , illustrates this point. Samples made with FFA release nanoparticles though samples made with FFB do not. For such a high cross-linking ratio, the polymeric network is heterogeneous with zones of pure polymer network and pockets of ferrofluid. The zones of pure polymeric network are so dense that the diffusion of free nanoparticles through them is impossible. Nanoparticles can only be released during swelling if they can follow an open percolating path. This happens in FGA samples ($R_S > 1$) not in FGB ($R_S \sim 1$) where the heterogeneities are less pronounced. It allows to sort out two families of ferrogel structures.

If $R_S < 1$, the polymeric network is weakly modified by the nanoparticles and the ferrogel is homogeneous. This is the situation observed whatever Φ_{synth} , as long as $R_{II} < 0.5$. For $R_{II} \sim 0.5$, the polymeric network becomes slightly heterogeneous. There are some nanoparticles able to rotate within the ferrogel. This is the situation for FGA at $\Phi_{\text{synth}} = 7\%$ and a cross-linking ratio CL = 0.5%.

If $R_S \geq 1$, the system is heterogeneous. At low R_{II} , there may be some nanoparticles able to rotate within the ferrogel in ferrofluid pockets. This is the situation for FGB at $\Phi_{\text{synth}} = 3\%$ and CL = 2%, with $R_{II} \sim 0.2$. For $R_{II} > 0.2$ the polymeric network is very heterogeneous at the synthesis. The pockets of ferrofluid percolate to form continuous zones, free of polymer, within the ferrogel, that can empty during the swelling process. This explains the large release ratio observed with FGB ferrogels at CL = 2% for $\Phi_{\text{synth}} = 5\%$ and $\Phi_{\text{synth}} = 7\%$. This is also the reason why FGA ferrogels at CL = 2% and $\Phi_{\text{synth}} \geq 5\%$ break down during swelling.

Apart from the conditions that play on the homogeneity of the ferrogel during the synthesis, a very important feature of the ferrogels is evidenced here whatever their homogeneity. It is an attractive interaction between the nanoparticles and the polymer chains that induces a *strong adsorption of the nanoparticles on the polymer network*. The existence of an attractive interaction is clearly proved by the reduction of the swelling ratio of the ferrogel compared to the swelling ratio of

the hydrogel at the same cross-linking ratio CL. Without interaction between the nanoparticles and the polymeric chains, the addition of nanoparticles bearing a negative charge in the hydrogel would have increased the chemical potential of water inside the gel and thus the swelling of the hydrogel (like in a dialysis bag). As one observes exactly the opposite behavior even at low Φ_{synth} , it points out an attractive part in the sum of interaction between nanoparticles and polymer chains. When Φ_{synth} is increased in the network, the effect increases and the swelling ratio G_{equil} of the ferrogel decreases (see Figure 4).

These attractive interactions existing between iron oxide nanoparticles and the PAM chains in the ferrogels are ascribed to hydrogen bonds between the carbonyl groups of the polymer (each monomer bearing a primary amide function) and hydroxyl functions at the iron oxide surface, either Fe-OH directly at the solid surface or hydroxyl end-groups from the citrate ligands coating the nanoparticles. The hypothesis of H-bonds formation between PAM and many oxides was proposed long ago.²³ It was evidenced by techniques like the shift of infra-red absorption bands of the polymer²⁴ and the shift of the iso-electric point of the oxide surface measured by potentiometric titration and zetametry.²⁵ Such H-bonds were also invoked in the kinetics of adsorption of PAM onto the haematite ($\alpha\text{-Fe}_2\text{O}_3$) surface, which has a chemical reactivity close to maghemite ($\gamma\text{-Fe}_2\text{O}_3$).²⁶ The completely different mechanism of complexation by direct chelation of Fe^{III} ions by polar groups of the polymer is very unlikely in our case of ferrogels, because the iron oxide surface is already covered by a dense layer of citrates, which offer many hydroxyl moieties to establish H-bonds with the polymer.

A second experimental evidence of this interaction is given by the observation of blocked nanoparticles just after synthesis, remaining blocked all along the swelling process, even if the mesh is larger than the nanoparticles ($R_s < 1$). As a release is geometrically possible, the remaining nanoparticles are thus all adsorbed to the polymeric network and decorate it. Depending on the synthesis conditions they are then decorating either a homogeneous polymer network ($R_s < 1$) or the pockets (empty or full of ferrofluid) of a heterogeneous network ($R_s \sim 1$). The birefringence experiment in large fields gives an indication about the binding energy of the nanoparticles to the polymeric network : it is larger than $2kT_{\text{amb}}$, a hydrogen bond being of the order of $4kT_{\text{amb}}$. Figure 11 quantifies the adsorption of the nanoparticles on the polymer chains through the Φ_{synth} -dependence of the ratio $\Phi^{\text{block}}/\varphi_{\text{AM}}$, volume fraction of blocked nanoparticles over polymeric volume fraction. It collects for homogeneous networks the previous observations of Tables 4 and 5:

- $\Phi^{\text{block}}/\varphi_{\text{AM}}$ remains constant before swelling and at swelling equilibrium, as adsorbed nanoparticles cannot diffuse outside the gel,
- the cross-linking ratio has no influence on $\Phi^{\text{block}}/\varphi_{\text{AM}}$, as the adsorption process is indeed related to an interaction between the polymeric chains and the nanoparticles,
- $\Phi^{\text{block}}/\varphi_{\text{AM}}$ only depends on Φ_{synth} and saturates at large Φ_{synth} , this plateau being consistent with SANS experiments on FGA at CL=0.5% and $\Phi_{\text{synth}} = 7\%$ that shows that the covering of the polymeric network by nanoparticles is close to saturation.

In the two heterogeneous swollen samples FGB at CL=2% and large Φ_{synth} , the values of $\Phi^{\text{block}}/\varphi_{\text{AM}}$ can no longer be located on the absorption curve of figure 11 because a large

part of the heterogeneous network is not accessible to the nanoparticles and does not participate to the adsorption process (see diamonds at large Φ_{synth} in Fig. 11).

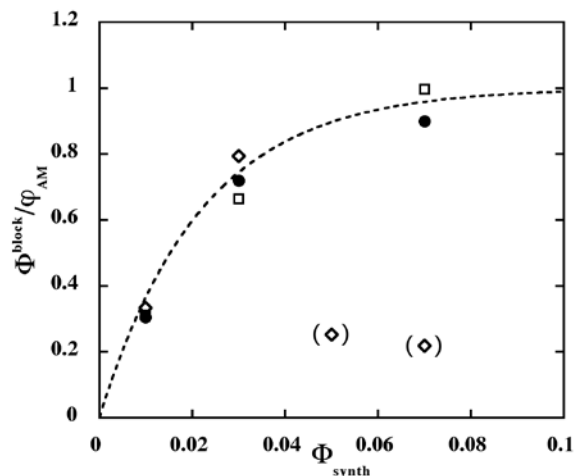


Figure 11 – **Nanoparticle adsorption** - Volume ratio of nanoparticles with respect to polymer in the synthesis conditions $\Phi^{\text{block}}/\varphi_{\text{AM}}^{\text{synth}}$ (full circles: FGA - CL = 0.5%) and at swelling equilibrium $\Phi^{\text{block}}/\varphi_{\text{AM}}^{\text{equil}}$ (open squares : FGA – CL = 0.5%; open diamonds : FGB – CL = 2%) versus initial volume fraction of nanoparticles Φ_{synth} .

Nanoparticle adsorption also limits the nanoparticle release during swelling, however it is not always the only cause for a low release. Because of local heterogeneities in the polymeric network, pockets of ferrofluid, with nanoparticles able to rotate, can be entrapped in the ferrogel even after swelling (see for example FGB at CL=2% and $\Phi_{\text{synth}} = 3\%$).

This strong adsorption of the nanoparticles on the polymeric chains can explain the large mechanical reinforcement obtained in ⁹ with FGA ferrogels at CL=1%. In such ferrogels, the synthesis conditions were chosen to get a ferrogel decorated by the nanoparticles with an homogeneous structure ($R_s < 1$ and $R_{\text{FI}} < 1$). It leads to a relative increase of the Young modulus $E^{\text{FG}} - E^{\text{HG}}/E^{\text{HG}}$ varying exponentially with $\Phi_{\text{equil}}/\varphi_{\text{AM}}$ (see inset of Figure 12) reaching for $\Phi_{\text{synth}} = 7\%$ ($\Phi_{\text{equil}} = 3\%$) a value 10 times larger than that of the corresponding hydrogel. It means that the number of cross-links mediated by the nanoparticles is then 10 times larger than the BAM cross-links.

Let us note as a last point that in the ferrogel (as it is true in the hydrogel – see SI) the Young modulus at swelling equilibrium is directly proportional to $\Pi_{\text{mix}}^{\text{FG}}$ the osmotic pressure of mixing of all the components of the system. By subtracting $\Pi_{\text{mix}}^{\text{HG}}$ the mixing osmotic pressure of the pure hydrogel (see SI), it is then possible to deduce the osmotic pressure $\Pi_{\text{mix}}^{\text{nanop}}$ associated to the mixing of the nanoparticles in the system “polymer + solvent”. These determinations for FGA at CL=1% are plotted in figure 12 as a function of Φ_{equil} . Adjusted by a Carnahan-Starling formalism (see SI), a much larger repulsion range ($\delta = 12\text{nm}$) is found with respect to that of Π^{FF} of the initial ferrofluid FFA ($\delta = 4.5\text{ nm}$). In these homogeneous ferrogels, the nanoparticles are distributed as in a dispersion of very strongly repulsive Hard-Spheres, this effective repulsion being mediated by the polymer chains on which the nanoparticles are adsorbed. This let suspect only weak effects from an applied magnetic field, as in standard strongly repulsive ferrofluids²⁷, a softening of the structure

being necessary to make them observable. As in ferrofluids, such effects could be induced if the ionic strength is increased either in the synthesis conditions or in the swelling bath.

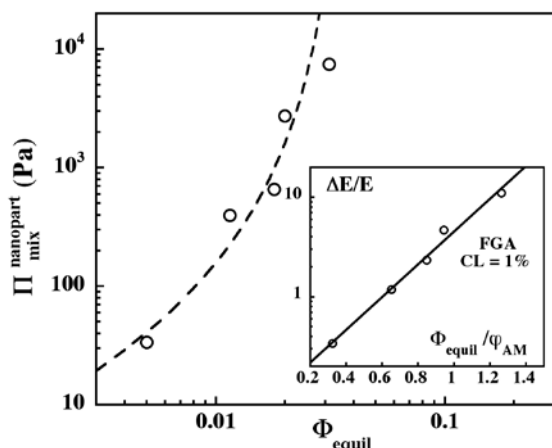


Figure 12 – **Homogeneous ferrogels FGA at swelling equilibrium CL=1%** - Nanoparticle contribution $\Pi_{\text{nanopart}}^{\text{mix}}$ to mixing pressure of ferrogels versus Φ_{equil} (open circles – dashed line: its Carnahan-Starling adjustment). Inset: Reduced variations $(E^{\text{FG}} - E^{\text{HG}})/E^{\text{HG}}$ of the elastic modulus (from ⁹) in these swollen ferrogels as a function of $\Phi_{\text{equil}}/\phi_{\text{AM}}$ in a log-log representation. The full line is an exponential fit.

Summary and perspectives

Magnetic nanocomposite materials of low Young modulus are here synthesized by dispersing maghemite nanoparticles in a PAM polymeric network. We show how the local structure of a ferrogel can be tuned from a very homogeneous structure to a very heterogeneous one by the synthesis conditions. These local structures are elucidated here thanks to the combined use of physico-chemical determinations, of magneto-optical dynamic birefringence and of static Small Angle Neutron Scattering. These measurements reveal an adsorption of the nanoparticles on the polymeric chains, which allows keeping some nanoparticles inside the swollen gels. This adsorption limits the swelling process and decorates the polymeric network with nanoparticles, which explains the reinforcement observed in ⁹ with these ferrogels at CL = 1% because entrapped nanoparticles behave as supplementary cross-linkers in the network. If the ratio R_s (defined as the nanoparticle effective volume over the polymeric mesh one in the homogeneous hydrogel) is too large the system becomes very heterogeneous and decorated pockets are observed. If $R_s < 1$, a homogeneous network decorated at the spatial scale of the polymeric mesh is observed for $R_{\text{II}} \leq 1$. When the ratio of volume fraction of nanoparticles to volume fraction of polymer $\Phi_{\text{equil}}/\phi_{\text{AM}}$ reaches 1, the polymeric array is saturated. If $R_s \geq 1$, it is no longer possible to form homogeneous ferrogels during the synthesis. The ferrogels are formed by connected networks of polymers with a tight mesh size without nanoparticles coexisting with pockets free of polymers decorated by nanoparticles. It becomes then difficult to keep the properties of the ferrogels under control.

We have thus shown how it is possible to monitor the mechanical properties of the ferrogels. As the key point of the monitoring of the properties is the interaction between the different partners of this complex system, we will investigate in a further work how the salinity of the medium modifies this

interaction and how it can change the properties of these swollen ferrogels.

Acknowledgements

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Supporting Information

Static and dynamic structural probing of swollen Polyacrylamide ferrogels

J.A. Galicia^{1,2}, F.Cousin³, E. Dubois¹, O. Sandre¹, V. Cabuil¹, R. Perzynski^{1,*}

¹ Université Pierre et Marie Curie – PECSA – UMR 7195 CNRS – UPMC – ESPCI, case 51, 4 Place Jussieu, 75252 Paris cedex 05 – France

² Fac. de Ing. Química, Benemerita Universidad Autónoma de Puebla, Edif 147/102 Ciudad Universitaria, 18 sur y San Claudio, Puebla, Pue. Mexico

³ Laboratoire Léon Brillouin, UMR 12 CNRS-CEA, CE-Saclay, 91191 Gif-sur-Yvette - France

* corresponding author,

e-mail : regine.perzynski@upmc.fr

1 Hydrogels

1.1 - Synthesis

The hydrogels are obtained by a free radical polymerization as described in part 2a of the main article replacing the ferrofluid by an aqueous solution of trisodium citrate (Na_3Cit) at a concentration $8.10^{-3} \text{ mol.L}^{-1}$. The range (0.5-2%) of cross-linker ratio is chosen for the following reasons :

- (i) Below CL = 0.5%, the gels are too soft to be manipulated
- (ii) Above CL = 2%, PAM hydrogels are known to present local heterogeneities of cross-linkers.^{1,2,3}

1.2 Swelling of the hydrogel

At the end of the synthesis, the hydrogel is out of thermodynamical equilibrium. Placed inside a water bath at the same concentration $[\text{Na}_3\text{Cit}]$ as the synthesis medium, it absorbs a large volume of water. The swelling equilibrium is reached when the hydrogel mass does not evolve anymore (typically after 3 weeks). Before reaching equilibrium, the bath is changed several times in order to accelerate the equilibration process. When equilibrium is reached, the swollen gel is weighed (m_{swollen}), then dried at 70°C during 12 hours, and weighed again (m_{dried}). m_{dried} is very close to the mass of polymer m_{polymer} because the polymerization yield is measured equal to 98%. This allows to determine the swelling ratio $G_{\text{HG}}^{\text{equil}}$ at equilibrium through $(m_{\text{swollen}} - m_{\text{dried}})/m_{\text{polymer}} = m_{\text{H}_2\text{O}}/m_{\text{polymer}}$. $G_{\text{HG}}^{\text{equil}}$ depends on the degree of cross-linking CL (see table SI.1). It is related to the volume ratio of polymer at equilibrium through $\phi_{\text{AM}}^{\text{equil}} = \rho_{\text{H}_2\text{O}}/\rho G_{\text{HG}}^{\text{equil}}$ with $\rho/\rho_{\text{H}_2\text{O}} = 1.35$, ρ being the polymer density. The same formula stands at the end of the synthesis : $\phi_{\text{AM}}^{\text{synth}} = \rho_{\text{H}_2\text{O}}/\rho G_{\text{HG}}^{\text{synth}}$ where $\phi_{\text{AM}}^{\text{synth}}$ and $G_{\text{HG}}^{\text{synth}}$ are the volume ratio of polymer and the swelling ratio in the preparation state (after cross-linking and before swelling).

1.3 –Swelling pressure of hydrogels and mesh size of the hydrogel polymeric array

Once $G_{\text{HG}}^{\text{equil}}$ is measured, it is possible to calculate the initial swelling pressure $\Pi_{\text{swl}}^{\text{HG}}$ (at polymer volume fraction $\phi_{\text{AM}} = \phi_{\text{AM}}^{\text{synth}}$) following Flory-Rehner theory knowing the Flory parameter χ . It is also possible to calculate the number of monomers N_c between two cross-links and to estimate the mesh size of the polymeric network ξ_{equil} either from the volume density of cross-links or from the gyration radius of a polymer branch between two cross-links, that give very similar results. All the results are summed up in table SI.1 for the three cross-linking ratios CL used here.

CL (%)	$G_{\text{HG}}^{\text{synth}}$	$\phi_{\text{AM}}^{\text{synth}}$ (%)	$G_{\text{HG}}^{\text{equil}}$	$\phi_{\text{AM}}^{\text{equil}}$ (%)	$N_c = M_c / M$	ξ_{equil} ξ_{Θ} (nm)	ξ_{synth} (nm)	$\Pi_{\text{swl}}^{\text{HG}}$ (kPa)
0.5	25.9	2.78	74.4	0.99	$1.55 \cdot 10^3$	24.0, 23.0	16.8	3.60
1	25.4	2.83	54.0	1.35	$8.3 \cdot 10^2$	17.5, 16.5	13.4	3.52
2	24.9	2.88	38.9	1.87	$4.2 \cdot 10^2$	12.5, 12.0	10.8	2.93

Table SI.1- **Characteristics of the hydrogels** – CL cross-linking ratio; $G_{\text{HG}}^{\text{synth}}$ and $\phi_{\text{AM}}^{\text{synth}}$: swelling ratio and polymeric volume ratio of the hydrogel determined immediately after synthesis; $G_{\text{HG}}^{\text{equil}}$ and $\phi_{\text{AM}}^{\text{equil}}$: swelling ratio and polymeric volume ratio at swelling equilibrium; N_c is the number of monomers between two cross-links, M_c being the molar mass of polymer between cross-links deduced at equilibrium from equ.(SI.4) and M the molar mass of monomer AM; ξ_{equil} is the mesh size of the hydrogel at swelling equilibrium deduced from equ.(SI.5), ξ_{Θ} the mesh size of PAM in a Θ solvent; ξ_{synth} is the mesh size of the hydrogel immediately after synthesis, $\Pi_{\text{swl}}^{\text{HG}}$ the initial swelling pressure of the hydrogel at $\phi_{\text{AM}} = \phi_{\text{AM}}^{\text{synth}}$.

The thermo-dynamical equilibrium of a neutral polymeric array, with a homogeneous distribution of reticulation nodes is ruled by a balance between osmotic and elastic forces. The swelling pressure of the hydrogel Π_{swl} is given by ^{4,5} :

$$\Pi_{\text{swl}}^{\text{HG}} = -\frac{1}{V_1}(\mu_1^{\text{gel}} - \mu_1^{\text{bath}}) = \Pi_{\text{mix}}^{\text{HG}} - \Pi_{\text{el}}^{\text{HG}} \quad (\text{SI.1})$$

where μ_1^{gel} (resp. μ_1^{bath}) is the chemical potential of water inside the hydrogel (resp. inside the bath), V_1 is the molar volume of the aqueous solvent (= $18 \text{ cm}^3/\text{mol}$), $\Pi_{\text{mix}}^{\text{HG}}$ is the contribution coming from entropic and enthalpic effects due to the polymer/solvent mixing, $\Pi_{\text{el}}^{\text{HG}}$ is the elastic pressure associated to the stretching of the polymeric chains with respect to their reference molten state (without solvent). We forget here any contribution coming from electrostatic interactions between chains. The mixing pressure writes :

$$\begin{aligned} \Pi_{\text{mix}}^{\text{HG}} &= -\frac{R_o T}{V_1} (\ln(1 - \phi_{\text{AM}}) + \phi_{\text{AM}} + \chi \phi_{\text{AM}}^2) \\ &\approx \frac{R_o T}{V_1} \left(\frac{1}{2} - \chi \right) \phi_{\text{AM}}^2 \quad \text{if } \phi_{\text{AM}} \ll 1 \end{aligned} \quad (\text{SI.2})$$

where R_o is the Perfect Gas constant, T the temperature in Kelvin, ϕ_{AM} the volume fraction of polymer inside the hydrogel and χ the Flory parameter which is here equal to 0.47.^{6,7}

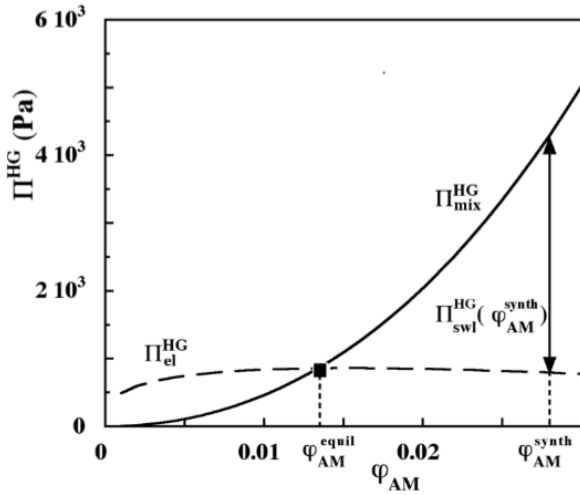


Figure SI.1 – **Various pressures of hydrogel and elastic modulus** – Mixing pressure Π_{mix}^{HG} (equ.(SI.2) - full line) and elastic pressure Π_{el}^{HG} (equ.(SI.3) - dashed line) as a function of the polymer concentration ϕ_{AM} . In the synthesis conditions, $\phi_{AM} = \phi_{AM}^{synth}$ and the swelling pressure $\Pi_{swell}^{HG} = \Pi_{mix}^{HG} - \Pi_{el}^{HG}$. At swelling equilibrium, $\phi_{AM} = \phi_{AM}^{equil}$ and $\Pi_{mix}^{HG} = \Pi_{el}^{HG}$ (solid square). It is proportional to the elastic modulus of the swollen hydrogel at equilibrium. A coefficient 0.67 is found while comparing to the measurement at CL = 1% of ref⁸.

The elastic contribution is related to the entropy of the stretched chains. Supposing an isotropic swelling with an homogeneous distribution of nodes in the hydrogel, we obtain :

$$\Pi_{el}^{HG} = R_o T v_c \left[\phi_{AM}^{synth} \left(\frac{\phi_{AM}}{\phi_{AM}^{synth}} \right)^{1/3} - \frac{1}{2} \phi_{AM} \right] \quad (SI.3)$$

where $v_c = \rho/M_c$ is the volumic number of nodes in the dry hydrogel (in mol/vol. of polymer), M_c being the molar mass between cross-links.

When the system is out of equilibrium, equation (SI.1) governs the swelling of the hydrogel. Figure SI.1 illustrates the ϕ_{AM} -dependence of Π_{mix}^{HG} (full line) and Π_{el}^{HG} (dashed line) at CL = 1%. Just after synthesis, Π_{mix}^{HG} given by equation (SI.2) with $\phi_{AM} = \phi_{AM}^{synth}$ is much larger than $\Pi_{el}^{HG}(\phi_{AM}^{synth})$ □s it corresponds to a deformation still small with respect to the melt. In that case, because Π_{swell}^{HG} is positive, the hydrogel absorbs water from the swelling bath. As the hydrogel swells, ϕ_{AM} decreases, Π_{mix}^{HG} decreases and Π_{el}^{HG} slightly increases, the hydrogel swells less and less. When $\Pi_{swell}^{HG} = 0$, that is when $\Pi_{mix}^{HG} = \Pi_{el}^{HG}$, the equilibrium is reached, $\phi_{AM} = \phi_{AM}^{equil}$ and the swelling stops.

The volume fraction of polymer inside the hydrogel at the end of the synthesis is almost the same for all the samples, the slight variation in table SI.1 resulting from the different CL. The volume ratio ϕ_{AM}^{equil} is deduced from the measurement of the swelling ratio G_{HG}^{equil} . It allows to obtain M_c at equilibrium (see table SI.1) through:

$$M_c = V_1 \rho \frac{\left[\frac{1}{2} \phi_{AM}^{equil} - \phi_{AM}^{synth 2/3} \phi_{AM}^{equil 1/3} \right]}{\ln(1 - \phi_{AM}^{equil}) + \phi_{AM}^{equil} + \chi \phi_{AM}^{equil 2}} \quad (SI.4)$$

We can thus deduce the number of monomers $N_c = M_c / M$ between two cross-links, $M = 71$ g/mol being the molar mass of monomer AM. We obtain also the mesh size ξ_{equil} at swelling equilibrium from :

$$\xi_{equil} = [N_a v_c \phi_{AM}^{equil}]^{1/3}. \quad (SI.5)$$

Let us note that the state of the system being close to Θ conditions ($\chi = 0.47 \sim 1/2$) the mesh size ξ_{equil} at swelling equilibrium is closer to $\xi_{\Theta} = aN^{1/2}$ than to the Flory mesh size $\xi_F = aN^{3/5}$, $a = 0.58$ nm being the length of monomer AM.^{9,10}

The experimental evidence that the structure of hydrogels is homogeneous (in the synthesis conditions and at swelling equilibrium) is provided by the fact that the effective concentration of cross-links at swelling equilibrium ($\sim 1/N_c$) is found proportional to CL. The mesh size after the synthesis is here given by $\xi_{synth} = \xi_{equil} (G_{HG}^{synth} / G_{HG}^{equil})^{1/3}$.

2 Ferrofluids

2.1 Synthesis

The magnetic nanoparticles are synthesized by co-precipitation in an aqueous ammonia solution of $FeCl_2$ and $FeCl_3$ salts that leads to colloidal magnetite which is fully oxidized to maghemite by $Fe(NO_3)_3$ in acidic medium.¹¹ The experimental conditions have been chosen in order to prepare particles with an average diameter of the order of 10 nm. A size-sorting process allows then a fractionation of the population of nanoparticles according to their diameter.¹² Finally the initially positively charged nanoparticles are coated with citrate species by addition of tri sodium citrate (Na_3Cit) in order to have negative surface charges associated to ionized carboxylate groups for pH ranging between 6 to 10. The citrate coated particles yield to stable dispersions in water at pH 7, the electrostatic interparticle repulsion preventing the nanoparticle aggregation. The adsorption equilibrium of citrate gives a residual ionic strength due to unadsorbed (free) citrate species Cit^{3-} and their Na^+ counterions. To ensure the citrate coating, one needs to keep free citrate salt concentration $[Na_3Cit]$ above a given minimal value, which depends on pH.¹³ At pH 7, the adsorption plateau corresponds to a free citrate concentration inside the solution $[Na_3Cit] \geq 2 \cdot 10^{-3} \text{ mol.L}^{-1}$.

2.2 SANS experiments :

The size and structure of FFA and FFB dispersions have been determined by SANS experiments performed in LLB (Saclay, France) on the PAXY spectrometer, in the wave-vector range $0.0065 \text{ \AA}^{-1} \leq q \leq 0.15 \text{ \AA}^{-1}$. In pure light water, the intensity after subtraction of the incoherent background is largely dominated by the nuclear contribution of the particles.¹⁴ As the nanoparticles are roughly spherical, the intensity $I(q, \Phi)$ scattered by a dispersion of volume fraction Φ writes :

$$\frac{I(q, \Phi)}{\Phi} = \Delta \rho^2 F(q) S(q, \Phi) \quad (SI.6)$$

where $\Delta \rho^2 = 5.67 \cdot 10^{21} \text{ cm}^{-4}$ is the neutronic nuclear contrast of the nanoparticles with respect to H_2O , $F(q)$ is the form factor of the nanoparticles and $S(q, \Phi)$ the structure factor of the colloidal dispersion. In the dilute regime and if the interparticle interactions are negligible, the structure factor of the dispersions is equal to 1. The scattered intensity is then proportional to $F(q)$.

Figure SI.2a presents the SANS scattered intensity (divided by Φ) for sample FFB at various Φ and low ionic strength $[\text{Na}_3\text{Cit}] = 8.10^{-3} \text{ mol.L}^{-1}$. The intensity at $\Phi=0$ is proportional to $F(q)$ and gives the size and shape characterization deduced from such an experiment. The plateau at low q in the Guinier range ($q < 2.10^{-2} \text{ \AA}^{-1}$) obtained at the lowest volume fraction confirms that for $\Phi < 1\%$, the interactions between particles can be almost neglected in the experimental conditions. At large q ($q > 6.10^{-2} \text{ \AA}^{-1}$) in the so-called Porod regime, $I(q, \Phi)/\Phi$ roughly decreases as q^{-4} whatever Φ , which is characteristic of solid particles with a sharp interface. Figure SI.2a presents the best fit of the form factor of the FFB particles, assumed spherical with a lognormal distribution of median diameter $d_o^{\text{scat}} = 7 \text{ nm}$ and polydispersity $\sigma^{\text{scat}} = 0.26$ (see table SI.2 for FFA characteristics).

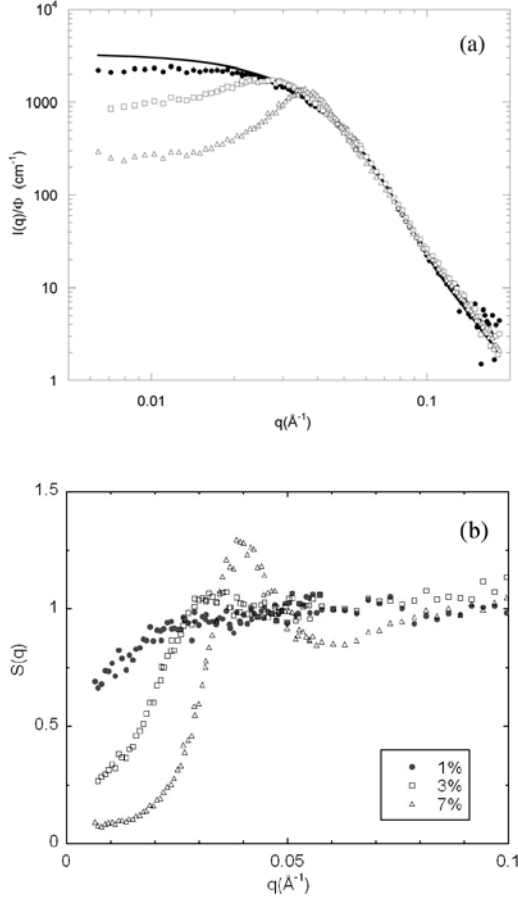


Figure SI.2 SANS probing of FFB ferrofluids at various Φ - The full lines correspond the form factor of the nanoparticles (see text and table 2). (a) Reduced scattering intensity $I(q, \Phi)/\Phi$ versus q at various Φ ; (b) Structure factor of the nanoparticles $S(q, \Phi)$ versus q at various Φ .

For volume fractions $\Phi > 1\%$, the inter-particle interactions are no longer negligible and the structure factor $S(q, \Phi)$ in equation (SI.6) is no more equal to 1. Figure SI.2.b provides the structure factor $S(q, \Phi)$ of three dispersions at volume fractions $\Phi = 1\%$, 3% and 7% , based on the same nanoparticles (FFB) at $[\text{Na}_3\text{Cit}] = 8.10^{-3} \text{ mol.L}^{-1}$. In the thermodynamic limit (for $q \rightarrow 0$), the structure factor tends to the compressibility of the system. Figure 2 shows that it decreases as the volume fraction increases, which means that the interparticle interaction is repulsive on average. The maximum of $S(q)$ at q_{max} , associated to the most-probable distance $l_{\text{mp}} = 2\pi/q_{\text{max}}$ between scattering entities inside the dispersion, increases in amplitude and simultaneously moves towards higher q_{max} values. If the interparticle interaction is repulsive on average, the nanoparticles are homogeneously dispersed inside the dispersion. Then the most-probable distance $2\pi/q_{\text{max}}$ is also

equal to the mean interparticle distance l_{mean} , related to the volume fraction Φ of the dispersion and the median diameter d_o of particles through $l_{\text{mean}} = d_o \sqrt{\frac{\pi}{6\Phi}}$. For $\Phi = 3\%$, l_{mp} is $\sim 196 \text{ \AA}$ ($q_{\text{max}} \sim 0.032 \text{ \AA}^{-1}$) and l_{mean} is $212 \text{ \AA}$. For $\Phi = 7\%$, l_{mp} is $\sim 161 \text{ \AA}$ ($q_{\text{max}} \sim 0.039 \text{ \AA}^{-1}$) and l_{mean} is $160 \text{ \AA}$. The good agreement between those two estimates in our ferrofluid dispersions shows that there are strong repulsive interaction between our nanoparticles at $[\text{Na}_3\text{Cit}] = 8.10^{-3} \text{ mol.L}^{-1}$.

	d_o^{scat} nm	σ^{scat}	d_o^{bir} nm	σ^{bir}	δn_o	τ_o μs	α	δ nm	δ nm
FFA	9.2	0.35	11.5	0.25	0.1	5.4	0.93	12	4.5
FFB	7.0	0.26	9.1	0.2	0.07	1.8	0.95	11	5

Table SI.2 - **Characteristics of the ferrofluid nanoparticles** – Parameters deduced by an adjustment to a log-normal distribution of diameters, of SANS (d_o^{scat} , σ^{scat}) and static birefringence (d_o^{bir} , σ^{bir}) measurements; δn_o is the intrinsic anisotropy of the nanoparticles¹⁵; τ_o characteristic relaxation time and α stretched exponent are deduced from the stretched exponential adjustment of birefringence relaxation using equ. (SI.8); d average nanoparticle diameter and δ electrostatic screening length are deduced from a Carnahan-Starling adjustment of the Φ -dependence of ferrofluid osmotic pressure $\Pi(\Phi)$ as in¹⁶.

2.3 Osmotic equilibrium of Ferrofluids

In the ferrofluid dispersions used here, the magnetic nanoparticles, due to their citrate coating, are in strong repulsive interaction. The osmotic pressure Π^{FF} due to the nanoparticle/solvent mixing can be described in terms of repulsive effective Hard-Spheres following the Carnahan-Starling formalism¹⁶:

$$\frac{\Pi^{\text{FF}} V}{\Phi kT} = \frac{1 + \Phi + \Phi^2 + \Phi^3}{(1 - \Phi)^3} \quad (\text{SI.7})$$

where $V = \frac{\pi}{6} d^3$, d being an averaged nanoparticle diameter. In the limit $\Phi \rightarrow 0$, the right hand term is equal to 1 and equation (SI.7) reduces to the Perfect Gas law, also valid in the limit of low Φ if the balance of interparticle interaction is nul (i.e. if the second virial of the osmotic pressure equals zero). Here the balance is repulsive and the repulsions are taken into account in the right hand term of equation (SI.7) replacing Φ by an effective volume fraction Φ_{eff} , which includes the range of the repulsion.¹⁶ Indeed the repulsion is strong enough to consider the nanoparticles as Hard-Spheres with an effective diameter $d + 2\delta$, where δ is the electrostatic screening length. It leads to an effective volume fraction $\Phi_{\text{eff}} = \Phi (1 + 2\delta/d)^3$. Figure 2 of the main text presents such adjustments for the two ferrofluids used here. We obtain $d = 12 \text{ nm}$ and $\delta = 4.5 \text{ nm}$ for FFA (resp. $d = 11 \text{ nm}$ and $\delta = 5 \text{ nm}$ for FFB).

2.4 Magnetic and magneto-optical properties

Each nanoparticle bears a magnetic moment $\vec{\mu}$ which is of the order of 10^4 Bohr magnetons and an optical anisotropy axis $\vec{u}$. Its modulus $|\vec{\mu}| = m_s V_{\text{magn}}$ is proportional to the magnetic volume V_{magn} of the nanoparticles and to m_s the magnetization of the nanoparticles material. Under a large applied field $\vec{H}$, this magnetic moment $\vec{\mu}$ orientates along the field direction. In a liquid dispersion of 10 nm -sized $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles, the magnetic torque also rotates mechanically the core of the nanoparticle together with its optical axis. At equilibrium in

large fields, the optical axis $\vec{H}$ becomes parallel to $\vec{H}$. The alignment of $\vec{H}$ along $\vec{H}$ confers to the dispersion a macroscopic magnetization M . The alignment of the optical axis $\vec{H}$ of all the nanoparticles gives a macroscopic optical birefringence Δn to the dispersion. At saturation, all the magnetic moments and all the optical axes are aligned along $\vec{H}$, then $M = M_s = m_s \Phi$ and $\Delta n = \Delta n_s = \delta n_o \Phi$, δn_o being the intrinsic optical anisotropy parameter of the nanoparticles.¹⁷ For intermediate fields and low Φ 's, M and Δn are well described by a Langevin formalism with in particular $\Delta n = \Delta n_s L_2(\zeta)$, where $L_2(\zeta) = 1 - 3L(\zeta)/\zeta$, $L(\zeta) = \tanh(\zeta)/\zeta$ and $\zeta = \frac{\mu_o \mu H}{kT}$ being respectively the Langevin function and the Langevin parameter. However, the shape of $\Delta n(H)$ is modified by the size distribution of the nanoparticles.¹⁷ In ferrofluids at low Φ 's, the experimental measurements of magneto-optical birefringence as a function of the applied field enable the determination of $(d_o^{bir}, \sigma^{bir})$ by comparison to the theoretical Langevin formalism taking into account a log-normal size-distribution (see table SI.2 and best fit of $\Delta n(H)$ for FFA in fig.6).

The magneto-optical birefringence $\Delta n(H)$ is measured as in¹⁵ up to a few 100 kA/m. Figure 6 of main text presents the variations of $\Delta n(H)$ normalized by its volume fraction Φ for a sample based on FFA nanoparticles at $[Na_3Cit] = 8 \cdot 10^{-3} \text{ mol.L}^{-1}$ for $\Phi = 1\%$ and the corresponding fit. The maximum value Δn_s of Δn is proportional to the volume fraction Φ , the proportionality coefficient δn_o being dependent on the nanoparticle size distribution.¹⁵ The values of δn_o for FFA and FFB are given in Table SI.2. Figure 6 of main text presents also the reduced variations of $\Delta n(H)/\Delta n_s$ at $\Phi = 7\%$. The shape of the curve is almost independent on Φ in our range of volume fractions expressing the weak influence of interparticle interactions on magneto-optical birefringence in the present repulsive regime.

The ferrofluid solutions can be also probed from a dynamic point of view. Using the same experimental device as the one described in¹⁸, we analyze the optical rotational answer of the colloid to a pulse of magnetic field of small amplitude ($H = 4.8 \text{ kA/m}$) and duration ($\sim 100 \text{ ms}$). The magnetic particles, being suspended in a liquid, align along the field direction during the pulse. It leads to a birefringence which, in low-field approximation, writes $\Delta n_{LF} = \delta n_o \Phi \zeta^2 / 15$. Here $\zeta \leq 0.14$ and Δn_{LF} is at most of the order of 10^{-5} . The temporal analysis of the birefringence relaxation at the cut-off of the field is performed in terms of a stretched exponential decay of optical intensity $I(t)$ (see figure SI.3a):

$$I(t) = I_o e^{-\left(\frac{t}{\tau_o}\right)^\alpha} \quad (\text{SI.8})$$

with $I(t) \propto \sin \varphi$, φ being the (here time-dependent) phase-lag of the birefringent sample. We can determine three physical quantities : the initial intensity I_o , the characteristic relaxation time τ_o and the stretched exponent α . Providing that the phase-lag φ of the birefringent sample is small enough, the intensity I_o is proportional to Δn_{LF} . The condition $\varphi = 2\pi e \Delta n_{LF} / \lambda \ll 1$, where e is the thickness of the optical sample cell (here of the order of $100 \mu\text{m}$) and λ the optical wave-length (here = $0.633 \mu\text{m}$), is easily fulfilled ($\varphi \leq 10^{-2}$). Thus we obtain in the liquid suspension:

- for the intensity I_o of the magneto-optical pulse

$$I_o \propto \Phi \zeta^2 \propto \Phi V_{\text{mag}}^2 \quad (\text{SI.9})$$

- for the time τ_o , which is proportional to the characteristic time of rotational diffusion:

$$\tau_o = \frac{\eta V_H}{kT} \quad (\text{SI.10})$$

with η the viscosity of the liquid carrier and V_H the hydrodynamic volume of the nanoparticles.

- for the exponent α , which is related to the width of the distribution of relaxation times in the dispersion

$$\alpha \approx 1 \quad (\text{SI.11})$$

for samples based on nanoparticles of thin distribution of sizes. This α value is decreasing towards 0.6 in polydisperse or agglomerated liquid dispersions^{18,19} and may reach a value of 0.3 in arrested thixotropic gels¹⁸ or glass-forming systems¹⁹.

The stretched-exponential nature of the relaxation of the magneto-optical signal is illustrated in the inset of figure SI.3a for sample FFA at $\Phi = 3\%$ and $[Na_3Cit] = 8 \cdot 10^{-3} \text{ mol.L}^{-1}$. The almost linear variation of $-\ln(I/I_o)$ as a function of time t in this log-log representation, shows that there is only one stretched exponential relaxation process involved in the decay of intensity. The adjusted straight line crosses the horizontal axis $-\ln(I/I_o) = 1$ at $t = \tau_o = 5.4 \mu\text{s}$. It has a slope $\alpha = 0.93$. In our range of Φ and within our experimental accuracy, this relaxation time is found here independent of Φ . Table SI.2 gives τ_o (denoted τ_o^{FF}) and α , obtained for the two kinds of samples based on FFA and FFB nanoparticles.

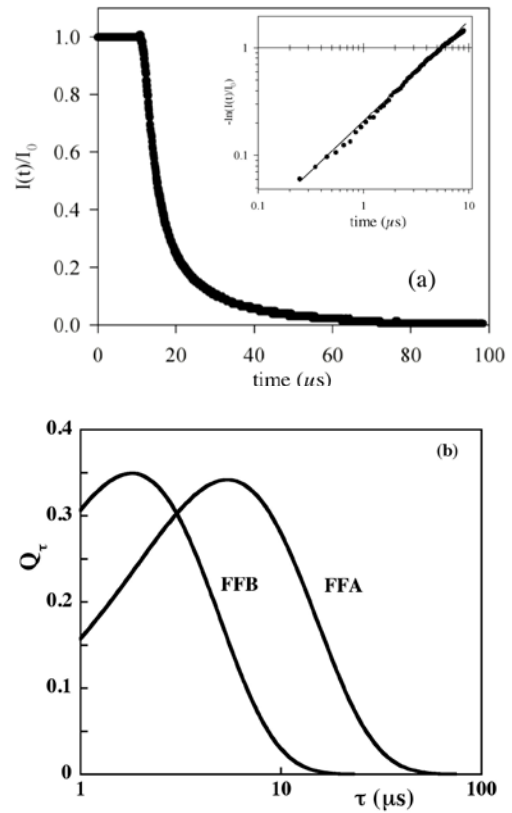


Figure SI.3 – *Dynamic birefringence of ferrofluids*

a – Relaxation of the optical intensity due to the birefringence of the dispersion under applied magnetic field (FFA with $[cit] = 8 \cdot 10^{-3} \text{ mol/L}$ and $\Phi = 3\%$). The inset illustrates that the decay is a stretched exponential by plotting $-\ln(I/I_o)$ as a function of time t in a log-log representation.

b – Distribution of characteristic relaxation times for FFA and FFB ferrofluids as obtained from Equ. SI.13.

In table SI.2, the values of α are rather close to 1 but different from 1, it expresses that the relaxation is non-exponential. Such a non-exponential relaxation is usually asserted to the existence of a distribution of times τ inside the ferrofluid. In these liquid solutions it is associated to the polydispersity in hydrodynamic size of the nanoparticles. This temporal relaxation can be inverted like in ²⁰. It leads to a distribution $Q(\ln(\tau))$ of relaxation times τ such that :

$$I(t) = \int I_o(\tau) e^{-\frac{t}{\tau}} Q(\ln(\tau)) d(\ln(\tau)) \quad (\text{SI.12})$$

Providing the approximations $I_o(\tau) \approx I_o \approx \text{cst}$ for a given sample, $e^{-t/\tau} = 1$ for $t < \tau$ and $e^{-t/\tau} = 0$ for $t > \tau$, it comes ¹⁹ :

$$Q(\ln \tau) = -\frac{d(I(t)/I_o)}{d(\ln t)} = \alpha \left(\frac{\tau}{\tau_o} \right)^\alpha e^{-\left(\frac{\tau}{\tau_o} \right)^\alpha} \quad (\text{SI.13})$$

Figure SI3.b plots these distributions of characteristic times (denoted Q_τ all along this text) for the two kinds of nanoparticles employed here FFA and FFB. Note that in our experimental range of Φ and $[\text{Na}_3\text{Cit}]$ the obtained distributions are independent of Φ and $[\text{Na}_3\text{Cit}]$.

3 – Rotational diffusion of nanoparticles within ferrogels probed by dynamical birefringence

Such a dynamical probing may be also performed in ferrogels. Here below the index “FG” stands either for “synth” or “equil” depending on the state of the ferrogel either in the synthesis conditions or at swelling equilibrium.

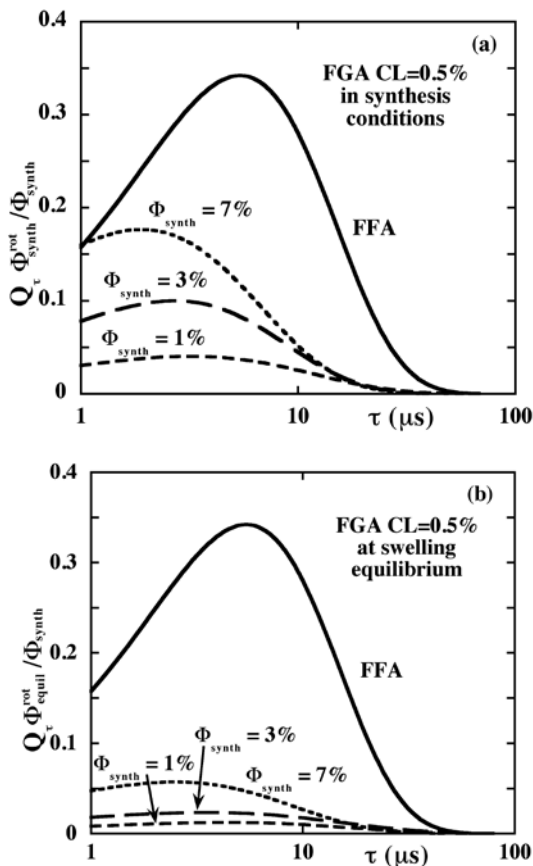


Figure SI.4 - **Dynamic birefringence ferrogels FGA - CL = 0.5%** – Reduced distribution of characteristic times $Q_\tau \Phi_{FG}^{\text{rot}} / \Phi_{\text{synth}}$ inside ferrogels at various Φ_{synth} , in the synthesis conditions (a) and at swelling equilibrium (b) as compared to pure ferrofluid FFA.

Performing two experiments, one with a ferrogel and one with its corresponding ferrofluid, at the same volume fraction $\Phi_{\text{FF}} = \Phi_{\text{FG}}$ and with the same sample thickness e , it is also possible to compare the respective initial intensities I_o^{FG} and I_o^{FF} . I_o^{FG} is here much lower than I_o^{FF} and at most equal to $I_o^{\text{FF}}/10$. Using equations (SI.10) and (SI.11), and with the hypothesis that the ratio of magnetic volumes is proportional to the ratio of hydrodynamic volumes, we obtain :

$$\frac{I_o^{\text{FG}}}{I_o^{\text{FF}}} = \frac{\Phi_{FG}^{\text{rot}}}{\Phi_{\text{FF}}} \left(\frac{\tau_o^{\text{FG}}}{\tau_o^{\text{FF}}} \right)^2 \quad (\text{SI.14})$$

Here Φ_{FG}^{rot} is the volume fraction of rotating nanoparticles inside the ferrogel in the low-field experiment of birefringence relaxation. The ratios $I_o^{\text{FG}}/I_o^{\text{FF}}$ and $\tau_o^{\text{FG}}/\tau_o^{\text{FF}}$ being both measured, it is possible using equation (SI.14) to deduce $\Phi_{FG}^{\text{rot}}/\Phi_{FG}$ the proportion (in volume) of nanoparticles rotating inside the ferrogel in this low field experiment. It is also possible to calculate the volume fraction Φ_{FG}^{block} of nanoparticles blocked in low field using $\Phi_{FG}^{\text{block}} = \Phi_{FG} - \Phi_{FG}^{\text{rot}}$. They are given in tables 2, 3 and 5 of main text for the various samples probed here. Figure SI.4 illustrates for FGA ferrogels at CL = 0.5%, the reduced distributions of characteristic times $Q_\tau \Phi_{\text{synth}}^{\text{rot}} / \Phi_{\text{synth}}$ obtained in non swollen samples at various Φ_{synth} (see Figure SI.4a) and the corresponding distributions $Q_\tau \Phi_{\text{equil}}^{\text{rot}} / \Phi_{\text{synth}}$ obtained in swollen samples at various Φ_{synth} (see Figure SI.4b). In these figures, the factors $\Phi_{\text{synth}}^{\text{rot}}/\Phi_{\text{synth}}$ and $\Phi_{\text{equil}}^{\text{rot}}/\Phi_{\text{synth}}$ allow a quantitative visualization of the nanoparticles which are rotating in the ferrogel with respect to those initially introduced.

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Doxorubicin Loaded Magnetic Polymersomes: Theranostic Nanocarriers for MR Imaging and Magneto-Chemotherapy

Charles Sanson,^{a,b} Odile Diou,^{a,b} Julie Thévenot,^{a,b} Emmanuel Ibarboure,^{a,b} Alain Soum,^{a,b} Annie Brûlet,^{c,d} Sylvain Miraux,^{e,f} Eric Thiaudière,^{e,f} Sisareuth Tan,^{g,h} Alain Brisson,^{g,h} Vincent Dupuis,^{i,j} Olivier Sandre,^{a,b,i,j,*} and Sébastien Lecommandoux^{a,b*}

^a Université de Bordeaux/IPB, ENSCBP, 16 avenue Pey Berland, 33607 Pessac Cedex, France

^b CNRS, Laboratoire de Chimie des Polymères Organiques (UMR5629), Pessac, France

^c CEA Saclay, LLB, 91191 Gif sur Yvette, France

^d CNRS, Laboratoire Léon Brillouin (UMR12), Gif sur Yvette, France

^e Université de Bordeaux, RMSB, 146 rue Léo Saignat, 33676 Bordeaux, France

^f CNRS, Résonance Magnétique des Systèmes Biologiques (UMR5536), Bordeaux, France

^g Université de Bordeaux, IECB, 33402 Talence Cedex, France

^h CNRS, Chimie et Biologie des Membranes et des Nanoobjets (UMR5248), Talence, France

ⁱ UPMC Université Paris 6, 4 place Jussieu, 75005 Paris, France

^j CNRS, Physicochimie des Electrolytes, Colloïdes et Sciences Analytiques (UMR7195) Paris, France

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*CORRESPONDING AUTHORS: Email: olivier.sandre@ipb.fr Tel: +33 (0)5-4000-3695, Fax: +33 (0)5-4000-8487,

Email: lecommandoux@enscbp.fr, Tel: +33 (0)5-4000-2241, Fax: +33 (0)5-4000-8487

ABSTRACT: Hydrophobically modified maghemite (γ -Fe₂O₃) nanoparticles were encapsulated within the membrane of poly(trimethylene carbonate)-*b*-poly(L-glutamic acid) (PTMC-*b*-PGA) block copolymer vesicles using a nanoprecipitation process. This formation method gives a simple access to highly magnetic nanoparticles (MNPs) (loaded up to 70 wt %) together with a good control over the vesicles size (100 to 400 nm). The simultaneous loading of maghemite nanoparticles and doxorubicin was also achieved by nanoprecipitation. The deformation of the vesicle membrane under an applied magnetic field has been evidenced by small angle neutron scattering. These superparamagnetic hybrid self-assemblies display enhanced contrast properties that open potential applications for Magnetic Resonance Imaging. They can also be guided in a magnetic field gradient. The feasibility of controlled drug release by radio-frequency magnetic hyperthermia was demonstrated in the case of encapsulated doxorubicin molecules, showing the viability of the concept of magneto-chemotherapy. These magnetic polymersomes can be used as efficient multifunctional nano-carriers for combined therapy and imaging.

KEYWORDS: block copolymer vesicles, polymersome, nanoprecipitation, superparamagnetic iron oxide nanoparticles, maghemite, magnetic hyperthermia, magneto-chemotherapy, multifunctional, MRI contrast agent, doxorubicin, theranostics

Over the past decades, nanopolymeric therapeutics has proven to improve the effectiveness of cancer treatments in animal experiments.^{1–4} During this period, progresses in modern polymer (physico)-chemistry have enabled the design of polymeric carriers with ever higher levels of complexity featuring addressable chemically reactive functions, defined chain architectures, and controlled morphologies and sizes.

When applied to the field of drug delivery, these features allow achieving and combining several desirable properties such as high drug loading content, controlled release, increased circulation half-life and targeting of pathological areas or specific cell receptors. Polymer nanomedicines have the potential to increase the shelf life of chemotherapeutics before administration and to improve their efficacy after administration.^{5–7} A direct consequence of the latter is the reduction in the dosing concentration and frequency of administration of the drug, hence the minimization of toxic side effects on healthy tissues, which are currently a major problem in chemotherapy. Among the different classes of polymeric nanomedicines, block copolymer vesicles also termed polymersomes offer an attractive structure for drug delivery applications.^{8–12} This block copolymer self-assembly in a closed bilayer has fostered a considerable attention since both hydrophilic and hydrophobic drugs can be loaded either in the aqueous lumen or in the membrane core respectively,^{13, 14} thanks to a thick membrane that imparts long-term stability to the object. After drug loading, vesicle disruption inducing drug release can be either triggered by an environmental stimulus, such as pH, temperature, light, or oxidation,^{15–17} or can be the consequence of polymer hydrolytic or enzymatic degradation.^{18, 19} Even though a wide variety of polymer nanocarriers for drug delivery has shown efficient entrapment and controlled release of drugs *in vitro*, the evaluation of their biodistribution *in vivo* has become possible by non invasive methods. To address this issue, one strategy consists in incorporating imaging probes together with the drugs into the polymer nanoparticles. These dual polymer nanocarriers for simultaneous cancer imaging and treatment open the field to “theranostic nanomedicines”, combining diagnostic and therapeutic components in an all-in-one nanoparticle.^{20, 21} Imaging probes to be loaded can belong to different families such as visible^{22, 23} and NIR fluorochromes,²⁴ radiotracers^{25–27}

or inorganic nanoparticles such as quantum dots,^{28–30} gold nanoparticles,^{31–35} or magnetic nanoparticles (MNPs).^{36, 37} Among the different MNPs, the so called “ultra-small superparamagnetic iron oxide” (USPIO) particles are synthetic $\gamma\text{-Fe}_2\text{O}_3$ or Fe_3O_4 nanometric grains in a perfectly dispersed state (*i.e.* unclustered individual nanoparticles).³⁸ As described in numerous review articles^{39–43}, iron oxide MNPs also called USPIOs are commonly used as negative contrast-enhancing agents in MRI, enabling high spatial resolution acquisition, three-dimensional, non-invasive imaging of the human body. Hydrophilic “stealth” polymers are often employed to enhance the stability and biocompatibility of the MNPs *in vivo* by hindering their further aggregation and by an anti-fouling effect against proteins of the immune system called opsonins.^{42, 44} Besides the MRI contrast enhancement properties attributed to their ability to distort strongly the magnetic field lines,⁴⁵ USPIOs can be used to kill cancer cells by their heating effect in radio-frequency magnetic fields. Hyperthermia (or thermal ablation) is identified as a promising approach in cancer therapy, particularly in combination with chemo- or radiotherapy.⁴¹ A promising hyperthermia route for treating deep tumors consists in concentrating MNPs around and inside the tumor site and increasing the temperature locally through conversion into heat of the energy from an external alternating magnetic field in the range of radio frequencies 100 kHz – 1 MHz. This magnetic hyperthermia led to an intense research activity both on the optimization of the conditions of treatment (power, concentration, geometrical parameters...) ^{46–48} and on the characteristics of the USPIOs themselves (chemical nature, distribution of sizes...).^{39, 49–57}

For this purpose of obtaining multifunctional drug vectors, hydrophilic USPIOs have been loaded at first in the aqueous compartment of liposomes.^{58, 59} Under a permanent magnetic field, magnetic liposomes deform into elongated ellipsoids, as it was evidenced for giant unilamellar vesicles.^{60–62} Interesting studies dealt with much smaller magneto-liposomes analogous in sizes to the pegylated lipid vectors of the DOXILTM formulation of the anticancer drug doxorubicin hydrochloride (DOX). To combine magnetism and thermo-sensitivity, DOX was encapsulated into magnetic vesicles with a lipid membrane initially in the gel state and becoming fluid at a temperature reachable by magnetic hyperthermia.^{63, 64} The application of a RF magnetic field led to massive release of encapsulated DOX since the magnetic hyperthermia was sufficient to reach locally the main chain phase transition temperature of the bilayer, thereby increasing the membrane permeability.⁶⁵ Recently, an analogous study with small hydrophobic USPIOs embedded in the membrane of liposomes evidenced the release triggered by a RF magnetic field of a fluorescent dye used as a model of hydrophilic drug.⁶⁶ The possibility to target a solid tumor by using magneto-liposomes and an extracorporeal magnet to guide them has also been reported.^{64, 67, 68} Despite the tremendous results obtained with liposomes, the morphology of lipid/MNP systems strongly varies with MNP and lipid concentrations.⁶⁹ They also suffer from the classical issue of instability associated with lipid bilayers,⁷⁰ which incite to use of polymersomes as an alternative to liposomes. We have shown for the first time that hydrophobic USPIOs can be loaded into polymersome membranes of PB-*b*-PGA and that a reversible variation of the membrane thickness can be induced by the application of a magnetic field.^{71–73} Later, Förster *et al.* induced the bridging of adjacent bilayers and formed multi-lamellar hybrid polymersomes by incorporating hydrophobic USPIOs into PI-*b*-PEO bilayers at a feed weight ratio up to 20 % sufficiently large to guide the vesicles by a magnetic field gradient.⁷⁴

In the present article, we describe a convenient procedure to prepare well-defined magnetic polymersomes featuring a hydrophobic internal membrane core made of the biodegradable block poly(trimethylene carbonate) (PTMC) and

a polypeptide biocompatible corona of poly(L-glutamic acid) (PGA). Having synthesized USPIOs with the appropriate characteristics (size and hydrophobic coating), those were embedded together with the efficient antitumor drug doxorubicin hydrochloride (DOX) into the membrane of dual-loaded vesicles by one-step nanoprecipitation. This process allowed reaching quantitative loading contents and controlling the final sizes with low polydispersity. The two-dimensional confinement of USPIOs inside the vesicular membrane was evidenced by small angle neutron and light scattering techniques and observed by atomic force and transmission electron microscopy. The magnetic membrane of the PTMC-*b*-PGA polymersomes was shown to be reversibly deformable under a permanent magnetic field. The release of DOX under local hyperthermia conditions induced by an oscillating RF magnetic field was also evidenced as a proof of concept of magneto-chemotherapy with magnetic polymersomes.

RESULTS AND DISCUSSION

Characteristics of the USPIOs

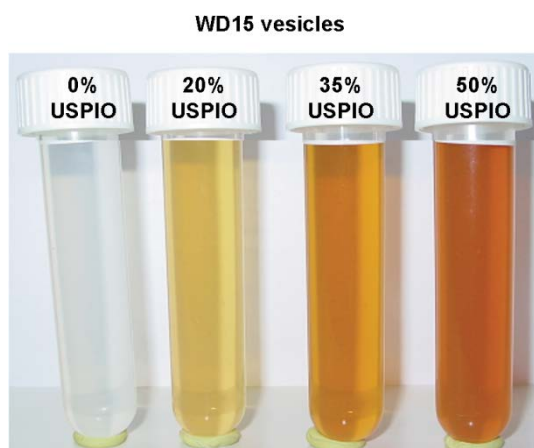
After synthesis⁷⁵, size fractionation⁷⁶ and surfactant coating⁷⁷, the radius of gyration and the hydrodynamic radius of the USPIO nanoparticles used in this work were $R_G^{\text{USPIO}} = 3.05 \pm 0.06$ nm and $R_H^{\text{USPIO}} = 4.7 \pm 0.07$ nm as measured respectively by SANS and DLS. The ratio $R_G^{\text{USPIO}}/R_H^{\text{USPIO}} = 0.65$ is not far from the theoretical value 0.775 for dense spherical particles,^{78, 79} the gap being reasonably ascribed to the contribution of the surfactant layer to R_H^{USPIO} only. Those sizes are in good agreement with the distribution measured by VSM (Supporting Information, S-d),^{80, 81} described by a Log-normal law of median diameter $D_{\text{mag}}^{\text{USPIO}} = 6.3$ nm, width $\sigma = 0.22$ and weight averaged diameter $D_w = 7.5$ nm. Concerning the magnetic hyperthermia capability, a specific loss power (SLP) of 14 W/g was reported for USPIOs synthesized by the same route and of analogous distribution of diameters ($D_{\text{mag}}^{\text{USPIO}} = 6.7$ nm, $\sigma = 0.20$, $D_w = 7.7$ nm) but at larger frequency ($f_{\text{RF}} = 700$ kHz) and much higher field intensity H_0 (24.8 kA/m).⁵⁴ Using its expected variation with these parameters ($\sim f_{\text{RF}} H_0^2$), we estimate a SLP value of 0.07 W/g in the conditions of biocompatible RF field used in this work ($f_{\text{RF}} = 500$ kHz and $H_0 = 2.12$ kA/m).

Characteristics, structure and stability of USPIO-loaded vesicles

In a previous study, the conditions of nanoprecipitation with the PTMC-*b*-PGA block copolymer were varied: choice of THF or DMSO as good solvent of the blocks, order (solvent into water or reverse) and duration of the addition...⁸² The influence of each experimental parameter was rationalized in order to finely tune the sizes and PDI of the vesicles. In addition, the PTMC block was shown to be semi-crystalline with an apparent melting temperature in vesicles near 34°C (lower than value at 37°C in the bulk) that has a strong influence on the size of the vesicles and on their interactions.⁸³ In the present study, we checked that incorporating inorganic nanoparticles at the nanoprecipitation step did not affect the self-assembly process of the diblock copolymer and that vesicular morphologies were still obtained. The conditions were selected according to the low PDI obtained, the compatibility of the organic solvent and of the obtained vesicles' sizes with *in vivo* applications. The copolymer was first dissolved in DMSO with or without $\gamma\text{-Fe}_2\text{O}_3$ USPIOs. Then water was added (up to 90 % of the final volume) at a controlled flow rate to trigger self-assembly. As the flow rate strongly influences the final size of the vesicles,⁸² we considered two sets of conditions: an almost instantaneous addition (5 seconds) leading to small vesicles ($R_H = 45\text{--}67$ nm) denoted WDi and a 15 minutes-addition leading to larger ones ($R_H = 187\text{--}202$ nm) denoted WD15. The characteristics of

nanoparticles' dispersions prepared by either one or the other of these conditions at increasing USPIO feed weight ratios (FWR) are shown in Table 1.

Table 1. Size and polydispersity index (PDI) of nanoparticles' dispersions prepared with increasing feed weight ratios of USPIO relatively to copolymer. Vesicles were prepared by nanoprecipitation in DMSO by adding water either in 5 s (WDi) or in 15 min (WD15). The image is a macroscopic view of samples.



Sample code	FWR (%)	R_H (nm)	PDI
WDi-0	0	67	0.07
WDi-20	20	50	0.14
WDi-35	35	45	0.16
WDi-50	50	47	0.16
WDi-70	70	52	0.18
WD15-0	0	202	0.05
WD15-20	20	196	0.09
WD15-35	35	195	0.20
WD15-50	50	187	0.22

The vesicles were found homogeneous in sizes, as observed by small PDI values in DLS. The loading of vesicles by USPIOs progressively increases the PDI (while remaining low) and slightly decreases R_H . This hydrodynamic size decrease (more pronounced for WDi than for WD15) and slight broadening of the sizes' distribution (for both nanoprecipitation speeds) are ascribed to a larger hydrophobic effect when the copolymer is combined with USPIOs coated by surfactants, and thus to a larger driving force for a faster self-assembling process. No aggregation occurred below a critical USPIO FWR. Beyond this threshold value, the hydrophobic USPIOs began to aggregate during nanoprecipitation forming ill-defined macroscopic clusters that rapidly migrated to the vial walls when approaching a magnet. This maximum FWR was

respectively 50 wt % for WD15 and 70 wt % for WDi. The larger threshold FWR with WDi vesicles compared to WD15 ones can be ascribed to a much faster kinetics of formation, thereby minimizing the probability of USPIOs' clustering before the completion of co-assembly with the copolymer. The maximum loading content of USPIOs in the membrane of the WDi-70 vesicles corresponds to a local volume fraction $\Phi_{USPIO}^{mb} = 12.1\%$. Interestingly, this is close to the reported value of 11% for the insertion of USPIOs in bilayers of polystyrene-*b*-polyacrylate, whereas larger volume fractions *e.g.* 21% lead to a morphological transition into micelles *via* nanoparticles' clustering.⁸⁴ Fully dispersed and stable suspensions were observed below and up to these maximum USPIO FWR values. In these conditions, the shelf life is longer than several months at room temperature. Static light scattering (SLS) measurements conducted on the WDi-50 sample strongly suggested a vesicular morphology. By drawing the Berry plot⁸⁵ over a scattering angular range from 50° to 150° and a concentration range from 0.2 to 1 mg/mL, we obtained the z-averaged radii of gyration ($R_{G,z}$) allowing to calculate the ratio $\rho = R_G/R_H$. While vesicles are characterized by ρ values close to 1, ρ values around 0.775 are expected for spherical micelles.⁷⁸ WDi-50 suspensions had a ρ value of 1.02 in good agreement indeed with vesicular self-assemblies.

A further insight to the exact morphology of USPIO-loaded PTMC₂₄-*b*-PGA₁₉ particles in either WDi or WD15 conditions was brought by small angle neutron scattering (SANS) experiments. Figure 1 represents the intra-aggregate structure factor $S_{intra}(q)$ of the USPIOs measured by SANS in a solvent mixture matching the neutron scattering length density of the copolymer. The shape of $S_{intra}(q)$ reflects both the interactions between the USPIOs inside the object and the overall shape of their aggregates in the attractive regime.^{86, 87} In the small q -regime, $S_{intra}(q)$ followed a power law with a slope approximately -2 typical of flat samples, supporting a vesicle-type morphology. In this q -region (Kratky-Porod regime), the thickness of the USPIO layer in the vesicle membrane can be calculated from the slope of $\ln[q^2 \times S_{intra}(q)]$ plotted vs. q^2 which is $-\delta^2/12$.⁸⁸ From the experimental data, we obtained respectively $\delta=13$ nm and $\delta=10$ nm for samples WD15-50 and WDi-70. The vesicular membrane thus contains no more than one or two layers of magnetic colloids. More precisely, the data were properly fitted using a hollow sphere form factor with respectively an internal radius $R=130$ nm and shell thickness $\delta=12$ nm for WD15-50, and $R=45$ nm lumen radius with membrane thickness $\delta=9$ nm for WDi-70. These radii deduced from SANS fits agree pretty well with the hydrodynamic radii on Table 1 measured by DLS. At large wave-vectors, the q^{-4} scaling law is typical of the Porod's regime expected for nanoparticles with a smooth interface. Moreover, $S_{intra}(q)$ presents a correlation peak around $8 \times 10^{-2} \text{ \AA}^{-1}$ (see Figure 1), associated to a most probable USPIO inter-particle distance $d_{max} = 2\pi/q_{max} = 7.8$ nm. Considering their weight-average diameter $D_w=7.5$ nm, we deduce that the USPIOs are closely packed inside the vesicular membrane for both WD15-50 and WDi-70 samples. The SANS curve of WDi-50 vesicles in D₂O where the neutron scattering contrast of the USPIOs is almost matched also exhibits this correlation peak (Supporting Information S-c), as explained by the close-packed structure of holes in the copolymer membrane confining the USPIOs at a high local volume fraction.

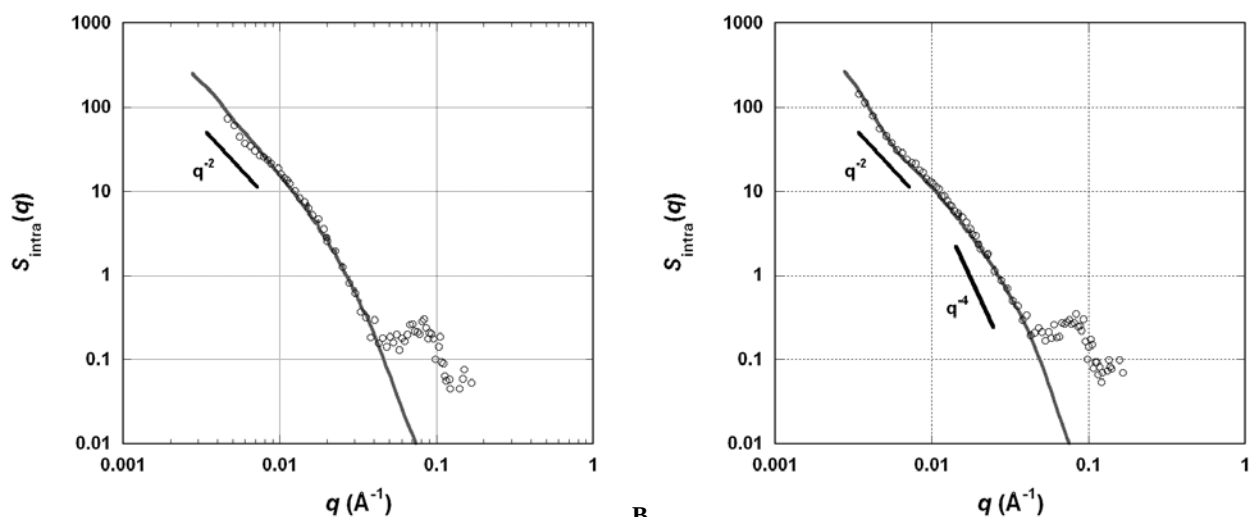


Figure 1. SANS curves of PTMC₂₄-*b*-PGA₁₉ vesicles WD15-50 (**A**) and WDi-70 (**B**), centrifuged then dispersed at 10 mg/mL in a mixture H₂O:D₂O (65.6/34.4 v/v). Experimental intra-aggregate structure factors $S_{\text{intra}}(q)$ of USPIOs are plotted as open circles. The solid lines represent the simulated form factors respectively for hollow spheres of mean radius $R=130$ nm (PDI=0.17) with membrane thickness $\delta=12$ nm (PDI=0.3) for WD15-50 (**A**), $R=45$ nm (PDI=0.35) with $\delta=9$ nm (PDI=0.3) for WDi-70 (**B**).

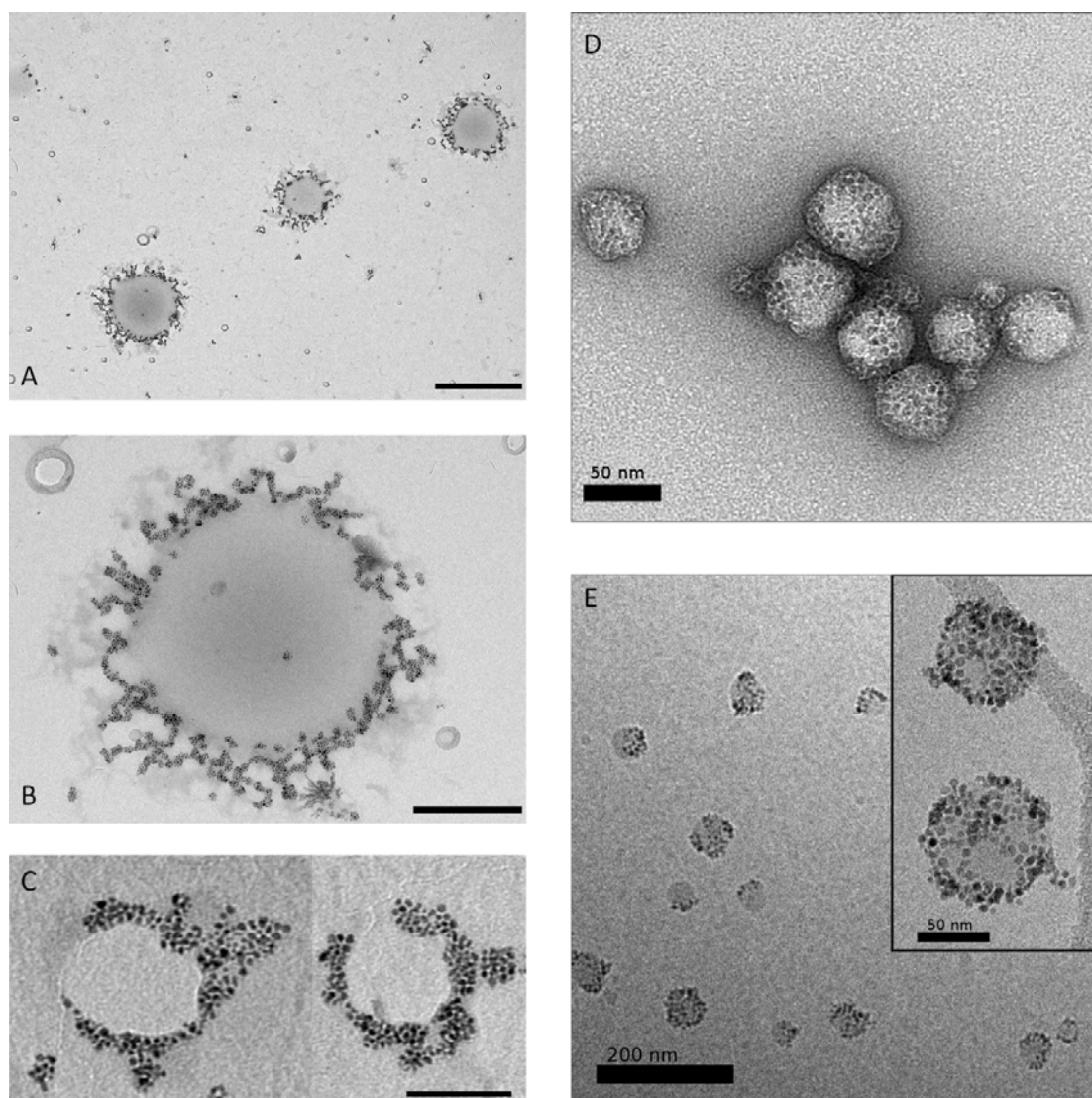


Figure 2. TEM images of USPIO-loaded vesicles prepared by nanoprecipitation. (**A**) Low magnification picture of WD15-50 vesicles (scale bar 1 μm); (**B**) Close-up view of a WD15-50 vesicle containing ~ 1500 USPIOs as measured by image analysis (scale bar 300 nm); (**C**) WDi-70 vesicles spreading on the substrate, which enables counting ~ 190 USPIOs on the left and ~ 220 USPIOs on the right (scale bar 100 nm); (**D**) Image of negatively stained WDi-50 vesicles, showing a group of vesicles laying intact on the carbon substrate (scale bar 50 nm); (**E**) Cryo-TEM image showing homogeneously dispersed WDi-50 vesicles (scale bar 200 nm). Inset: close-up view of two vesicles showing a mantle of respectively ~ 80 and ~ 110 close-packed USPIOs with some uncovered areas (scale bar 50 nm).

To summarize our SANS results, we found membrane thicknesses equal to 12.5 ± 0.5 nm and 9.5 ± 0.5 nm (either by scaling law or by form factor fitting) for WD15-50 and WDi-70 vesicles respectively. Due to the chosen $\text{H}_2\text{O}/\text{D}_2\text{O}$ solvent matching the copolymer scattering, these values represent the thickness of the USPIOs' layer only. In D_2O solvent were the neutron scattering signal originates both from the magnetic contrast of iron oxide and the nuclear contrast of the copolymer, we measured a total membrane thickness 29.1 ± 0.6 nm from Kratky-Porod's plots of the data reported in the ESI file (figure S-c) for WDi vesicles independently of their iron oxide content (from 0 to 50% FWR), in accordance with the value 30 ± 2 nm reported for the total membrane thickness of non magnetic vesicles made of PTMC₂₄-*b*-PGA₁₂ with a similar PTMC block of molar mass $M_n = 2750$ g/mol.⁸² The measurement by SANS of the hydrophobic bilayer thickness for WDi vesicles well compares to the value $\delta = 9.6$ nm measured by cryo-TEM for polymersomes made of poly(ethylene oxide)-*b*-polybutadiene (noted EO₂₆-BD₄₆ or OB2) with a total molar mass of 3600 g/mol and a hydrophilic fraction of 28%, thus a hydrophobic block mass of 2600 g/mol close to the one of PTMC here.⁷⁰ The 25% increase of hydrophobic thickness for WD15 vesicles is ascribed to the swelling of vesicles' membranes by the incorporation of USPIOs, which was presumably not possible for WDi ones due to their much higher curvature.

The WD15-50 and WDi-70 samples were further observed by TEM (Figure 2) and AFM (Figure 3) to confirm the vesicular morphology. TEM images mainly show the arrangement of the USPIOs because of the low electron scattering density of the copolymer compared to iron oxide. For both nanoprecipitation conditions (WD15 and WDi), hollow structures made of a close-packed arrangement of USPIOs were observed. The diameters measured on the TEM images 2B and 2C are around 750 nm and 150 nm respectively for WD15-50 and WDi-70 vesicles, which is larger than two times their hydrodynamic radii reported on Table 1 (374 nm and 104 nm respectively). This apparent discrepancy is ascribed to the total spreading of the vesicles onto the carbon substrate. The drying step during sample preparation and the strong wetting on substrates presumably induced the rupture of membranes, which explains the presence of fragments as well as not entirely closed structures.^{71–73} Unlike images A, B and C of Figure 2 that were obtained by spraying the samples onto the grids, image D originates from a more gentle protocol combined with staining (see Experimental) that led to vesicles sitting intact on the substrate. Both images D and E (cryoTEM) show apparent diameters much closer to the light scattering results, undoubtedly confirming the proposed structure. However we chose to show images A, B and C in spite of the spreading effect, because the flattening of the membrane onto the substrate enables to count the USPIOs per vesicle much easily than with the projection of intact spherical vesicles (D and E).

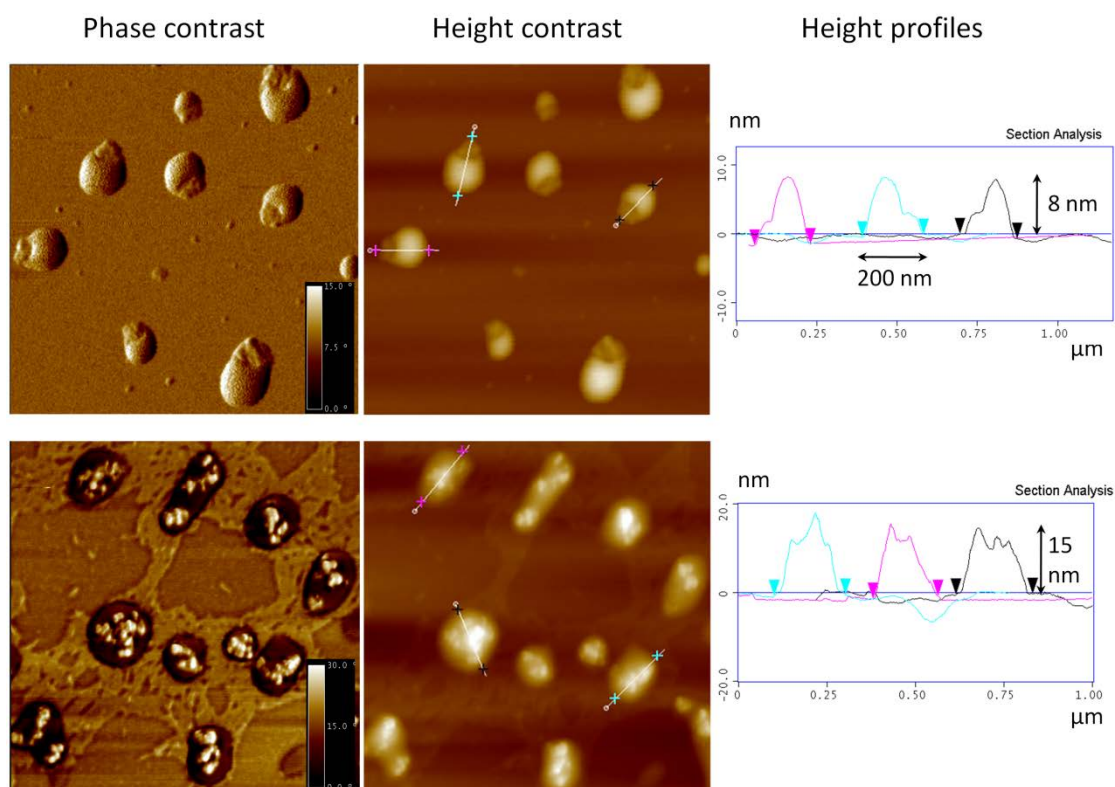


Figure 3. Tapping ModeTM AFM phase and height images of 1×1 μm surfaces of PTMC₂₄-*b*-PGA₁₉ vesicles prepared by nanoprecipitation WDi without magnetic nanoparticles (upper panel) and with 50 wt % USPIO WDi-50 (lower panel). The average heights are measured on the right by cross-sections.

The WDi vesicles were also observed by AFM with and without the presence of 50 wt % USPIO. AFM phase images of empty vesicles (WDi-0) showed spherical vesicles, which aqueous interior leaked out due to drying and strong adsorption onto the freshly cleaved mica surface. When USPIOs were incorporated into the membrane (WDi-50), those presented multiple bright spots. The contrast of phase AFM pictures being proportional to the surface toughness,^{89, 90} we identify

those bright spots with the hard inorganic USPIOs embedded within the soft polymer matrix and spatially distributed over the vesicular surface as large patches. The average thicknesses of membranes spread on mica were analyzed on the AFM height images. These profiles revealed that the presence of USPIOs increase the thickness from 8 nm to 15 nm, the difference being very close to the weight average inorganic diameter $D_w = 7.5$ nm. If the vesicles were adhering intact on the mica substrate,

simply deflated by soft drying conditions, one would expect to measure an inorganic thickness equivalent to two layers of USPIOs (respectively from the top and the bottom of the closed membrane). But the AFM images correspond to a single membrane thickness instead, which indicates that the vesicles had burst and spread onto the substrate, as depicted schematically in Supporting Information S-h.

To conclude on the SANS, (cryo)TEM and AFM results, these measurements show that due to their hydrophobic coating, the USPIOs are confined in 2 dimensions within the membranes, between the two leaflets of the copolymer bilayer.

Magnetization, migration and deformation under magnetic field of USPIO-loaded vesicles

As for the magnetization properties, the magnetization curves of both individual USPIOs and USPIO-loaded vesicles were fitted according to Langevin's law of paramagnetism, each USPIO being a giant magnetic monodomain with an average magnetic dipole of $8200 \mu_B$, which is also the approximate number of Fe^{3+} ions per USPIO (see Supporting Information S-d). On figure S-5, the VSM curve of WD15-50 vesicles loaded with 50 wt % USPIO exhibits a plateau value $M_s/\Phi m_s$ allowing to determine a concentration 0.43 g/L of USPIOs very close to the expected one (0.429 g/L , taking into account the USPIO FWR and the dilution effect during dialysis). Like previously stated, the vesicular dispersions were homogeneous, as attested by the detection of neither aggregates by DLS nor clusters of bare USPIOs moving when approaching a permanent magnet. Altogether, these results suggested a 100 % loading efficiency for the USPIOs at feed weight ratios lower than 50 % for WD15 and 70 % for WDi vesicles.⁹¹ Thus one can use mass conservation to deduce the values of the volume fraction Φ of USPIOs inside the membranes, on the basis of their initial weight ratio (FWR) relatively to the copolymer and their respective mass densities $d_{USPIO}=5.1 \text{ g/cm}^3$ and $d_{copol}=1 \text{ g/cm}^3$. An estimate of the mean number of USPIOs per vesicle of radius R_H and membrane thickness δ can be calculated according to:

$$N_{\text{USPIO/vesicle}}^{\text{USPIO}} = \frac{V_{\text{mb}} \times \Phi_{\text{USPIO}}^{\text{mb}}}{V_{\text{USPIO}}} \quad (1)$$

where the volume of membrane is calculated by $V_{\text{mb}} = 4\pi R_H^2 \delta$ using geometrical values obtained by DLS and SANS respectively and V_{USPIO} is averaged over the size distribution law of diameters ($\pi D_w^3/6$). These calculations for each set of experiments gathered in Table 2 lead to estimated numbers of USPIOs in pretty good accordance with the values observed on the TEM pictures of Figure 2.

Table 2. Characteristics of USPIO-loaded vesicles: feed weight ratio, hydrodynamic radius R_H , local volume fraction Φ of USPIOs in membrane and their number N per vesicle calculated by equation (1) with respectively $\delta=13 \text{ nm}$ for WD15 samples and $\delta=10 \text{ nm}$ for WDi ones (SANS values).

Sample code	USPIO FWR (%)	$\Phi_{\text{USPIO}}^{\text{mb}}$ (%)	R_H (nm)	$N_{\text{USPIO/vesicle}}^{\text{USPIO}}$
WD15-20	20	3.8	196	1085
WD15-35	35	6.4	195	1830
WD15-50	50	8.9	187	2340
WDi-20	20	3.8	50	55
WDi-35	35	6.4	45	75
WDi-50	50	8.9	47	110
WDi-70	70	12.1	52	190

The migration of the WD15-50 vesicles under a controlled magnetic field gradient was also used to estimate differently the number of confined USPIOs averaged on a large population of vesicles. The assessment of the ability of magnetic polymersomes to be attracted and concentrated at a specific location *in vivo* is also particularly relevant. Compared to magnetophoresis experiments with objects of sizes around $10 \mu\text{m}$ such as giant liposomes or biological cells,^{62, 92-95} a supplemental difficulty arose from the low value of the Peclet's hydrodynamic number, which means that the magnetophoretic motion of the vesicles was not significantly larger than their Brownian motion (see the videos supplied as ESI). Usually, magnetophoretic measurements with such colloidal particles prone to thermal agitation are done by measuring light absorption profiles as a function of time and space.⁹⁶⁻⁹⁹ In the present work, we chose alternatively a statistical method to infer the average drift velocity V_{drift} and the diffusion constant D_{vesicle} by following a large number of individual trajectories, as once described in a study of Brownian colloids in a liquid crystal.¹⁰⁰

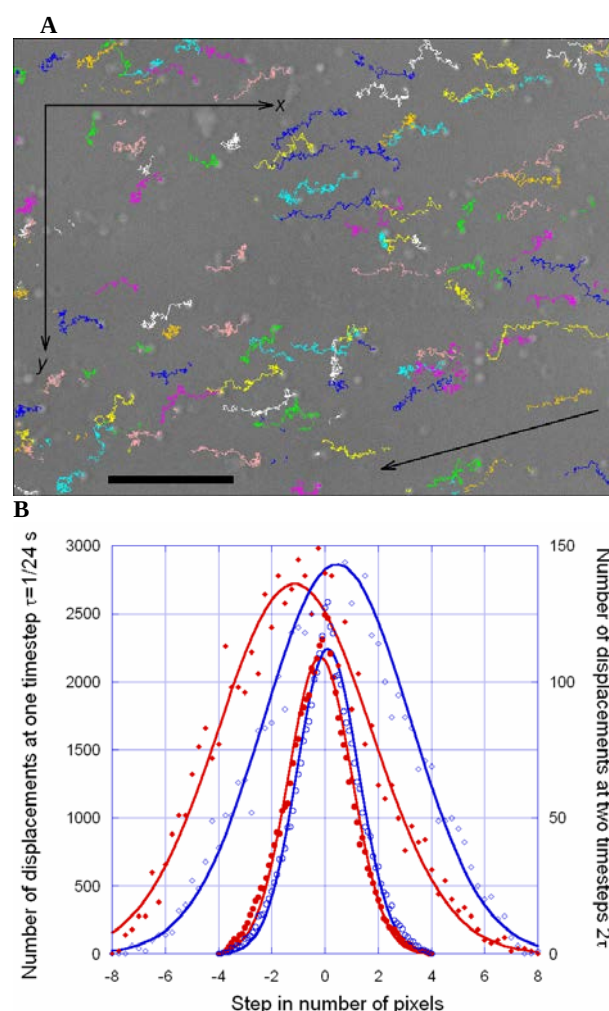


Figure 4. Video microscopy snapshot (inverted bright field image) superposed with the trajectories (A) during 10s of WD15 PTMC₂₄-b-PGA₁₉ vesicles loaded with 50 wt % of USPIOs. The vesicles were discriminated from the background by image analysis with the ParticleTracker plugin for ImageJ. The magnetophoretic mobility of the vesicles is visualized by the shifts between the histograms (B) of displacements in the x (red markers) and the y (blue markers) directions for durations respectively of $\tau=1/24=0.042 \text{ s}$ (circles) and 2τ (diamonds). The arrow indicates the direction of magnetic field gradient of intensity $dB/dz=18.5 \text{ T/m}$. The scale bar represents $20 \mu\text{m}$.

The Brownian motion appears isotropic with a unique translation diffusion constant $D_{\text{ves}}=1.11 \mu\text{m}^2/\text{s}$. The Stokes-Einstein's formula gives a hydrodynamic radius deduced by video-microscopy $R_{\text{H}}^{\text{video}}=196 \text{ nm}$ comparable to the value obtained by DLS. Due to an imperfect alignment of the magnetic field gradient with the x axis, we had to extract both coordinates of the magnetophoretic motion to calculate the total drift velocity:

$$\|\vec{V}_{\text{drift}}\| = \sqrt{V_x^2 + V_y^2} = 1.09 \mu\text{m/s} \quad (2)$$

This experimental value is compared to the theoretical estimate obtained by balancing the forces acting on a spherical magnetic vesicle at steady state in an external magnetic field gradient, which are the magnetophoretic force F_B , and the viscous drag, F_v , acting against it. The two forces are given by:

$$F_B = m \frac{dB}{dz} \quad (3)$$

$$\text{and} \quad F_v = 6\pi\eta R_H V_{\text{drift}} \quad (4)$$

where m is the magnetic moment of the vesicle,¹⁰¹ dB/dz is the gradient of the magnetic field, η is the viscosity of the solvent, R_H is the hydrodynamic radius of the vesicle, and V_{drift} is the velocity of the particle. From the exact balance of the magnetic and the viscous forces, we calculate a theoretical magnetophoretic velocity $V_{\text{drift}} \approx 0.5 \mu\text{m/s}$ for the WD15-50 vesicles under a field gradient $dB/dz=18.5 \text{ T/m}$. The factor around one-half between the expected drift velocity and the value $V_{\text{drift}}=1.09 \mu\text{m/s}$ measured experimentally cannot be ascribed to the statistical noise because the uncertainties of the average displacements were estimated at 0.4% and 1.6% for the histograms at τ and 2τ time steps containing respectively 64519 and 3920 data points. As a tentative explanation, we know from a reported work on giant magnetic liposomes that the drag coefficient is enhanced compared to Stokes' formula (4) if the vesicles were deformed by the field into high aspect ratio ellipsoids during their migration.¹⁰² Another correction compared to the drag coefficient of a solid sphere originates also from the viscous dissipation inside the fluid magnetic membrane, for instance if it was subjected to a "caterpillar" or a "crawling" motion.¹⁰³ In addition to these pure hydrodynamic effects, the measured V_{drift} higher than its expected value might be explained by an underestimate of the numbers of USPIOs per vesicle appearing in Table 2. This would occur for example in the case of a non negligible amount of "blank vesicles" that were undetected but increased the average LC of iron oxide inside the magnetic vesicles above the USPIO/copolymer ratio (FWR) used for nanoprecipitation.

Apart from estimating the magnetic payload of the vesicles, the magnetophoresis experiment is also relevant to estimate their efficiency for magnetic guiding both *in vivo* and *in vitro*. Their magnetophoretic mobility in the vicinity of a strong NdFeB magnet is indeed of the same order of magnitude than

values $\approx 1 \mu\text{m/s}$ reported by studies that evidenced the enhanced uptake of magnetic nanocarriers by cell cultures under field gradients^{104, 105}. For *in vivo* experiments, it was hypothesized that the accumulation of magnetic colloids injected in the main blood stream at a specific region under magnetic field requires that their migration is faster than the blood velocity in the smallest vessels alimentering the tumor.⁹⁸ However, the guiding of magnetic stealth liposomes injected in the caudal vein of mice by a strong permanent magnet applied directly on a solid tumor was evidenced, even though their drift velocity was $10 \mu\text{m/s}$ only.⁶⁸ A model experiment consisting in attracting clusters of MNPs of sub-micron diameters (330 nm) by a permanent magnet while they were circulating in a flow loop showed that they were efficiently deposited at the surface of the capillary near the magnet even with a stream velocity as high as 1 cm/s .¹⁰⁶ Therefore we believe that the USPIO loaded vesicles WD15-35 or WD15-50 are good candidates for such magnetic targeting applications, whereas the WDi vesicles might be too small and contain an insufficient number of USPIOs.

The magnetic response of PTMC₂₄-*b*-PGA₁₉ vesicles with their membrane filled by 50 wt % (WD15-50) or 70 wt % USPIO (WDi-70) was also studied by anisotropic SANS under an applied magnetic field. Vesicles were dispersed in light water (H₂O) in order to work in almost pure nuclear contrast conditions under field. The magnetic contrast of the $\gamma\text{-Fe}_2\text{O}_3$ USPIOs in H₂O being much lower than the nuclear contrast, the anisotropy of the SANS signal was not simply due to magnetization but reflects the spatial organization of the USPIOs and their possible rearrangement under magnetic field. The SANS patterns of WD15-50 vesicles are shown on Figure 5 at increasing field intensities up to 1 T.

The scattering patterns became clearly anisotropic when a magnetic field was applied to the sample. The lines of iso-intensity in the $3 \times 10^{-3} - 3 \times 10^{-2} \text{ \AA}^{-1}$ q -range were elliptical, elongated perpendicularly to the field direction. One possible scenario compatible with this asymmetry consists in the deformation of the hollow spheres formed by the USPIOs into either oblate or prolate ellipsoids symmetric by rotation along the field direction. However, one should keep in mind that the observed q -range corresponds to the length scale of the membrane thickness rather than to the whole size and shape of the vesicles.⁷¹⁻⁷³

In order to study this shape anisotropy more quantitatively, the scattering patterns were averaged in angular sectors around two directions parallel ($^{\parallel}$) and perpendicular ($^{\perp}$) to the magnetic field. Examples of the resulting intensity curves are plotted on Figure S-2 (Supporting Information S-b). By comparing the difference $q^{\perp} - q^{\parallel}$ in these two directions relatively to the wave vector q^0 obtained by an isotropic averaging at the same intensity value, an anisotropy factor could be calculated for each sample and each magnetic field intensity (Table 3).

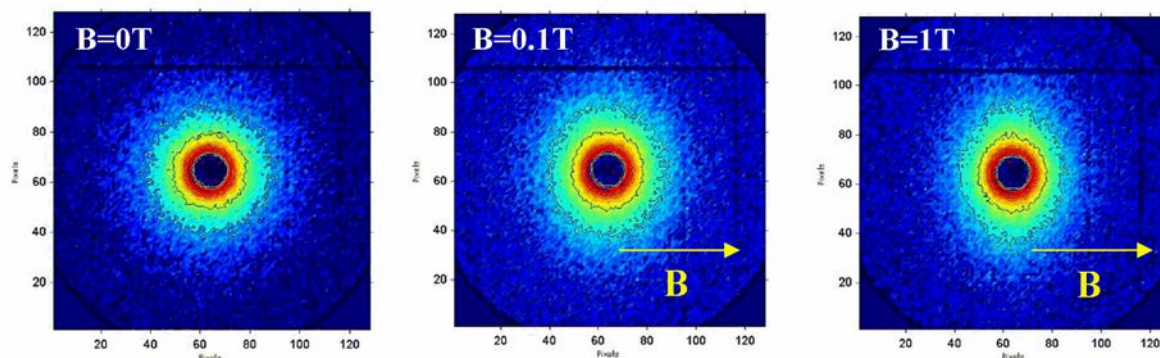


Figure 5. Anisotropic SANS patterns of WD15-50 in H₂O in the q range $3 \times 10^{-3} - 3 \times 10^{-2} \text{ \AA}^{-1}$ under a magnetic field (horizontal) of intensity $B=0 \text{ T}$ (left), $B=0.1 \text{ T}$ (middle) and $B=1 \text{ T}$ (right). Each color corresponds to an iso-intensity range.

Table 3. Anisotropy factor of WD15-50 and WDi-70 vesicles calculated from anisotropic averaging of their SANS patterns at 6 cm^{-1} iso-intensity under increasing magnetic field intensities.

B (T)	$(q^{\perp} - q^{\parallel}) / q^0 (\%)$	
	WD15-50	WDi-70
0.1	11.3	6.8
0.6	24.4	12.7
1	26.8	12.0

The calculated anisotropy factors confirmed the increase of membrane anisotropy with the applied magnetic field already visible on the SANS patterns. The anisotropy factor increased mainly between 0.1 and 0.6 T for both vesicular dispersions and remained almost constant up to 1 T. The plateau value reached at 0.6 T is ascribed to the saturation of the magnetic moment of a vesicle above $B \approx 0.7\text{ T}$ as observed on the magnetization curve (Figure S-5). It is worth noticing that the anisotropy parameter is smaller for WDi-70 than for WD15-50 vesicles, as explained by the number of USPIOs per vesicle (12 times smaller for WDi-70 than for WD15-50) and by the vesicle size (4 times smaller). Presumably due to their smaller size associated with a higher membrane curvature, WDi-70 vesicles are less prone to magnetic deformation than the much larger WD15-50 ones.

USPIO-loaded vesicles as contrast agents for MRI

The efficiency of MRI contrast agents based on USPIO is usually assessed by measuring the T_1 (longitudinal) and T_2 (transverse) relaxation times of the proton spins relaxations. Then the relaxation rates $1/T_1$ and $1/T_2$ are plotted versus total iron concentration in mM and the resulting slopes ($\text{s}^{-1}\text{mM}^{-1}$) called respectively r_1 and r_2 relaxivities can be used to compare different samples of USPIO differing by their size, dispersity, local concentration, aggregation state or any other parameter like the confinement in either a hydrophilic or a hydrophobic environment. In particular the encapsulation of USPIOs in hydrophobic polymers hampers the diffusion of water protons in the vicinity of USPIOs, which results in poor T_1 contrast enhancement,¹⁰⁷ so that we can infer the same effect in our systems where the USPIOs are buried within a hydrophobic membrane. In addition, r_1 decreases rapidly as a function of the applied magnetic field (*i.e.* the Larmor's resonance frequency) while r_2 reaches a plateau value due to the so-called "secular term" in its theoretical expression.^{45, 108} The T_1 and T_2 relaxation times were measured on a 4.7 T (200Mz) research MRI system. Their inverse values $1/T_1$ and $1/T_2$ were plotted as a function of iron molar concentration for PTMC-*b*-PGA vesicles prepared in WDi condition with USPIOs' FWR ranging from 20 to 70 wt %. The r_1 and r_2 relaxivities deduced from the slopes are reported on Table 4. To assess the experimental sensitivity, the solutions were diluted by a factor from 20 to 500: no vesicle size variation was observed by DLS under such dilutions. While r_1 values are weak and almost constant ($\sim 3\text{ s}^{-1}\text{mM}^{-1}$), r_2 values are of the same order of magnitude ($\sim 100\text{ s}^{-1}\text{mM}^{-1}$) and even larger than for commercial T_2 contrast agents: these are either individual (AMI-227, Ferumoxtran/Sinerem)¹⁰⁹ or clustered (AMI-25, Feridex/Endorem)^{110, 111} USPIOs coated with a Dextran polymer, the latter being denominated SPIOs because of their larger size. The clustering of USPIOs inside objects at a high

loading density is indeed known to enhance the negative contrast for T_2 -sequence IRM images compared to individual USPIOs, as reported for hydrophobic USPIOs in micelles^{107, 112, 113} or hydrophilic ones in liposomes¹¹⁴ or electrostatic coacervates with charged polymers.¹¹⁵ However, the articles that link the experimental r_2 values to theory are rather scarce: they dealt with SPIOs made of magnetite cores aggregated by Dextran of varying sizes¹¹⁶ and more recently with individual USPIOs coated by a controlled thickness of silica.¹¹⁷ In the present work, we note a steeper slope on Figure 6-A for WDi-35 ($r_2=134\pm 2\text{ s}^{-1}\text{mM}^{-1}$) as compared to WDi-20 ($r_2=81\pm 1\text{ s}^{-1}\text{mM}^{-1}$). These relaxivities are both larger than the value $r_2=39\pm 2\text{ s}^{-1}\text{mM}^{-1}$ reported for USPIOs individually dispersed in water synthesized by the same aqueous route and of the same size distribution.¹¹⁵ For 50 wt % USPIOs in the vesicles, the transverse relaxivity reached $r_2=173\pm 7\text{ s}^{-1}\text{mM}^{-1}$, but then it saturates near this value at $r_2=182\pm 4\text{ s}^{-1}\text{mM}^{-1}$ for the highest loading content 70 wt %. On the theoretical point, A. Roch *et al.* predicted such a plateau of r_2 when increasing the size of clusters of USPIOs.¹¹⁶

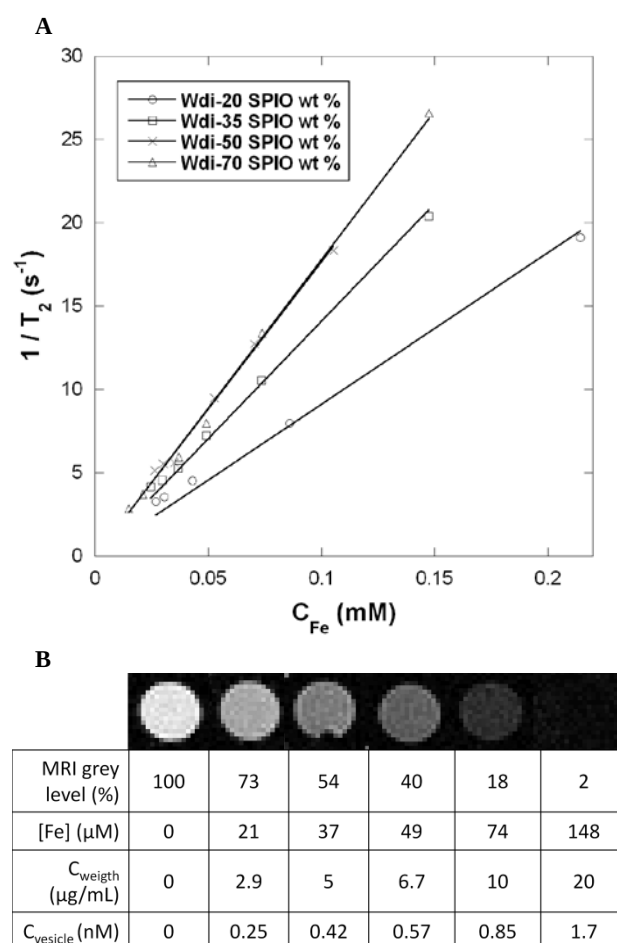


Figure 6. (A) Transverse relaxation rates ($1/T_2$, s^{-1}) as a function of iron concentration (mM) for PTMC₂₄-*b*-PGA₁₉ vesicles (WDi) loaded with 20, 35, 50 and 70 USPIO wt %. The slopes give the r_2 value, respectively 81 ± 1 , 134 ± 2 , 173 ± 7 and $182\pm 4\text{ s}^{-1}\text{mM}^{-1}$; (B) T_2 -weighted MRI images extracted from T_2 measurements experiment (4.7 T; multiple spin-echo 2D imaging sequence; $T_R=10000\text{ ms}$; inter echo-time, 5 ms; number of echo images, 256; FOV, $50\times 50\text{ mm}$; matrix, 128×128 ; slice thickness, 1 mm) of WDi-70 vesicles at different dilution factors. The table gives the molar concentrations of iron ions, the total weight concentrations and the equivalent molar concentrations of vesicles.

To evidence the effect of USPIO-loaded vesicles on T_2 -weighted MR images, Figure 6-B shows MR images of wells containing increasing concentrations of WDi-70 vesicles. A remarkable darkening (*i.e.* negative contrast enhancement) appeared even at low vesicle concentration. The MRI detection limit, defined as the copolymer concentration at which the MRI signal intensity decreases to 50 % of that of pure water,¹⁰⁷ was measured at 6.7 $\mu\text{g/mL}$ for WDi-70 vesicles. Since the molar mass of the vesicles measured by SLS is 1.182×10^7 g/mol (Supporting Information S-i), the above sensitivity limit corresponds to a vesicle concentration of approximately 0.57 nM, which is one order of magnitude lower than the 5 nM reported for magnetic micelles¹⁰⁷ and, to our knowledge, the lowest value ever reported. For applications such as the evaluation of the bio-distribution or the targeting efficiency of a drug conveyed in nano-carriers, the concentration of ferric ions may not be the most relevant parameter for the radiologists. Therefore the r_1 and r_2 relaxivities are also expressed in Table 4 according to the concentration of vesicles in nM to facilitate the comparison with other nano-particular contrast agents. Unlike

the relaxivities per ferric ion which saturate, their values per WDi vesicle increase monotonously with the magnetic FWR inside the membrane, at an almost constant hydrodynamic size.

Table 4. Longitudinal (r_1) and transverse (r_2) relaxivities of USPIO-loaded WDi vesicles when used as contrast agents for MRI at 4.7 T, deduced from the linear fits of the relaxation rates $1/T_1$ and $1/T_2$ versus molar concentrations both of ferric ions (in mM) or of vesicles (in nM). The number of Fe^{3+} per vesicle is the product of the number of USPIOs per vesicle (Table 2) by 8200 Fe^{3+} per USPIO on average.

Sample code	R_H (nm)	$N_{\text{Fe}^{3+}/\text{vesicle}}$	$r_1 _{\text{Fe}^{3+}\text{ion}}$ ($\text{s}^{-1}\text{mM}^{-1}$)	$r_1 _{\text{vesicle}}$ ($\text{s}^{-1}\text{nM}^{-1}$)	$r_2 _{\text{Fe}^{3+}\text{ion}}$ ($\text{s}^{-1}\text{mM}^{-1}$)	$r_2 _{\text{vesicle}}$ ($\text{s}^{-1}\text{nM}^{-1}$)
WDi-20	50	4.5×10^5	2.8 ± 0.02	1.3 ± 0.01	81 ± 1	37 ± 0.4
WDi-35	45	6.2×10^5	3.6 ± 0.08	2.2 ± 0.05	134 ± 2	83 ± 1.2
WDi-50	47	9.0×10^5	3.6 ± 0.2	3.3 ± 0.2	173 ± 7	156 ± 6
WDi-70	52	1.6×10^6	3.5 ± 0.1	5.5 ± 0.2	182 ± 4	283 ± 7

Doxorubicin loading and release by macroscopic heating

To determine the feasibility of magnetically controlled drug release, a dual loading of USPIOs and of doxorubicin was carried out. The nanoprecipitation was performed at pH 10.5 in order to deprotonate the DOX ($\text{pK}_a \sim 8.3$), thus maximizing the loading content ($\text{LC} = 34\%$ without size variation at 50% FWR) and extending the release duration as described in a previous work.¹¹⁸ For each vesicular dispersion, the USPIO feed weight ratio (FWR) was fixed at a value lower than the maximum USPIO loading (namely 50 wt % for WDi and 35 wt % for WD15) so that space was left in the membrane for DOX entrapment. The DOX FWR in the nanoprecipitation mixture was then progressively increased. A DOX FWR of 20% was selected for both vesicular types since a drug loading at this level did not alter the self-assembly of the vesicles significantly: Table 5 shows indeed a moderate variation of their hydrodynamic size (R_H decreases by 16% for WD15 and increases by 8% for WDi) and an unchanged surface charge. A larger 30% DOX FWR can be sustained by WD-15 vesicles without any size change, but for the smaller WDi-50 vesicles it leads to a two-fold size increase, presumably due to their larger curvature energy already invoked to explain their lower deformability under static magnetic field.

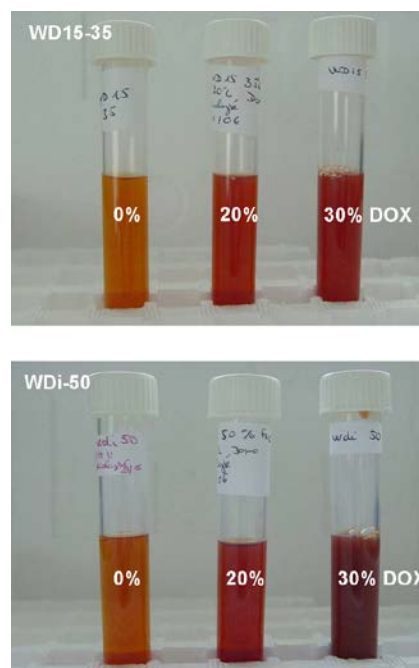


Table 5. Doxorubicin feed weight ratio (FWR), hydrodynamic size, polydispersity index and ζ potential of WD15-35 and WDi-50 vesicles. The pictures show the corresponding sample tubes.

Sample Code	DOX FWR (%)	R_H (nm)	PDI	ζ (mV)
WD15-35	0	152	0.15	-39.6
	20	124	0.23	-39.3
	30	128	0.16	nd*
WDi-50	0	56.5	0.22	-40.8
	20	61	0.15	-42.0
	30	137	0.20	nd*

*not determined

After nanoprecipitation with dual-loading in DOX and USPIOs, an extensive dialysis against a large volume (4 L) of Tris buffer (pH 7.4, 30° C, ionic strength 150 mM) during 4h allowed to reduce the pH back to 7.4 and to completely remove the unbound drug and DMSO. As for the colloidal stability of these dual loaded vesicles, their ζ potential remained strongly negative (~ -40 mV). Therefore the corona of PGA chains was unaffected, which excludes the precipitation of the USPIOs and of the drug onto the hydrophilic chains and proves their embedment deeply inside the vesicular PTMC-*b*-PGA membrane. The loading content (LC) and loading efficiency (LE) of DOX were determined by spectrophotometry. Values obtained for both vesicular dispersions with or without USPIOs are gathered in Table 6. A DOX LC around 10 wt% was found in all cases, independently of the presence of USPIOs in the membrane. Finally, the colloidal stability of the WDi vesicles was tested in MEM cell culture medium with fetal bovine serum (10% v/v FBS), and no change in size was observed for 24 hours.

Table 6. Influence of USPIO feed weight ratio on the DOX loading content and efficiency into WDi and WD15 vesicles. The FWR is in wt % relatively to copolymer in DMSO before nanoprecipitation. The LC is measured by spectrophotometry after nanoprecipitation and dialysis. The LE is the yield LC/FWR.

Sample code	USPIO FWR (%)	DOX FWR (%)	DOX LC (%)	DOX LE (%)
WD15	0	20	12.5	74
	35	20	9	52
WDi	0	20	9.5	47.5
	50	20	12	70

Comparing precisely the DOX loading efficiency between dual loaded vesicles and non magnetic ones, we observe that LE decreases by 22% for WD15 vesicles, whereas it increases by 22.5 % for WDi ones. As a result, the insertion of USPIOs and DOX appears competitive in the case of the larger WD15 vesicles, certainly due to a lack of space in the membrane (the difference between Φ_{mb}^{USPIO} and its maximal value being only 2.5 %, see Table 2). On the opposite, the incorporation seems cooperative for the smaller WDi vesicles. Such synergetic effect of dual loading has already been mentioned for

copolymer micelles, for which the LC of DOX could be enhanced from 3 to 12 wt % by the co-encapsulation with hydrophobic USPIOs.³⁶

Subsequently, *in vitro* release studies from the several prepared vesicular dispersions were monitored in various conditions by comparing the absorbance at $\lambda_{max}=485$ nm with the DOX absorbance calibration curve (after background correction). The release kinetics *in vitro* at 37 °C of WDi-50 and WD15-35 vesicles fed with 20 wt % DOX appear almost similar. As seen on Figure 7 indeed, a plateau at around 50 wt % of released DOX was reached in both cases after one day. As stated in a previous work on the physicochemical conditions to optimize the loading and release of DOX with PTMC-*b*-PGA vesicles (but for a DOX LC of 34 wt % 3 times larger than in the present work and without USPIOs),¹¹⁸ temperature has a strong influence on the kinetics as well as on the amount of drug released: the plateau values at 5°C, 20°C, 37°C and 45°C were found respectively equal to 5%, 30%, 60% and 85% of the initial DOX load in the vesicles. This temperature sensitivity is presumably due to the semi-crystalline nature of the PTMC blocks inside membranes evidenced once by microcalorimetry⁸³ and in this work by birefringence measurement (see Supporting Information S-e).

On Figure 7, only 15 % of DOX was released after 6 hours at 23°C compared to 45 % released after the same time at 37 °C thus above the melting temperature of PTMC in the membrane of vesicles.

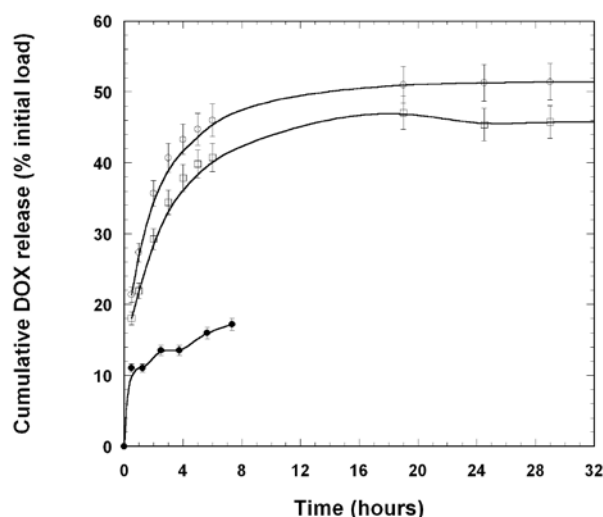


Figure 7. *In vitro* release kinetic profiles of USPIO/DOX dual loaded vesicles with initial 20% DOX FWR obtained for WDi-50 (○) and WD15-35 (□) vesicles at 37 °C and for WDi-50 vesicles at 23 °C (●). In all cases, the release medium was Tris 10 mM (pH 7.4, ionic strength 150 mM).

Doxorubicin release by magnetic hyperthermia

Having in mind this thermo-sensitivity of the release rate of DOX *in vitro* from dual-loaded PTMC-*b*-PGA vesicles, we studied the effect of an excitation by an oscillating magnetic field of the USPIOs confined in the membranes. Submitted to a strong radio-frequency field, USPIOs are known to dissipate heat originating from friction losses of their magnetic dipoles according to two different relaxation modes: Néel's relaxation consisting in the flips of each dipole between the "easy axes" of the crystalline structure and the Brownian rotational diffusion of the USPIO grains in the solvent of viscosity η . According to

a commonly accepted model,⁵⁰ the specific loss power under a field of frequency f_{RF} and mean intensity H_0 expressed in W/g writes:

$$SLP(f_{\text{RF}}, H_0) = \frac{\pi f_{\text{RF}}}{d} \chi''(f_{\text{RF}}) H_0^2$$

$$\text{with } \chi''(f) = \mu_0 \frac{m_s^2 V_{\text{USPIO}}}{3k_B T} \frac{f_{\text{RF}} \tau_{\text{eff}}}{1 + (f_{\text{RF}} \tau_{\text{eff}})^2} \quad (5)$$

Here $\chi''(f_{\text{RF}})$ is the loss term of the dynamic susceptibility of an USPIO with specific magnetization m_s , mass density d and volume V_{USPIO} . The effective relaxation time τ_{eff} corresponds to the fastest mode between the two mechanisms participating to thermal dissipation. Both of them can be expressed as functions of the particle volume:

$$\tau_{\text{Néel}} = \tau_0 \times \exp(K_a V_{\text{USPIO}} / k_B T) \quad (6)$$

$$\text{with } \tau_0 \approx 10^{-9} \text{ s and } K_a \approx 10^4 \text{ J/m}^3$$

$$\text{and } \tau_{\text{Brown}} = 3\eta V_{\text{USPIO}} / k_B T \quad (7)$$

Although it does not take into account the possible variation of relaxation times with the magnetic field intensity,¹¹⁹ this model correctly describes the strong dependence of the SLP with the size distribution of a suspension of USPIOs⁵³ and gives an optimal diameter about 14–15 nm. When USPIOs are confined in a viscous environment as in lipid compartments inside biological cells (endosomes), membrane, the Brownian relaxation mode can be neglected.^{54, 120}

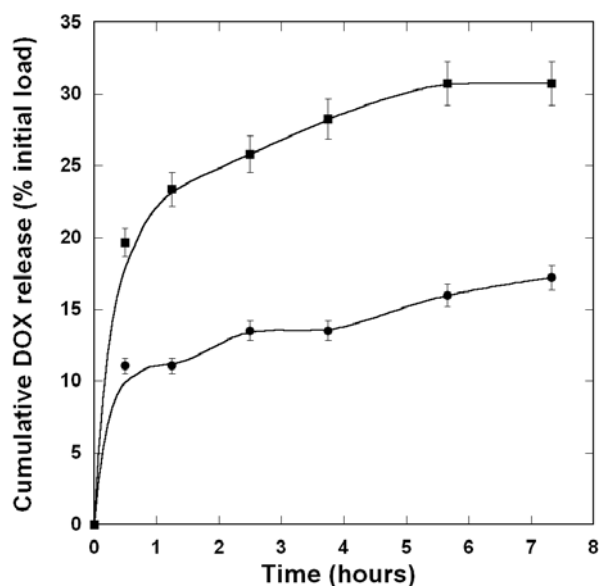


Figure 8. Influence of a RF oscillating magnetic field on the *in vitro* release kinetics of WDi-50 vesicles at constant bath temperature (23 °C); ●: B=0 T; ■: AC magnetic field (f_{RF} =500 kHz, B_0 =2.65 mT).

Figure 8 displays the kinetic profiles at constant temperature 23 °C with and without the application of an oscillating magnetic field of frequency f_{RF} =500 kHz and mean field intensity H_0 =2.12 kAm⁻¹. Although these field conditions might appear weak, they were already over passing by more than a factor two the upper dose of RF irradiation $f_{\text{RF}} \times H_0 < 4.85 \times 10^8$ Am⁻¹s⁻¹ recommended for a human being.¹²¹ After 7 hours, the DOX release content is multiplied by a factor 2 under RF field compared to the same vesicles with USPIOs embedded in the membrane but kept away from the coil. The heat produced *via* Néel's relaxation is believed to increase the fluidity of the semi-crystalline polymeric membrane, increasing dramatically the diffusion of the encapsulated DOX out of the membrane. It should be stressed that we have not observed any macroscopic heating of the vesicular dispersion.¹²² Even if the global

temperature of the suspension remained almost unchanged, we infer that a local temperature raise around 7°C took place in the close vicinity of the membrane (*i.e.* at the nanometric scale). The approximately two-fold enhancement of DOX release rate under the RF magnetic field (Figure 8) was indeed of the same type than the three-fold enhancement observed when the vesicular suspension was heated macroscopically by 14°C thus above the melting temperature of the PTMC blocks (Figure 7). A control experiment performed by placing the WDi-50 vesicles during several hours under a constant magnetic field of intensity B_0 =0.4 T showed that a static deformation of the vesicles had no impact on the release rate of DOX, thereby confirming the necessity to excite the USPIOs at a frequency in the RF-range close to their Néel's relaxation in order to detect an effect on the membrane permeability of the vesicles.

CONCLUSIONS

In the present study, the formation of new hybrid vesicular self-assemblies from the biodegradable PTMC-*b*-PGA copolymer and hydrophobically coated γ -Fe₂O₃ nanoparticles has been investigated. Hybrid vesicles have been obtained by one-step nanoprecipitation, leading to high loading content of magnetic nanoparticles (up to 70 wt %) in the membrane together with a good control over vesicles' size and dispersity. The vesicular morphology was elucidated by combining light and neutron scattering techniques together with electronic and atomic force microscopy. These magnetic vesicles exhibited a long-term colloidal stability and showed suitable properties for biomedical applications: being guided by an external magnetic field gradient created by a small permanent magnet, they also showed an important contrast enhancement in Magnetic Resonance Imaging with a particularly low (sub-nanomolar) detection limit. Dual encapsulation of magnetic nanoparticles with doxorubicin in the biodegradable vesicular matrix is very promising as a versatile method to prepare multifunctional drug nanocarriers. The drug release rate could indeed be enhanced twice under the application of a RF oscillating magnetic field producing a local hyperthermia at the scale of the membranes.

The well-known hyperthermia effect of USPIOs was utilized here in a softer and gentler manner of action on the polymersomes' membrane permeability than by thermoablation, which is based on the melting temperature of a semi-crystalline polycarbonate block. In future studies, we will enhance the RF-triggered release effect by using USPIOs with larger diameters (*e.g.* by a factor 2), which are known to exhibit much higher specific loss powers (~100 W/g or more). Apart from a higher thermal dissipation acting on the membrane fluidity and hence on the diffusion constants, those larger USPIOs will be partially ferrimagnetic, *i.e.* with a magnetic anisotropy energy $E_a > k_B T$. This might introduce another mechanism of membrane permeation, by direct rotation of the grains at the frequency of the oscillating magnetic field. Such a mechanism would be reminiscent of the "molecular drill" effect¹²³⁻¹²⁵ predicted long ago for lipid bilayers under mechanical stress by adsorption onto a corrugated surface.

To summarize, by exhibiting biocompatibility of the polymeric matrix, ease of preparation, contrast enhancement in MRI and triggered release under RF oscillating field, those hybrid vesicles are good candidates for the magneto-chemotherapeutic treatment of cancer. This work evidenced for the first time the concept of multi-functional polymersomes to combine imaging and therapy, opening new avenues to improve cancer treatments and to understand their mechanisms. The impact of such theranostic systems on tumor regression is currently under investigation.

EXPERIMENTAL DETAILS

Materials and syntheses

Polymer, drug and buffers. PTMC₂₄-*b*-PGA₁₉ diblock copolymer was synthesized by ring-opening polymerization (ROP) of γ -Benzyl-L-glutamate *N*-carboxyanhydride initiated by an amino functionalized PTMC macroinitiator upon a previously published method.⁸² All the experiments were conducted on a PTMC₂₄-*b*-PGA₁₉ ($M_n = 4900$ g/mol) block copolymer which presents a hydrophilic weight fraction of 50 wt % and a molar mass dispersity of 1.15. The solvent for nanoprecipitation (DMSO) was used without prior purification. Doxorubicin hydrochloride (CAS: 25316-40-9) was supplied by Discovery Fine Chemicals (Wimborne, UK). DOX was reconstituted in DMSO, stored at 5 °C and used within one month. Sodium chloride, Tris-HCl and Tris base were provided by Sigma.

Iron oxide nanoparticles. Superparamagnetic nanoparticles of maghemite (γ -Fe₂O₃), also called USPIOs, were synthesized by alkaline coprecipitation of iron(II) and iron(III) salts⁷⁵ and sorted according to their size by fractionated phase separations.⁷⁶ Briefly, the ionic strength was increased to screen the electrostatic interactions between the nanoparticles and obtain successive fractions of narrower size distribution, as measured all along the sorting process by vibrating sample magnetometry (VSM) and on the final sample by scattering techniques (SLS, DLS and SANS). For dispersion in CH₂Cl₂, the surface of the nanoparticles was grafted by the anionic surfactant Beycostat NB09 (CECA, Arkema group, France) used to disperse inorganic pigments in aromatic and chlorinated oils (but insoluble in aliphatic solvents), which is a mixture of mono- and diesters of phosphoric acid. The grafting procedure (30 min at 60°C, 20 mol% relatively to iron) was previously described.⁷⁷

Preparation of empty, USPIO loaded and DOX/USPIO dual-loaded vesicles

Carbonate buffer (pH 10.5, 50 mM, 4.5 mL) was added onto PTMC₂₄-*b*-PGA₁₉ (5 mg) dissolved in DMSO (0.5 mL) under magnetic stirring (1000 rpm) in a plastic tube (1.5 cm diameter), leading to a homogeneous dispersion of vesicles. A syringe pump controlled the water flow rate during injection. Two addition durations (5 seconds and 15 minutes respectively) of water solution into DMSO were used in order to tune the final average vesicle size. The resulting samples were respectively called WDi (for “instantaneous”) and WD15. The organic solvent was then removed by extensive dialysis against 4 L Tris buffer replaced at least twice (10 mM Tris, pH 7.4, 25 °C ionic strength 150 mM).

USPIO loading was performed at different feed weight ratios (FWR) ($\text{wt}_{\text{USPIO}}/\text{wt}_{\text{copolymer}}$) using the same nanoprecipitation method. A negligible volume of USPIO suspension in CH₂Cl₂ (e.g. $V_{\text{CH}_2\text{Cl}_2}/V_{\text{water}}=0.55\%$ for $\text{wt}_{\text{USPIO}}/\text{wt}_{\text{polymer}}=50\%$) was added into the DMSO/copolymer solution prior to the addition of water. For DOX/USPIO dual-loaded vesicles, doxorubicin hydrochloride was at first solubilized in the DMSO/copolymer solution at 2 mg/mL before mixing with the USPIOs. After water addition, organic solvent and free DOX were removed by dialysis for 4h with a membrane of 3500 g/mol MWCO against 4 L Tris buffer (10 mM Tris, pH 7.4; 30 °C, ionic strength 150 mM). The doxorubicin loading content (LC) was determined after vesicle rupture using sonication in a mixture containing 80 % volume of DMSO. This solvent mixture induced the aggregation of USPIOs that were then separated by centrifugation (1h, 10000 rpm). Then the titration of DOX was performed from the UV absorbance at $\lambda_{\text{max}}=485$ nm using the known value for doxorubicin in a DMSO/Tris buffer (80/20 v/v) mixture as calibration (see Supporting Information S-g).

Experimental Methods

Dynamic light scattering (DLS) and static light scattering (SLS) were performed using an ALV Laser goniometer, which consisted of a 35 mW HeNe linear polarized laser with a wavelength of 632.8 nm and an ALV-5000/EPP Multiple Tau Digital correlator with 125 ns initial sampling time. The samples were kept at constant temperature (25 °C) during all the experiments. The accessible scattering angle range ranged from 30° up to 150°. However, most of the dynamic measurements were carried out at 90°. Aliquots of the samples (1 mL in a 10 mm diameter cylindrical glass cell) were immersed in a filtered toluene bath. The data acquisition was done with the ALV-Correlator Control software and the counting time for DLS was fixed for each sample at 30 s. To perform light scattering in static mode, the differential refractive index increment dn/dc of PTMC₂₄-*b*-PGA₁₂ vesicles in buffer was measured over a concentration range from 0.2 to 1 mg/mL by means of a differential refractometer (Wyatt Optilab rEX) operating at a wavelength of 658 nm and at 25°C. A dn/dc value of $0.3454 \pm 6 \cdot 10^{-4}$ mL/g was obtained for WDi vesicles loaded with 50 wt % USPIOs, which is larger than the value $dn/dc=dn/d\Phi d^{\text{USPIO}}=1.08/5.1=0.21$ mL/g reported for pure USPIOs coated with the same Beycostat surfactant.¹²⁶ The mean hydrodynamic radii and polydispersity indexes (PDI) were determined using the 2nd order cumulant analysis.

Isotropic Small Angle Neutron Scattering (SANS) measurements were performed on the PAXY spectrometer of the Laboratoire Léon Brillouin (CEA-Saclay, France) equipped with a two dimension detector made of 128×128 cells. We used two configurations: the first one with a sample-to-detector distance of $D=6.7$ m and a neutron wavelength of $\lambda=10$ Å to cover a q range of $2.5 \cdot 10^{-3} - 2.5 \cdot 10^{-2}$ Å⁻¹; the second one with $D=2$ m and $\lambda=6$ Å to cover a q range of $2 \cdot 10^{-2} - 0.2$ Å⁻¹. Full angular averaging of the detector cells at constant q was realized for the scattering patterns with the PASINET software available at www-llb.cea.fr.

The samples were prepared by nanoprecipitation, centrifuged and redispersed in the desired mixture of hydrogenated and deuterated solvents at a final concentration of 10 mg/mL. Three solvents were used in order to match the scattering length densities of the various components of the loaded magnetic polymersomes and to focus the contrast on selected features (see Supporting Information S-a). The magnetic scattering length density of the USPIOs estimated from the magnetization at saturation M_s and the volume of the nanoparticles was $\rho_{\text{mag}}^{\text{USPIO}} \approx 10^{10}$ cm⁻². One solvent was pure H₂O, which allowed observing mainly the nuclear scattering of USPIO but also in a reduced way the copolymer signal. Pure D₂O almost matched the nuclear signal of the USPIOs: this scattering intensity revealed the fluctuation of the polymeric membrane together with the magnetic scattering of the USPIOs. Finally, the use of a H₂O/D₂O (65.6/34.4 v/v) mixture matching the copolymer scattering length density enabled to focus on the nuclear scattering of the USPIOs only. The calculated contrast of neutrons scattering-length densities between γ -Fe₂O₃ and this H₂O/D₂O mixture was $\Delta\rho = 5 \cdot 10^{10}$ cm⁻². SANS measurements were done in 5 mm thick quartz cuvettes for D₂O or 1 mm thick ones for H₂O and H₂O/D₂O solvents to minimize the incoherent scattering. All the scattered intensity curves were corrected from the incoherent background of their proper solvents. They have been also normalized by the incoherent signal delivered by a 1 mm gap water sample in order to account for the efficiency of the detector cells. Absolute values of the scattering intensity, $I(q)$ in cm⁻¹, were obtained from the direct determination of the number of neutrons in the incident beam and the detector cell solid angle.^{127, 128}

Here we mainly discuss the SANS signal obtained with USPIO loaded polymersomes' suspensions in the H₂O/D₂O mixture, which matches the copolymer. Following a method used for other kinds of nanocomposites made from colloids or

micelles,^{86, 87} the SANS curves of the USPIO loaded vesicles were divided by the volume fraction Φ_{USPIO} and by the form factor of the USPIO nanoparticles measured independently on a dilute solution. This procedure yields the intra-aggregate structure factors $S_{\text{intra}}(q)$ of the USPIO nanoparticles, which tell us about their spatial arrangement into aggregates of a given geometry (micellar, vesicular, fractal...). The calculated form factor of hollow shells took into account their radius, membrane thickness, dispersity and the experimental resolution of the spectrometer.⁸⁸

Anisotropic SANS measurements. The sample was placed between the poles of an electromagnet producing a homogeneous magnetic field at the sample position, as checked by a Hall probe (Walker scientific). The solvent used was pure H₂O which does not match the nuclear scattering length density of the copolymer but is insuring a negligible magnetic scattering of iron oxide ($\Delta\rho = 1.4 \cdot 10^{10} \text{ cm}^{-2}$ between H₂O and the magnetic scattering length density of iron oxide). Nevertheless, the nuclear contrast of the USPIO ($\Delta\rho = 7.5 \cdot 10^{10} \text{ cm}^{-2}$) remained still three times larger than the one of the copolymer ($\Delta\rho = 2.5 \cdot 10^{10} \text{ cm}^{-2}$). The scattering intensity being proportional to the square of the contrast, we can neglect the contribution arising both from the copolymer and from the magnetic moments of the USPIOs in the total scattered intensity. An anisotropic analysis was applied to the scattering patterns obtained under magnetic field. To obtain anisotropic curves with a good statistics, the intensity on the 2D-detector was averaged in angular sectors either $[-30^\circ; 30^\circ]$ along the field direction where the scattered intensity was weaker, and thus called $I^{\parallel}(q)$, or $[-15^\circ; 15^\circ]$ around the perpendicular direction and denoted $I^{\perp}(q)$.

Magnetization measurements. The magnetization curves of the maghemite USPIOs and of the USPIO-loaded vesicles were determined using a home-made vibrating sample magnetometer (VSM) under an applied magnetic field up to 0.93 Tesla. From the shape of the magnetization *versus* field intensity curve $M(H)$, the size distribution of the magnetic cores was obtained by convolving the first order Langevin's law of paramagnetism $L_1(\xi) = M/\Phi m_s = \coth(\xi) - 1/\xi$ with $\xi = \mu_0 m_s \pi D^3 H / 6 k_B T$ (k_B is the Boltzmann constant and μ_0 the vacuum magnetic permeability) with a Log-normal probability law of median diameter $D_{\text{mag}}^{\text{USPIO}}$ and width σ , defined as the standard deviation of the distribution $\text{Ln}(D/D_{\text{mag}}^{\text{USPIO}})$.^{80, 81}

Magnetic birefringence. The setup that has been described precisely¹²⁹ was improved for temperature control. Briefly, it consisted in an electromagnet used to magnetically induce a macroscopic birefringence in a magnetic colloid made of birefringent magnetic nanoparticles (or made of nano-objects filled with such MNPs). This induced birefringence was then measured by sending a linearly polarized He/Ne laser beam (10 mW) through the birefringent sample and analyzing the transmitted light with a second polarizer and a photodiode. A photo-elastic modulator and a lock-in amplifier were used to increase the setup sensitivity, the resulting AC and DC signals being related respectively to the levels of birefringence and dichroism under the applied magnetic field. To perform measurements at various controlled temperatures, the glass cell containing the sample was put in a specifically designed copper cell, which temperature was regulated using a Pt100 temperature probe and Peltier devices connected to a current source and externally controlled by a PC using NI LabVIEW.

Magnetophoresis. A magnetophoretic experiment consists in measuring the constant velocity reached by magnetic objects in a magnetic field of increasing intensity (spatial gradient), applying on them a magnetic force balanced by a viscous drag one.^{62, 92, 93, 95, 97, 104} In our case, a drop of an aqueous vesicle solution was placed between a glass slide, a 200 μm spacer and a cover slip to prevent evaporation and convection. This cell was mounted on the stage of an inverted optical microscope (Leica DM-IL). A strong NdFeB magnet of 22 mm diameter

and 10 mm thickness (Aimants Calamit, France) was held by a clip 6 mm away from the centre of the focus plane of the microscope. Bright field optical microscopy images taken with a 40X objective were recorded with a digital camera (Infinity3-1U, Lumenera, Ottawa, Ontario, Canada) enabling pixel-binning to enhance the recording rate. The magnetophoretic trajectories of about 280 vesicles exhibiting a biased thermal motion toward the magnet were recorded at video rate (24 frames/s). Three sequences (each containing 240 frames of 800x600 pixels) were analyzed off-line using the "ParticleTracker" plug-in developed by the MOSAIC group at ETH Zürich for the free image processing software ImageJ with the following parameters: Kernel radius= 6, Cutoff radius= 0.0, Percentile= 0.6, Displacement= 5.0, Linkrange= 120).¹³⁰ Each stack of 240 frames (10 s duration) necessitated a computing time of 16 min with a 64-bit desktop PC with 4Gb RAM. For theoretical calculations of the number of USPIOs per vesicle from the average drift velocity, a magnetic field gradient $\text{dB}/\text{dz} = 18.5 \text{ mT/mm}$ and an average magnetic flux density $B_0 = \mu_0 H_0 = 174 \text{ mT}$ were used as reported for an identical magnet.⁹³

MRI Relaxometry. For different USPIO-vesicle formulations, T_1 and T_2 relaxivities were measured at 4.7 T ($f_{\text{Larmor}} = 200 \text{ MHz}$) on a research MRI system (Bruker Biospec 47/50, Ettlingen, Germany) at 20°C. The transverse T_2 measurements were acquired using a multiple spin-echo 2D imaging sequence ($T_R = 10000 \text{ ms}$; inter echo-time, 5 ms; number of echo images, 256; FOV, 50x50 mm; matrix, 128x128; slice thickness, 1 mm). The longitudinal relaxation times T_1 were obtained out using an inversion-recovery 2D imaging sequence (increment of inversion delay: 34 ms with 456 increments) followed by a RARE imaging sequence (RARE Factor: 8; T_R/T_E^{eff} : 10000/7.7 ms; FOV: 50x50 mm; matrix: 128x128; slice thickness: 1 mm). The relaxivity values r_1 and r_2 were calculated by linear fits of the relaxation rates $1/T_1$ and $1/T_2$ (s^{-1}) vs. iron concentration (mM) or vesicle concentration (nM).

Iron titration. The total iron concentration (mol/L) was determined by atomic absorption spectroscopy (AAS) with a Perkin-Elmer Analyst 100 apparatus after degrading the USPIO-loaded vesicles in boiling HCl (35%). The volume fraction of iron oxide was deduced from the molar mass (159.7 g/mol) and mass density (5.1 g/cm³) of $\gamma\text{-Fe}_2\text{O}_3$, i.e. numerically $\Phi_{\text{USPIO}} (\% \text{ v/v}) = 1.577 [\text{Fe}] (\text{mol/L})$.

Electrophoretic mobility. Empty and loaded vesicles were analyzed with a ZetaSizer NanoZS (Malvern Instruments, Worcestershire, UK). The electrophoretic mobility (μ) was converted into zeta potential (ζ) using Smoluchowski's approximation, which is valid since the vesicles are all much larger than the Debye length κ_D^{-1} of the buffers ($\kappa_D D_H \gg 1$). All the measurements were performed at 25°C and the data were at least the average of triplicate values.

Transmission electron microscopy. TEM images were recorded on a Hitachi H7650 microscope working at 80 kV equipped with a GATAN Orius 11 Megapixel camera. Samples were prepared by spraying a 1 mg/mL solution of the vesicles onto a copper grid coated with carbon (200 mesh) using a homemade spray tool.

TEM with negative staining. USPIO-loaded polymer vesicles (0.04 mg/mL in water) were adsorbed on a carbon-coated EM grid and negatively stained with 1% uranyl acetate. TEM was performed with a CM120 (FEI) microscope.

Cryo-TEM imaging. USPIO-loaded polymer vesicles (2 mg/mL in water) were deposited on an EM grid coated with a perforated carbon film. After draining the excess liquid with a filter paper, grids were quickly plunged into liquid ethane and mounted onto a Gatan 626 cryoholder. TEM was performed with a Tecnai F20 (FEI) microscope operated at 200 kV. The images were recorded with a 5 Megapixel USC1000-SSCCD camera (Gatan).

Atomic force microscopy. AFM images were recorded in air with a Nanoscope IIIa microscope operating in dry Tapping-mode. The probes were commercially available silicon tips with a spring constant of 42 N/m, a resonance frequency of 285 kHz and a typical radius of curvature in the 10–12 nm range. Freshly cleaved mica was used as sample substrate materials. For the observation of empty and USPIO loaded vesicles, sample solutions in water at concentrations of 0.01 mg/mL and 0.1 mg/mL respectively were deposited on the substrate (20 μ L) and dried under vacuum at 40 °C for 12 hours.

In vitro DOX release. The required quantity of drug-loaded vesicles was poured into a dialysis tubing (Spectra/Por Float-A-Lyzer, 50 000 g/mol MWCO, 10 mm diameter, 10 mL volume). The dialysis membrane filled with 5 mL of DOX loaded polymersomes was introduced into a bath of 50 mL buffer (10 mM Tris, pH 7.4, ionic strength 150 mM). At each sampling point, sink conditions were maintained by replacing 2 mL of the outer medium reservoir by fresh buffer. Because of the known sensitivity of DOX to degradation, the amount of released drug was calculated by the difference between the initial drug content and the drug remaining at each sampling point in the suspension of vesicles. More precisely, a spectrophotometric measurement at $\lambda_{\text{max}} = 485$ nm was performed on an aliquot taken inside the dialysis bag. To take into account absorption by iron oxide and turbidity, the DOX concentration was calculated from the measured absorbance using a calibration curve in water after subtracting the absorbance value of similar USPIO-loaded vesicles. Another method consisted in redispersing the vesicles inside the aliquots into individual components (USPIOs, molecular DOX and copolymer unimers) in a DMSO:Tris (80:20) mixture before measuring the absorbance. Their calibration curves being provided as Supporting Information S-g, both methods led to comparable results, attesting the reliability of the measurements.

In vitro DOX release under an oscillating RF magnetic field. We used a RF generator built at the ICMCB laboratory in Pessac, France.^{131, 132} An alternating magnetic field with $f_{\text{RF}} = 500$ kHz frequency and mean field intensity $H_0 = 2.12$ kA/m (induction $B_0 = \mu_0 H_0 = 2.65$ mT) was generated by a 28-turn pancake coil (20 cm height) cooled by a water circulation. The frequency was adjusted by a Celes inductor C97104 (Celem Passive Components, Israel). The electrical current was provided by a wave generator (FI1202, Française d'Instrumentation, France) connected to a power amplifier (AR Worldwide 800A3, 10kHz–3MHz, EMV, France). The vesicles were prepared as usual then diluted by a factor 2. A dialysis bag filled with half of the dispersion (4 mL) was placed inside a plastic cylindrical vessel filled with 30 mL Tris buffer, fitting inside the coil of the above described setup. The release profile of the other half was performed in the same conditions of volumes, vessels and ambient temperature of the room (23 °C), but kept far away from the magnetic field as a control experiment of release without RF field. In particular, both reservoirs of release medium were not stirred to avoid any parasitic heating due to the presence of a magnetic bar inside the RF magnetic field.

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Supporting Information Available

Contrasts and treatments of the SANS data of $\gamma\text{-Fe}_2\text{O}_3$ USPIOs embedded within the membrane of poly(trimethylene

carbonate)-*b*-poly(L-glutamic acid) (PTMC-*b*-PGA) vesicles. Shape anisotropy of the vesicles and variation of their membrane thickness under magnetic field. SANS study of magnetic vesicles in D_2O . Magnetization of USPIO-loaded vesicles. Magnetic birefringence of USPIO-loaded vesicles. Calibration curves for DOX titration by spectrophotometry. Additional AFM images of bare and USPIO-loaded vesicles. Static light scattering (Zimm plot) of USPIO-loaded vesicles. Movies of the pure Brownian motion of vesicles (without magnetic field) and of their drift motion by magnetophoresis (under a field gradient). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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101. The magnetic moment of the vesicle m is directly related to μ^{USPIO} , the average magnetic moment of each of the encapsulated USPIO particles and to their degree of orientation under a mean field intensity value B_0 given by Langevin's first order function $L_1(\xi)$ with $\xi=\mu^{USPIO}B_0/k_B T$ according to $m=N^{USPIO} \times \mu^{USPIO} \times L_1(\xi)$, where N^{USPIO} is the number of USPIO per vesicle. The magnetic moment per nanoparticle μ^{USPIO} is simply the product of the specific magnetization m_s by V_{USPIO} . For the mean magnetic field intensity $B_0=174$ mT in this magnetophoretic experiment, the USPIO-loaded vesicle magnetization M corresponds to 55 % of its saturation value M_s ($L_1(\xi)=0.55$, as seen on Figure S-4).
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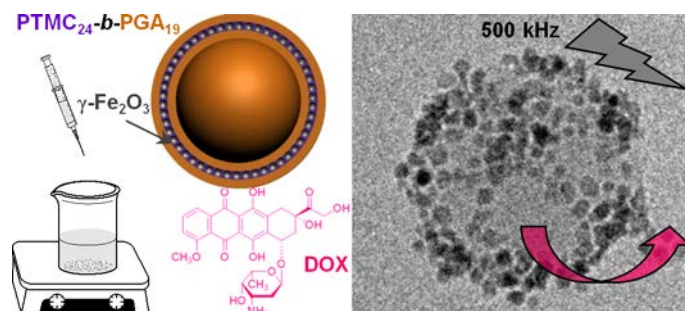
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TOC Graphic



Incorporation of magnetic nanoparticles into lamellar polystyrene-*b*-poly(*n*-butyl methacrylate) diblock copolymer films: influence of the chain end-groups on nanostructuration

Siham Douadi-Masrouki^{a, b, c, 1}, Bruno Frka-Petescic^{a, b, c, 2}, Maud Saved^{d, e, 3}, Bernadette Charleux^{d, e, 4}, Valérie Cabuil^{a, b, c, 5}, Olivier Sandre^{*, a, b, c, 6}

^a UPMC Univ Paris 6, UMR 7195 Physicochimie des Electrolytes, Colloïdes et Sciences Analytiques – 4 place Jussieu, case 51 75005 Paris France

^b Centre National de la Recherche Scientifique, UMR 7195 PECSA, 75005 Paris France

^c ESPCI ParisTech, UMR 7195 PECSA, 75005 Paris France

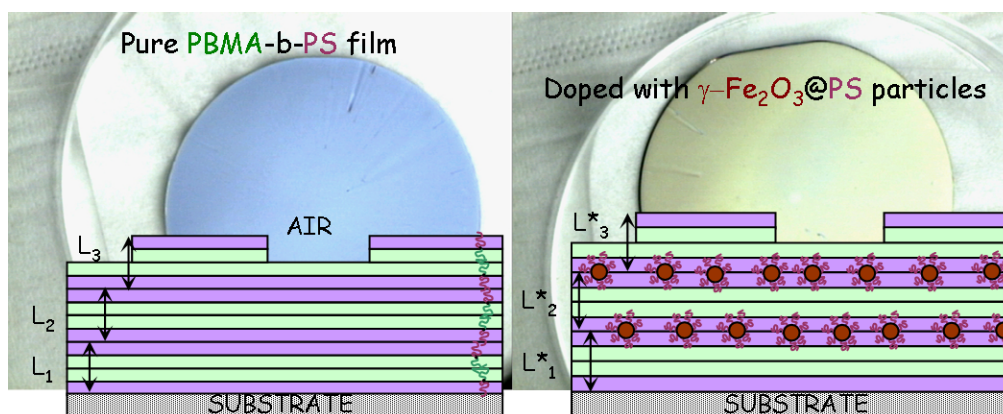
^d UPMC Univ Paris 6, UMR 7610 Laboratoire de Chimie des Polymères – 4 place Jussieu, case 185 75005 Paris France

^e Centre National de la Recherche Scientifique, UMR 7610 LCP, 75005 Paris France

*corresponding author: olivier.sandre@upmc.fr, tel: +33-5-4000-3695, fax: +33-5-4000-8487

Abstract: In this article, we present new samples of lamellar magnetic nanocomposites based on the self-assembly of a polystyrene-*b*-poly(*n*-butyl methacrylate) diblock copolymer synthesized by atom transfer radical polymerization. The polymer films were loaded with magnetic iron oxide nanoparticles covered with polystyrene chains grown by surface initiated-ATRP. The nanostructuration of the pure and magnetically loaded copolymer films on silicon was studied by atomic force microscopy, ellipsometry, neutron reflectivity and contact angle measurement. The present study highlights the strong influence of the copolymer extremity – driven itself by the choice of the ATRP chemical route – on the order of the repetition sequences of the blocks in the multi-lamellar films. In addition, a narrower distribution of the nanoparticles' sizes was examined as a control parameter of the SI-ATRP reaction.

Keywords: Polymer Synthesis, Polymer Physical Chemistry, Polymer Composite Materials.



Graphical Abstract: Sketches of the nanostructure of lamellar films of PBMA425-*b*-PS490 block copolymer either pure or doped with magnetic nanoparticles coated by a PS brush and macroscopic pictures of the films on silicon wafers (respectively nb. 2 and 13 in Table 6).

¹ siham.douadi@gmail.com

² bruno.frka-petescic@upmc.fr

³ Present address: Université de Pau et des Pays de l'Adour – CNRS, UMR 5254 IPREM Equipe Physique et Chimie des Polymères – Technopole Hélio parc, 2 Av P. Angot, 64053 Pau France, maud.save@univ-pau.fr

⁴ Present address: Université Claude Bernard Lyon 1 – CNRS – CPE, UMR 5265 Chimie, Catalyse, Polymères et Procédés – CPE Lyon Bât 308, 43 Bd du 11 Novembre 1918, 69616 Villeurbanne France, bernadette.charleux@lcpp.cpe.fr

⁵ valerie.cabuil@upmc.fr

⁶ Present address: Université Bordeaux 1 – CNRS – Institut Polytechnique de Bordeaux, UMR 5629 Laboratoire de Chimie des Polymères Organiques – 16 Av Pey Berland, 33600 Pessac France, olivier.sandre@ipb.fr

1. Introduction

In order to design new materials with multi-functional properties, chemists elaborate composite materials combining an organic matrix with inorganic fillers. While being homogeneous at the macroscopic scale, those materials usually contain characteristic internal structures of different geometries (points, cylinders, planar or curved surfaces...) that can exhibit a long-range order (*e.g.* lamellar or cylindrical mesophases) responsible for the improvement of physical properties (mechanical resistance, special anisotropy, response to specific stimuli...). One elegant way to engineer a nanostructured matrix loaded with inorganic nanoparticles somehow organized at a large scale relies on their co-assembly with block copolymers (BCP) into complex architectures.¹ Our work focus on the case of lamellar materials where the alternating layers are made of diblock copolymers and iron oxide magnetic nanoparticles (MNP), which open new applications as optical coatings with specific reflection or guiding properties. An ordered polymer matrix with a lamellar morphology at the mesoscopic scale can be obtained by depositing a melt of a symmetrical diblock copolymer onto a flat substrate. In spite of the weak incompatibility of polystyrene (PS) and poly(*n*-butyl methacrylate) (PBMA), the existence of ordered layers has been shown for molten films of symmetrical PS-*b*-PBMA diblock copolymers of different molar masses provided that they are annealed at an appropriate temperature.² According to the literature on thin nanocomposite films, one can maintain a lamellar morphology while doping certain blocks of the BCP if the volume fraction of inorganic nanoparticles is sufficiently low and if their diameters are smaller than a fraction of the pure BCP lamella thickness (typically 20-30%).³ In addition, the surface of the nanoparticles must be grafted by a polymer of the same nature as one of the blocks, as it was shown for gold nanoparticles with various BCPs.⁴ When using MNPs, such hybrid lamellar materials are of particular interest for technological applications, because they combine the low processing temperature of the polymer matrix (compared to standards in electronics industry) with the outstanding properties of magnetic fillers. On the one hand, MNPs interact strongly with electromagnetic waves over a broad spectrum of wavelengths: magnetic birefringence, circular dichroism and Faraday rotation in the UV-Vis range,⁵ Brown's and Néel's relaxations in radiofrequency (kHz-MHz) magnetic fields inducing hyperthermia,⁶ ferromagnetic resonance (FMR) causing a strong absorption of microwaves (GHz).⁷ On the other hand, a long-range order as multi-layers of MNPs could select propagation modes of the waves and give rise to Bragg-type reflection also called "optical-band gaps" or "photonic crystals".⁸ In this article, we focus our attention on the insertion into a lamellar symmetrical BCP film of iron oxide MNPs, exhibiting superparamagnetism. Those MNPs behave indeed as "soft magnets", each of them bearing a large magnetic moment (typically 10^4 Bohr's magnetons) that orients in an external magnetic field but retains no magnetization in a zero field due to thermal agitation.

This work differs from reported studies that dealt with metallic MNPs (FePt alloy) deposited at the surface of thin Polystyrene-*b*-poly(methyl methacrylate) films:⁹ on the one hand the PS-*b*-PMMA lamellae were not parallel but perpendicular to the substrate, on the other hand the FePt MNPs were not confined by a chemical coating but rather trapped physically by corrugations of the surface. The goal of those studies was indeed to take benefit from a lamellar period of 30 nm to control the surface density of thermally blocked magnetic dipoles of the FePt face-centered tetragonal phase¹⁰ (above 500°C) in order to get magnetic storage materials with increased capacity. Other early studies on thin films of symmetrical polystyrene-*b*-poly(*n*-butyl methacrylate) copolymers have underlined the importance of the conditions of film deposition (*e.g.* substrate smoothness) and annealing (temperature, hydrostatic pressure and time length) on the quality of the lamellar structure. Their authors examined the defects at the top surface of the films^{11,12}, the width and height of the scattering peak¹³ and the lateral correlation length ξ of the nanostructure.¹⁴ Later studies described lamellar magnetic nanocomposites made from PS-*b*-PBMA and iron oxide MNPs synthesized separately and then co-assembled by depositing a mixture in solution onto a flat substrate and annealing the films above the *T_g* of the PS blocks.¹⁵⁻¹⁸ Other teams studied the alternative route that consists in using a preformed lamellar structure of BCP as the template for the synthesis of MNPs either from a vapour¹⁹ or a liquid phase²⁰, as it was also proposed for an isotropic PS matrix²¹ or for poly(acrylic acid)-*g*-poly(*n*-butyl acrylate) cylindrical brushes²². The studies on PS-*b*-PBMA were continued for doped films by the quantification of the distortion by the MNPs of the lamellar order (through the decrease of ξ)²³ and by the kinetics of growth of defects at the film surface for undoped ones.²⁴ Until now, all the articles on nanostructuration with PS-*b*-PBMA share the common feature of utilizing copolymers synthesized by anionic polymerization. The most recent article on PS-*b*-PBMA revealed the strong influence of the chemical nature of the chain end-groups on its phase diagram.²⁵ More precisely, maleic anhydride end-groups induce the perforation of the lamellae above 245°C, which is not observed for carboxylate end-groups. An important question raised by our present work is the influence of end-groups resulting from the polymerization scheme (controlled radical *vs.* anionic polymerization) onto the wetting properties of the lamellae both on the hydrophilic substrate and at the top layer. Whereas the synthesis of PS-*b*-PBMA diblock copolymers by anionic polymerization is well documented,²⁶⁻²⁸ only one article described the synthesis of PS-*b*-PBMA diblock copolymers by controlled radical polymerization (CRP).²⁹ Among the CRP methods, atom transfer radical polymerization (ATRP) is a convenient pathway to synthesise well-defined methacrylic based copolymers such as PMMA-*b*-PS 30 or Pi-BMA-*b*-PS.³¹ We report here on the synthesis of well-defined PBMA-*b*-PS symmetric diblock copolymers synthesized by ATRP in bulk. The challenge consisted in synthesizing well-defined PBMA-

b-PS diblock copolymers *via* ATRP with sufficient high molar masses to reach the segregated state. In most of the previous studies, the MNPs made of maghemite iron oxide were coated with a PS brush of molar mass 13000 g.mol⁻¹ obtained by a “grafting onto” technique³² using sulfonate-terminated PS chains also synthesized by anionic polymerization and end-functionalized by 1,3-propane sultone.³³ Here we describe an efficient synthesis of the same type of inorganic magnetic nanoparticles grafted with a PS shell by surface initiated polymerization also called “grafting from”. Due to the popular success of ATRP as a versatile controlled radical polymerization technique, the coating of iron oxide nanoparticles with different polymers using surface initiated (SI)-ATRP has been described recently by other groups.^{34–36} The add-on value of our study consists in polymerizing PS from the surface of MNPs with only moderate aggregation issue and inserting them into the lamellae of PBMA-*b*-PS while keeping the long-range order as evidenced by several surface techniques such as ellipsometry, contact angle measurement and neutron reflectivity. The synthesis of PBMA-*b*-PS by ATRP offers us the opportunity to study the influence of a halide chain end-group onto the lamellar ordering of the diblock copolymer, in comparison with the previous studies^{11–18, 23–27, 29} where the PS-*b*-PBMA samples were synthesized by anionic polymerization, leading to H-terminated chains²⁷ (see Scheme 1 in the Electronic Supplementary Material file).

2. Experimental

2.1 Materials

Styrene (S) and *n*-butyl methacrylate (BMA) (Aldrich) were distilled prior to use in order to remove the inhibitor. *N,N',N'',N'''*-pentamethyldiethylenetriamine (PMDETA), copper (I) bromide CuBr, copper (II) bromide CuBr₂, copper (I) chloride CuCl, copper (II) chloride CuCl₂ (Aldrich), benzonitrile, ethyl-2-bromoisobutyrate (EBrIB) (Aldrich), hexane and toluene were used as received.

2.2 Synthesis of PBMA-*b*-PS diblock copolymer

Synthesis of PBMA macroinitiator by ATRP. A solution of BMA (15 g, 10.5 mmol), PMDETA (46 mg, 0.26 mmol), and EBrIB (51 mg, 0.26 mmol) was degassed by nitrogen bubbling during 30 minutes. The degassed solution was transferred under a nitrogen flow *via* a double-tipped stainless steel needle into the polymerization flask containing the copper halide. The reactor was immediately immersed in an oil bath thermostated at 100°C for the required polymerization time. The polymer solution was diluted in dichloromethane and passed through a neutral alumina column to remove the copper catalyst. The recovered PBMA homopolymer solution was precipitated in a large excess of methanol, and dried under vacuum at room temperature.

*Synthesis of the PBMA-*b*-PS diblock copolymer by ATRP.* A solution containing the PBMA macroinitiator (5 g, 0.11 mmol), styrene (11.13 g, 10.7 mmol) and PMDETA (19 mg, 0.11 mmol) was prepared prior to be degassed by nitrogen bubbling during 30 minutes. The introduction of the copper halide (CuCl) under nitrogen flow into the polymerization flask thermostated at 100°C marked the time zero of the polymerization. After the required time of reaction, the crude polymer solution diluted with dichloromethane was passed through a neutral alumina column. The recovered diblock copolymer was subsequently precipitated into methanol and the residual PBMA block was removed by extraction with hexane.

2.3 Synthesis of γ -Fe₂O₃@PS nanoparticles

Aqueous synthesis of γ -Fe₂O₃ nanoparticles.

Superparamagnetic nanoparticles made of maghemite (γ -Fe₂O₃) were synthesized in water according to Massart's procedure.³⁷ At first, magnetite Fe₃O₄ nanocrystals (also called ferrous ferrite FeO.Fe₂O₃) were prepared from an alkaline coprecipitation of a quasi-stoichiometric mixture of iron +II (1 mole) and iron +III (1.4 mole) salts in an acidic medium (HCl pH≈0.4). One litre of a concentrated ammonia solution (20 %) was quickly added onto the acidic iron salts mixture, which produced a black solid suspension almost instantaneously. Those Fe₃O₄ nanoparticles were first acidified with 0.36 L of nitric acid (52%) then oxidized by adding a ferric nitrate solution (0.8 mole). After 30 min at boiling temperature, the suspension had turned to a red colour characteristic of maghemite γ -Fe₂O₃ nanoparticles. After washing steps in acetone and diethyl-ether to remove the excess ions, the nanoparticles are readily dispersed in water and form a true “ionic ferrofluid”, made of maghemite nanoparticles with a positively charged surface, which remain in a monophasic state under the application of a magnetic field of arbitrary value. Those crystals exhibit a Log-Normal distribution of diameters with an average around 7 nm and a standard deviation σ about 0.4, as measured by magnetometry (ESI-Figure 1). This aqueous ferrofluid is then coated by oleic acid to obtain an oily ferrofluid,³⁸ which is a dispersion of magnetic nanoparticles stabilized by steric repulsions in an organic medium (n-hexane) compatible for the synthesis of γ -Fe₂O₃@PS nanoparticles. Briefly, the grafting reaction was performed by mixing the aqueous MNPs with 20 mol% of ammonium oleate (firstly obtained by deprotonation of oleic acid with ammonia) for 30 min at 70°C. The resulting precipitate was washed 3 times with methanol and finally with diethyl ether in order to remove the excess surfactant and all traces of water until redispersion in n-hexane. Once coated by oleate, the MNPs exhibit a low hydrodynamic diameter in n-hexane (between 26 and 36 nm depending on the samples as measured by DLS in dilute suspensions) with a solid content that can be concentrated up to 30 % w/w, the solid weight being composed at 75±5% of iron oxide as evidenced by iron titration and gravimetry.

In the following experiments, we have used either maghemite nanoparticles with a relatively high size polydispersity ($\sigma=0.4$) or suspensions which have been treated with a size-sorting procedure by fractionated phase-separation enabling to decrease the standard deviation down to $\sigma=0.2$ and that will be named thereafter “monodisperse” magnetic nanoparticles.³⁹

Synthesis of γ -Fe₂O₃@PS nanoparticles by surface-initiated polymerization (SI-ATRP).

As described previously, an ATRP initiator was coated onto the γ -Fe₂O₃@oleate nanoparticles *via* a ligand-exchange reaction.⁴⁰ More precisely, the surfacted ferrofluid (28.3 mmol iron) was mixed with 2-bromo-2-methylpropionic acid (8.5 mmol BrMPA) in *n*-hexane (30mL). The atmosphere of the mixture was replaced with nitrogen by freeze-thawing and pumping cycles using a Schlenk tube equipped with a magnetic stirrer and a Rotaflow valve. After 72 h of stirring at room temperature under inert atmosphere, a magnetic precipitate was obtained. After several washes with *n*-hexane to remove oleic acid, the BrMPA-functionalized γ -Fe₂O₃ nanoparticles were readily dispersed in styrene. To achieve SI-ATRP, the mixture of γ -Fe₂O₃@BrMPA, styrene and PMDETA was carefully degassed by nitrogen bubbling during 30 min. Then it was transferred through a cannula into the polymerization flask already containing copper bromide. The reactor was immersed in an oil bath thermostated at 100°C for the required polymerization time. The product was diluted in dichloromethane and filtrated through a Fluoropore™ membrane to remove the copper catalyst⁴¹ or simply washed by water. After this purification step, the γ -Fe₂O₃@PS core-shell nanoparticles were precipitated in a large excess of methanol and dried out on a Büchner. The brownish powder could be readily dispersed in dichloromethane or toluene for characterization or further utilization.

Raw and grafted nanoparticles were characterized using vibrating sample magnetometry (VSM), atomic absorption spectroscopy (AAS), transmission electron microscopy (TEM), and dynamic light scattering (DLS) experiments. For analysis purpose, aliquots of each synthesis of γ -Fe₂O₃@PS nanoparticles were degraded into iron ions by hot treatment in an acidic mixture of HNO₃/HCl that leaves the PS chains intact. Then the polymer was separated by phase transfer into dichloromethane and precipitated in an excess of methanol, in order to be analyzed by size-exclusion chromatography (SEC).

2.4 Analytical techniques

¹H NMR (250 MHz) analyses were performed in CDCl₃ in 5 mm tubes at room temperature using an AC250 Bruker spectrometer. The monomer conversion (x) was calculated from the proton NMR spectrum of the crude polymer solution using the vinylic protons of the monomer (5 – 6 ppm) and the characteristic peak of the polymer.

Size exclusion chromatography (SEC) was performed in THF at a flow rate of 1 mL.mn⁻¹ to measure the number- (M_n) and the weight- (M_w) average molar masses and the molar-mass dispersity index (M_w/M_n). The SEC apparatus (TDA model 302 from Viscotek) was equipped with two mixed bead columns (PLgel, mixed C, 5 μ m) and one guard column (PLgel, 5 μ m, 100 Å) thermostated at 40°C. Three detectors ran online: a light scattering (LS) detector (2 angles at 90° and 7°, λ = 670 nm), a viscosimeter (Wheaston bridge) and a refractive index (RI) detector. SEC with triple detection yields the absolute values of the molar masses without the need of standards, once the refractive index increments (dn/dc) of PBMA and PBMA-*b*-PS were measured respectively at 0.077 and 0.125 as the slopes of RI measurements at 40°C *vs.* concentration in THF. Our measured value of dn/dc for PBMA is very close to the value 0.075 reported for high molecular weight PBMA synthesized by reverse ATRP in miniemulsion.⁴² In the case of PBMA, the most commonly used SEC technique calibrated by polystyrene standards led to underestimated molar masses: $M_{n, \text{triple}}/M_{n, \text{standard}} = 1.31 \pm 0.01$.

Vibrating sample magnetometry (VSM) was used to record the magnetization curve $M(H)$ with a home-made apparatus for field values up to 0.9 Tesla, above which a saturation plateau M_{sat} was reached. The shape of the normalized curve $M(H)/M_{\text{sat}}$ was well fitted by a Langevin's function typical for superparamagnetism, taking into account the size distribution of the nanoparticles.⁴³ This distribution was well described by a Log-normal function with two parameters: the median diameter d_0 and the distribution width σ . The magnetization at saturation is related to the volume fraction Φ by $M_{\text{sat}} = \Phi \cdot m_s$ where $m_s = 3 \times 10^5$ A/m is the specific magnetization of colloidal maghemite, which is lower than for the bulk oxide.⁴⁴

Atomic Absorption Spectrophotometry (AAS). The iron concentration was obtained by AAS using a Perkin Elmer AAnalyst 100 apparatus. Before titration, the magnetic nanoparticles (γ -Fe₂O₃ or γ -Fe₂O₃@PS) were brought up to ebullition in concentrate hydrochloric acid (35%) or a mix of hydrochloric and nitric acids until the total dissolution of the nanoparticles into iron (III) ions. The conversion law from the molar concentration of ions in the titrated aliquot to the volume fraction in the original suspension is simply $\Phi(\text{v/v } \%) = 1.577 \cdot [\text{Fe}]$ (mol/L) as deduced from the molar mass (159.7 g.mol⁻¹) and the mass density (5.1 g/cm³) of maghemite.

Transmission electron microscopy (TEM). The morphology of the particles was determined by TEM using a JEOL 100 CXII (UHR) microscope working at 80kV. To study the γ -Fe₂O₃@PS core-shell nanoparticles, we used a short exposition (15 s) of the grids to RuO₄ vapour as a contrast agent specific to the aromatic molecules of the PS shell.⁴⁵ This agent was produced freshly by reacting 0.2g RuCl₃ with 10mL of aqueous NaClO 5.25%.⁴⁶

Dynamic light scattering (DLS). Hydrodynamic diameters (d_h) were measured with a Malvern NanoZS ZetaSizer working at an angle of 173° in n-hexane (RI $n=1.375$ and viscosity $\eta_{25^\circ\text{C}}=0.28$ cP) for the MNPs coated by oleic acid and in dichloromethane (RI $n=1.432$ and viscosity $\eta_{25^\circ\text{C}}=0.389$ cP) for the MNPs after grafting by SI-ATRP of PS. The correlograms were fitted using a multi-modal algorithm provided by the manufacturer.

2.5 Typical procedure for sample preparation

Silicon wafers (n-doped type from Siltronix Inc., 2" diameter, 355–405 μm thickness) were cleaned as according to the following procedure. At first, silicon substrates were sonicated during 3 minutes in chloroform prior treatment with a freshly prepared "piranha" solution (70/30, v/v, 95% concentrated H_2SO_4 / 30% aqueous H_2O_2) at 100°C for 10 minutes. Silicon substrates were rinsed with distilled water, ultrapure water and subsequently dried under a nitrogen stream until no trace was visible (otherwise the rinsing step was repeated). The films were deposited by spin-coating 750 μL of a toluene solution at 6000 rpm (Süss Microtec Delta-10TT) onto freshly cleaned silicon wafers. The solvent mixtures contained PBMA-*b*-PS and magnetic nanoparticles at a total concentration (if no other specified value) $C = 20$ g/L with an inorganic volume fraction Φ (defined as the volume of Fe_2O_3 divided by the total dry volume of iron oxide and polymer) between 0 and 0.25%. The films were annealed at 150°C i.e. 50°C above the glass temperature of PS, a temperature estimated to be sufficient for self-assembly ($\chi_{150^\circ\text{C}}\approx 0.04$ see 3.1) but 30°C below the order-disorder transition (UCOT) of PBMA-*b*-PS of $M_n\approx 10^5$ g.mol $^{-1}$ according to literature.² In addition, the risk of polymer degradation was limited by annealing it under vacuum for a period of 48 or 72 hours at most.

2.6 Characterization methods for copolymer films

Atomic Force Microscopy (AFM).

The surface of the composite thin films after annealing was characterized by AFM. The images were taken either with a Nanoscope III (Digital Instruments, Veeco) in the dry Tapping ModeTM or with a Picoscan apparatus (Molecular Imaging, Agilent) in the Acoustic AC ModeTM. The images (topography and phase) were imported by ImageJ using a plugin available at <http://rsb.info.nih.gov/ij/plugins/afm.html> to analyze the features of the film surface quantitatively.

Ellipsometry was carried out in the Laboratoire de Physico-Chimie des Polymères et des Milieux Dispersés (ESPCI, Paris – France) with a SE400 Sentech Instrument apparatus operating at a wavelength $\lambda = 632.8$ nm. The ellipsometric angles Ψ and Δ were measured and fitted using the Fresnel equations of light reflection by an infinite silicon substrate (refractive indexes $n_s = 3.874$ and $k_s=0.0016$) covered by 20 Å of SiO_2 oxide ($n_o=1.46$) and a layer of adjustable refractive index and thickness values. The measurement was repeated at 5 different locations on each wafer in order to get standard deviation values.

Neutron Reflectivity (NR) was performed in the Laboratoire Léon Brillouin (LLB, CEA Saclay – France) on the time-of-flight reflectometer EROS. The reflectivity curve was recorded at a constant angle 0.93° for a neutron wavelength λ varying between 2.7 and 26 Å, thus covering a scattering vector range $q = 4\pi/\lambda \sin\theta$ between 0.078 and 0.75 nm $^{-1}$. The reflectivity curves were fitted with the SimulReflec software accessible at <http://www-llb.cea.fr> calculating a model reflectivity curve by iterations of the thicknesses t_i and the coherent neutron scattering length densities (SLD) Nb_i . The initial configuration consisted in an infinite silicon medium ($Nb_{\text{Si}}=2.14\times 10^{-6}$ Å $^{-2}$) covered with a thin (1.3 nm) layer of oxide ($Nb_{\text{SiO}_2}=3.32\times 10^{-6}$ Å $^{-2}$) and a succession of PS-PBMA/PBMA-PS bilayers ($Nb_{\text{PS}}=1.41\times 10^{-6}$ Å $^{-2}$ and $Nb_{\text{PBMA}}=6.32\times 10^{-7}$ Å $^{-2}$). An integer number of bilayers (e.g. 3, 4 or 5) were chosen according to the total film thickness measured independently by ellipsometry.

Contact angle. The free surface energy of the thin films was calculated by contact angle measurements, using the Owens-Wendt method with droplets of two liquids of different polarisabilities (water and ethylene glycol).⁴⁷

3. Results and discussion

3.1 Synthesis of symmetric PBMA-*b*-PS diblock copolymer by ATRP

In this work, the requirements for the diblock copolymer synthesis were driven by the expected lamellar self-assembly of the copolymer within the film. At first, a similar degree of polymerization of each block had to be obtained ($N_{\text{PS}}\approx N_{\text{PBMA}}$). Then the condition to reach the ordering transition writes $\chi N > 10.5$ for a symmetrical diblock copolymer, χ being the Flory-Huggins parameter describing the interaction of the two types of segments at the annealing temperature.⁴⁸ We estimate $\chi_{140^\circ\text{C}}=0.045$ from a small angle neutron scattering study of binary blend mixtures of 10%-d(uterated)-PS and PBMA homopolymers⁴⁹ that leads to $\chi/V_0=2.5\times 10^{-4}$ Å $^{-3}$ (taking an average molecular volume $V_0=(V_{\text{PS}}V_{\text{PBMA}})^{1/2}\approx 180$ Å 3). We know also $\chi_{160^\circ\text{C}}=0.037$ from a value published for d-PS and PMMA,⁵⁰ a system close to ours. We deduce that the minimum degree of polymerization N of each block should be around 300.

We first investigated the synthesis of the poly(n-butyl methacrylate) (PBMA) homopolymer by ATRP, which was subsequently used as the macroinitiator for styrene polymerization. The experimental conditions for synthesis of both PBMA macroinitiators and PBMA-*b*-PS diblock copolymers are reported in Table 1. The ATRP mechanism is based on the activation-deactivation equilibrium between dormant and active species which is established through a redox reaction using the copper-based catalyst. As depicted in equation 1, the ATRP kinetics of a specific monomer is mainly governed by the $k_p.K$ ratio, with k_p the propagation rate constant and K the equilibrium constant.

Table 1. Experimental conditions for the synthesis of PBMA macroinitiator and PBMA-*b*-PS diblock copolymers by ATRP. ^a

Expt	Monomer	Initiator	Nature of catalyst	[M]:[Initiator]:[CuX]:[CuX ₂]:[PMDETA]	Polym. time (h)
1	BMA	EBrIB	CuBr/CuBr ₂	400:1:0.8:0.2:1	1.5
2	BMA	EBrIB	CuCl/CuCl ₂	400:1:0.8:0.2:1	2
3	S	PBMA-Br (from expt 1)	CuCl	665:1:1:1	15
4	S	PBMA-Cl (from expt 2)	CuCl	690:1:1:1	16

^a Experimental conditions: T = 100°C; [BMA] = 6.2 mol.L⁻¹; [S] = 8.7 mol.L⁻¹

From the values of $k_p K$ previously published⁵¹ ($k_p K = 1.1 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ for MMA and $3.6 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$ for styrene polymerization at 90°C using EBrIB as initiator and 4,4'-(di-5-nonyl)-2,2'-bipyridine as copper ligand), the more reactive methacrylic monomer was logically polymerized as the first block in order to enhance the initiation efficiency of the macroinitiator for the second block, hence limiting the amount of residual first block.

$$\ln \frac{[M]_0}{[M]} = k_p K \cdot \frac{[Cu^{II} X_2][RX]}{[Cu^I X]} t \quad \text{Eq. (1)}$$

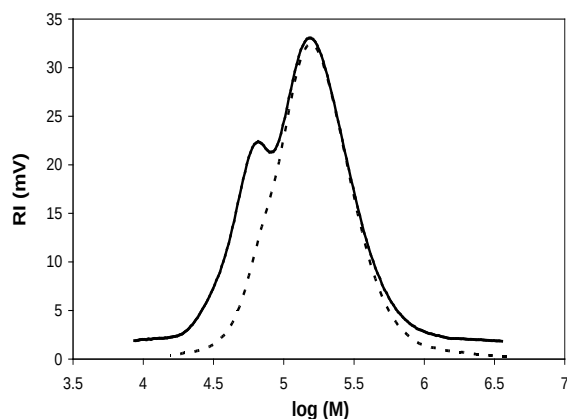
The ATRP of BMA was carried out at 100°C using 20 mol% of CuX₂ and 80 mol% of CuX as copper catalyst. The control over the BMA polymerization was observed whatever the nature of the copper halide (Br or Cl), as shown by low molar-mass dispersity indices ($M_w/M_n < 1.2$) and the correct matching between theoretical and experimental molar masses (Table 2).

Table 2. Analysis results of the PBMA homopolymer synthesis.

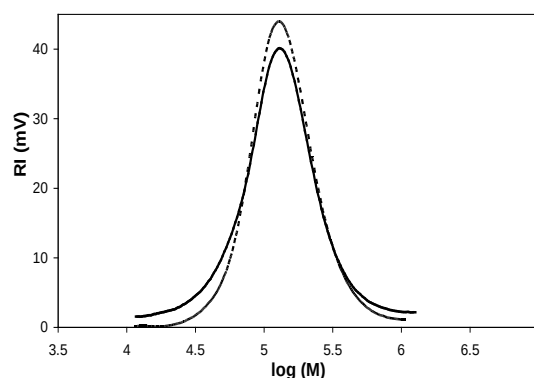
Expt	Conversion (%)	M_n theoretical ^a g.mol ⁻¹	M_n SEC g.mol ⁻¹	M_w/M_n	f^b
1	87	49 680	57 520	1.19	0.86
2	84	47 975	60 860	1.18	0.79

^a $M_n^{\text{theoretical}} = M_{\text{EBrIB}} + [\text{BMA}]_0 / [\text{EBrIB}]_0 \times \text{conversion} \times M_{\text{BMA}}$

^b Initiator efficiency, $f = M_n^{\text{theoretical}} / M_n^{\text{SEC}}$



(a)



(b)

Figure 1. SEC of the PBMA₄₀₅-*b*-PS₄₆₀ (a, expt 3) and the PBMA₄₂₅-*b*-PS₄₉₀ (b, expt 4) diblock copolymers before (bold line) and after (dashed lines) hexane extraction.

Nevertheless, the nature of the copper halide catalyst affected the proportion of PBMA dead chains as highlighted by the SEC traces of the PBMA-*b*-PS diblock copolymers depicted in Figure 1. Indeed, the presence of residual PBMA homopolymer polluting the PBMA-*b*-PS diblock copolymer was noticeable when using the bromide-terminated PBMA macroinitiator (experiment 3) whereas pure diblock copolymer was recovered from the PBMA macroinitiator synthesized in the presence of the copper chloride catalyst (experiment 4). The higher proportion of PBMA dead chains is in accordance with the higher activation rate constant (k_a) of copper bromide-based catalyst in comparison with k_a of the copper chloride-based catalyst.⁵² The clear shift of the SEC trace of the diblock copolymer (Figure 2) together with the narrow molar mass distribution of the copolymer (Table 3) highlighted the control of the PBMA-*b*-PS diblock copolymer synthesis up to high molar masses ($\approx 100\,000 \text{ g.mol}^{-1}$). In conclusion, we successfully synthesized well-defined copolymers PBMA₄₀₅-*b*-PS₄₆₀ (exp. 3) and PBMA₄₂₅-*b*-PS₄₉₀ (expt. 4) which fulfilled the initial requirements, *i.e.* the synthesis of a symmetrical diblock copolymer with an overall degree of polymerization up to 915.

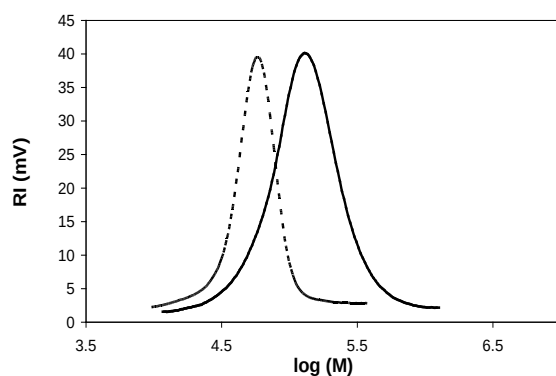


Figure 2. SEC of the PBMA₄₂₅ macroinitiator (dashed lines, expt 2) and the PBMA₄₂₅-*b*-PS₄₉₀ diblock copolymer (bold line, expt 4).

Table 3. Analysis results of the PBMA-*b*-PS diblock copolymer synthesis.

Expt	Conversion (%)	M_n theoretical ^a g.mol ⁻¹	M_n SEC ^b g.mol ⁻¹	M_n SEC ^c g.mol ⁻¹	M_w/M_n ^c	f ^d	M_n NMR ^e g.mol ⁻¹
3	53	94 175	85 400	105 400	1.38	0.9	102 740
4	77	116 115	102 400	111 900	1.44	1.0	114 250

^a $M_n^{\text{theoretical}} = M_n(\text{PBMA}) + [S]_0 / [\text{PBMA}]_0 \times \text{conversion} \times M_S$

^b M_n of the crude diblock copolymer.

^c Macromolecular characteristics of the di-block copolymer recovered after extraction of the residual PBMA homopolymer with hexane.

^d f is the initiator efficiency: $f = M_n^{\text{theoretical}} / M_n^{\text{SEC}}$ using M_n^{SEC} of the diblock copolymer free of any residual PBMA macroinitiator.

^e $M_n^{\text{NMR}} = M_{\text{EBrIB}} + \text{DP}_{\text{BMA}} \times M_{\text{BMA}} + \text{DP}_S \times M_S$, with DP the average degree of polymerization. DP_{BMA} was calculated using M_n^{SEC} of the macroinitiator and DP_S was calculated on the basis of the proton NMR integrations of both monomer units.

3.2 Synthesis of $\gamma\text{-Fe}_2\text{O}_3$ @PS nanoparticles

This study necessitated to synthesize magnetic nanoparticles and disperse them as inorganic fillers in the targeted nanocomposite films. Those MNPs could be brought either as a dry powder or as a ferrofluid, *i.e.* a stable colloidal dispersion. Our aim was to obtain nanocomposite films well ordered as lamellae with the inorganic fillers embedded in some of the layers only. This purpose could not be reached by starting from a dry powder because irreversibly aggregated nanoparticles would not disperse properly into polymer lamellae. To confine MNPs within the layers of the diblock copolymer and keep a lamellar order, it was necessary to coat those nanoparticles with a thin polymer layer of the same nature as one block of the copolymer. This strategy to obtain nanocomposite materials was once called *ex-situ synthesis* of the MNPs and *co-assembly* with the BCPs.

Among the different available magnetic nanoparticles, we focused our attention on those made of $\gamma\text{-Fe}_2\text{O}_3$ (maghemite), which is a pure iron^{III} oxide that belongs to the spinel crystallographic structure. The TEM pictures showed “rock-like” nanoparticles, which can be approximated as spheres having diameters between 5 and 12 nm (see Figure 3). Their size distribution was rather broad as shown by the Log-normal fit with median value $d_0 = 6.8$ nm and standard width $\sigma = 0.39$, as determined by VSM (ESI-Figure 1). In this work, we also examined the advantage of using MNPs with a fairly narrower size distribution. A size sorting procedure by successive phase separations enabled to decrease the width down to $\sigma = 0.25$ for a median $d_0 = 6.2$ nm.³⁹ The TEM micrographs show the visible difference between

unsorted “polydisperse” and size-sorted “monodisperse” $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles (Figure 3).

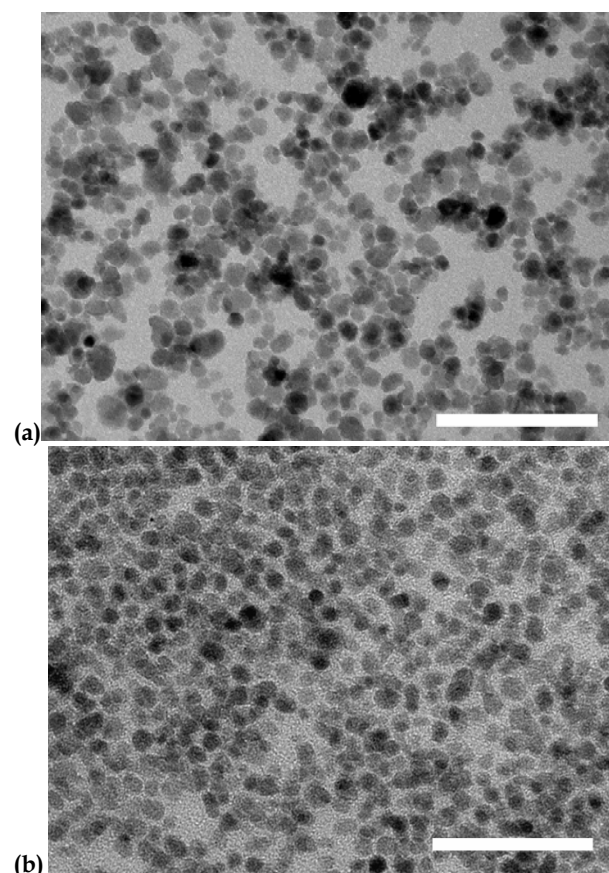


Figure 3. TEM micrographs of polydisperse (a) and monodisperse (b) $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles. The scale bar length is 50 nm in both cases.

Table 4. Experimental conditions to synthesize γ -Fe₂O₃@PS MNPs by SI-ATRP (at 100°C).

Expt.	γ -Fe ₂ O ₃ d_0 (nm) / σ^a	[Styrene] mol.L ⁻¹	[St]/[γ -Fe ₂ O ₃ @BrMPA]/ [CuBr]/[PMDETA]	Polym. time (min)
γ -Fe ₂ O ₃ @PS1	6.8 / 0.39	7.84	20:1:1:1	30
γ -Fe ₂ O ₃ @PS2	6.2 / 0.25	7.89	20:1.5:1:1	30
γ -Fe ₂ O ₃ @PS3	7.0 / 0.23	7.30	10:1.5:1:1	45

^a Two parameters of a Log-normal distribution for the magnetic core's diameters obtained by fitting the VSM curves with Langevin's law.

Table 5. Analysis results of the γ -Fe₂O₃@PS nanoparticles synthesis.

Expt.	dh (nm) ^a	Mn PS SEC ^b (g.mol ⁻¹)	Mw/Mn ^b	% w/w γ -Fe ₂ O ₃ ^c	% w/w PS ^d
γ -Fe ₂ O ₃ @PS1	144	45 820	2.45	27	73
γ -Fe ₂ O ₃ @PS2 ^e	210 / 60	23 840	1.98	1.3	98.7
γ -Fe ₂ O ₃ @PS2 ^f	280 / 50	19 330	1.77	7.4	92.6
γ -Fe ₂ O ₃ @PS3	130	15 560	1.41	79	21

^a Hydrodynamic diameter of filtrated solutions measured by DLS (two values indicate a bimodal distribution).

^b Macromolecular data obtained by SEC after separation from iron oxide (see 2.3 and 2.4).

^c Obtained after titration of iron by absorption spectrometry.

^d Deduced by difference % w/w PS = 100% - % w/w γ -Fe₂O₃.

^e CuBr removed by filtration after polymerization.

^f CuBr removed by water rinsing.

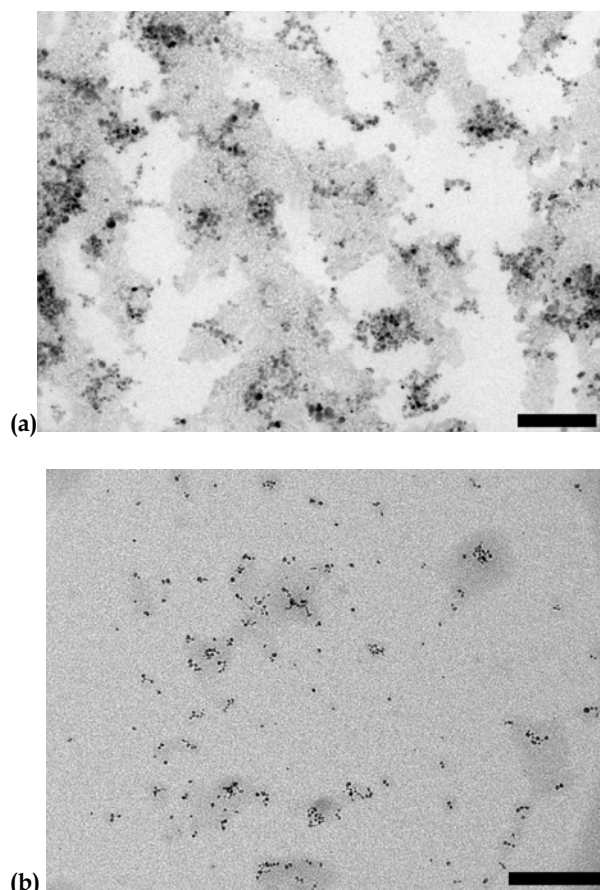


Figure 4. TEM micrographs of “polydisperse” γ -Fe₂O₃@PS1 (a) and “monodisperse” γ -Fe₂O₃@PS2 (b) nanoparticles. The scale bar length is 200 nm in both cases. The grey areas around the black inorganic cores are obtained by coloration of the PS aromatic rings by RuO₄.

We used those two types of nanoparticles to investigate a possible effect of the particle size distribution on the control of polymerization during the “grafting from”

synthesis to get γ -Fe₂O₃@PS nanoparticles. The experimental conditions for three syntheses of PS-grafted nanoparticles are reported in Table 4. Because of washing steps after the ligand-exchange, the presence of free initiator in excess is unlikely. Thus the actual molar ratio of initiator *vs.* catalyst might be smaller than the theoretical values of 1 or 1.5.

After synthesis, we obtained large aggregates of core-shell nanoparticles ($d_h > 150$ nm) that required filtration to obtain smaller aggregates (Table 5). Nevertheless, the TEM micrographs of those particles showed that the broadly distributed maghemite nanoparticles led to large aggregates, while the “monodisperse” nanoparticles led to much fewer small aggregates in coexistence with isolated nanoparticles coated by PS corona (Figure 4). This aggregation effect observed during the SI-ATRP polymerization of styrene may be ascribed to termination reactions between growing chains initiated from the functionalized nanoparticles surface. According to Matyjaszewski, such termination reactions very probable in bulk-polymerization could be eliminated by performing SI-polymerization in a dilute dispersed phase instead⁵¹ or under high pressure around 6 kbar.⁵³

Furthermore, monodisperse nanoparticles γ -Fe₂O₃@PS3 gave a better-defined SEC trace after de-grafting of the chains than the polydisperse ones γ -Fe₂O₃@PS1 (Figure 5). This indicates that a narrow distribution of diameters of the magnetic cores is somehow related to the quality of CRP and the final state of aggregation. In the literature, several studies about SI-ATRP at the surface of iron oxide MNPs clearly evidenced the living character of polymerization but without leading systematically to a low degree of aggregation for the core-shell products. A. Kaiser *et al.* used polydisperse Fe₃O₄ (magnetite) MNPs with a size distribution analogous to our sample 1 and also coated with BrMPA for the SI-ATRP of styrene in

toluene at three monomer concentrations.³⁶ In spite of the heterogeneous phase (the samples exhibiting a culot and a supernatant with $d_h \approx 200\text{nm}$), molar-mass dispersity indices were always below 1.3 with M_n up to $90000\text{ g}\cdot\text{mol}^{-1}$. The authors showed that the aggregation of MNPs clearly reduces the level of available initiator, providing PS chains of higher molar masses.

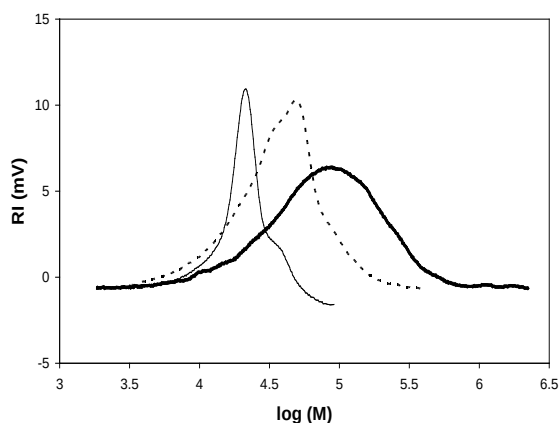


Figure 5. SEC curves of the polymer chains after degradation of the inorganic cores of $\gamma\text{-Fe}_2\text{O}_3\text{@PS1}$ (bold line), $\gamma\text{-Fe}_2\text{O}_3\text{@PS2}$ (dashed lines) and $\gamma\text{-Fe}_2\text{O}_3\text{@PS3}$ (thin line).

In the present work, we observe also that a higher experimental M_n is concomitant with a broader molar mass distribution M_w/M_n (Table 5). Therefore a lower concentration of grafted initiator is involved to create chains, producing a lower amount of control agent (*i.e.* Cu(II)Br_2) by the so-called “persistent radical effect”.⁵⁴ Another reported study strengthens the idea that a lower size-dispersity of the magnetic cores improves the control of radical polymerization: S. M. Gravano *et al.* used indeed maghemite $\gamma\text{-Fe}_2\text{O}_3$ MNPs made by iron carbonyl decomposition,³⁴ for which we estimate that the width parameter σ of a Log-normal distribution is below 0.1, to compare with 0.2 at best for MNPs synthesized by aqueous route. Having grafted a 2-bromopropionate ester on them, the authors studied the SI-ATRP of styrene in the bulk on those highly monodisperse magnetic cores. The perfect 1st-order and living character of the polymerization being evidenced by the authors, the PS chains were short and controlled ($M_n < 28000\text{ g}\cdot\text{mol}^{-1}$ for 50% conversion) with a particularly narrow molar mass distribution ($M_w/M_n < 1.1$). However, this article exhibits a TEM image of large (hundreds of nm) aggregates of MNPs embedded in a PS matrix looking like our Figure 4a.

In conclusion, we synthesized $\gamma\text{-Fe}_2\text{O}_3\text{@PS}$ core-shell nanoparticles by surface-initiated polymerization (also called “grafting from”) using ATRP. Although aggregates persisted, we slightly improved the control of the radical polymerization (compared to our first attempt on Table 5) and decreased the proportion of aggregates by using iron oxide nanoparticles more homogeneous in sizes for the SI-ATRP synthesis. Even though more efforts would be necessary to explain quantitatively a surface curvature effect when nano-sized particles are used for SI-ATRP

(*e.g.* by measuring the grafting density of BrMPPA initiator on several batches of MNPs of different distributions of diameters), the evidenced relationship between the characteristics of inorganic synthesis (distribution of diameters) and of polymer synthesis (molar mass dispersity indexes) adds valuable information about that domain.

3.3. Formation and characterization of pure copolymer films Lamellar order of pure copolymer films.

At first, we studied pure copolymer films without nanoparticles in order to check their lamellar order. According to the literature, films made of symmetrical copolymers annealed in a range of temperatures above the T_g of their blocks and between the upper (UCOT) and lower (LCOT) critical ordering transitions² self-assemble into regular lamellae. Such an organization leads to characteristic defects at the film surface because the top layer is rarely complete. Depending on the initial thickness of deposited copolymer (never strictly equal to an integer number of the lamellar period), these defects appear either as “islands” or “holes”.^{11, 12}

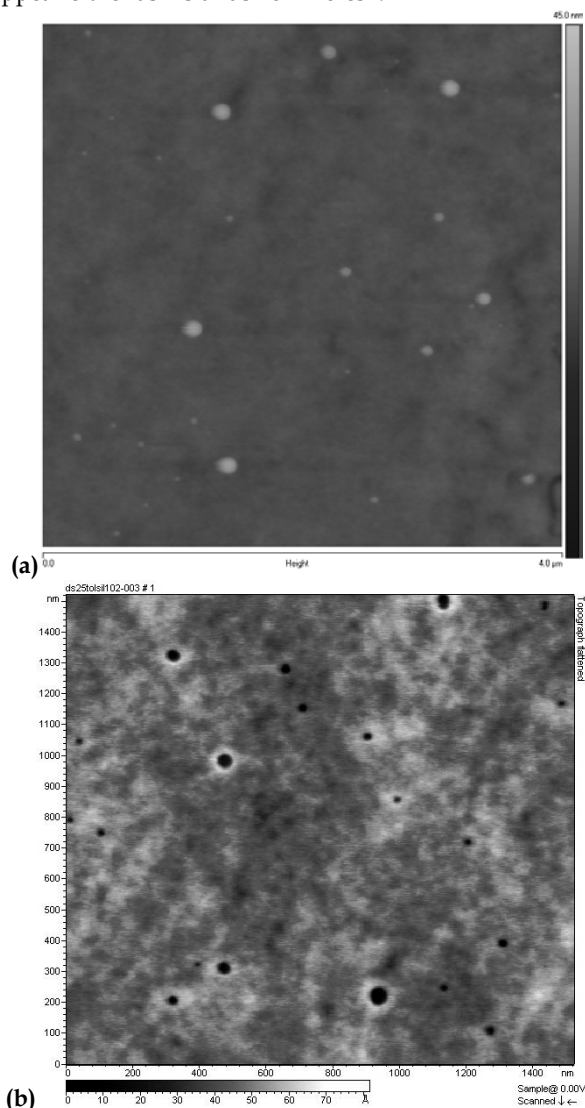


Figure 6. AFM height images of pure PBMA425-*b*-PS490 films leading either to “islands” (a) or “holes” (b) at their top surface depending on thickness. Statistical analyses of

the heights, depths and diameters of those surface defects are provided as ESI-Figure 2.

Both types of characteristic defects were detected by AFM with PBMA₄₂₅-*b*-PS₄₉₀ films by varying the deposited volume and substrate area (Figure 6). In the case of the islands, the statistical analysis of this AFM topographical picture provided us an average height $H_0=16.37\pm0.73$ nm that we can identify with half the value of the lamellar spacing^{11, 12, 15, 24, 55} to get $L_{AFM}=32.7\pm1.5$ nm. Ellipsometry data gave us the global thickness of the films with an indication about their roughness (standard deviation), *e.g.* 122.5 $\pm$ 1.1 nm for the undoped film (nb. 2 on Table 6). The average optical refractive index $n=1.540\pm0.004$ for the pure copolymer is reasonable for a thin organic layer on silicon.

Neutron reflectivity (NR) enabled us to study the system at a much more mesoscopic scale. A flat un-structured film such as ours right after spin-coating gives a

reflectivity profile that decreases globally as q^{-4} while being modulated by a regular oscillation (Kiessig fringes) with a constant period related to the film thickness. After annealing, we observed an overshoot (*i.e.* an amplification of the first maximum compared to the following ones) which is a direct evidence of the lamellar order (Figure 7(a)).⁵⁷ Considering this highest peak at a wave vector $q_{max}\approx0.166$ nm⁻¹ as due to quasi-Bragg reflection on the lamellae, we estimated the average lamellar period by $L_{ave}=2\pi/q_{max}\approx37.9$ nm for the undoped film, which is 16% larger than from AFM. For such nanostructured films, the super-oscillations of the NR curve replacing the regular Kiessig fringes before annealing enabled to build a scattering length density (SLD) profile with a total thickness of the film of $L_{tot}=L_1+L_2+L_3=118$ nm in accordance with ellipsometry and with quite a regular period (Figure 7(b)).

Table 6. Analysis results by ellipsometry and NR of the thin films of PBMA₄₂₅-*b*-PS₄₉₀ at a volume fraction Φ in MNPs after annealing at 150°C.

Film nb.	Ann. time (h)	Φ (v/v) γ -Fe ₂ O ₃	Thickness ^a (nm)	RI ^a	L_1^b (nm)	L_2^b (nm)	L_3^b (nm)	L_4^b (nm)	L_5^b (nm)	L_{tot}^c (nm)	L_{ave}^d (nm)	$N_{layer}=\frac{L_{tot}}{L_{ave}}$
2	48	0	122.5 $\pm$ 1.1	1.540 $\pm$ 0.004	30.0	42.2	45.4	-	-	117.6	37.9	3.1
5	48	0.025%	114.6 $\pm$ 0.8	1.562 $\pm$ 0.005	31.2	45.3	41.5	-	-	117.9	39.3	3.0
8	72	0.05%	108.1 $\pm$ 1.4	1.642 $\pm$ 0.013	32.2	48.0	31.5	-	-	111.7	42.2	2.6
9	72	0.10%	109.4 $\pm$ 3.5	1.607 $\pm$ 0.023	32.8	48.0	33.3	-	-	114.0	41.1	2.8
10	72	0.15%	106.6 $\pm$ 0.4	1.660 $\pm$ 0.017	32.8	48.7	30.0	-	-	111.5	43.8	2.5
11	72	0.25%	116.6 $\pm$ 5.2	1.606 $\pm$ 0.030	-	-	-	-	-	-	-	-
12	72	0.025%	50.2 $\pm$ 0.7	1.533 $\pm$ 0.007	-	-	-	-	-	-	-	-
13	72	0.025%	184.0 $\pm$ 4.9	1.536 $\pm$ 0.004	34.0	46.1	49.0	54.5	-	183.6	41.9	4.4
14	72	0.025%	248.4 $\pm$ 17.4	1.727 $\pm$ 0.097	45.4	46.3	46.6	46.2	44.4	228.9	41.1	5.7

^a Thickness, roughness (standard deviation) and refractive index measured by ellipsometry.

^b L_i is the thickness of the i^{th} bilayer PS-*b*-PBMA/PBMA-*b*-PS as measured from the SLD profile fitting the neutron reflectivity curve.

^c Total film thickness deduced from the fitting SLD profile $L_{tot}=\sum L_i$.

^d Average bilayer thickness deduced from the quasi-Bragg peak $L_{ave}=2\pi/q_{max}$ of the NR curve. The five values in bold cases can be fitted by a linear regression $L_{ave}=L_0(1+p\Phi)$ with $L_0=38.6$ nm and $p=89$ as described by B. Hamdoun *et al.*^{16, 56}

Considering the thickness of the film and the average lamellar period, we can calculate the average number of bilayers by $N_{layer}=L_{tot}/L_{ave}$. This number being close but slightly above an integer (here $N_{layer}=3.1$), the top surface of this film exhibit island-type defects on the AFM picture of Figure 6(b). In addition, the average lamellar period of this film is about 38 nm *i.e.* 31% larger than the value 29.5 $\pm$ 0.5 nm measured in previous studies with films prepared with a PS-*b*-PBMA sample of molar mass $M_w=82000$ g.mol⁻¹ synthesized by anionic polymerization with $M_w/M_n=1.05$ that was used for several structural studies of incorporation of MNPs.¹⁵⁻¹⁸ Taking into account the 46% increase of M_n for our PBMA₄₂₅-*b*-PS₄₉₀ synthesis compared to this reference sample, one calculate a scaling law $L_{ave}\sim M_n^{0.67}$. It may be fortuitous, but this value 31%/46%=0.67 is close to the theoretical exponent 0.643 for the scaling law of the lamellar period of symmetrical BCPs *vs.* molar mass⁵⁸ that was firstly

studied experimentally by Hadzioannou *et al.* who measured a larger exponent (0.79) for PS-*b*-PI copolymers.⁵⁹ We gathered our measurement with all the published values of the lamellar period of (sometimes deuterated) PS-*b*-PBMA films of M_n varying from 64000 to 650000 g.mol⁻¹ measured by several methods (Small Angle X-ray^{2, 25} and Neutron¹³ Scattering, X-ray⁶⁰ and Neutron Reflectivity^{14, 17, 18, 23, 24}, AFM^{15, 24, 55} and optical interference microscopy¹²) on a master curve (ESI-Figure 3). This bibliographic study leads to a scaling law exponent around 0.69, very close to the exponent 0.643 predicted by Helfand due to compaction of the chains in the lateral direction into dense bilayers under surface tension and thus stretching in the direction normal to the lamellae.⁵⁸ For unilamellar vesicles with hydrophobic amorphous blocks like polybutadiene, which are a particular case of the lamellar state of BCPs, Eisenberg showed that the scaling exponent of bilayer thickness *vs.*

molar mass varies from 1/2 for the lower masses (*e.g.* <10000 g.mol⁻¹) to 2/3 for the larger ones.⁶¹ With PBMA-*b*-PS around 100000 g.mol⁻¹, we are certainly in the latter regime and thus the chains are more extended than the conformation of random coils hypothesized by Hamdoun in his theoretical model of nanocomposite BCP/MNP thin films.⁶²

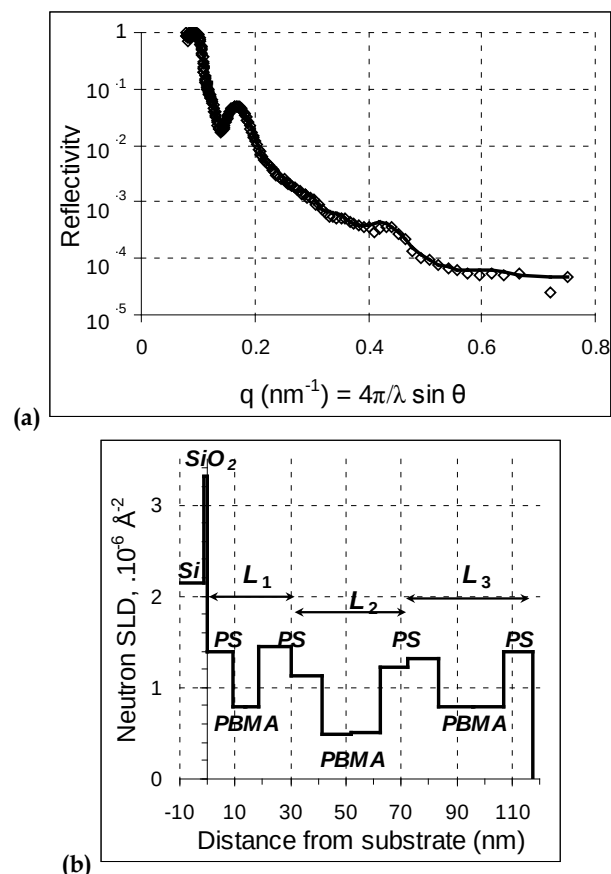


Figure 7. (a) Neutron Reflectivity spectrum of a PBMA₄₂₅-*b*-PS₄₉₀ copolymer film and (b) calculated SLD profile deduced from the fitting curve.

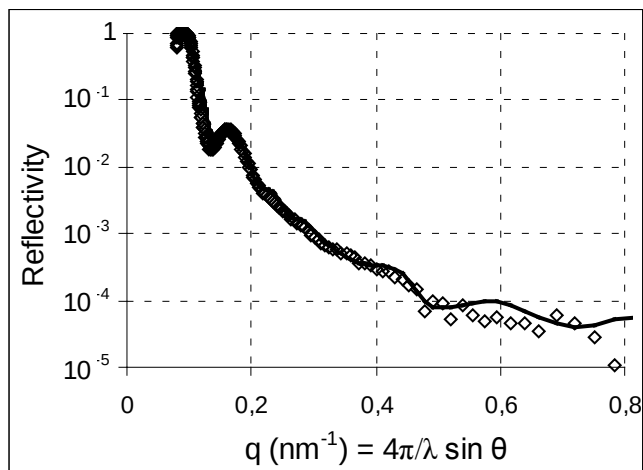
Interpenetration and sequence order of the blocks. NR provides other information in addition to the lamellar period. The calculated neutron SLD profile on Figure 7(b) enables superposing a calculated reflectivity curve (solid line) to the experimental points of the pure film (diamonds on Figure 7(a)). The lower contrast between the calculated Nb_{PBMA} (increase) and Nb_{PS} (decrease) compared to their initial values before curve fitting (pure homopolymers) shows that the copolymer blocks may not be totally phase-separated. By looking at the SLD profile, one notices also that the first bilayer thickness L_1 (the closest to silicon) is significantly lower than the two following ones, an effect ascribed to interpenetration of the PBMA blocks. More importantly, the sequence order of the alternating PS and PBMA blocks in the lamellae is different from the previously reported systems.^{17, 18, 23} Contrary to these studies showing the PBMA blocks always at the interfaces due to a lower surface energy, all our SLD profiles correspond to the PS blocks in contact with both the air and the substrate. The opposite hypothesis for the alternating layers starting from the silicon chip with PBMA blocks led systematically to a

lower fitting quality of the simulations to the experimental NR curves. We ascribe this different sequence order to the modification of chemical end-groups of the copolymers used here. In the previous studies indeed, the diblock PS-*b*-PBMA copolymers were always synthesized by anionic polymerization. In the present work, the PBMA-*b*-PS copolymer was synthesized by ATRP, implying a halide initiator / catalyst system and therefore different chemical end-groups. Contact angle measurements were carried out to test this explanation by calculating the interfacial energy of those films (top solid surface/air). Films of either pure PBMA (expt 2 in Tables 1 and 2) or PS (also synthesized by ATRP) homopolymers and of the PBMA₄₂₅-*b*-PS₄₉₀ copolymer were prepared in the same conditions as all films studied by NR (in particular the same amount of deposited polymer onto the substrate). While the values of surface energy with air were similar for the PS homopolymer (66.01 mJ.m⁻²) and for the diblock copolymer (63.53 mJ.m⁻²), the surface energy was much lower for the PBMA homopolymer film (7.46 mJ.m⁻²). In the case of PS-*b*-PMMA synthesized by anionic polymerization, only a slight difference of surface energies (less than 1%) was reported between the PS and the PMMA blocks, which enables a fine tuning of the anchoring of the lamellae between parallel and perpendicular orientation to the substrate.^{9, 63} For PBMA-*b*-PS prepared by ATRP, there is on the contrary a strong contrast of surface energies between the two blocks. Those wetting measurements confirm the profiles deduced from neutron reflectivity with PS layers of the diblock copolymer in contact with air. This phenomenon is counter-intuitive when one takes into account only the surface energy with air which is much lower for PBMA. As shown indeed for blends of PS and PBMA homopolymers in the same range of molecular weights (around 10⁵ g.mol⁻¹), the interface is enriched in PBMA compared to bulk and the top surface is always made of a thin layer (below 1 nm) of pure PBMA, as expected from its lowest surface tension with air.⁶⁴ In the case of a thin film of diblock PBMA-*b*-PS copolymer, the total interfacial energy contains not only the surface tension with air but also the interfacial tension with the substrate. Considering the ATRP route chosen to synthesize our copolymer (Table 1 and ESI-Scheme 1), we expect that brominated or chlorinated chemical groups are located at the end of the PS block (but not of the PBMA one that was polymerized first). Therefore a polarisability increase might be sufficient to lower the interfacial energy between the silicon substrate and the PS layers compared to the PBMA ones. Once the first block in contact with Si is determined by higher polar interactions, the nature of the top surface layer is constrained by the total thickness L_{tot} of the film. This scenario might explain the inversion of the nature of the blocks in contact with the wafer and with air compared to previously published results.

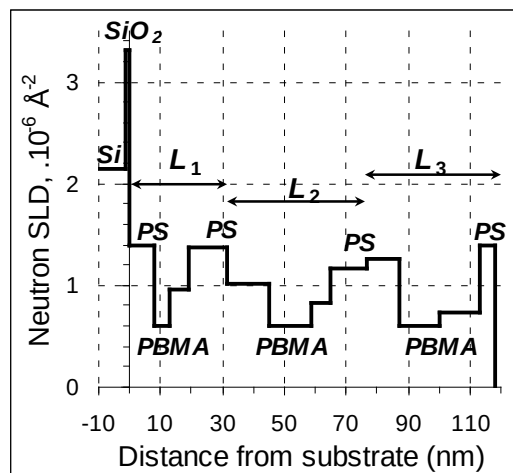
3.4. Formation and characterization of films doped with magnetic nanoparticles

Similar analyses by AFM, NR and ellipsometry were carried out with PBMA₄₂₅-*b*-PS₄₉₀ thin films doped with MNPs from the “polydisperse” batch γ -Fe₂O₃@PS1. Five values of the iron oxide volume fraction were studied for increasing amounts of nanoparticles between $\Phi=0.025\%$ and $\Phi=0.25\%$ (films nb. 5, 8, 9, 10, 11 on Table 6). The

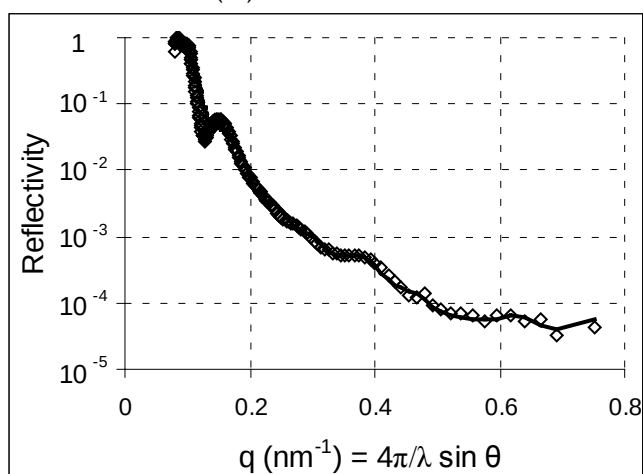
lamellar order was preserved up to $\Phi = 0.15\%$, as evidenced by the super-oscillation (overshoot) of the NR curves typical of a lamellar period (Figure 8). A volume fraction of iron oxide in the film $\Phi = 0.25\%$ was above the threshold value at which the lamellar order becomes distorted.



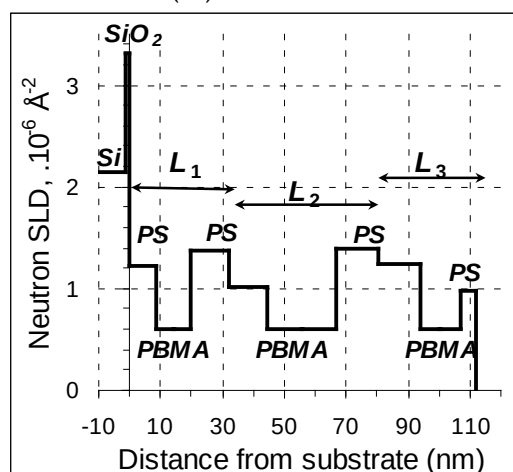
(a1)



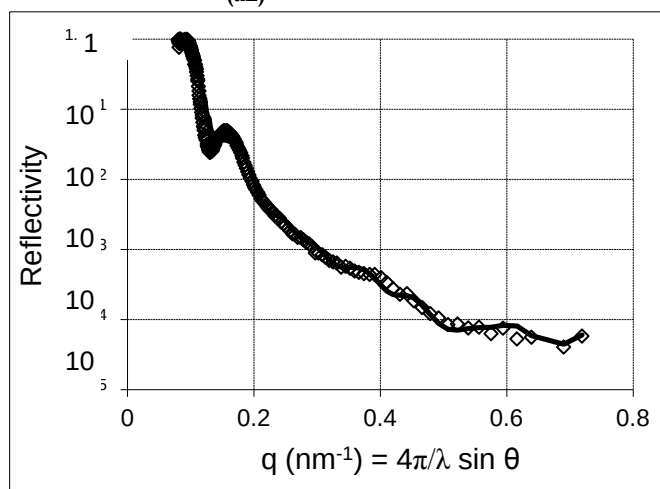
(b1)



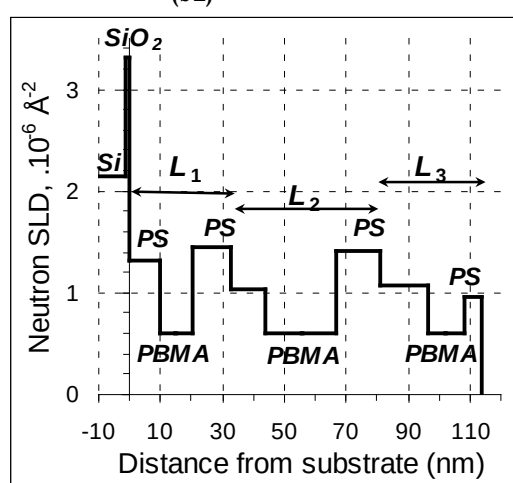
(a2)



(b2)



(a3)



(b3)

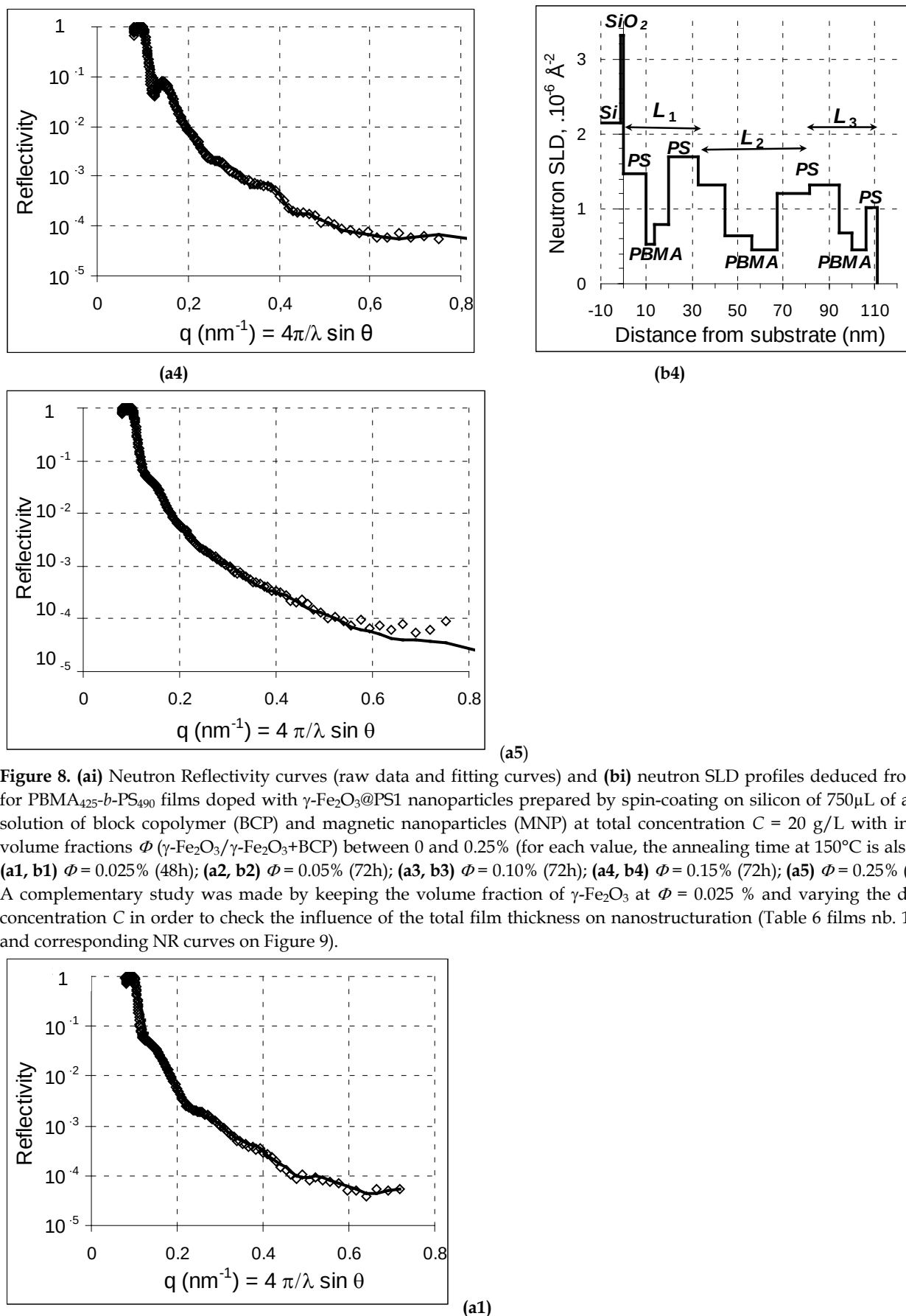


Figure 8. (ai) Neutron Reflectivity curves (raw data and fitting curves) and **(bi)** neutron SLD profiles deduced from the fit for PBMA₄₂₅-*b*-PS₄₉₀ films doped with $\gamma\text{-Fe}_2\text{O}_3$ @PS1 nanoparticles prepared by spin-coating on silicon of 750 μL of a toluene solution of block copolymer (BCP) and magnetic nanoparticles (MNP) at total concentration $C = 20 \text{ g/L}$ with increasing volume fractions ϕ ($\gamma\text{-Fe}_2\text{O}_3/\gamma\text{-Fe}_2\text{O}_3\text{+BCP}$) between 0 and 0.25% (for each value, the annealing time at 150°C is also given): **(a1, b1)** $\phi = 0.025\%$ (48h); **(a2, b2)** $\phi = 0.05\%$ (72h); **(a3, b3)** $\phi = 0.10\%$ (72h); **(a4, b4)** $\phi = 0.15\%$ (72h); **(a5)** $\phi = 0.25\%$ (72h). A complementary study was made by keeping the volume fraction of $\gamma\text{-Fe}_2\text{O}_3$ at $\phi = 0.025\%$ and varying the deposited concentration C in order to check the influence of the total film thickness on nanostructuration (Table 6 films nb. 12, 13, 14 and corresponding NR curves on Figure 9).

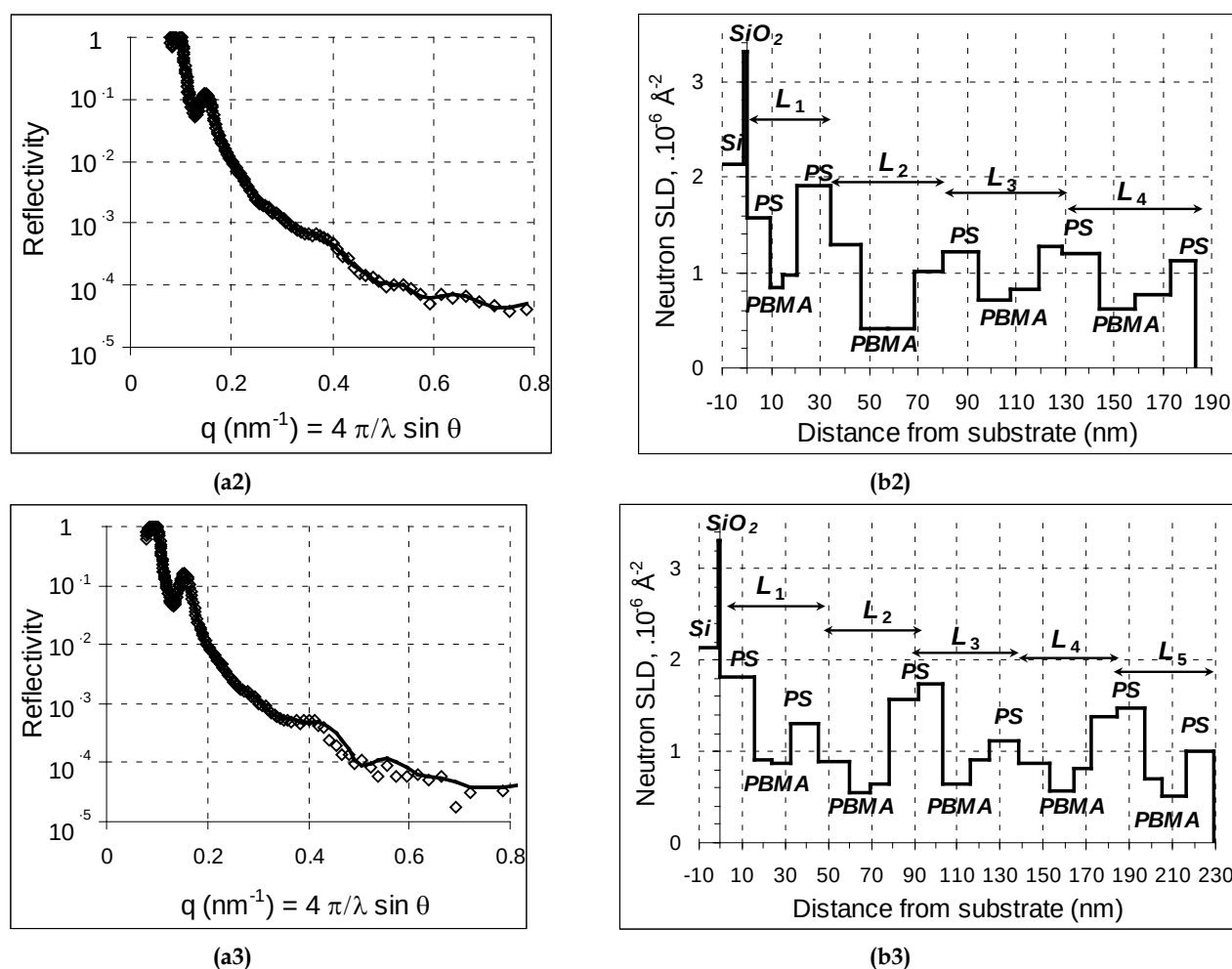


Figure 9. (a1) Neutron Reflectivity curves (raw data and fitting curves) and (b1) neutron SLD profiles deduced from the fit for PBMA₄₂₅-b-PS₄₉₀ films doped with γ -Fe₂O₃@PS1 nanoparticles at $\Phi = 0.025\%$ for varying total concentration C of the solution deposited on silicon (annealing time at 150°C is always 72h): (a1) $C = 10$ g/L ; (a2, b2) $C = 30$ g/L ; (a3, b3) $C = 40$ g/L.

Swelling of the lamellar period by the PS-functionalized MNPs.

As long as the lamellar order was preserved, the incorporation of MNPs into the films had a visible effect on the quasi-Bragg peak that was shifted to slightly lower q_{max} values. This corresponds to an almost monotonous increase of the average lamellar period in the doped case from $L_{\text{ave}}=39.3$ nm for the minimum volume fraction $\Phi=0.025\%$ to $L_{\text{ave}}=43.8$ nm for the maximum one $\Phi=0.15\%$. Following the model by B. Hamdoun *et al.*,^{16, 56} we compute a linear regression $L_{\text{ave}}=L_0(1+p\Phi)$. With this formalism, a value $p=1/3$ corresponds to the case of “small nanoparticles” (*i.e.* entropy driven) confined between adjacent PS layers, while “large nanoparticles” that swell the PS blocks homogeneously would yield $p=1$. In our study, we find $p\approx 89$, which is much larger than the experimental value $p=0.27$ reported for lamellar nanocomposites prepared from PS-*b*-PBMA and MNPs coated by PS chains both synthesized by anionic polymerizations.^{16, 17} This discrepancy might originate from the much higher organic content of the γ -Fe₂O₃@PS nanoparticles in our syntheses (Table 5). With a fraction 73 % w/w of PS chains for our “polydisperse” sample, we calculate that the magnetic cores represent only 7.1%

v/v of the MNPs. Thus we can recalculate the true volume fractions of the MNPs including their organic shells $\Phi_{\text{cor}}\approx\Phi/0.071$ for all the doped films. Within this correction, the slope becomes $p_{\text{cor}}=6.3$ which is still above 1, maybe as an effect of the slight aggregated state of the MNPs. By looking carefully at the NR measurements of the doped films, we remark that the second step of the SLD profiles is almost always above the value of pure PS (except for the thickest film nb. 14 where the maximal value of Nb is at the third step of the profile). As the SLD of iron oxide is well above polymer values ($Nb_{\text{Fe}_2\text{O}_3}=7\times 10^{-6}$ Å⁻²), we deduce that this local raise of SLD might evidence the confinement of the MNPs between the PS blocks of the first (L_1) and the second (L_2) bilayer (and between the second and the third for the thickest films with five bilayers). This irregular repartition of the MNPs not penetrating indistinctively all the space between PS layers might be related to a contribution of sedimentation during the self-assembly process. To complete this study on the insertion of MNPs into lamellae of PBMA-*b*-PS, AFM pictures of the doped film nb. 10 (with the highest load $\Phi=0.15\%$ of MNPs) and of the thicker film nb. 13 are shown on ESI-Figure 2. They exhibit a few differences with the AFM pictures of the undoped film (Figure 6). At

first a much higher density of cracks are present compared to the surface of the pure BCP film. However this roughness remains of the order of a few nm at most (4.2 ± 0.7 nm), otherwise the NR curves of doped films would not present oscillations. Another salient feature is the presence of dark disks on the phase image only, contrary to a pure BCP film showing dark disks – assimilated with holes – on both the height and the phase images. The contrast of phase AFM pictures being proportional to the surface toughness,⁶⁵ we can assign those dark regions to rubbery PBMA domains, much softer than the glassy PS surface. This observation is coherent with the NR and contact angles analyses, that both concluded that the top surface was mainly composed of a PS layer. On the opposite, the streaks on the height image appear on the phase image brighter than the PS background. This might be an indication of the presence of a hard layer of inorganic MNPs beneath the PS layer, somehow responsible for the appearance of cracks. A closer look to those streaks enabled to visualize the BCP lamellae directly, *i. e.* with an orientation of the layers parallel to the top surface (ESI-Figure 2). This phenomenon indicates some distortion of the layers induced by the MNPs, even though most of the surface area covered by the film remains in the same orientation as the pure copolymer.

Refractive index enhancement and optical reflectivity. In addition to the overall thickness of the films, the ellipsometric measurements provide their average refractive index independently. For a composite multi-layer nanostructure, RI is expected to exhibit a complex variation (*i.e.* varying with several parameters such as the total thickness of the coating, the number and the nature of the layers...). Nevertheless, we observe a monotonous behavior for the series of films at approximately constant thickness 110 ± 4 nm and varying inorganic volume fraction Φ between 0.025% and 0.15% v/v. We see indeed that the values of n increase linearly (1.56, 1.61, 1.64, 1.66) as a function of the average bilayer thickness L_{ave} (39.3, 41.1, 42.2, 43.8 nm), which is not the case as a function of Φ . Concerning the capability of those magnetic multi-lamellar films as photonic materials, we calculate the theoretical peak of their optical reflectivity⁸ occurs at a wavelength $\lambda_{max} = 4nL_{ave} \approx 290$ nm for the maximum loading in MNPs (film nb. 10 on Table 6). If we intend to use our system in the visible instead of the ultra-violet range, we need to shift λ_{max} to *e.g.* 500 nm. If such a factor around 1.7 was obtained only by the increase of the lamellar period, L_{ave} should reach ≈ 75 nm. This would necessitate synthesizing a PBMA-*b*-PS sample of molar mass $M_n \approx 270000$ g.mol⁻¹ according to the scaling law plotted on ESI-Figure 3. Although challenging, this goal seems possible to reach by ATRP. The doping by maghemite MNPs will favor the further decrease of this threshold M_n , because they were shown to increase both the lamellar period and the refractive index. By taking into account the observed empirical law that the refractive index of films doped with MNPs varies linearly with the average bilayer thickness, we expect λ_{max} to scale

with L_{ave}^2 . Therefore a 30% increase of the period would be sufficient to reach a maximum $\lambda_{max} \approx 500$ nm in the central part of the visible spectrum. Such a bilayer thickness $L_{ave} \approx 57$ nm could be reached for a molar mass $M_n \approx 190000$ g.mol⁻¹, as estimated from the master curve plotted from the bibliographic study.

5. Conclusions

A well-defined nearly symmetrical PBMA-*b*-PS diblock copolymer with a number-average molar mass as high as 112 000 g.mol⁻¹ and a polydispersity index as low as 1.4 has been successfully synthesized by controlled radical polymerization *via* ATRP. At first, the lamellar ordering of thin films formed with this diblock copolymer after annealing has been evidenced by AFM by the presence of “islands” and “holes” type’s defects on top of the films. Our samples differ from the previously reported systems in two main points: the polymers were synthesized by atom transfer radical polymerization rather than anionic polymerization and the nanoparticles were coated by a “grafting from” method rather than “grafting onto”. These differences of the chemical route modifying the end-groups of the polymer chains are invoked to explain an order inversion of the repetition sequences in the multi-lamellar films on flat silicon substrates, as revealed by neutron reflectivity and checked by contact angle measurements and AFM phase analysis. In other words, the chemical end-groups of the copolymer have a strong influence on the surface energies of the lamellae at the interfaces with both substrate and air.

In parallel, magnetic iron oxide nanoparticles with a coating of PS chains were prepared. As for the copolymer, these chains were synthesized by ATRP, with the supplemental difficulty of initiating the polymerization at a nanometric surface. Their molar mass dispersity was decreased down to 1.4 thank to the choice of magnetic cores with a narrower distribution of diameters. The presence of clusters presumably due to coupling reactions between growing chains from adjacent nanoparticles could not be totally eliminated. A recent study showed that the clustering effect of MNPs also occurs in a homogeneous matrix of PS,⁶⁶ even though they were previously properly dispersed in solution. If the average size of the clusters remains below a threshold value of the order of the lamella thickness (*e.g.* 50 nm) and if they are not too dense, they can be flattened between the lamellae and remain compatible with the self-assembly process. The TEM images of Figure 4 that are projections on the flat surface of the copper grid show indeed aggregates that do not seem thicker than one layer of MNPs.

The introduction of PS-grafted γ -Fe₂O₃ magnetic nanoparticles into the thin films leads to nanocomposites with a preserved lamellar structure, opening possibilities for applications as reflectors in different spectral ranges. In particular, ellipsometry shows a noticeable increase of the optical refractive index that encourages studying further those multi-lamellar magnetic films with a polymer matrix as a material for optical waveguides. Our long-term prospect is to take benefit from the orientation

properties under magnetic field of such nanocomposite thin films for technical applications as responsive mirrors, specific wave absorption or reflection media in either the visible or hyper-frequencies spectrum. In particular, we plan to study the optical reflectivity of these magnetic nanocomposite films under the application of a constant magnetic field of the order of 1 Tesla: the induced magnetization of MNPs confined in 2-d layers associated with the rubbery behavior of PBMA in the “sandwich” nanostructure could lead to a modulation of the lamellar period through a magnetostriction mechanism.⁶⁷ Such an effect would lead to photonic materials that could be controlled by a magnetic field. Other applications related to electronics industry could be thought of, the prerequisite of keeping temperature below *e.g.* 450°C during the whole nanostructuration process to be integrated with semiconductors being fulfilled for the types of BCPs and MNPs that we chose. Those applications necessitate further physical studies (*e.g.* microwave absorption measurements) of the pure lamellar BCP films and of the samples doped with MNPs.

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Universal Scaling Law to Predict the Efficiency of Magnetic Nanoparticles as MRI T2-Contrast Agents

Yves Gossuin^{*,†}, Jean-François Berret,[‡] Quoc L. Vuong,[†] Jérôme Fresnais,[¶] and Olivier Sandre^{*,‡}

[†]Université de Mons, Biological Physics Department, 20 Place du Parc, 7000 Mons, Belgique

[‡]Université Denis Diderot Paris-VII, CNRS UMR7057, Matière et Systèmes Complexes, 10 rue Alice Domon et Léonie Duquet, 75013 Paris, France

[¶]UPMC Univ Paris 06, CNRS UMR7195, Physicochimie des Colloïdes et Sciences Analytiques, 4 place Jussieu, 75005 Paris, France

[‡]Université de Bordeaux, CNRS UMR5629, Laboratoire de Chimie des Polymères Organiques, ENSCBP, 16 Avenue Pey Berland, 33607 Pessac, France

*corresponding authors: yves.gossuin@umons.ac.be, olivier.sandre@ipb.fr

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Supporting Information available free of charge *via* the Internet at <http://pubs.acs.org>

ABSTRACT: Magnetic particles are very efficient Magnetic Resonance Imaging (MRI) contrast agents. In the recent years, chemists have unleashed their imagination to design multi-functional nanoprobes for biomedical applications including MRI contrast enhancement. In this study, we focus on the direct relationship between the size and magnetization of the particles and their nuclear magnetic resonance relaxation properties, which condition their efficiency. Experimental relaxation results on maghemite particles exhibiting a wide range of sizes and magnetizations are confronted to previously published data and to well established relaxation theories with a good agreement. This allows us to derive the experimental master curve of the transverse relaxivity versus particle size and to predict the MRI contrast efficiency of any type of magnetic nanoparticles. This prediction only requires the knowledge of the size of the particles and their saturation magnetization. For maghemite single crystals, the information needed to predict the relaxation efficiency – namely the size and magnetization of the crystal – could be obtained through an adequate fitting of a single magnetization curve. For larger contrast agents made of several magnetic cores assembled into various geometries (dilute fractal aggregates, dense spherical clusters, core-shell micelles, hollow vesicles...), one needs to know the cluster size, the crystal magnetization and the intra-aggregate volume fraction occupied by the magnetic materials relatively to the whole (hydrodynamic) sphere. Finally a calculation of the maximum

achievable relaxation effect – and the size needed to reach this maximum – is performed for maghemite single nanoparticles, dense clusters of maghemite nanoparticles, core-shell (oxide layer around a metallic core) and zinc-manganese ferrite nanoparticles.

Magnetic contrast agents allow a high sensitivity for the early detection of different pathologies and the tracking of magnetically tagged cells *in vivo* through molecular and cellular imaging.¹ In the various magnetic nanoparticles or magnetic hybrids proposed so far, authors have interpreted their relaxivity results compared to literature and specifications of commercial products by referring to different notions such as: nature and size of the Ultra-small Superparamagnetic Iron Oxide (USPIO) grains, effects of clustering (several USPIOs per contrast agent), of a non magnetic shell or of a hydrophobic membrane, of water permeability... In this work, we propose a general treatment of all literature data with a rigorous method using only two (for individual USPIOs) or three (for clusters or hybrids) parameters: diameter and magnetization of the whole particle, and volume fraction of magnetic materials inside.

Values of the literature were confronted to our own products, based on USPIO nanoparticles made of maghemite (γ -Fe₂O₃) synthesized in water according to Massart's procedure² (see Supporting Information). The nanoparticles were readily dispersed in water and form a true "ionic ferrofluid". The iron oxide surface bears positive charges due to

adsorption of protons in acidic media, in that case a dilute HNO_3 solution at pH between 1.2 and 1.7. Such ionic ferrofluids remain in a monophasic state under the application of a magnetic field of arbitrary value. On the microscopic scale, those crystals exhibit a Log-Normal distribution of diameters of parameters $d_0=7$ nm and $\sigma=0.38$, as measured by vibrating sample magnetometry (VSM).³ Maghemite nanoparticles with such a broad dispersity were treated with a size-sorting procedure based on fractionated phase-separation.⁴ After magnetic sedimentation on a strong ferrite magnet to accelerate demixtion, a concentrate could be readily separated from the supernatant. By repeating the phase-separation protocol on both the concentrate and the supernatant, we obtained four new fractions at second level of refined distribution of sizes, and so on after a third and fourth level. To take into account the residual polydispersity, we estimated a weight-averaged diameter characteristic of each sample by calculating the 4th and 3th order moments of the Log-normal distributions deduced by VSM: $d_w = \langle d^4 \rangle / \langle d^3 \rangle = d_0 \exp(3.5\sigma^2)$. This choice of the characteristic size enabled comparing samples of varying size distributions (even for a same median size d_0). It also fairly compares to the mean diameter observed on the electron microscopy pictures and to twice the gyration radius that can be obtained by a common experiment (Guinier's plot) from several scattering techniques (light, X-rays or neutrons). After the size-sorting process, the fractions of interest were coated either by coordination bonding of surface iron ions with tri-sodium citrate (Na_3Cit) ligand⁵ or by electrostatic complexation with sodium polyacrylate (PAA_{2k} or PAA_{5k}) using a precipitation-redispersion protocol.⁶ Superparamagnetic Iron Oxide (SPIO) particles consisting in spherical clusters of a limited number of individual crystals were also prepared by a coacervation protocol with oppositely charged hydrophilic copolymers controlled by salinity.⁷ Experimental details for the size sorting and coating procedures of the USPIOs and their controlled clustering into SPIO coacervates are described in the Supporting Information.

Literature data for pure maghemite nanoparticles,^{8,9} maghemite core-silica shell particles,¹⁰ maghemite or magnetite nanoparticles clustered by polymers,¹¹⁻¹⁶ or encapsulated in lipid vesicles,^{17,18} pristine iron/iron oxide core-shell nanoparticles,¹⁹ and manganese-ferrite nanoparticles clusters^{20,21} were also used to complete our relaxation analysis. Except for very small ones (below 4 nm) which can be used as T_1 positive contrast agents,²² magnetic particles are only effective as T_2 negative contrast agents since at clinical imaging fields (at least 1.5T), their longitudinal relaxation rate R_1 is tremendously low. This was checked for our samples – by recording the evolution of R_1 on a large range of magnetic fields – confirming the small effect of the particles on water longitudinal relaxation (Figure S5 in Supporting Information). On the opposite, water transverse relaxation rate R_2 was strongly enhanced by the presence of magnetic particles with no variation *versus* magnetic field values between 0.47 and 1.41 T (Figure S6). For maghemite particles of diameter d and saturation magnetization M_v (corresponding to the total magnetic moment divided by the particle volume and expressed in the SI unit, A/m), the value of R_2 is given either by the outer sphere (OS) theory – also called

motional average regime – or by the static dephasing regime (SDR).

Particles in the OS relaxation regime: $\Delta\omega\tau_D < 1$

In this case the relaxation is given by

$$R_2 = \frac{1}{T_2} = \frac{16}{45} f \tau_D (\Delta\omega)^2 \quad \text{Eq. (1)}$$

where f is the volume fraction occupied by the particles in the suspension, $\Delta\omega = \gamma\mu_0 M_v / 3$ is the angular frequency shift experienced by a proton at the equator of the particle, $\gamma = 2.67513 \times 10^8 \text{ rad.s}^{-1}.\text{T}^{-1}$ the gyromagnetic factor of the proton, $\mu_0 = 4\pi 10^{-7} \text{ T.m.A}^{-1}$ the magnetic permeability of vacuum and $\tau_D = d^2 / 4D$ the translational diffusion time of the protons in the magnetic field inhomogeneities created by the particles (D being the water translational diffusion constant and d the particle diameter).²³ To obtain the transverse relaxivity r_2 – defined as R_2 normalized by the equivalent iron concentration $[\text{Fe}]$ in mM – the volume fraction f should be expressed in terms of iron concentration. Relaxivity is used in the literature to compare the efficiency of contrast agents. Indeed the iron concentration is easy to measure (by UV-Vis absorption or by atomic emission spectroscopy), which explains the use of the relaxivity concept as a normalized relaxation rate, even if normalization by the volume fraction would be more logical from a theoretical point of view as shown by Eq. (1). The relation between f and $[\text{Fe}]$ is obtained from the molar mass and the mass density of the magnetic oxide (here maghemite):

$$f = [\text{Fe}] \frac{M_{\text{Fe}_2\text{O}_3}}{2\rho_{\text{Fe}_2\text{O}_3}} = 1.57 \times 10^{-5} [\text{Fe}] \quad \text{Eq. (2)}$$

with the molar mass $M_{\text{Fe}_2\text{O}_3} = 0.1597 \text{ kg.mol}^{-1}$ and the mass density $\rho_{\text{Fe}_2\text{O}_3} = 5100 \text{ kg.m}^{-3}$ of maghemite expressed in SI units to obtain the iron concentration in mol.m^{-3} equivalent to mmol.L^{-1} . Then equation (1) writes:

$$r_2 = \frac{R_2}{[\text{Fe}]} = \frac{2}{405} \frac{M_{\text{Fe}_2\text{O}_3}}{\rho_{\text{Fe}_2\text{O}_3}} \frac{\gamma^2 \mu_0^2 M_v^2 d^2}{D} \quad \text{Eq. (3)}$$

which is valid only if the Redfield condition is fulfilled:

$$\Delta\omega\tau_D < 1 \quad \text{Eq. (4)}$$

This is the case for rather small particles of pure maghemite, or for larger entities whose overall magnetization is small compared to that of pure maghemite, for example for dilute clusters or vesicles containing a few iron oxide nanoparticles, or for isolated magnetic cores wrapped by a rather large impermeable coating. For maghemite particles respecting Eq. (4), the relaxivity can be divided by M_v^2 in order to isolate the dependence on the diameter of the particles. This leads, when replacing the different constants by the maghemite corresponding values, to

$$\frac{r_2}{M_v^2} = a_{\text{theo}} d^2 = 5.9 \times 10^{-12} d^2 \quad \text{Eq. (5)}$$

using $D = 3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$ as water diffusion constant at 37°C. To verify this relationship, we have represented r_2/M_v^2 for our samples (r_2 in $\text{s}^{-1}.\text{mM}^{-1}$) *versus* diameter on a log-log scale (Figure 1). The saturation magnetization M_v (A.m^{-1}) was obtained from the fits of the magnetometry curves with a precise knowledge of the solid volume fraction f in the suspension from an

independent titration of iron (also necessary to deduce the relaxivities). Data from literature corresponding to various ferrite nanoparticles and to clusters in the OS regime are also presented on Figure 1.

For individual USPIO nanoparticles, the weight average diameter (nm) obtained by magnetometry was used (d_w). For the clusters and the nanoparticles surrounded by an impermeable non magnetic coating (e.g. silica or polymer), the plotted diameter was either measured by electron microscopy (d_{TEM}) or by dynamic light scattering (d_H) depending on availability in the cited references.

For clusters and hybrids, we introduce the intra-aggregate volume fraction of magnetic materials ϕ_{intra} to derive a corrected relaxivity $r_2' = r_2 \times \phi_{intra}$ that needs to be used for the calculation of $r_2 \times \phi_{intra} / M_v^2$ to properly compare the values of relaxivities between different systems. This correction provides relaxation

data for clusters as if they were presenting the same volume fraction of particles as in a 1 mM iron maghemite USPIO particles' suspension. Indeed, the normalization by M_v^2 is theoretically justified for the same total volume fraction of particles f in the suspension (including both coating and iron oxide parts), not for the same iron concentration, even though the relaxation efficiency is usually expressed in terms of relaxivity per equivalent mM of iron atoms. For example with clusters containing $\phi_{intra}=10\%$ in volume of maghemite nanoparticles, the volume fraction f of clusters in a 1 mM [Fe] suspension will be 10 times larger than for a 1 mM suspension of the dispersed maghemite nanoparticles. The measured relaxivities should thus be divided by ten to normalize the relaxation effect to the same volume fraction of magnetic particles.

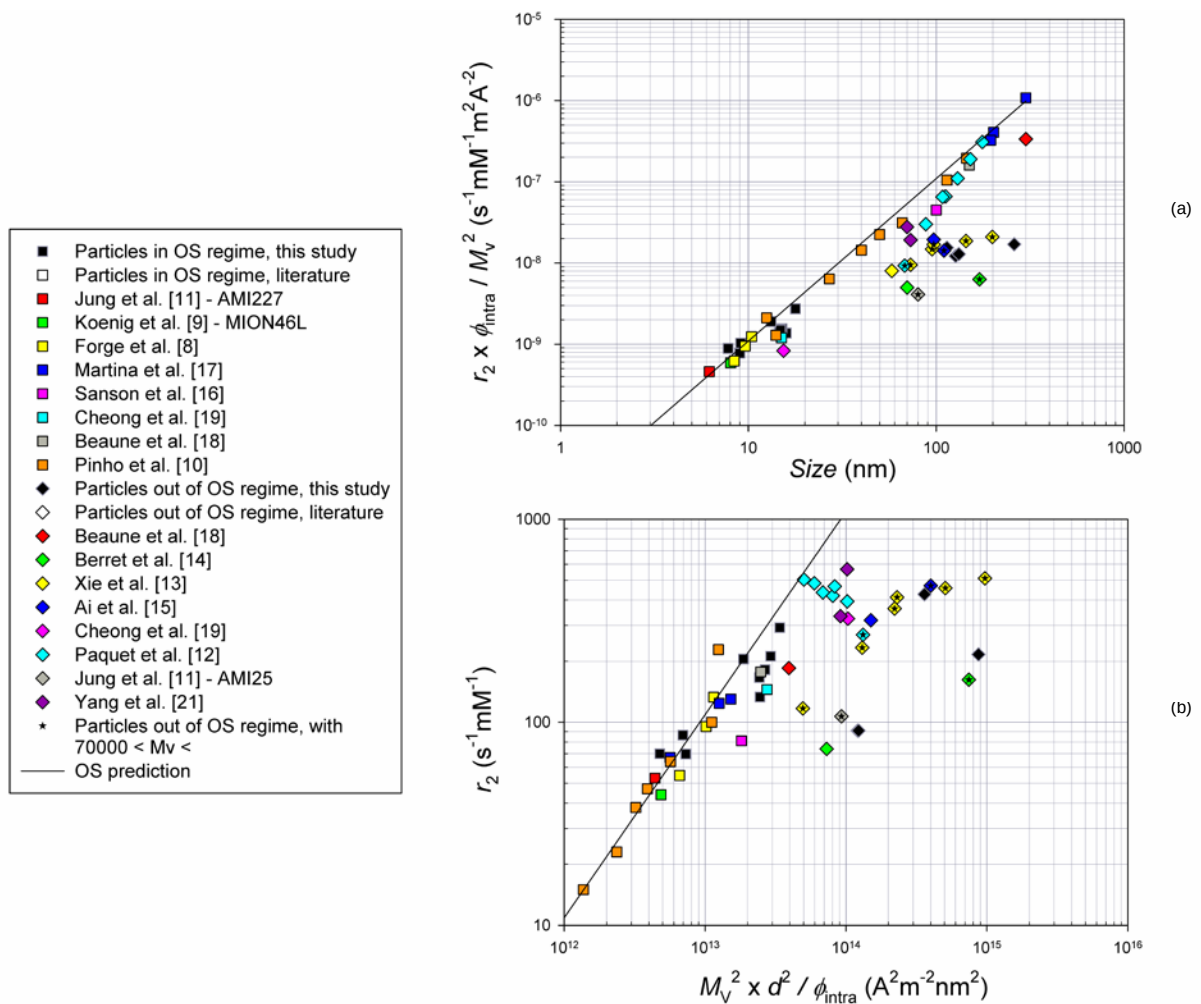


Figure 1. (a) Transverse relaxivity at high field ($\geq 1T$) and at $37^\circ C$ with the appropriate weighting by the intra-aggregate volume fraction ϕ_{intra} and normalization by the square of the magnetization enabling to compare USPIOs (single cores) and SPIOs (clusters) on the same curve. The solid line is Eq. (6). Samples were divided into two categories: OS regime when $\Delta\omega\tau_D < 1$ (square symbols) and out of OS regime when $\Delta\omega\tau_D > 1$ (diamonds). (b) Raw values of the transverse relaxivity at high field ($\geq 1T$) appearing linear with the squares of the magnetization and of the diameter divided by the intra-aggregate volume fraction for individual USPIOs (for which $\phi_{intra}=100\%$) and clusters (either of low size or dilute) in the OS regime i.e. satisfying Eq. (4). We can also introduce the specific magnetization of the magnetic cores in the cluster m_s and relate it to the whole body magnetization $M_v = \phi_{intra} \times m_s$. Then the transverse relaxivity r_2 becomes linear with $m_s^2 \times d^2 \times \phi_{intra}$ in the OS regime. The other clusters corresponding to $\Delta\omega\tau_D > 1$ exhibit a lower r_2 than the power law and saturate below a plateau value given by Table 1.

Figures 1a and 1b show the experimental data of samples from this study and from literature. Figure 1a presents the normalized relaxivity, obtained as described above, while Figure 1b presents directly the relaxivity in order to ease the comparison with measured values. Both figures show that samples respecting Eq. (4) are indeed quantitatively following a quadratic dependence on diameter over almost two decades (6–300 nm):

$$\frac{r_2}{M_v^2} = a_{\text{exp}} d^2 = 11 \times 10^{-12} d^2 \quad \text{Eq. (6)}$$

where a_{exp} was obtained by a one-parameter quadratic fit. The agreement between a_{exp} and a_{theo} values is fairly good, since the size distribution of the nanoparticles – although contained in the volume averaged diameter – is expected to influence relaxation²⁴ and thus the actual value of prefactor a . By using this relationship, it is now possible to predict the transverse relaxivity at magnetic fields above 1 T and at 37°C of any sample of particles or clusters in the OS relaxation regime whose diameter (d_w , d_{TEM} or d_H), magnetic content $\overline{\mu_{\text{FeO}_3}}$ and saturation magnetization M_v are known, prior to any NMR or MRI measurement. For particles in the OS regime whose relaxivities were measured at another temperature than 37°C, one can simply use a correction factor corresponding mainly to the tabulated variation of viscosity, reflecting the change of diffusion constant of water molecules.

Particles out of the OS relaxation regime: $\Delta\omega\tau_D > 1$

For particles whose size or magnetization implies $\Delta\omega\tau_D > 1$, another model should be used: the Static Dephasing Regime (SDR), with a predicted relaxation rate of

$$R_2^* = \frac{1}{T_2^*} = \frac{2\pi}{3\sqrt{3}} f \Delta\omega \approx R_2 \quad \text{Eq. (7)}$$

With the same transformation as above, one obtains

$$r_2^* = \frac{R_2^*}{[\text{Fe}]} = \frac{\pi}{3\sqrt{3}} \frac{M_{\text{FeO}_3}}{\rho_{\text{FeO}_3}} \frac{\gamma \mu_0 M_v}{3} \approx r_2 \quad \text{Eq. (8)}$$

In fact, the SDR model is not taking into account the effect of the refocusing pulses used in all T_2 measurement sequences. Equations (7) and (8) are only exact for R_2^* and r_2^* respectively. Nevertheless, they also provide a good estimate of the maximum reachable values of r_2 and R_2 when $5 < \Delta\omega\tau_D < 20$.²⁵

When designing a contrast agent, the size and magnetization of the particle should be chosen to satisfy this condition in order to obtain the highest R_2 . Above the upper limit $\Delta\omega\tau_D \approx 20$, R_2 is no more approximated by the SDR, since the refocusing pulses used in the measurement sequence become effective. In this third relaxation regime described by the Partial Refocusing Model,²⁶ $R_2 < R_2^*$ and it depends tremendously on the echo time.

For particles with characteristics corresponding to $1 < \Delta\omega\tau_D < 5$, the outer sphere model is already no more valid and a transition between OS and SDR relaxation is observed. The SDR predicted relaxation rate is reached only when $\Delta\omega\tau_D \approx 10$. This transition is observed on Figure 1a for dense

clusters of maghemite particles of similar M_v (comprised between 7×10^4 and 10^5 A.m^{-1} , symbols with a star). For these samples, the value of $r_2 \times \phi_{\text{intra}} / M_v^2$ increases with size then approaches a plateau value. For more diluted clusters, the points should follow another curve since Eq. (8) predicts that different M_v values will lead to different plateau values. The normalization by M_v^2 only makes sense indeed for particles respecting the OS condition Eq. (4) and thus it is difficult to build an empirical function able to predict the relaxation rates for magnetic particles over the whole range of diameter and magnetization. Therefore the different samples out of the OS regime appear as a cloud of points on Figure 1. Only a tendency can be obtained for particles with M_v between 7×10^4 and 10^5 A.m^{-1} (symbols with a star inside).

The point corresponding to iron/iron oxide core-shell particles is slightly below the data corresponding to particles in the OS regime with similar sizes (see supporting information). This is logical since for this sample $\Delta\omega\tau_D = 1.5$, a value corresponding to the transition between OS and SDR relaxation regimes. Even though r_2/M_v^2 is smaller than the OS prediction, the raw value r_2 is twice as large as for a pure iron oxide USPIO of same outer diameter. However the comparison is somehow complicated by the different stoichiometry and density of both compounds (pristine iron in the core and iron oxide in the shell), implying different volume fractions f for iron oxide and core-shell particles with identical iron concentration: respectively $f = 1.57 \times 10^5$ for iron oxide and $f = 10^5$ for $\text{Fe@Fe}_3\text{O}_4$ at 1 mM equivalent [Fe], so that the increase of specific magnetization ($M_v = 6.6 \times 10^5 \text{ A.m}^{-1}$ or 115 emu/g) is somehow counterbalanced by the decrease of molar volume. Nevertheless, increasing the specific magnetization of USPIOs by an appropriate choice of their metal composition is always an option to optimize the properties as MRI contrast agents (as long as toxicity is not introduced by metals). It was recently proved indeed that an appropriate composition of zinc and manganese ferrite enables reaching $r_2 = 860 \text{ s}^{-1} \cdot \text{mM}^{-1}$.²⁷

The relaxation enhancement effect of magnetic particles is expected to present a maximum value for a given size. It is well known from computer simulations and also supported by the shape of the curve obtained for dense clusters. This maximum occurs when the system is completely in the SDR. As previously stated, this is the case when $\Delta\omega\tau_D \approx 10$. It is thus possible to estimate, using Eq. (8), the maximum efficiency of different types of magnetic particles as well as the optimal size at which this maximum should be reached. The results, obtained by using the expressions of $\Delta\omega$ and τ_D , are presented in Table 1.

Type of contrast agent	M_v $A.m^{-1}$	d_{optimal} nm	r_2^{max} $s^{-1}.mM^{-1}$
Maghemite nanoparticles	3.5×10^5	55	750
Clusters of maghemite nanoparticles at a volume dilution of 5 ($\phi_{\text{intra}}=20\%$ maghemite fraction v/v)	7×10^4	120	750
Iron/iron oxide core-shell particles	6.6×10^5	38	1200
$(Zn_{0.4}Mn_{0.6})Fe_2O_4$ nanoparticle	8.75×10^5	35	1860

Table 1. Magnetizations, optimal diameters and maximum relaxivities for different types of magnetic particles.

The first important information obtained from Table 1 is that the maximum relaxivity is the same for maghemite nanoparticles and clusters of maghemite particles, only the optimal size of particles needed to reach the maximum is different (from the combination of equations with the condition $\Delta\omega\tau_D \approx 10$). In the case of USPIOs, such a diameter of 55 nm remains below the maximum size of magnetic (Weiss) monodomains,²³ but it will be a challenge to reach experimentally in a proper dispersed (colloidal) state because of strong inter-particle attractions. On the contrary, different SPIO clusters described in the literature plotted on Figure 1b already approached this maximum relaxivity of $750 s^{-1}.mM^{-1}$,^{13,28,29} although not yet reached experimentally with γ - Fe_2O_3 or Fe_3O_4 cores, according to the authors' knowledge. Secondly, as expected,^{19,30} core-shell or special ferrite particles with a higher saturation magnetization than pure iron oxide could allow obtaining relaxivities up to 1200–1860 $s^{-1}.mM^{-1}$. Finally, it should be stressed that this optimum relaxation will only be approached by particles presenting a rather narrow size distribution centered on the optimal size, since smaller (in the OS relaxation regime) and larger particles (following the Partial Refocusing Model) will present reduced relaxivities and lower the mean transverse relaxivity of the sample. This may explain why some samples are not presenting high relaxivities although their mean size is close to the optimal size.

To conclude, by validating simple principles of the theory of proton relaxometry on a wide range of experimental systems, this communication proposes a unified method to predict the transverse relaxivity r_2 of MRI contrast agents at clinical field based on materials (M_v) and geometrical (d , ϕ_{intra}) parameters. The scaling laws $r_2 = a_{\text{exp}} M_v^2 d^2 / \phi_{\text{intra}}$ and $r_2 = a_{\text{exp}} m_s^2 d^2 \times \phi_{\text{intra}}$ corresponding to the Outer Sphere model and the maximum predicted r_2 at the cross-over between OS and SDR regimes offer practical tools to the chemists who aim at optimizing the relaxometric properties for MRI in the design of particles more complex than the commercially available T_2 contrast agents, such as multi-modal probes or theranostic nanovectors.

ASSOCIATED CONTENT

Supporting Information. SI.1 Synthesis of maghemite particles. (a) Maghemite nanoparticles. (b) Coatings. (c) Synthesis of the clusters. SI.2 Characterization of the nanoparticles. (a) Magnetometry. (b) Transmission Electron Microscopy. (c) Dynamic Light Scattering. (d) NMR Relaxometry SI.3 Data used for Figure 1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Authors

*yves.gossuin@umons.ac.be, olivier.sandre@ipb.fr.

Author Contributions

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ABBREVIATIONS

USPIO, ultra-small superparamagnetic iron oxide; SPION, superparamagnetic iron oxide nanoparticle; MRI, magnetic resonance imaging; OS, outer sphere; MAR, motional average regime; SDR, static dephasing regime; DLS, dynamic light scattering; VSM, vibrating sample magnetometry; SI, système international d'unités.

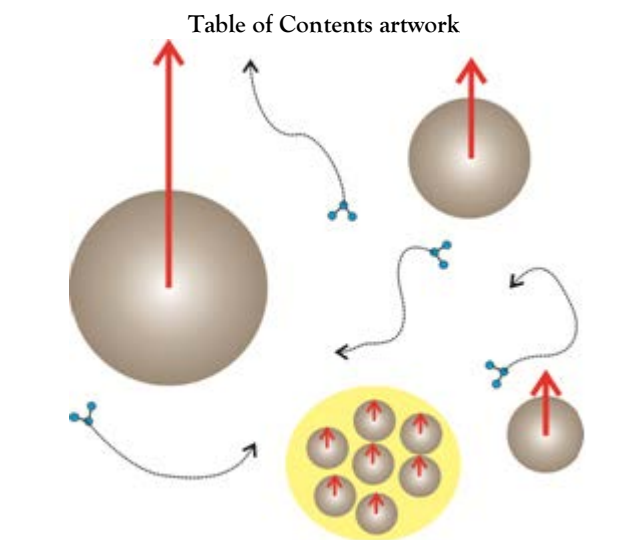
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SYNOPSIS TOC. This study focuses on the uppermost importance of the size and magnetization of magnetic nanoparticles on their performance as MRI contrast agents. Experimental relaxation and magnetometry results on maghemite particles are used for the drawing up of a master curve, which allows the prediction of the efficiency of future nanoparticles or dense clusters of nanoparticles. A calculation of the optimal size for T_2 contrast agents of different natures is also performed.



Phase behavior of nanoparticles in a thermotropic liquid crystal

Cristina Da Cruz, Olivier Sandre, Valérie Cabuil*

Laboratoire des Liquides Ioniques et Interfaces Chargées, Unité Mixte de Recherche 7612 Centre National de la Recherche Scientifique / Université Pierre et Marie Curie,

Case 63, 4 Place Jussieu, 75252 Paris cedex 05 FRANCE

* corresponding author

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ABSTRACT: In this work we describe the outstanding behavior of a nanocomposite system composed of the thermotropic liquid crystal 5CB doped with nanoparticles of the magnetic iron oxide maghemite ($\gamma\text{-Fe}_2\text{O}_3$). We show that the I-N transition is associated to a reversible gathering of nanoparticles inside droplets of ferronematic phase coexisting with a non magnetic nematic host phase.

Composite systems composed of a liquid crystal (LC) doped by particles have been the subject of numerous famous works in the past¹ and in the last years.² LC ordering induces topological defects^{3,4} and additional long-range forces⁵ among colloidal particles. These forces can result in the formation of supermolecular structures,^{6,7} cellular structures^{8,9} and even soft solids.¹⁰

Due to their anisotropic properties, liquid crystals can be orientated under magnetic or electric fields.¹¹ However, the intensity of a magnetic field necessary to align a LC can be too high (> 0.1 Tesla) for practical applications. This limitation could be overcome with *ferronematic* liquid crystals (FNs) obtained by doping a nematic matrix with ferromagnetic particles. Well before their real experimental synthesis, ferronematics were first introduced theoretically by Brochard and de Gennes¹ to increase the magnetic anisotropy of LCs and to lower the magnetic field intensities required for their orientation. The authors also predicted that a strong anchoring of the mesogen molecules onto the particles would result in a ferromagnetic behavior of the matrix (*i.e.* a hysteretic magnetization curve).¹

Since then, research on lyotropic¹²⁻¹⁵ or thermotropic^{16,17} ferronematics have focused on the decrease of the critical field of the so-called Fredericks transition, which originates from the competition between field-induced and surface-induced orientations of the nematic director. Until now, the poor solubility of the magnetic grains in thermotropic LCs has restricted their study to either large (0.5 μm long) magnetic needles¹⁶ or to spherical magnetic nanoparticles (with typical dimensions of 100 Å) but volume fractions of 0.05% at most¹⁷.

Ferrofluids are colloidal suspensions of magnetic nanoparticles that remain stable under whatever intensity of magnetic field or magnetic field gradient.¹⁸ So-called ionic ferrofluids^{19,20} are composed of electrically charged magnetic grains in water, which electrostatic repulsion prevents from flocculation. A second type called oily ferrofluids is composed of surfactant coated magnetic nanoparticles, for which the stability of the colloidal solution is provided by a

steric repulsion which counterbalances Van der Waals and magnetic dipolar attractions. Ferrofluids in various organic solvents can be derived from ionic ferrofluids by coating the grains with appropriate surfactants.²¹ The typical concentration of magnetic grains in a ferrofluid is larger than 10^{16} grains / cm^3 . The average magnetic moment of a grain is of the order of 10^4 Bohr magnetons (also the approximate number of iron ions per nanoparticle).

Herein we describe the formulation of a composite material that results from the incorporation of magnetic nanoparticles into a matrix made of a thermotropic liquid crystal (LC) exhibiting a nematic phase at room temperature. A special attention is paid to the dispersion state and to the phase behavior of the magnetic nanoparticles in the matrix in relation with the phase behavior of the LC.

Previous works mentioned dispersing a magnetic powder made of nanometric grains directly into a liquid crystal and then sonicating¹⁷. Instead of this method, we chose to mix the LC with a ferrofluid constituted of a dispersion of magnetic nanoparticles in a volatile solvent in order to reach higher concentrations in the LC and a proper dispersion state. The choice of the surfactant preventing particles from aggregation is of uppermost importance.

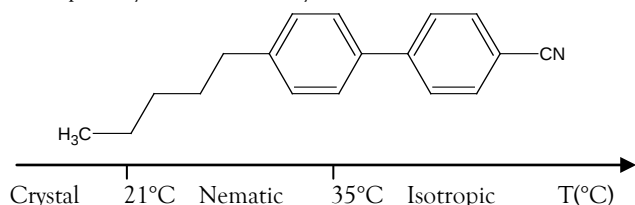
Materials

The nematic liquid crystal used for this work is 5CB *i. e.* 4-pentyl-4'-cyano-biphenyl presented in **scheme 1**, which exhibits a nematic phase between 21°C and 35°C.

The magnetic nanoparticles are prepared by alkaline co-precipitation of ferric and ferrous salts following a procedure described previously.²⁰ The obtained magnetic oxide is maghemite $\gamma\text{-Fe}_2\text{O}_3$. The surface of the nanoparticles is grafted by Beycostat NE (BNE),^{21,22} which is a commercial anionic surfactant composed of a mixture of mono- and diesters of phosphoric acid, the chain being an alkylphenol containing nine ethoxy groups. We choose Beycostat NE to coat the nanoparticles because of its affinity for aromatic solvents, whereas oleic acid used in some publications leads to a systematic aggregation of the magnetic nanoparticles. BNE is

adsorbed directly onto the precursor cationic maghemite nanoparticles in nitric acid medium. After rinsing some of the ungrafted BNE in excess in a mixture of methanol/ether, the dispersion of the nanoparticles is achieved in dichloromethane. These magnetic nanoparticles exhibit some polydispersity in diameters ranging from 5 to 12 nm. (see supporting information)

Scheme 1: Molecular structure and polymorphism of 5CB, the liquid crystal here in study.



Preparation of ferronematic samples

The ferronematics are prepared by simply adding the magnetic nanoparticles suspended in dichloromethane to the

liquid crystal in nematic phase at room temperature and then waiting for the solvent to evaporate. The iron concentration in the LC is measured by Atomic Absorption Spectroscopy (AAS) at $\lambda = 480$ nm after degradation of iron oxide nanoparticles into ferric ions by concentrated hydrochloric acid.²³

Optical observations

First we see by eye a biphasic state at room temperature, that disappears when we heat carefully our ferronematics until crossing the phase transition to isotropic state. At this $T_{(N-I)}$ temperature and above, a stable dispersion of the nanoparticles is obtained. This homogeneous mixture resulting from the heating to the isotropic phase stays homogeneous even when submitted to a strong magnetic field (around 1 Tesla) as long as temperature remains above $T_{(N-I)}$. Therefore, it can be considered as a true ferrofluid with the isotropic LC as solvent. Optical microscopy experiments are carried out in order to observe the microphase behavior of the mixture.

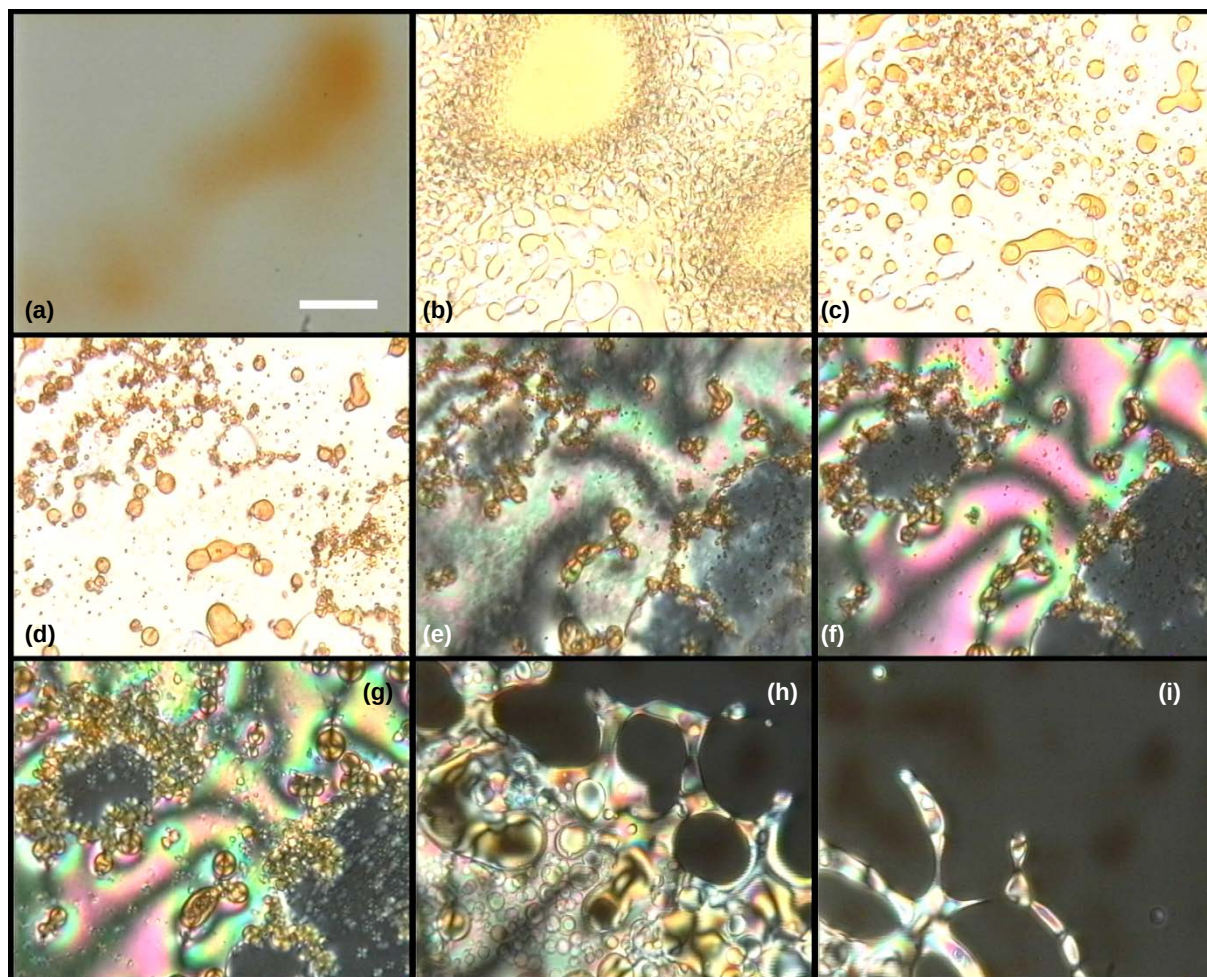


Figure 1: Observation of the microphase separation of a typical ferronematic sample of this study by optical microscopy under parallel (a, b, c, d,) or crossed polarizers (e, f, g, h, i). Pictures (a), (h) and (i) are above $T_{(N-I)}$ and all the other ones are below. The scale is 50 μ m. (a): isotropic phase above $T_{(N-I)}$ in an almost completely homogeneous state, the orange color being slowly disappearing; (b): sudden apparition of an interface at $T_{(N-I)}$; (c): formation of round droplets; (d): shrinking of the droplets and concentrating of the magnetic materials inside; (e): observation of the strings of droplets superposed with the disclination lines of the nematic matrix; (f): observation of black crosses inside the droplets; (g): growth of the droplets when the temperature is increased; (h): above $T_{(N-I)}$, dissolution of the droplets into the nematic and advance of the isotropic (black) phase; (i): homogeneous isotropic state almost everywhere.

The FN samples are observed under crossed polarizers using an upright microscope (Leitz, 20X magnification) equipped with a homemade Peltier temperature-controlled stage. Some

of the warm isotropic mixture is deposited between two glass slides where it cools down and then it is heated again above the nematic-isotropic transition point. The phenomenon

occurring on cooling below the I-N transition appears under the microscope as the formation of dark orange microdroplets, which means rich in iron oxide nanoparticles. In a reversible way, droplets disappear abruptly on heating above $T_{(N-I)}$, and then are replaced by a diffuse cloud of nanoparticles vanishing slowly (**figure 1a**). On decreasing again temperature below the I-N transition, the microphase separation again occurs in a reversible way and at the same temperature. After a fluctuating interface has appeared suddenly from the initially homogenous matrix (**figure 1b**), the new phase concentrates into droplets, that rapidly shrink and turn into a darker color (**figure 1c**). We observe also that the neighboring droplets originating from the rupture of a larger drop remain aligned together (**figure 1d**). Under crossed polarizers (**figure 1e**), the localization of those strings appears somehow superposed with the Schlieren texture. In other words, the droplets of ferromagnetic phase below $T_{(N-I)}$ are expelled towards the defect lines of the predominant LC (which exhibit typical colors of a

nematic LC under crossed polarizers). We notice also that the diameter of the droplets is controlled by the cooling rate of the quench. Qualitatively, the faster is the cooling, the smaller are the droplets (on average). By careful examination of the droplets under crossed polarizers (**figure 1f**), we become aware that nematic disclination lines exist also inside the droplets (black crosses), meaning that there are not made of isotropic phase. When the sample is heated again, the sizes of the droplets start to increase (**figure 1g**). Then we see the front of isotropic phase (black under crossed polarizers) advancing against the nematic region (**figure 1h**), until it invades the whole space (**figure 1i**). Furthermore, it is important to point out that no matter how many temperature cycles are applied to the samples, the magnetic nanoparticles never form a solid flocculate, which means that some LC acts as a solvent inside the droplets.

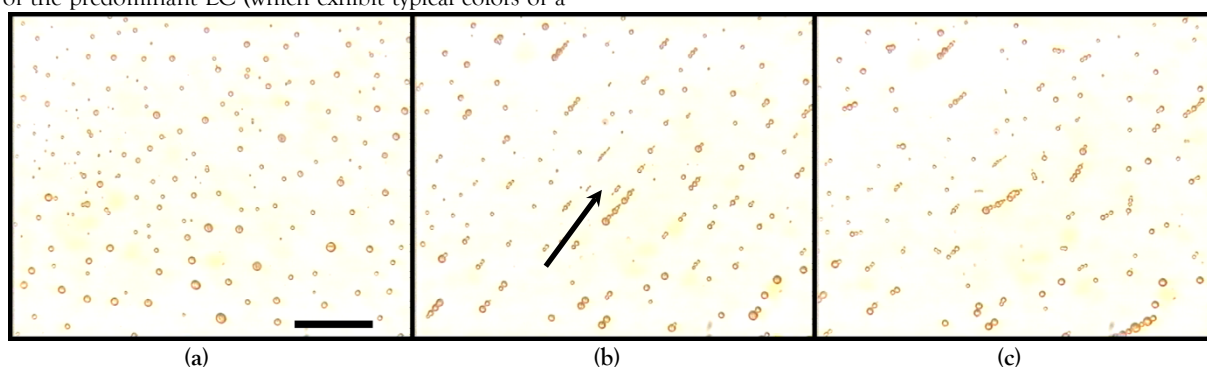


Figure 2: Application of a magnetic field ($B=0.03$ Tesla) to the droplets after the demixing. Polarizers are parallel. The scale bar is 50 μm . (a): Before application of the field, spherical droplets are present, with diameters ranging from 1 to 7 μm (with a maximum of the distribution around 4 μm); (b): after 9 min of field application, most droplets are found within chains formed by magnetic dipolar attractions; (c): after switching off the field, the droplets remain attached but the orientation of the chains have started to randomize.

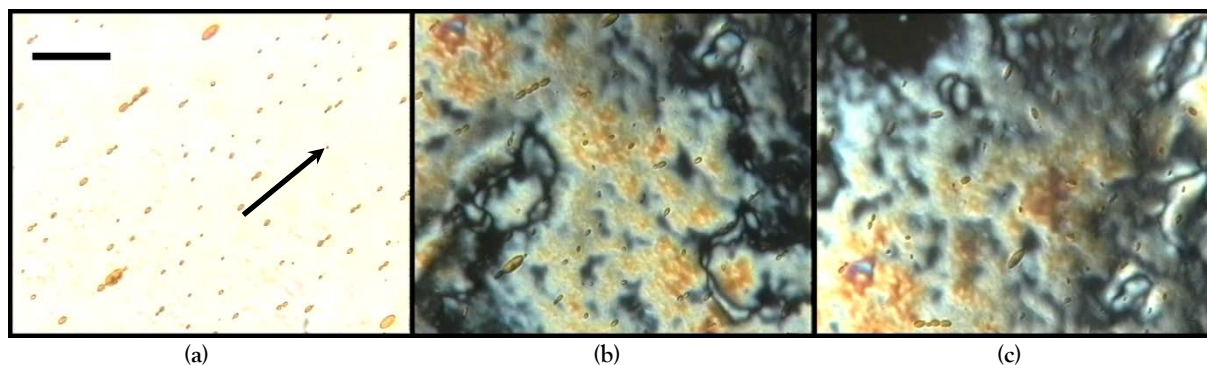


Figure 3: Application of a magnetic field ($B=0.03$ Tesla) since the beginning of the demixing below $T_{(N-I)}$ observed under parallel (a) and crossed polarizers (b, c). (a): the droplets are not only forming chains, but they are also elongated as prolate ellipsoids; (b): under crossed polarizers, the orientation of the chains appears independent of the Schlieren texture of the nematic environment; (c): after release of the magnetic field, both chaining and ellipsoidal deformation persist, but they exhibit random orientations.

Following the variance rule, the existence of a bi-phasic state below $T_{(N-I)}$ is a direct consequence of a multi-component system. Such a case was reported for hydrophilic silica particles (5 nm diameter) in a 5CB host,²⁴ that do not disperse in the nematic phase but are captured by the N-I interface at the transition. In that example, a complete exclusion of the silica particles is observed below the I-N transition, leading to a precipitate forming a “cellular” structure. Such a network formation below $T_{(N-I)}$ has also been reported recently for the same 5CB LC loaded with sterically stabilized colloids made of a polymer (PMMA), of different diameters ranging from 100 nm to 1 μm .²⁵ By using NMR spectroscopy, the authors of this article proved that even 10°C below $T_{(N-I)}$, 10% of the 5CB

molecules were still in the isotropic phase coexisting with the nematic phase, which they explain by a tri-component system (LC / particles / solvent residue). Here on the contrary, the magnetic nanoparticles accumulate inside non isotropic droplets. The formation of the nematic order below $T_{(N-I)}$ tends here to confine the nanoparticles into concentrate ferromagnetic droplets in coexistence with the apparently unmodified and empty nematic phase.

Microscopic observations of the 2-phase behavior are also made under an applied magnetic field. At first we cool from isotropic to nematic phase at 2 °C/min without any field: small spherical droplets form as seen on **figure 2a**. Under the application of a magnetic field of low intensity (0.03 Tesla)

parallel to the plane of the sample, the droplets which are close enough to attract each other gather into chains containing between two and fifteen droplets (**figure 2b**). When the field is released, chains persist for a while but display rotational Brownian motion as can be seen on **figure 2c**. Chains also appear flexible as droplets within a given chain can move around and eventually escape from the chain. If we wait longer, all the chains disappear. When heating to isotropic, all droplets disappear and nanoparticles disperse themselves again homogeneously in the isotropic LC.

Another kind of experiment consists in applying the same magnetic field all along the transition from the isotropic to the nematic state. In that case, the droplets not only chain but although significantly elongate (**figure 3a**), meaning that they are both magnetic and deformable. However, we never observe droplets coalescing under magnetic dipolar attraction. Observed under crossed polarizers (**figure 3b**), the nematic host phase still exhibits disclination lines, which is a clue that it is poor in magnetic nanoparticles and does not benefit from their orienting properties. Contrary to the strings of droplets under zero field connected to the Schlieren texture, this chaining under a magnetic field is ascribable to field-induced dipolar attractions between the highly magnetic droplets. After field release, the chains persist and the droplets keep their elongated shape (**figure 3c**), but they start to rotate randomly under Brownian motion.

The interpretation of these results is delicate and needs some discussion compared to previously published works on mixtures of nematics with either solid or liquid colloids. With a somehow analogous system consisting of droplets of an aqueous ferrofluid mixed with an immiscible nematic LC,²⁶ Poulin et al. have observed the spontaneous chaining of the droplets by the interplay of surface and elastic forces, in the absence of magnetic field. This effect is not dictated by the magnetic interactions, and therefore is observed also for pure water droplets as long as an appropriate surfactant is used to determine a normal boundary condition of the LC molecules at the surface of the droplets.²⁷ When the anchoring energy dominates over the elastic energy (associated to distortions of the nematic director), each droplet associated to a so-called "hedgehog" defect interacts with the other droplets by (i) long range (dipolar type) attraction and (ii) short range repulsion (of elastic nature, presumably due to the thin nematic layer squeezed when droplets become closer). The situation in the present work is different because 5CB constitutes both the nematic host matrix and the solvent of the dispersed ferrofluid phase. Therefore we can imagine that the boundary conditions at the surface of the droplets is rather planar than normal. At least we know that the interfacial cost of the droplets in the nematic matrix must be low, because they are easily deformed into ellipsoids by a weak magnetic field. This point is corroborated by the absence of coalescence, which would be favored by a larger interfacial tension.

To summarize those optical microscopy experiments, we observe: i) a biphasic behavior below $T_{(N-I)}$ with a nematic LC phase poor or empty of nanoparticles and droplets of ferrofluid phase rich in nanoparticles; ii) a sensitivity to a weak magnetic field causing the deformation and/or the chaining of the droplets along the field direction, but no coalescence.

DSC experiments

Mixtures with different volume fractions of nanoparticles in 5CB are studied by Differential Scanning Calorimetry in order to determine if the biphasic behavior observed optically leads to an endothermic peak, which is a thermodynamic signature of the nematic to isotropic transition. The curves of the heat flows vs. temperature are collected with a Perkin-Elmer DSC 7 calorimeter coupled with a cryostat at different rates of temperature change.

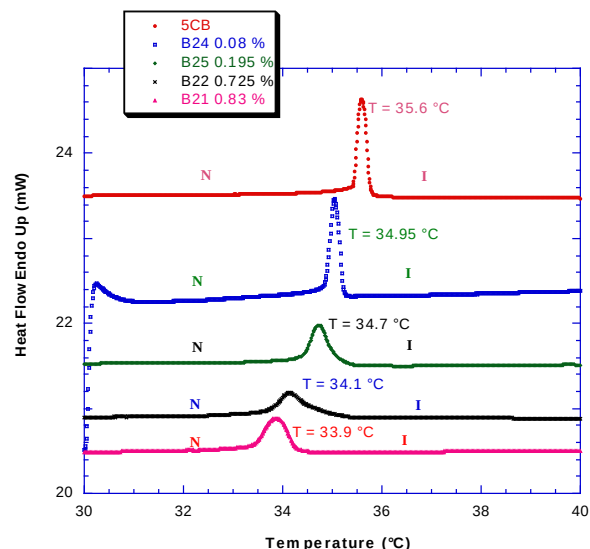


Figure 4: DSC thermograms on heating at 1°C/min for 5CB ferrofluid with different volume fractions of maghemite nanoparticles.

We report here only the results on heating at 1 °C/min, which is also the rate also used in the SAXS experiments. From these thermograms (**figure 4**), we see that increasing the quantity of nanoparticles incorporated in the liquid crystal matrix causes a decrease of the range of the nematic phase existence together with a widening of the clarification peak. From this series of peaks, $T_{(N-I)}$ measured precisely by DSC is plotted vs. the volume fraction Φ of nanoparticles on **figure 5**.

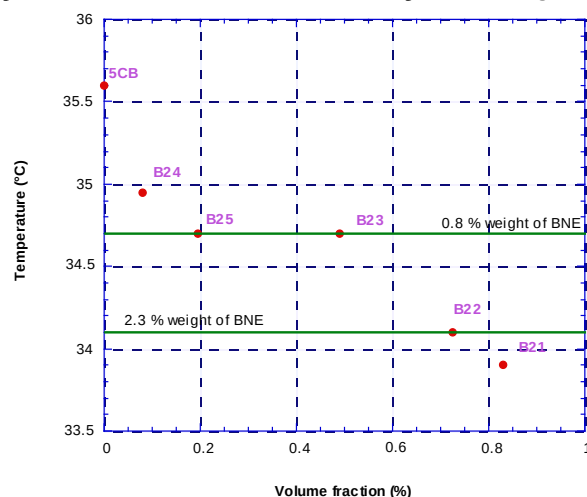


Figure 5: Dependence of the phase transition temperature $T_{(N-I)}$ from the nematic phase to the isotropic phase (determined on heating at 1 °C/min) with the volume fraction on nanoparticles. The horizontal lines represent the two control experiments made without nanoparticles but with the indicated concentration of BNE surfactant.

We see that the decrease of $T_{(N-I)}$ starts from the lowest value of Φ , which proves that no threshold concentration of particles is required for the 2-phase behavior. From the almost

linear decrease of $T_{(N)}$ as a function of Φ , we conclude that some chemical species is more soluble in the isotropic phase than in the nematic phase and perturbs the nematic phase enough to be considered as an impurity. Two possibilities can be considered: on the one hand, a small amount of nanoparticles can be directly soluble inside the nematic matrix (in addition to the droplets which contain most of the nanoparticles); on the other hand, the nanoparticles could be perfectly insoluble in the nematic phase and only the surfactants desorbed from the nanoparticles enter inside the nematic phase. To decide which phenomenon really occurs, we analyze the thermogram of 5CB without nanoparticles but with some added BNE surfactant inside. The transition to isotropic phase occurs at 34.1°C and 34.7°C respectively with 2.3 % and 0.8 % in weight of BNE, which are the same temperatures as B22 and B25 samples respectively. These values fit really well with the estimates of the final proportion of free BNE inside the ferronematics by dilution of the initial ferrofluid, which contains about 5 wt % BNE for a volume fraction of nanoparticles $\Phi = 1.2$ %. To be entirely sure that the decrease of $T_{(N)}$ is due the presence of BNE only, we separate the nematic phase from the magnetic isotropic phase by decantation of the droplets on a strong magnet (-0.5 Tesla). The DSC thermogram of the entire ferronematic and of this separated N phase showing exactly the same $T_{(N)}$ temperature lower than for pure 5CB, we are allowed to conclude that the free BNE is the only parameter that regulates this decrease of transition temperature. As a conclusion, we have proved that the magnetic nanoparticles do not contribute to this decrease of $T_{(N)}$, for which only the free surfactant is responsible. This fact is corroborated by the absence of iron titrated by AAS inside the nematic phase isolated from the droplets by magnetic separation of a ferronematic. If any nanoparticles existed in this nematic phase, the corresponding $[Fe]$ would be less than $10^{-5} \text{ mol L}^{-1}$ (sensitivity of AAS). Let us note that previous studies on ferronematics with other magnetic nanoparticles coated with surfactants at lower concentrations ($< 0.1 \text{ vol } \%$) reported a monophasic behavior with no variation of the clarification temperature.

SAXS experiments

Optical microscopy has indicated that the I-N transition is concomitant with the apparition of a dispersed phase dense in magnetic nanoparticles in coexistence with the nematic host phase containing very few particles or even not at all. Such phase separation can be studied using small angle neutron or X-ray scattering, two techniques that are well suited to study the structure and interactions between nanoparticles in ferrofluids and materials derived from them. SAXS is appropriate here because the contrast between the nematic matrix and the ferric oxide is quite important, so that the signal of 5CB can be considered as a background scattering (**supporting information**). The cross-correlations between nanoparticles in ferronematics are pointed out by comparing the intensity scattered by the composite systems to the one of the dilute ferrofluid, behaving as an ideal gas. SAXS experiments are performed on two samples at two different volume fractions Φ (equal to about 0.2 and 0.7 % respectively) and at different temperatures to follow the interactions between nanoparticles as a function of temperature. These experiments were done in the LURE laboratory using synchrotron radiation (beamline D24, Orsay, France). The samples are introduced in Lindemann X-ray glass capillaries of

1 mm inner diameter placed horizontally in a Mettler F82 temperature stage. Different scattering patterns in transmission mode are recorded as a function of temperature with increasing or decreasing rates of $\pm 1 \text{ }^{\circ}\text{C/min}$. The distance from the sample to the detector was 1750 mm enabling to cover the following q -range : $9 \cdot 10^{-3} - 2 \cdot 10^{-1} \text{ } \text{\AA}^{-1}$. Each SAXS curve is the average of four independent frames, each obtained with an acquisition time of 100 s.

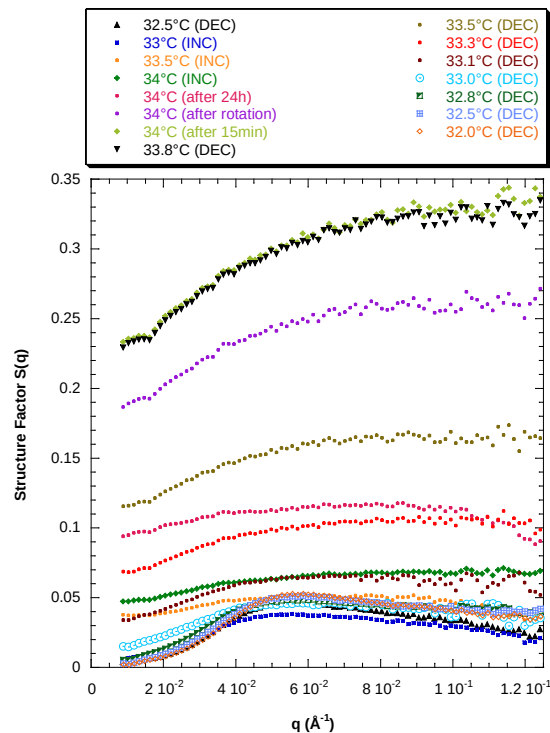


Figure 6: Curves showing the dependence of the structure factor of nanoparticles at different temperatures with a rate of $1 \text{ }^{\circ}\text{C/min}$ as a function of increasing (INC) or decreasing (DEC) temperature for the more concentrated sample (volume fraction = 0.725 %). * capillary turned around its symmetry axis N. B.: We notice from the graphs that the intensity is higher when the sample is in the isotropic state and decreases regularly when reaching the isotropic-nematic transition. The opposite phenomenon is observed in the reverse way. We think that it is due to the sedimentation of the droplets to the bottom of the capillary, because they are rich in nanoparticles and thus with a higher mass density than the nematic phase below T_C . That would result in a lower concentration of nanoparticles analyzed in the beam, and so a lower scattered intensity. As control experiments, we let first the sample at rest for 24h and we observe the intensity slowly increasing by spontaneous homogenization; then, we rotate the capillary by 180° around its axis, and we check that the intensity further increases by a factor three (see curves at 34°C). This variation of nanoparticles concentration in the beam due to sedimentation of bi-phasic samples is also responsible for the difference between the plateau value $S(q)$ at large q and the expected value of 1.

First of all we determine the form factor $I_{FF}(q)$ in a stable dilute ferrofluid, which represents intra-particle density correlations due to their shape convolved with the polydispersity of sizes. The background signal $I_{BK}(q)$ without capillary is also recorded. To take in consideration the contribution $I_{LC}(q)$ of the liquid crystal to the signal $I_{FN}(q)$ of the ferronematics, we record the intensity scattered by pure 5CB at different temperatures between 33°C and 36°C . The

signals obtained at all temperatures are strictly identical and thus can be averaged together. In the studied q -range $I_{LC}(q)$ appears independent of temperature on each side of N-I transition because the signature of the nematic order occurs at higher q values. In our experiments, the liquid crystal only contributes to the high- q background and thus can be subtracted (**supporting information**) to extract the signal of nanoparticles only. All transmitted X-ray intensities T_{Bk} , T_{LC} , T_{FF} and T_{FN} , are measured with an air ionization chamber. As a result, the structure factor is calculated at all temperatures using the following equation:

$$S(q) = \frac{\frac{I_{FN}(q)}{T_{FN}\Phi_{FN}} - \frac{I_{LC}(q)}{T_{LC}}}{\frac{I_{FF}(q)}{T_{FF}\Phi_{FF}} - \frac{I_{Bk}(q)}{T_{Bk}}} \quad \text{where } \Phi_{FF} \text{ and } \Phi_{FN} \text{ are the}$$

volume fractions of nanoparticles in ferrofluid and ferronematic respectively, measured as previously explained **Erreur ! Signet non défini.**

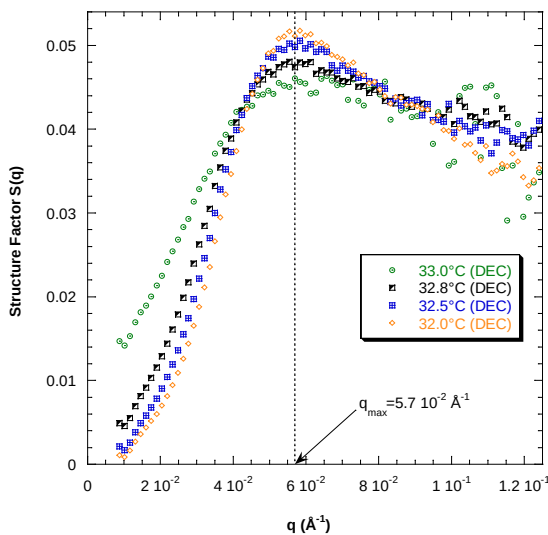


Figure 7: Zoom close to the Isotropic-Nematic transition for the more concentrated sample (volume fraction = 0.725 %).

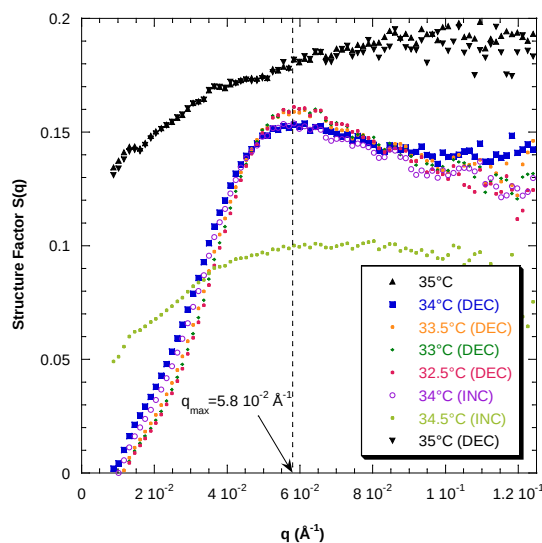


Figure 8 : Curves showing the dependence of the structure factor of nanoparticles at different temperatures with a rate of 1 °C/min on increasing (INC) or decreasing (DEC) temperature for the less concentrated sample (volume fraction = 0.195 %).

By this way, the structure factor of the nanoparticles is determined as a function of temperature for two samples with a volume fraction of nanoparticles of 0.725 % and 0.195 % respectively. These SAXS experiments being sensitive mainly to the nanoparticles, they provide a signature of the I to N transition specific to the nanoparticles, pointing out the effect of the transition of the LC matrix on their spatial organization.

At high temperature where the sample is in the isotropic and homogeneous state, $S(q)$ is nearly flat except at lowest q values where it slightly decreases, which tells us about repulsive interactions between nanoparticles (low compressibility). When temperature is decreased below a threshold T_C , a correlation peak appears suddenly. From the top of this peak at the abscissa q_{max} , we deduce the more likely distance between the particles $d_{max} = 2\pi/q_{max}$. Thus we obtain an estimate of the

local volume fraction $\Phi_{local} = \frac{\pi d_0^3}{d_{max}^3}$, d_0 being the characteristic

diameter of the nanoparticles that we estimate by d_0^{SAXS} obtained from the form factor (see Supporting information).

For the more concentrated sample (0.7 %) a local volume fraction of 17 % is obtained and for the less concentrated one (0.2 %), 18 %. The value of Φ_{local} does not vary significantly with the average Φ measured by iron titration and is much higher than Φ . Therefore we deduce that Φ_{local} measured by SAXS is the volume fraction of nanoparticles inside the droplets observed by optical microscopy below $T_{(N-I)}$. As a consequence, the T_C temperature defined as the appearance of the correlation peak is ascribable to the appearance of the droplets and therefore directly related to $T_{(N-I)}$ of the ferronematic.

For a given sample, reproducible results for T_C were obtained when increasing and decreasing temperature several times, independently of the sample history. Besides, the two samples do not indicate the same temperature T_C : for the more concentrated sample $T_C = 32.8\text{--}33^\circ\text{C}$ (**figure 7**), whereas for the less concentrated sample, $T_C = 34\text{--}34.5^\circ\text{C}$ (the range of transition is not broader but we recorded less spectra around the transition, **figure 8**). This dependence of T_C with the average concentration of particles is in agreement with the variation of the clarification temperature obtained by DSC thermograms : $T_{(N-I)} = 34.1^\circ\text{C}$ at $\Phi = 0.7\%$ and $T_{(N-I)} = 34.7^\circ\text{C}$ at $\Phi = 0.2\%$ (**figure 5**). Even though the slight differences (0.4–1 °C) between T_C and $T_{(N-I)}$ are likely ascribable to an experimental difference between the temperature stages used for each method, we cannot conclude for sure to the equivalence of T_C and $T_{(N-I)}$. Nevertheless, the results of these two techniques converge to the same conclusion that both temperatures T_C and $T_{(N-I)}$ decrease simultaneously. These temperature shifts are related to the concentration of free BNE soluble in the nematic phase.

CONCLUSION

Colloidal dispersions of small particles in nematic liquid crystals are an interesting type of soft matter. The difference from ordinary colloids arises from the orientational ordering of the calamitic molecules and the resulting structure in the colloid. In this work, we present a detailed study of the phase behavior of 5CB with incorporated magnetic nanoparticles by different techniques including optical microscopy, DSC, and SAXS. We have found a multi-scale organized system: magnetic nanoparticles of maghemite confined inside ferronematic droplets, themselves dispersed in a nematic host. By

introducing a large amount of magnetic nanoparticles in a nematic host, we have found indeed a phase separation between droplets rich in nanoparticles and a continuous nematic LC empty of nanoparticles and containing unadsorbed surfactant. This 2-phase behavior has never been reported in previous works, certainly because authors used much lower particles concentrations as ours (Φ of 5×10^{-4} % at most against $\Phi > 0.2$ % here). We have demonstrated that the transition temperatures $T_{(N)}$ measured by DSC and T_C by SAXS are both affected by the presence of nanoparticles and surfactant concentration in a similar way, which is a decrease. The DSC measurements show a decrease of the $T_{(N)}$ temperature linear with Φ as well as a widening of the corresponding peak. These two elements lead us to conclude on the partial solubilization of some species in the liquid crystal in the nematic phase, which we have identified to be pure surfactant. Concerning the possible discrepancy between T_C associated to the presence of droplets and $T_{(N)}$ related to the nematic host phase, experiments could be attempted in future on an experimental set-up combining SAXS and DSC simultaneously. Other perspectives to be explored could be: using the dipolar forces between the magnetic droplets under field to probe the elasticity of the nematic matrix; checking the existence of a hysteresis loop in the magnetization curve of ferronematics as predicted theoretically,¹ obtaining true homogeneous ferronematics by controlling the anchoring of the LC on the nanoparticles. This last goal could be reached by grafting mesogenic molecules resembling to the matrix. More generally, the role of the interaction between the “surfactant” molecules covering the nanoparticles and the thermotropic molecules of the matrix is of prime importance and will be clarified in a forthcoming study; this needs to coat the magnetic nanoparticles by several types of molecules that will interact differently with 5CB.

Supporting information:

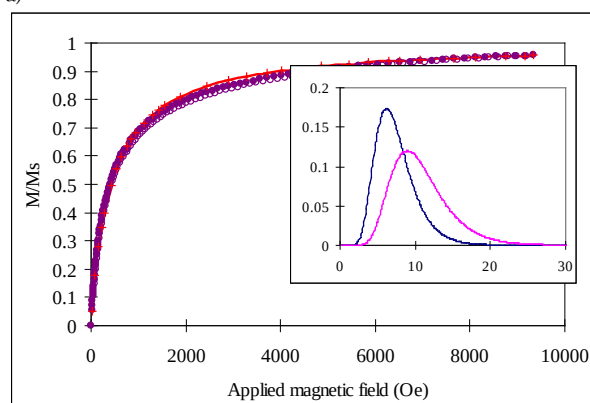
The distribution of the diameters of the nanoparticles used in this study is determined by fitting either the magnetization curve or the form factor of the dilute ferrofluid. As ferrofluids are giant paramagnetic liquids indeed, their magnetization curve versus the applied magnetic field follows a Langevin's law,^{28,29} as long as their interactions are negligible i. e. for small volume fractions $\Phi < 1$ %. The measured magnetization is highly sensitive to the distribution of diameters of the magnetic nanoparticles in the sample,^{30,31} for which we assume a Log-normal distribution law calculated from:

$$P(d) = \frac{1}{\sqrt{2\pi}\sigma d} \exp \left[-\frac{1}{2\sigma^2} \left(\ln \frac{d}{d_0} \right)^2 \right]$$

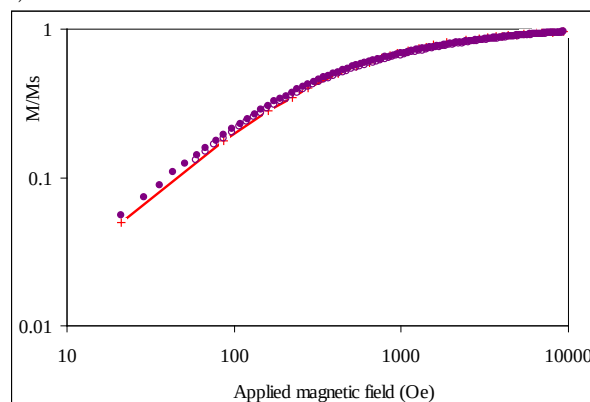
with a characteristic diameter $d_0 = \exp \langle \ln d \rangle$ and a standard deviation σ . Combining the Langevin's law with the distribution $P(d)$ provides us with two parameters d_0 and σ used to fit the experimental curves. The same Log-normal distribution law is also used to compute the theoretical form factor of the dilute ferrofluid, assumed to be a polydisperse assembly of spherical nanoparticles. By adjusting the magnetization curve and the experimental form factor of the ferrofluid used in this study, the characteristic diameters $d_0^{\text{mag}} = 6.3$ nm and $d_0^{\text{SAXS}} = 7.6$ nm are found respectively, both with $\sigma = 0.4$ corresponding to a relatively broad distribution. The discrepancy between the two methods originates from the

magnetically disordered shell at the surface of the grains, which contributes to X-rays scattering but not to magnetization. In other words, the magnetic core of the nanoparticles is somehow smaller than their geometrical volume.

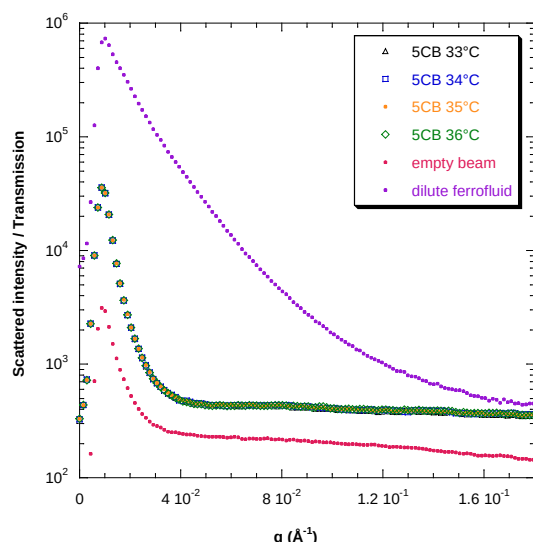
a)



b)



Supporting information 1: Magnetization curve of the ferrofluid in dichloromethane measured with a homemade vibrating sample magnetometer. The magnetization M is normalized by the value at saturation $M_s = 42$ Oe is plotted vs. the applied magnetic field H (Oe). The plateau value corresponds to a volume fraction $\Phi = M_s/m_s = 1.2\%$ where $m_s = 3400$ Oe is the specific magnetization of colloidal maghemite.³² The two parameters d_0 and σ describing the size polydispersity enable to fit the magnetization at both high (a) and low (b) fields.



Supporting information 2: SAXS intensities corrected by sample transmission as a function of the scattering vector q for the empty beam, the dilute ferrofluid and the liquid crystal 5CB alone at different temperatures around T_{NI} .

Acknowledgements:

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Synthesis of iron oxide nanoparticles in a microfluidic device: preliminary results in a coaxial flow millichannel

Ali Abou Hassan,^{*a} Olivier Sandre^a, Valérie Cabuil^a and Patrick Tabeling^b

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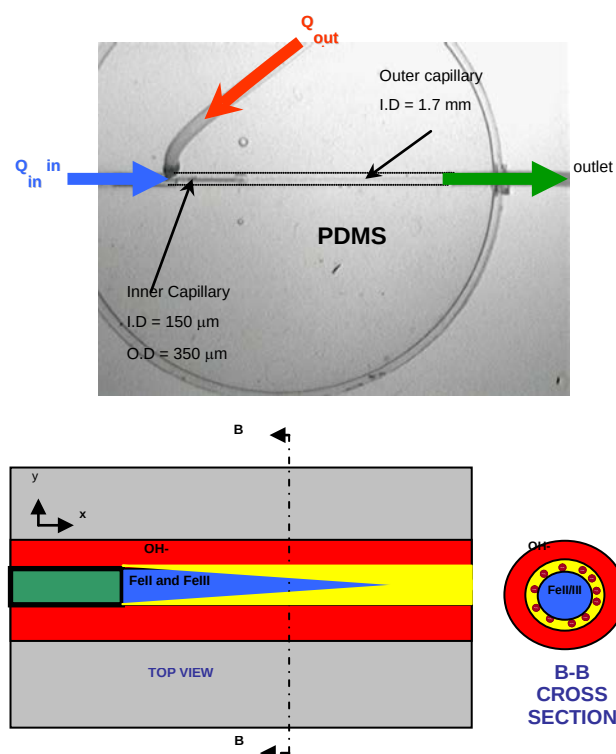
⁵ A millimetric coaxial flow device operating under laminar flow has been designed to study the synthesis of iron oxide nanoparticles in a millichannel. The flow rate of the different reagents has been adjusted all over the experiments so that magnetic and stable colloidal iron oxide particles with a size less than 7 nm have been prepared continuously.

¹⁰ Miniaturization of chemical reactions and the development of “lab on a chip” (LOC) technology has gained significant importance in the recent years.¹ One predicts indeed that small dimensions of vessels lowers the diffusion times of chemical species, so that mass transport is improved in microstructured reactors.² Scaling down reactors dimensions is proposed as an opportunity to improve size and composition control in the field of materials synthesis.³ Local variations in reaction conditions such as concentration and temperature are minimized and therefore control of both nucleation and particle growth can be used to improve monodispersity in nanoparticles synthesis.⁴

As an example, the synthesis of iron oxides nanoparticles has attracted much attention motivated by their wide range of applications. Colloidal iron oxides are used as single bit-elements in high density magnetic data storage arrays,⁵ ionic ferrofluids,⁶ and in the biomedical field for example as contrast enhancement agents for Magnetic Resonance Imaging,⁷ or for hyperthermia,⁸ etc... Because the properties of these nanocrystals strongly depend on their dimension, the synthesis of monodisperse nanoparticles is one of the most challenging problems.⁴ At the beginning of the 1950's V. K. Lamer proposed a mechanism based on a nucleation-growth process to describe the synthesis of nanoparticles.^{5, 6} Since then, various chemical synthetic strategies have been reported to obtain monodisperse nanoparticles with different shapes.¹⁰ Recently, several groups designed microreactors and reported the improvement of inorganic and metallic nanoparticles synthesis. However the synthesis of inorganic materials has been focused on Quantum Dots as CdS¹¹ or CdSe¹², silica,¹³ titania,¹⁴ and titania/silica core-shell colloidal nanoparticles.¹⁵ There has been until now no reported study of the synthesis in microchannels of colloidal iron oxide nanoparticles from the following alkaline coprecipitation reaction:



⁴⁵ For this purpose, we designed a 3D millifluidic device performing the mixing of two coaxial flows of miscible fluids (Fig. 1), one containing the iron “precursor salts”, the other a strong base. The length of the capillary from the confluence



⁵⁰ Fig. 1 Coaxial flows device operating under laminar regime: image of the millifluidic device (top) and sketch of the coaxial streams (bottom) with the reaction zone (yellow) where the growth of nanoparticles occurs.

region to the outlet is 3cm, which corresponds to residence times between 4 and 10 sec depending on the two flow rates Q_{in} and Q_{out} . The outer capillary with 1.7mm diameter (ϕ) was shaped by the molding of a cylindrical tubing (Upchurch) in a Petri dish with Polydimethyl siloxane (PDMS, Sylgard 184) and subsequent removal when the resin is cured. The central capillary with 150 μ m of inner diameter (I.D.), 360 μ m of outer diameter (O.D.) was obtained by fixing a glass capillary (Plymicro[®], usually used for Capillary Electrophoresis) inside the tip of a micropipette (Gilson), which conical shape enables a precise centering. A (poly) tetrafluoroethylene (PTFE) tube (500 μ m I.D., 10cm) leading to a sample collection vial was

connected to the device outlet. By comparison with a 2D flow focusing device (*i.e.* a typical “Y-shaped” channel), the 3D coaxial flows setup offers two main advantages: on the one hand, it enables a precision positioning of the “precursors” flow at the centre of the channel in both longitudinal and lateral dimensions and on the other hand, it avoids adsorption of any precipitate species onto the PDMS walls which are totally wetted by the alkaline outer flow.

A solution of total iron salts concentration $c=10^{-2}$ mol/L with 0.5 as molar ratio Fe(II)/Fe(III) was prepared by mixing FeCl₃ and “fresh” FeCl₂·4H₂O salts in diluted and degased hydrochloric acid (pH~0.4). Diluted hydrochloric acid was used as Fe(II) ions are more stable in acidic medium so non iron hydroxide can precipitate. The tetramethylammonium hydroxide ((CH₃)₄NOH, TMAOH) at a concentration 0.172 mol/L was the alkaline solution. Both solutions were injected into the channel through two syringes actuated automatically (Harvard apparatus), the iron “precursors” solution was pumped into the inner capillary and the TMAOH base into the outer capillary of the channel. TMAOH was chosen prior to any other base as the TMA⁺ cations afford enhanced stability of colloidal oxide dispersion.¹⁶

The volumetric rate flows of both solutions (respectively Q_{in} and Q_{out}) were adjusted to avoid any turbulent vortex in the capillary.¹⁷ Owing to the laminar regime in the channel, mixing is controlled by the diffusion of species.¹⁸ The diffusion of iron complexes in aqueous solution is much slower than the diffusion of hydroxide ions OH⁻ and of the protons H⁺. The diffusion coefficient (*D*) is almost 1×10^{-9} m²/s for a single iron complex as [Fe(OH₂)₆]^{2/3+}^{19, 20} compared to $D \sim 5.3 \times 10^{-9}$ m²/s for OH⁻ and $D \sim 9.31 \times 10^{-9}$ m²/s for H⁺²¹ at 25°C in diluted solutions. Thus, the diffusion of iron complexes from the central acidic stream into the peripheral TMAOH stream flow is disregarded. On the contrary, the fast diffusion of H⁺ and OH⁻ leads to the prediction that the pH varies continuously from acidic in the central flow to basic at the periphery, the pH gradient being progressively smoothed out when moving towards the outlet of the channel. The coprecipitation reaction will therefore be likely confined in the neutral region near the channel centre. The excess of TMAOH will allow the dispersion of the particles after their synthesis, and avoid their adsorption onto the walls. The particles will be collected at the outlet without any problem of plugging. In order to stabilize the inner flow of iron solution before initiating the hydrolysis, we injected at first a HCl solution of same pH~0.4 in the outer stream. The outer flow is then switched to TMAOH solution. A few seconds after the outlet (time across the PTFE tubing), the reaction was “quenched” by fast solvent extraction to prevent any ageing of the nanoparticles in the aqueous solution. In practical, the collection vial containing a cationic surfactant dimethyldodecyl bromide (C₂₆H₅₆BrN, DDAB, C=0.02M) in cyclohexane was stirred at around 200 rpm with a glass helix stirrer.²² Nanoparticles, coated by DDAB in the cyclohexane phase are then collected and a stable colloidal suspension is obtained.

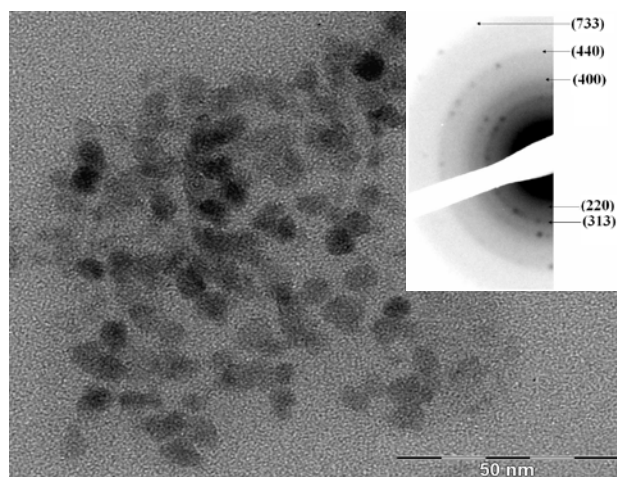


Fig. 2 TEM image of nanoparticles prepared in the channel (for flow rates $Q_{in}=100\mu\text{l/min}$ and $Q_{out}=400\mu\text{l/min}$) and electron microdiffraction pattern with Miller indices of $\gamma\text{-Fe}_2\text{O}_3$

Samples for Transmission Electron Microscopy (TEM) were prepared by placing a single drop of the resulting suspension onto a 200 mesh holey carbon-coated copper grid (SPI) which was then allowed to dry in air. The suspensions obtained by Fe(II)/Fe(III) coprecipitation were also analysed by Vibrating Sample Magnetometry (VSM).

The flow of TMAOH was fixed ($Q_{out}=400\mu\text{l/min}$) and the volumetric flow of the iron precursors mixture was increased ($10 < Q_{in} < 100\mu\text{l/min}$). Colour changed from brown to black, characteristic of magnetite Fe₃O₄. The suspensions obtained are always stable but the Dynamic Light Scattering (DLS) measurements of the aqueous solutions showed that the colloidal suspension exhibits the lowest hydrodynamic size ($d_H=120$ nm) for a ratio $Q_{out}/Q_{in} \sim 4$. TEM observation of the colloidal suspension in cyclohexane indicated that the nanoparticles produced in the channel are fairly spherical with an average size around 7nm. The evidence of their crystallinity is provided by the electron microdiffraction pattern in the inset of Fig. 2, which shows the presence of maghemite phase $\gamma\text{-Fe}_2\text{O}_3$.

Although suspensions obtained in cyclohexane are stable in zero magnetic field. They sediment in the presence of a magnetic field gradient (for example on a strong permanent magnet), which suggests a

magnetic character. This observation agrees with the VSM measurement on a stable unquenched (aqueous) suspension: the magnetization curve (Fig. 3) follows the Langevin law typical of superparamagnetism, calculated for an assembly of nanoparticles with a rather narrow distribution of diameters fitted by a Log-normal law of parameters $d_0=6\text{nm}$ and $\sigma=0.2$. The particles size deduced from the analysis of the magnetization curve shape are in good accordance with the TEM pictures.

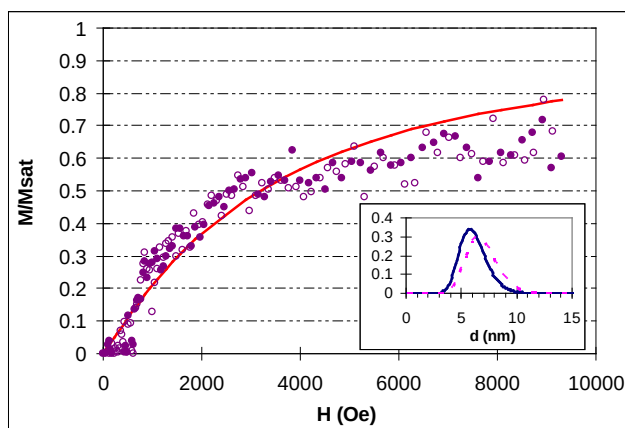


Fig. 3 Magnetisation curve of a stable suspension in water of nanoparticles produced in the millifluidic device. The inset curves represent the fitting Log-normal laws for the numbers distribution (solid line) and the volume distribution (dotted line) of diameters.

By measuring both the volume fraction of nanoparticles $\phi = 5.7 \times 10^{-5}$ (from iron titration by atomic spectroscopy) and the saturation magnetization $M_{\text{sat}} = 7.9 \text{ A/m}$ for the suspension, we deduce a specific magnetization $m_s = M_{\text{sat}}/\phi = 1.4 \times 10^{+5} \text{ A/m}$ for the materials, which is much below the bulk value of maghemite $\gamma\text{-Fe}_2\text{O}_3$ ($3.5 \times 10^{+5} \text{ A/m}$), but however not so far from the m_s value about $2.6 \times 10^{+5} \text{ A/m}$ usually obtained for nanoparticles of approximatively the same sizes prepared with the standard large scale synthesis.²³ Therefore we can deduce that compared to classical ones, nanoparticles prepared within few seconds in a millifluidic channel exhibit only a small decrease of ordering of their magnetic moments.

We have demonstrated that aqueous dispersions of superparamagnetic iron oxides nanoparticles can be prepared on chip in a millichannel reactor by providing a cylindrical geometry of the device, and the use of TMAOH as alkaline reagent. These results open the way to other experiments as online fonctionnalization, online detection and structural measurement and a further miniaturization of the reaction using smaller channels.

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^a Laboratoire Liquides Ioniques et Interfaces Chargées (LI2C), UMR7612 UPMC Univ Paris 06 / CNRS / ESPCI, Université Pierre et Marie Curie, 4 place Jussieu, case 51 75252 Paris cedex 5, France. Fax: +33 (1) 44273228; Tel: +33 (1) 44273166; E-mail: ali.abou_hassan@upmc.fr

^b Laboratoire "Microfluidics, MEMS & Nanostructures" (MMN), UMR7083 CNRS / ESPCI, Ecole Supérieure de Physique et de Chimie Industrielles de Paris, 10 rue Vauquelin 75231 Paris cedex 5, France

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Synthesis of Goethite by Separation of the Nucleation and Growth of Ferrihydrite Nanoparticles by Microfluidics**

Ali Abou-Hassan*, Olivier Sandre, Sophie Neveu and Valérie Cabuil

Continuous flow and droplets-based microreactors have been largely used for studying and optimizing chemical reactions.^[1–13] Several nanoparticles have been synthesized in microfluidic devices: Quantum Dots as CdS,^[5] CdSe,^[6] CdSe-ZnS core-shell nanoparticles,^[7] metallic nanoparticles as Pd, Co, Ag, Au,^[8] and oxides as γ -Fe₂O₃,^[9, 10] SiO₂,^[11] TiO₂,^[12] or SiO₂-TiO₂ core-shell nanoparticles.^[13] Compared to conventional bulk synthesis, microfluidics offers a better control of the reaction parameters (temperature, mass transfer...) which can lead to a better control of particles size.^[1–4] Goethite (α -FeOOH) is an iron oxy-hydroxide widely found in iron-rich soils.^[14] This clay mineral constitutes the natural ochre pigment and because of its elongated shape, synthetic goethite is often used as a precursor of α -Fe “hard magnet” particles for magnetic recording.^[15] Again because of this elongated shape, suspensions of anti-ferromagnetic goethite nano-laths exhibit an original magneto-optical effect and self-assemble spontaneously into a nematic liquid crystal phase above a threshold concentration.^[16] Different methods have been reported for the synthesis of acicular goethite particles principally based on aging of the ferrihydrite nanoparticles obtained by alkalisation of iron (III) salt solutions,^[17] but to date a microfluidic synthesis of goethite particles has not been demonstrated. Due to the importance of particles shape for the improvement of the magnetic properties or for the control of the particles assembly, controlling the synthesis conditions of these particles is important.

So-called “digital microfluidics” manipulating biphasic flows is currently very popular to produce microdroplets, behaving as microreactors for the synthesis of nanoparticles. However, it frequently uses oils and surfactants that can affect the nucleation-growth mechanisms of the particles. Compared to microdroplets reactors, continuous flow reactors are easier to handle and more representative of the conditions of bulk synthesis with improved homogeneity so as to offer a better reproducibility of the results.^[20] Another benefit provided by the continuous microfluidic systems is the ability to add reagents all over the channel length, without needing to synchronize with a dropping frequency as for microdroplets reactors.^[21] Mixing in the continuous flow reactors is diffusion-limited, occurring by diffusion of the chemical species at the confluence of the flows.^[22]

To overcome this limitation, hydrodynamic flow focusing based on the squeezing of a sample flow between sheath flows by using appropriate geometrical conditions has been demonstrated as a reliable method to reduce the diffusion distances and decrease the mixing times.^[23] By adjusting the volumetric rates respectively of sample and sheath flows, a stable central jet is achieved. Besides, the parabolic velocity profile due to the laminar Poiseuille flow is nearly flat in the central focalised jet flow and can be described as a plug flow, so that when the chemical reaction proceeds in the sample jet, one can expect to minimize the shear stress causing the Taylor-Aris dispersion effect during the nanoparticles synthesis.^[24]

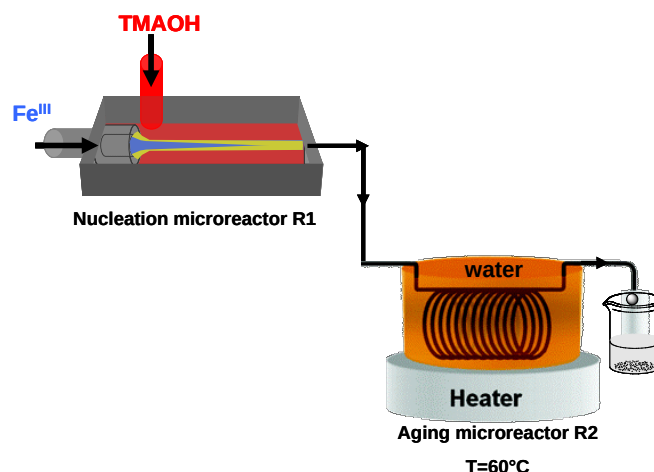


Figure 1. Schematic representation of the experimental setup used for the preparation of the ferrihydrite and goethite nanoparticles.

The aim of the present work is to describe a new method based on microfluidics allowing the acceleration and the control of the production of small goethite nanolaths. The idea is to separate the nucleation of the ferrihydrite nanoparticles from their growth into goethite particles. The nucleation of the primary nanoparticles of ferrihydrite is induced by diffusive mixing at room temperature in a microreactor based on a coaxial flows-geometry (R1; Figure 1). This mixing reactor described in a previous work^[9] is based on a three dimensional coaxial flows device of two streaming reagents. The 3D hydrodynamic geometry allows a rapid homogeneity of the reactants through focalisation and avoids technical problems such as channel clogging and fooling onto the reactor walls.

To induce the nucleation reaction in the R1 microreactor, the solubilised iron (III) chloride solution and the alkaline solution of tetramethyl ammonium hydroxide (TMAOH) are flushed respectively into the inner and the outer streams with the rate flows Q_{in} and Q_{out} . The rate flow of the different species can be continuously adjusted in order to produce a stable inner stream and different mixing times. Fast mixing is required to ensure a homogeneous and uniform chemical composition of the nucleation region in the microreactor.

[*] Prof. V. Cabuil, Dr. O. Sandre, Dr. S. Neveu and PhD A. Abou-Hassan.
UPMC Univ Paris 6, Laboratoire des Liquides Ioniques et Interfaces Chargées UMR 7612, équipe Colloïdes Inorganiques (LI2C)
Université Paris 6 (UPMC) Bat F(74), case 51
4 place Jussieu, F-75252 Paris Cedex 05 (France)
Fax: (+33) 1-44-27-36-75
E-mail: ali.abou_hassan@upmc.fr
Homepage : <http://www.pccsa.upmc.fr>

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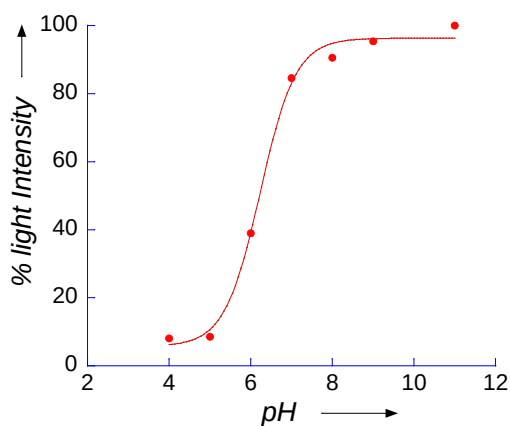


Figure 2. Fluorescence intensity of a 6 μM fluorescein solution at different pH normalized by its maximum value.

The mixing time is determined experimentally from a 3D-mapping of pH inside the channel R1. A detailed physical analysis including numerical simulations will be published elsewhere. In a first approximation, mixing time by flow focusing in the laminar regime is independent of the total volumetric flow rate Q_{tot} and depends only on the ratio of the outer to inner flow rates $Q_{\text{out}}/Q_{\text{in}}$ as reported initially by Scampavia et al.^[25] However, our preliminary results indicate that increasing Q_{tot} also has a strong effect on the polydispersity of the produced nanoparticles by decreasing the width of the residence time distribution. Experimentally, the quantitative analysis of the mixing time and the pH mapping in the nucleation reactor R1 is performed by labelling the acidic inner stream by a 6 μM fluorescein solution. Fluorescein is used as a pH probe since it can exist in several ionization forms, each one of which having distinct spectral properties.^[26] Scheme 1 and Figure 2 respectively show only the prevalent forms in aqueous solution and the experimental fluorescence intensity *versus* pH curve for 6 μM solution. By following the fluorescein emission intensity inside the mixing channel R1 by confocal laser scanning microscopy (CLSM), we obtain a cross-section plot of the pH jump along the focalised stream jet from which we get an estimate of the mixing time by assuming a linear relationship between time and position. After some trials, the best mixing time achieved in the micromixer R1 is about 80 ms for a ratio $Q_{\text{out}}/Q_{\text{in}} \sim 400$.

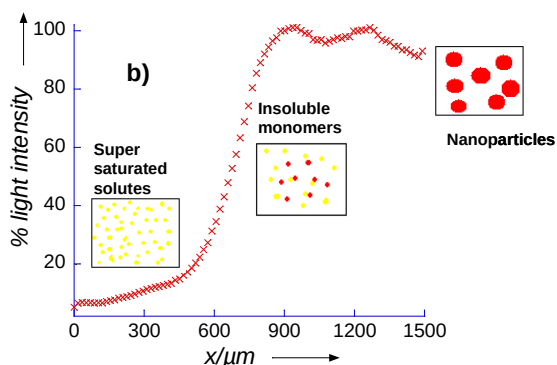
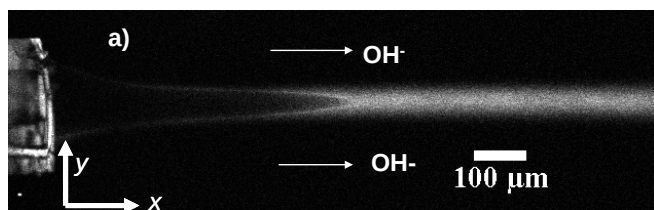


Figure 3. a) CLSM image during the mixing of the acidic inner stream labelled with 6 μM fluorescein solution with the outer stream of TMAOH solution. The flow rates are $Q_{\text{in}} = 0.5 \mu\text{l/min}$ and $Q_{\text{out}} = 200 \mu\text{l/min}$. b) average plot profile of the fluorescence intensity in the inner stream as a function of position in the channel during mixing, reflecting the pH variation.

Figure 3 shows the dynamical mixing in the focalised jet due to diffusion of the OH^- ions flowing in the outer stream into the fluorescein central stream. The fast mixing is inferred from the steep increase of the fluorescence intensity profile along the x axis corresponding to a pH jump from below to above the $\text{pK}_a \sim 6.2$ of fluorescein, before reaching a plateau value for an alkaline pH around 13 (due to the final concentration of TAMOH). As pH rises above neutrality, the solubility of the iron (III) species decreases and as soon as the supersaturation concentration is reached, nucleation of ferrihydrite occurs.

Before aging, a drop is collected at the outlet of R1 and analysed by Transmission Electron Microscopy (TEM). Figure 4a shows well defined spherical ferrihydrite nanoparticles (nanodots) for which particle-size measurements result into a 4 ± 1 nm average particle diameter. The electron diffraction pattern of a large selected zone confirms the presence of “2 lines” (meaning poorly crystalline) ferrihydrite.^[17] Moreover, high resolution TEM (HRTEM) measurements (Figure 4b) show that the nanoparticles are monocrystalline, exhibiting atomic planes with an interplanar distance of about 2.5 Å, which is consistent with ferrihydrite nanoparticles.

At the outlet of the R1 micromixer, the suspended ferrihydrite nanoparticles are directly injected into the microtubular aging coil R2 ($L=150\text{cm}$), which is continuously heated in a water bath at 60°C. Temperature profiles are calculated in order to determine the tubing length (hence the time) needed for the fluid to reach a steady state. Under the operating flow rate, the ferrihydrite solution reaches 60°C in $\sim 1\text{s}$, *i.e.* within the first centimeter after it entered the heated zone of the tubing. The effective resident time is about 15 min, as estimated from the tubing length along which the fluid has reached the stationary temperature of 60°C.

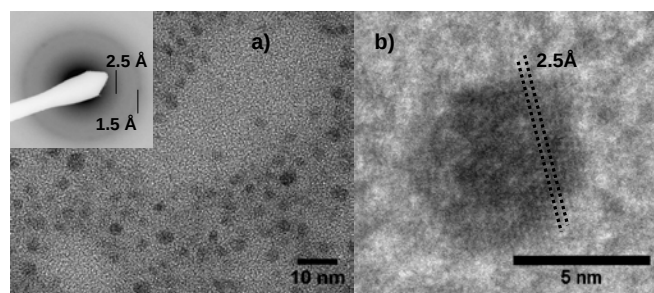


Figure 4. a) TEM picture of the sample taken after precipitation in the R1 microreactor (before aging) showing 4 ± 1 nm diameter ferrihydrite nuclei. The selected area diffraction pattern (inset) is typical of 2-lines ferrihydrite. b) HRTEM image of an individual ferrihydrite nanoparticle with a 2.5 Å lattice fringe.

After aging for 15 min under continuous flow through R2, goethite nanolaths under growth are observed with an average length $L=30 \pm 17$ nm and width $w=7 \pm 4$ nm ($N=100$ particles) yielding an axial ratio $L/w=4 \pm 4$ (Figure 5a). This short aging time appeared to be sufficient for the growth of fairly crystalline and anisotropic goethite nanoparticles differing only by smaller sizes compared to ones obtained after complete aging (one day at 60°C according to literature^[27]). Moreover, the presence of remaining ferrihydrite nuclei undergoing aggregation in the batch after 15 min and even after 24h at 60°C (date not shown) supports the idea that goethite nanoparticles were apparently formed here by the aggregation mechanism rather than by dissolution/reprecipitation.

We attribute (040) indexes to the lattice fringes with 2.5 Å spacing running parallel all along the length of the crystal (thereby an evidence of monocrystallinity) as imaged by HRTEM (Figure 5b). In addition, the particle of Figure 5b appears to be composed of nodules that are similar in size to the nanodots, which is consistent with the idea that growth proceeds by aggregation of nuclei that are aligned by the shear stress of the laminar flow.

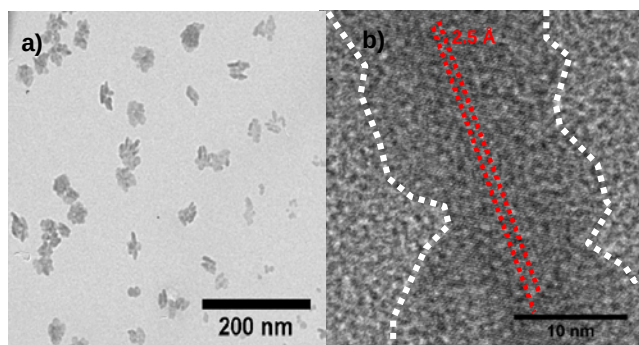


Figure 5. a) TEM image of the produced nanolaths after aging for 15 min in the R2 microtubular loop, at pH-13 and under laminar flow b) HRTEM image of a nanorod particle. Lattice fringe spacing is consistent with goethite. The dashed lines serve to highlight the morphology and texture of the particle.

In summary, we have demonstrated a significant acceleration of the synthesis of goethite nanoparticles from ferrihydrite nuclei by the use of a continuous-flows microfluidic system. The novelty of this approach lies in the fact that nucleation of the primary particles (ferrihydrite) and growth of the goethite nanoparticles are separated in two independent microreactors operating in different conditions. In the nucleation microreactor, the streaming reagents are mixed by molecular diffusion at room temperature in a flow focusing geometry. The homogeneity of the mixture is ensured by the fast mixing time that we estimated by fluorescence microscopy. Moreover, the technical problem of microchannel clogging due to precipitation onto the walls is avoided thanks to the 3D geometry. Compared to bulk synthesis that yields goethite nanoparticles with typically length $L=250$ nm, width $w=40$ nm and polydispersity index about 50% for both dimensions,^[28] our method of growth in a microfluidic aging channel minimizes local temperature gradients, ensures a regular laminar flow and finally leads to crystalline nanolaths with approximately the same values of aspect ratio and polydispersity index than in the bulk synthesis but smaller in sizes. Moreover, the short time required for aging (15 min for a velocity of 0.1 cm/s) compared to bulk synthesis (several hours or days) may originate from the small diameter of the aging reactor causing a shear that pre-aligns the primary ferrihydrite nanoparticles and speed up their oriented aggregation process. Beside the importance of the different applications of goethite, this work opens a new fundamental question about the use of microfluidics in the aging process of materials dispersed in a fluid carrier.

Experimental Section

The coaxial flows micromixer (R1) is obtained by molding in poly(dimethylsiloxane) (PDMS) and is described in details in the ref 9. The aging microtubular loop (R2) consists in a transparent PTFE tube of 1.7 mm inner diameter and 150 cm total length (Upchurch Scientific). Harvard Apparatus syringe pumps (pico 11 plus) controlled the flow rates. As starting materials for the precipitation of iron oxyhydroxide nanoparticles, we used FeCl_3 (27%, VWR),—tetramethyl ammonium hydroxide $(\text{CH}_3)_4\text{NOH}$ (97%, Sigma Aldrich), and hydrochloric acid HCl (37%, VWR) of analytical grade. For the CLSM images, fluorescein sodium salt $\text{C}_{20}\text{H}_{10}\text{Na}_2\text{O}_5$ (98.5%, Sigma

Aldrich) is used. 0.1M and 0.01 M Fe^{III} solutions are prepared by dilution of the commercial solution by 0.1M hydrochloric acid solution. TAMOH solutions at 0.1 M (pH13) are used rapidly after preparation to prevent carbonation. 6 μm fluorescein sodium solution is prepared by adding the fluorescein sodium salt to 0.1M hydrochloric acid. TEM and HRTEM images were recorded with a JEOL JEM 100CX transmission electron microscope operating at 100 kV with a point to point resolution of 0.3 nm. Confocal Laser Scanning Microscopy (CLSM) was performed on a Leica SP5 microscope with a 5X (0.11NA) objective. 488nm argon laser line was used for excitation and emitted fluorescence collected from 500 to 600nm

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