Élaboration de la silice magnétique colloïdale pour application en biologie moléculaire : extraction des acides nucléiques

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Elaboration de la Silice Magnétique Colloïdale Pour Application en Biologie Moléculaire : Extraction des Acides Nucléiques

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A…

Mon père

Ma mère et toute ma chère famille

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RÉSUMÉ

Le diagnostic moléculaire est un diagnostic basé sur l'analyse des acides nucléiques nécessite incontestablement la préparation d'échantillon. Cette préparation à pour objectif d'extraire des acides nucléiques d'un milieu généralement très complexe, de les purifier, de les concentrer voir les transporter dans des microsystèmes utilisés comme outils de diagnostic. Aujourd'hui, l'utilisation de nouvelles technologies et en particulier l'utilisation de supports solides ont permis de palier à un grand nombre de problèmes comparé aux méthodes conventionnelles. L'évolution de ces supports solides en particules colloïdales a permis de répondre à la demande des nouvelles technologies en apportant, une grande surface spécifique, une séparation rapide suite au caractère magnétique, un transport simple dans les microsystèmes et une chimie de surface modulable pour une bonne extraction de l'analyte recherchée.

Ainsi, l'objet de cette étude est la synthèse de la silice magnétique submicronique en taille pour l'extraction des acides nucléiques. La synthèse de la silice magnétique a été conduite en trois étapes. Dans un premier temps, la synthèse de ferrofluide organique a été réalisée par coprécipitation des chlorures ferriques et ferreux en basic avant transfert en milieu organique. La deuxième étape a été la préparation d'émulsions magnétiques stables, fortement magnétiques (pour une séparation rapide) et de taille relativement homogène et reproductible. Le ferrofluide préparé organique préparé a été émulsionné pour préparer une émulsion huile dans l'eau (O/W) en utilisant un tensioactif anionique. L'émulsion magnétique a été ensuite encapsulée par une écorce de silice via le procédé sol-gel. Le procédé d'encapsulation a été optimisé via une étude systématique et par une caractérisation physico-chimie et colloïdale complète de particules. La caractérisation morphologique des particules obtenues a montré une structure cœur magnétique et une écorce de silice parfaitement homogène.

Ces particules de silice magnétique ont été utilisées pour étudier l'adsorption des acides nucléiques (fragment d'ADN) en fonction du pH et de la salinité. Les résultats montrent une bonne capacité d'adsorption des acides nucléiques et également un bon relargage. Ce résultat encourageant montre que ces particules peuvent être utilisées dans le diagnostic moléculaire où l'extraction, la purification et la concentration des acides nucléiques sont très recherchées.

Preparation of Colloidal Silica Magnetic Particles for Molecular Biology Application: Nucleic Acids Extraction

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ABSTRACT

Currently, the genetics and DNA-based applications are developing very vast. All these applications such as gene therapy, diagnosis and PCR (polymerase chain reaction) require a previous step, which is the isolation and purification of genetic materials from their compartment. Taking care that the extraction method should produce the DNA in high purification state and in good conditions. The DNA extraction and purification are well known from long time ago, but these methods are time and organic solvents consuming. Nowadays, new nanotechnology-based techniques allow establishing rapid, efficient, environment respecting and cheaper isolation methods for DNA extraction.

Silica-coated magnetic emulsions as a form of core-shell were successfully synthesized for magnetic separation of DNA. Magnetic core is the separation tool and silica shell is the DNA capturer and releaser part.

The silica-coated magnetic emulsions synthesis was carried out in three steps. Starting from the organic ferrofluid synthesis by the coprecipitation of ferrous and ferric chlorides in aqueous ammonium hydroxide solution. The iron oxide nanoparticles were coated with oleic acid layer and redispersed in octane. Second step consisted of preparation of magnetic emulsion. The obtained ferrofluids used as oil to prepare oil in water emulsion (O/W) and SDS was used as surfactant. Then, the magnetic emulsion particles, the magnetic droplets, were coated with silica shell using the sol-gel process. The encapsulation was performed using TEOS as silica precursor and its hydrolysis was catalyzed by ammonium hydroxide. Particles characterization showed that the performed synthesis produced perfect core-shell particles.

These particles have been used to study the DNA binding in different conditions of pH, ionic strength and DNA concentration in solution. Results show good fixation and release of DNA molecules by the silica magnetic particles. In addition to the extraction results, the colloidal stability and speed separation of silica-coated magnetic particles, these particles can be recommended as strong DNA separation tools.

Abréviations

AEAPS	
AFM	Atomic force microscopy
AMPS	3-(2-aminoethylamino) propylmethyldimethoxysilane
APS	Ammonium Persulfate
APTES	3-aminopropyl triethoxysilane
APTMS	3-aminopropyl trimethoxysilane
BPEA	2-(2-bromopropionyloxy) ethyl acrylate
BSA	Bovine serum albumin
DLS	Dynamic Light Scattering
DMF	*N,N*-dimethylformamide
DNA	Deoxyribonucleic acid
DVB	Divinylbenzene
EDTA	Ethylene diamine tetra acetic acid
EPTES	N-(-ethylenamine) propylamine triethoxylsilane
ETR	Engineered Transferrin Receptor
FiTC	Fluorescein Isothiocyanate
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GOx	Glucose Oxidase
GPS	3-glycidoxypropyl trimethoxysilane
HRP	Horse radish peroxidase
HTEM	High-Resolution Transmission Electron Microscopy
Igepal®CO-520	Polyoxyethylene (5) nonylphenyl ether
Kcps	Kilo counts per second
KPS	Potassium Persulfate
ME	Magnetic Emulsion
MFH	Magnetic Fluid Hyperthermia
MICR	Magnetic Ink Character Recognition
MMS	Magnetic Microspheres
MNP	Magnetic Nanoparticle
MPS	3-(trimethoxysilyl) propyl methacrylate
MSCs	Mesenchymal Stem Cells
NP-5	Poly(ethylene glycol) nonylphenyl ether
NPs	Nanoparticles
PDADMAC	Poly(diallyldimethylammonium chloride)
PEG	Poly(ethylene glycol)
PFCs	Perfluorocarbons
pKa	Acid Dissociation Constant
PL	Photoluminescence
PMMA	Poly Methyl Methacrylate
PPY	Polypyrrole
PS	Polystyrene
PSS	Poly (sodium 4-styrenesulfonate)
PVP	Polyvinylpyrrolidone
QDs	Quantum Dots
RM	Reverse Microemulsion
RMI	Resonance Magnetic Imaging
RNA	Ribonucleic Acid
SA	Succinic Anhydride
SDS	Sodium Dodecyl Sulfate

SEM	Scanning Electron Microscopy
SI-ATRP	Surface Initiated Atom Transfer Radical Polymerization
Si-ME	Silica-coated Magnetic Emulsion
SiNPs	Silica Nanoparticles
SIP	Surface Initiated Polymerization
SPION	Superparamagnetic Iron Oxide Nanoparticles
TE	Tris-HCl-EDTA buffer
TEM	Transmission Electron Microscopy
TEOS	Tetraethyl Orthosilicate
TGA	Thermogravimetric Analysis
THPC	Tetrakis (hydroxymethyl) phosphonium chloride
TMOS	Tetramethylorthosilicate
UV	Ultraviolet
XRD	X-ray Diffraction
XPS	X-ray Photoelectron Spectroscopy

I. INTRODUCTION GENERALE

Le diagnostic médical à pour but de déceler dans un prélèvement biologique (sang, sérum, crachats, extrait cellulaire, etc.) la présence d'un analyte (ARN, ADN, urée, cholestérol, hormone, virus, bactéries etc.). Pour ce faire, la méthode généralement utilisée est basée sur la capture spécifique qui consiste à immobiliser sur un support solide une molécule biologique (antigène, anticorps, oligonucléotide etc.) capable de fixer spécifiquement l'analyte recherché. Les principales exigences pour la conception de tests de diagnostic performant repose sur la spécificité, la sensibilité, la rapidité et le coût. Pour y répondre, un large éventail de particules a été utilisé comme support solide de biomolécules. En effet, ces colloïdes offrent des avantages grâce à l'importante surface spécifique disponible, une fonctionnalisation variable et une séparation rapide (centrifugation, filtration, aimantation). Toutefois, jusqu'à présent, ce type de supports présente un inconvénient majeur en raison de leur surface incompatible avec l'activité enzymatique et pouvant induire de fortes interactions non-spécifiques, surtout dans le cas des échantillons biologiques complexes. La concurrence scientifique dans le domaine existe mais elle est principalement focalisée sur l'utilisation des techniques d'extraction en plusieurs étapes et non automatisable ce qui limite leurs succès. L'utilisation de particules dans le diagnostic biomédical en général et l'enrichissement d'échantillons commence a être exploitée ce qui induit une concurrence non seulement dans l'élaboration de supports magnétiques particulaires, mais également les applications qu'il en découle. En effet, des avancés technologiques ont été réalisées dans le domaine de la capture générique des acides nucléiques totaux. Il est à présent possible d'extraire, purifier, concentrer, amplifier et détecter les acides nucléiques de milieux complexes. En revanche, il est pratiquement impossible d'automatiser ce procédé sans faire appel aux particules magnétiques pour remplacer les techniques conventionnelles (extraction au phénol-chloroforme suivie d'une précipitation à l'éthanol).

Il est à noter que le succès d'un test dans le diagnostic biomédical réside non seulement dans l'élaboration d'outils de détection performants mais aussi dans la préparation des échantillons nécessitant impérativement un prétraitement. Dans le cas de tests basés sur la détection d'acides nucléiques, ce prétraitement consiste à libérer le matériel nucléique dans le milieu, le capturer et le purifier avant de pouvoir mettre en œuvre les étapes d'amplification et de détection. Ainsi, l'utilisation de particules de la silice magnétiques permettra non seulement d'augmenter la sensibilité de certains tests, mais aussi d'envisager leur automatisation ou leur intégration dans des microsystèmes où la rapidité de séparation magnétiques sous l'action d'un champ magnétique extérieur est un atout incontournable. Le développement de telles particules magnétiques nécessite la mise au point d'émulsions

magnétiques submicroniques et parfaitement caractérisées. L'efficacité de la capture des acides nucléiques, sera examinée en utilisant des échantillons modèles.

Aujourd'hui, le développement de la nanotechnologie, surtout dans le demain biomédical, a permis l'utilisation de nano et microparticules comme supports de biomolécules pour applications diagnostics et thérapeutiques : *in vivo* comme les agents de contraste [1], le traitement de cancer[2] et la délivrance de molécules actives[3] et *in vitro* pour comme supports pour l'extraction, la purification, la concentration de biomolécules ou tout simplement comme outil de détection dans le cas de diagnostic[4].

La silice particulaire et colloïdale est capable de présenter des interactions avec les macromolécules biologiques comme les acide nucléiques[5] et les protéines[6]. Ces interactions peuvent êtres réversibles dans des conditions du pH et de la salinité du milieu. Les particules de silice sont largement utilisées comme supports solides pour la séparation de biomolécules[7–9]. Les interactions impliquées dans l'adsorption des acides nucléiques ne sont pas très élucidées et ne peuvent êtres uniquement de type électrostatique suite à l'utilisation de quantités de sel très importantes (NaCl 4M ou $MgCl_2$ 0,2M). Il est à noter que l'ADN et la silice sont chargés négativement et pour favoriser l'adsorption de l'ADN il est nécessaire de réduire la porté des interactions répulsives et de favoriser par la même occasion la précipitation de l'ADN sur la silice et pour ce faire la forte salinité est indispensable. La quantité d'ADN adsorbé dépend donc du pH capable d'affect la charge de surface de la silice et la salinité du milieu d'adsorption[10]. Ainsi, plusieurs formes et tailles de nano et micro particules de silice ont été synthétisées et utiliser pour réaliser l'isolation et la purification des acides nucléiques[11,12]. Le développement de nouvelles technologies et en particulier les nanotechnologies a permis de moduler la surface des particules, d'apporter des spécificités via le greffage de polymères sur des particules primaires, modification chimique simple en utilisant une chimie classique et plus généralement via encapsulation de particules semences.

Généralement, l'encapsulation est une méthode très connue et très utilisée pour la modification des surfaces des particules. L'encapsulation peut être réalisée soit par polymérisation sur semence par un procédé de type sol-gel. Le Sol-Gel[13] est un procédé très efficace et largement utilisé pour encapsuler les nano et microstructures par une écorce de silice. Le principe de cette méthode est basé sur l'hydrolyse d'un agent précurseur l'orthosilicate de tétraéthyle (TEOS) en présence d'un catalyseur acide ou basique, comme l'hydroxyde d'ammonium. Le TEOS hydrolysé se condense pour former un polymère non organique, composé de dioxyde de silice SiO_2. La forme et le type de la couche SiO_2 varient selon le catalyseur utilisé[14] et la quantité de TEOS ajoutée[15].

Les particules encapsulées par une écorce de silice sont largement utilisées dans des applications diverses et variées. En effet, la présence de la silice à la surface des particules semences présente des avantages supplémentaires par exemple protéger les cœurs encapsulés, améliorer la stabilité colloïdale, augmenter la biocompatibilité, et la fonctionnalisation de la surface. Les nanoparticules d'oxyde de fer sont les particules les plus étudiées et encapsulées par une écorce de silice ce qui permet l'obtention de morphologie cœur-écorce. Les particules composites formées possèdent des propriétés magnétiques d'oxyde de fer composant le cœur et les propriétés de surface induites par la silice. Ces composites sont largement synthétisées et utilisées pour des applications biomédicales comme l'imagerie par résonance magnétique (IRM)[1], l'extraction des acides nucléiques[16], l'immobilisation des protéines[17,18], dans les biosenseurs[19] et la libération de molécules actives[20] et dans la biologie moléculaire[21].

L'objectif de la thèse :

L'objectif principal de cette thèse est d'élaborer des particules de silice magnétiques submicronique en taille pour l'extraction et la purification des acides nucléiques et utilisable dans le microsystème et les bio-nanotechnologies. Le cahier des charges imposé par les applications bio-nanotechnologies doit réponde aux exigences suivantes:

- des particules de silice magnétique de taille submicronique
- magnétique et super-paramagnétique
- bonne teneur en charge magnétique
- bonne stabilité colloïdale
- bonne stabilité chimique (pas de dégradation dans le temps)
- faible sédimentation
- bonne écorce de silice protectrice
- bonne adsorption des acides nucléiques
- bon rendement de purification des acides nucléiques

Pour répondre aux critères généralement imposés par le diagnostic in vitro, nous proposons l'élaboration de la silice magnétique en utilisant des émulsions magnétiques comme semences. Les émulsions magnétiques seront formulées après la préparation de ferrofluide organique stable et super-paramagnétique à base d'oxyde de fer. Cet oxyde de fer sera préparé par nanoprécipitation des sels ferreux et ferriques.

Afin de préparer des émulsions magnétiques sensibles au champ magnétique, la taille doit être impérativement supérieure à 100 nm et de préférence proche de 300 nm pour une séparation rapide sous l'action d'un simple champ magnétique. Ces émulsions magnétiques doivent présenter une bonne stabilité colloïde (ni coalescence et crémage). Ces émulsions une fois parfaitement caractérisées seront encapsulées par une écorce de silice. L'encapsulation

sera réalisée en utilisant le procédé sol-gel. Lors de cette encapsulation une attention particulière sera dédiée à la morphologie des particules composites, la composition chimique et les propriétés colloïdales. Une fois parfaitement caractérisées, les particules composites seront utilisées par la suite pour l'adsorption et la désorption des acides nucléiques. L'ensemble des résultats obtenus sont présentés dans ce manuscrit de thèse compose d'un premier chapitre consacré à l'état de l'art sur la synthèse des ferrofluides aqueux et organique, la formulation des émulsions magnétiques, le procédé sol-gel et son utilisation pour l'encapsulation des particules semences et en fin les applications biomédicales des particules encapsulées par une écorce de silice.

Le deuxième chapitre est concerné à la partie expérimentale portant sur la synthèse des ferrofluides organiques, la formulation d'émulsions magnétiques et l'encapsulation de ces émulsions par couche externe de silice. L'ensemble des dispersions élaborées a été caractérisé.

Le troisième chapitre présente également une étude expérimentale portant sur l'adsorption et la désorption de l'ADN sur les particules de silice magnétiques synthétisées. Une étude systématique a été réalisée en examinant l'effet de pH, la concentration en sel (Na Cl et MgCl2), la concentration initiale de l'ADN en solution et la cinétique d'adsorption.

Enfin, la thèse se termine par une conclusion générale résumant l'ensemble des résultats obtenus et présente également quelques perspectives.

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II. CHAPITRE 1
ETUDES BIBLIOGRAPHIQUE

Introduction :

Dans cette partie de notre thèse, l'état de l'art reporté dans la littérature sur les quatre thèmes principaux de la thèse a été traité sous forme d'articles de revues.

Le premier article présente les ferrofluides qui sont des colloïdes stables de nanoparticules magnétiques, principalement des oxydes de fer, dispersées dans un solvant porteur soit aqueux soit organique. Les nanoparticules magnétiques ont été synthétisées pour la première fois dans les années 1950. Ensuite, ces nanoparticules ont été utilisées dans des centaines d'applications diverses et variées. La première partie de la bibliographie s'intéresse aux ferrofluides, leurs méthodes de synthèse, leurs propriétés, les applications, industrielles et biomédicales. Cette étude est complétée par la formulation des émulsions magnétiques en utilisant les ferrofluides. Les émulsions magnétiques discutées sont soit directes ou inverses.

La deuxième revue traite l'encapsulation par une écorce de silice des différents types de nano et microparticules. Il est reporté que l'encapsulation se fait par deux méthodes principales : le sol-gel et la microémulsion inverse. La première méthode est basée sur l'hydrolyse et la polycondensation d'un précurseur de silice (orthosilicate de tétraéthyle ou TEOS) en présence d'un catalyseur. Et la seconde méthode (la microémulsion inverse) est fondée sur les mêmes principes, mais la technique est différente où l'hydrolyse et la polycondensation de TEOS se font dans les gouttelettes de l'émulsion inverse qui contiennent la particule à encapsuler, le TEOS et le catalyseur. L'article donne également des exemples sur les particules encapsulées comme des oxydes de fer, l'or, l'argent, les nanoparticules photoniques (quantums dots), les particules de latex (polystyrène, le polyméthacrylate) et d'autres particules. Ensuite, une attention particulière est portée sur les méthodes de fonctionnalisation de la couche de silice comme l'utilisation des agents de couplages de la silice ou le griffage chimique de polymères. Enfin, les techniques utilisées pour caractériser ce type de nano et micro structures et leurs propriétés principales.

La troisième revue présente les applications biomédicales des particules encapsulées par une écorce de silice. Parmi ces applications, l'immobilisation des protéines, l'extraction de l'ADN, la libération de gènes et de molécules actives, l'imagerie biomédicale in vivo et in vitro et des applications pharmaceutiques. Grâce à ces propriétés uniques la surface de la silice est devenue très importante dans le domaine de la nano-biotechnologie. La silice se caractérise par sa biocompatibilité, morphologie contrôlée, bonne stabilité chimique. En plus de ces avantages, la surface de la silice est aisément fonctionnalisable par de nombreux

procédés et groupes fonctionnels, ce qui augmente considérablement le nombre d'applications de ces particules hybrides ou composites.

Résumé :

Les ferrofluides : les méthodes de synthèse, propriétés et applications

Les ferrofluides ont été synthétisés début des années 1960s et tout de suite après, ces produits sont devenus indispensables dans un grand nombre d'applications. Cette étude bibliographique est focalisée sur les ferrofluides magnétiques, les méthodes de la synthèse, leurs propriétés et les applications, et enfin le concept de l'émulsion magnétique.

Les oxydes de fer ont été synthétisés tout d'abord par la réduction de taille, broyage, ensuite, d'autres méthodes ont été employées comme la synthèse chimique, la coprécipitation et la décomposition thermique. Un ferrofluide obtenu doit avoir obligatoirement le superparamagnétisme, c'est-à-dire que les particules magnétiques ont la propriété magnétique seulement sous l'effet d'un champ magnétique externe. Cette caractéristique de ferrofluides est très outil pour les futures applications dont le domaine industriel comme les peintures magnétiques, les encres magnétiques et les applications biomédicales comme agents de contraste pour l'imagerie biomédicales in vivo par la résonance magnétique, la théranostique (combinant la thérapie et le diagnostic) et les outils de séparation magnétique de biomolécules.

Les émulsions magnétiques ou les émulsions de ferrofluides ont été développées début des années 90s comme une nouvelle forme de ferrofluide. Elles se composent généralement de gouttelettes de ferrofluides organiques dispersées dans la phase continue aqueuse.

Ferrofluids: Synthesis and Applications

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Abstract

Discovery of the magnetic property of magnetic fluid or ferrofluid and its effects on the other materials open the wide varieties of applications in human daily life. Continually new applications require different forms of magnetic materials, which have significant attractions nowadays. This review highlights the ferrofluids synthesis methods; all known methods are cited whereas size reduction and chemical co-precipitation were detailed. The industrial and biomedical applications of ferrofluids and particles based on ferrofluids were discussed. Finally, the new form of ferrofluids represented as magnetic emulsion was introduced.

1. Introduction

The magnetization or the magnetic susceptibility is a value, positive or negative, indicates the magnetization degree of a material under the effect of magnetic field. Furthermore, the material response to a magnetic field is depending on its state, that means there is difference between the reaction bulk and nanoparticles under the magnetic filed effect. Nanoparticles with a radius below 150A° belong to "single domain particle", which mean that the particle is in a state of uniform magnetization at any magnetic field.[1] Moreover, both particles shape and size found to be greatly considerable factors when discussing the magnetic properties of particles.[2] Consequently, the nanoparticles also have paramagnetism property that they possess magnetism properties when, and only when, they are under the effect of magnetic field. Iron oxide nanoparticles are found to have superparamagnetic properties, which allow using these nanoparticles for wide range of applications.

A magnetic fluid, or known as ferrofluid, belongs to colloidal suspensions family and consists of stable colloidal fine nanoparticles in a carrier liquid. The magnetization properties are related to the nanoparticles, which are essentially consisting of iron oxides. But metallic particles, at nanometric size, possess a long-range magnetostatic attraction, thus they toward to the agglomeration and sedimentation. Therefore, the particles size should be less than 10 nm and a stabilizing agent is required to enhance the stability colloidal by reducing the attractive forces and raising the repulsive forces. The oleic acid is widely used as a stabilizing agent and many carrier liquids are employed in order to achieve a stable ferrofluid. Nowadays, ferrofluids are commercially available to satisfy the different applications in several fields such as magnetic inks,[3,4] paints[5] and biomedical applications.[6]

This review highlights the ferrofluids synthesis methods, the two most important: size reduction and chemical coprecipitation, and will cite the applications based on ferrofluids. Industrial applications, which include, magnetic recording media, magnetic inks and sealing by ferrofluids. And the newly adapted biomedical applications *in vivo* and *in vitro* such as RMI contrast agents, cells separation and nucleic acids extraction.

2. Synthesis methods

A magnetic fluid or ferrofluid is a colloid of fine ferromagnetic particles dispersed in liquid carrier and they can't be prepared by melting the ferromagnetic metals such as iron, cobalt and nickel, because the ferromagnetic materials lose their strong magnetic properties when heated above Curie temperature. Moreover, for all known ferromagnetic materials this temperature is always below the melting points.[7] Therefore, to prepare a good and useful ferrofluid it was necessary to synthesize fine ferromagnetic particles, coated them by an adsorption stabilizer layer and dispersed them in a suitable liquid. There are several methods used to produce ferromagnetic nanoparticles e.g., size reduction, chemical synthesis (coprecipitation and thermal decomposition methods), electro-decomposition techniques, evaporation of metal in liquid, and carrier liquid exchange.[8,9] Size reduction is the first method used and chemical synthesis is frequently used to prepare ferromagnetic nanoparticles so these two methods will be described in details.

2.1. Size reduction

First of all, the preparation process was via grinding the permanent magnets and ferromagnetic metals for more than 20 days in presence of oleic acid and organic solvent (heptane).[10] Thus, obtained particles were at an average size from 50 to 200 A° and it was the first time that such suspension considered as an apparently magnetically responsive continues liquid. Despite the long and hard work needed by this technique it was widely used to

synthesize ferrofluids. Rosensweig and coworkers used Papell's grinding method to prepare the ferrofluids and they extensively studied their physical,[11] thermal[12] and hydrodynamic[13–15] properties. Throughout the later years, the size reduction (grinding) method was using to prepare stable ferrofluids. Kaiser et al[16] published his work on the preparation and the properties of stable ferrofluid prepared by the same technique, in this paper they cited the most important works used grinding to prepare ferrofluids. Research and development in ferrofluids field are drastically attracted the researchers and the industry that was clear from the number of published papers and patents, whereas between the period of 1986 and 1990 more than 1000 patents and 1900 papers were published.[17]

2.2. Chemical synthesis

Due to the considerable interesting of ferrofluids and their wide applications it was necessary to improve the synthesis methods. Thus, the chemical synthesis, include precipitation from salt solution and thermal decomposition of metal carbonyls, were good, fast, easy, cheap and controlled ways for ferrofluids production. Although, practically they consist of two steps: first one is the nanoparticles preparation and the second is the dispersion in liquid carrier.

The chemical coprecipitation method is most conventional method to prepare ferrofluid magnetic nanoparticles. Generally, all these procedures begin with a mixture of $FeCl_3$ and $FeCl_2$ solution in water and then coprecipitation is performed by the addition of base, typically ammonium or sodium hydroxide. The reaction can be summarized as follow:

$$2FeCl_3 + FeCl_2 + 8NH_4OH \rightarrow Fe_3O_4 + 4H_2O + 8NH_4Cl$$

This process involves two stages: i) a short burst of nucleation when the concentration of the species reaches critical supersaturation and ii) a slow growth of the nuclei by diffusion of the solutes to the surface of the crystal. In order to produce monodisperse MNPs, the nucleation should be avoided during the period of growth[18]. The method is affected by different parameters like the type and concentration of salts (e.g., chlorides, sulfates, nitrates), temperature, pH and the addition rate of ammonia[18–21]. According to thermodynamics of this reaction, the precipitation of Fe_3O_4 should be completed at pH between 8 and 14 with a stoichiometric ratio of $Fe^{3+}$to Fe^{2+} of 2:1 under inert atmosphere at room temperature or at elevated temperature. Usually, ferromagnetic particles produced by this technique have an average size below 20 nm and they are composed of mixture of γ-Fe_2O_3 and Fe_3O_4 depending on the reaction conditions[22].

First preparation of magnetic fluids by coprecipitation of ferric and ferrous salts in aqueous solution was did by Reimers et al,[23] the reaction ratio fixed at 2:1 ($FeCl_3$:$FeCl_2$) and NH_4OH

28% used to precipitate the iron oxide nanoparticles. After the coprecipitation in a basic medium, the ferrofluids were subjected to surfactant-stabilized for colloidal stability. Fatty acid derivatives are most often used to stabilize these iron oxide nanoparticles either in organic or aqueous medium. Oleic acid is the most common dispersant agent used as non water-soluble adsorption layer, thus oleic acid-coated nanoparticles can be easily and highly dispersed in organic solvent.[24] The carboxyl groups at the chain end of oleic acid bind onto the nanoparticles surface, at pH alkaline, and the aliphatic chain form the hydrophobic layer that help the nanoparticles to disperse in non polar solvent.

On the other hand, ferrofluids attracted developers to use them in hydrophilic media that needed to coat the ferromagnetic nanoparticles by ionic layer. Massart et al,[25–28] prepared aqueous ferrofluids without surfactants in acidic medium, nitric acid is used to have ionic iron oxide nanoparticles. It has found that the size of precipitated particles can be controlled by adding the citrate ions to the reaction medium.[27] These ions, at a specific ratio, can control the particles size by inhibiting the growth process so raising the number of nucleation. Moreover, sodium dodecyl sulfate (SDS) was played the same role to control the particles size.[29] It was also reported that the addition of chelating organic anion (carboxylate or hydroxy carboxylate ions, e.g., citric, gluconic or oleic acid or polymer surface complexing agents (dextran, carboxydextran, poly(vinyl alcohol) or starch), stabilizer and/or reducing agents, during the formation of magnetite can help to control the size of nanoparticle.[18,19] The chelation of organic ions on the magnetic particle surface either prevents nucleation and then leads to larger particle or inhibits the growth of crystal nuclei, leading to small nanoparticle.

Although the coprecipitation process is a simple and cost-effective method that can produce a high yield of the magnetic ferrofluid and the reaction temperature, and time are lower than other methods, e.g., thermal decomposition and hydrothermal, the particles prepared tend to be polydisperse. The control of particle size distribution is quite limited because only kinetic factors affect the crystal growth.

To obtain highly monodispersed magnetic nanoparticles with a narrow size distribution, the thermal decomposition is required. Generally, the thermal decomposition of organometallic compounds followed by oxidation in high-boiling organic solvents containing stabilizing surfactant can lead to high-quality monodispersed magnetic nanoparticles, which usually required relatively higher temperatures and a complicated operation.[19,30] The organometallic compounds include $Fe(cup)_3$ (cup = *N*-nitrosophenyldroxylamine),[31] $Fe(acac)_3$ (acac = acetylacetonate),[32] or $Fe(CO)_5$.[33] Monodisperse γ-Fe_2O_3 nanocrystallites with size of 4-16 nm were prepared by thermal decomposition of $Fe(CO)_5$ in the presence of oleic acid at 100 °C by

controlling the experimental parameters.[33] The resulting iron nanoparticles were transferred to monodispersed γ-Fe_2O_3 nanocrystallites by controlled oxidation by using trimethylamine oxide as a mild oxidant. Besides ferrofluid magnetic nanoparticle with control over size and shape are obtained by the thermal decomposition method, the resulting nanoparticles are generally only dissolved in nonpolar solvent which is the drawback of this method.

3. Properties

In most of the great interest applications, the particles perform best when the size of the nanoparticles is below a critical value, which is dependent on the material but is typically around 10-20 nm. Then each nanoparticle becomes a single magnetic domain and shows superparamagnetic behavior when the temperature is above the so-called blocking temperature.[19] A single-domain particle is uniformly magnetized, i.e., all the spins align in the same direction. To synthesize single-domain particle, it is necessary to control the particle size and prevent its aggregation. The magnetic behavior of aggregated particles considerably differs from that of individual particle, hence, aggregation results in the uncontrolled and irreproducible behavior of magnetic material.[34] The characteristics of magnetic nanoparticles are usually displayed as Figure 1. The superparamagnetic material (curve 2) exhibits no remnant magnetization (M_r). The coercive force (the external field required to reduce the magnetization back to zero) (H_c), is zero and its magnetization (M) dependence on the external magnetic field (H) lacks the hysteresis loop. On contrary, the magnetic dipoles inside the ferromagnetic material always exist in the absence and presence of an external field and produce a hysteresis loop (figure1).[35] In addition, the particle magnetic moment in a superparamagnetic state reverses at time shorter than the experimental time scales.

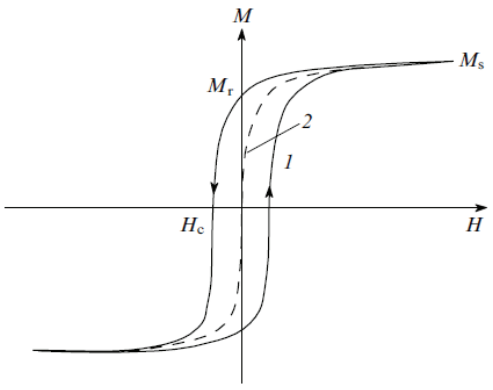

Figure 1 Magnetization curves of (1) ferromagnetic and (2) superparamagnetic materials. M_s is saturation magnetization, H_c is coercive force, H is the applied magnetic field, M is induced magnetization, M_r is remnant magnetization.[35]

Generally, the saturation magnetization (Ms) values found in nanostructured materials are usually smaller than the corresponding bulk phase, provided that no change in ionic configurations occurs. Accordingly, experimental value for M_s in ferromagnetic nanoparticles have been reported to span the 30-50 emu.g^{-1} range, lower than the bulk magnetic value 90 emu.g^{-1}.[30] In addition, an important parameter to describe superparamagnetic colloids is the blocking temperature, a transition point at which the thermal energy is comparable to the magnetic anisotropy energy or the energy barrier for spin reorientation. Below blocking temperature, the anisotropy of the particle blocks the free movement of the moment. Above the blocking temperature, the moment is free to align in an applied magnetic field and appears superparamagnetic. For instant, Hyeon et al[33] reported the blocking temperatures of the monodisperse γ-Fe_2O_3 nanocrystallites obtained by the thermal decomposition method depending on their particle diameter. The blocking temperatures of the γ-Fe_2O_3 nanocrystallites with particle diameter of 4, 13 and 16 nm were found to be 25, 185 and 290 K, respectively. Mehdaoui et al[36] synthesized ferromagnetic 16 and 11 nm Fe(0) nanoparticles of cubic shape. The M_s of bulk iron were 200 ± 10 and 178 ± 9 Am^2.kg^{-1} at 300K for particle diameter of 16 and 11 nm, respectively, just below the bulk value. Their coercive field Hc at 300K are 16 and 5mT, respectively. Under a fast switching magnetic field, magnetic nanoparticle can be generated thermal energy.[36–38] The frictions caused by the physical rotation of nanoparticles, Brownian relaxation, and magnetization reversal within nanoparticle, Neel relaxation lead to the loss of magnetic energy and generation of thermal energy. The power released by the nanoparticles is assessed by their specific adsorption rate (SAR). Increase in SARs above 1W.g^{-1} could be beneficial for several aspects of the hyperthermia applications.[38] The SAR measured on 16 and 11 nm nanocubes was 1690 ± 160 and 1320 ± 140W.g^{-1} at 300kHz and 66mT, respectively. Although very large SARs on ferromagnetic nanoparticles were obtained, the efficiency of magnetic nanocubes for hyperthermia applications was limited due to the presence of magnetic interactions.

4. Application

The ferrofluids and their synthesis techniques were the focus of attention of scientists soon after the first synthesis in 1960s. Few years later, tell 1980s, all published papers and patents took about the ferrofluids synthesis and properties.[10,13,15,16,23,25,26,39–41] Thus, the real applications of ferrofluids started in 1980s including magnetic inks[42], paints for recording taps,[43] sealing,[44] and more recently in biomedical applications[45] such as in resonance magnetic imaging (RMI)[46,47], therapeutic agents for cancer treatment[37,48,49] and bioseparation.

4.1. Industrial applications

The magnetic properties of ferrofluids were widely applied in data saving field, tapes for audio and video recording, magnetic cards, computer storage and informatics.[43,50] Primarily, magnetic pigments were produced using iron oxide[51–53] then magnetic inks[54] and paints were prepared from these pigments. The strong point in using magnetic inks that the magnetic characters can be recognized digitally, magnetic ink character recognition (MICR) is a technology adapted to read this type of characters.[55] Consequently, using this technique provides a high secure environment of work especially for banking and magnetic cards uses. Moreover, save time and efforts to transfer information into digital form.

Recording media are mainly based on magnetic films techniques[56], which are produced from magnetic fluids, to record and play back the information. Furthermore, the informatics developments nowadays need high capacity of storage and speed of data transfer, thus developing the magnetic recording media is drastically raised last years.[57–59]

Sealing and bearing by use of ferrofluids was invented by Furumura et al.[44] It was depending on the filing of engraved grooves on the surface of cylindrical magnet by ferrofluid, which will be retained in the gab between the magnet and rotary shaft. Then, this use of ferrofluids was commonly and extensively studied.[60–65] This technique is widely applied in industrial pump, vacuum systems and computer hard disk drives.[65] And more recently ferrofluids are applied as valves in microfluidics devices,[66] herein an organic ferrofluid was used to generate up to 5 kPa in a device composed of a pump and two valves as shown in figure 1.

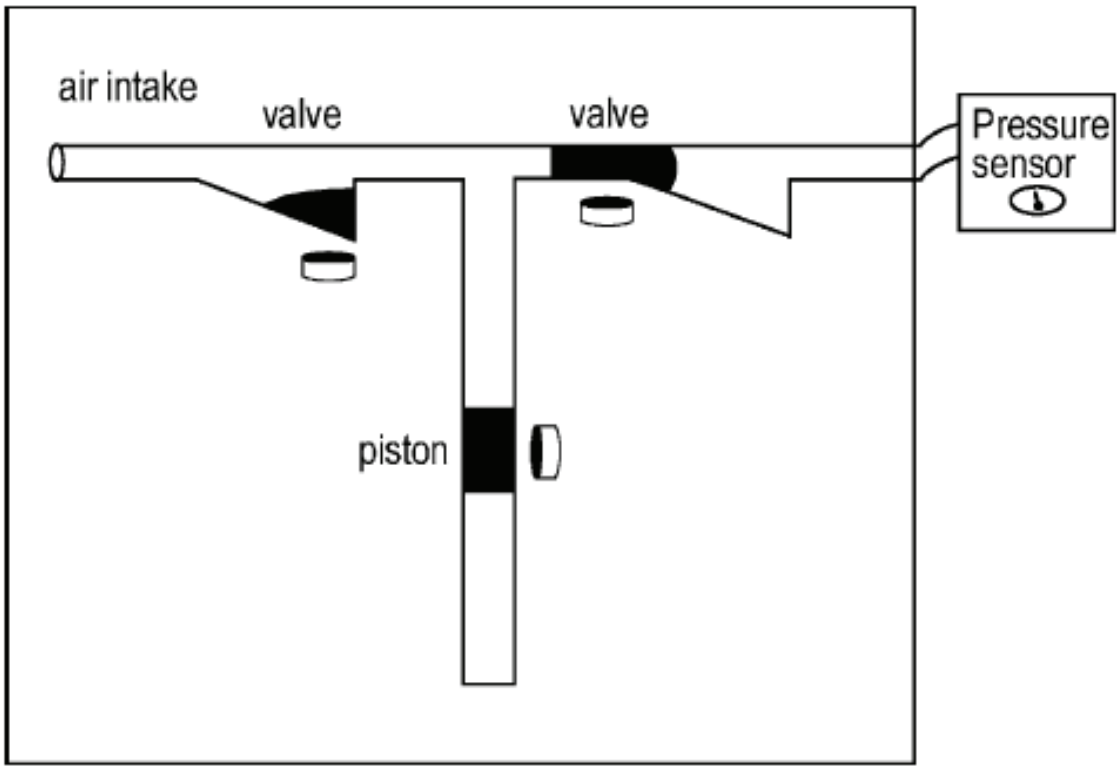

Figure 1: pumping system used ferrofluid and permanent magnets as a pump and valves.[66]

4.2. Biomedical applications

The nanotechnology is drastically developed in the last decade and it is widely applied in biomedical fields, especially for diagnosis and hyperthermia. On the other hand, the techniques of molecular biology are well known and developed. Thus the association between the nanotechnology and the molecular biology created high preferment nanostructures that show promotion characteristics such as rapidity, specificity and sensibility.

Ferrofluids in their industrial form aren't tolerant to be in contact with biological systems due to the cytotoxicity of magnetic fluids.[67] Therefore, coating methods were applied to modify the nanoparticles surfaces, for example with dextran[68–70] or albumin,[71–73] so these techniques expanded the use borders of magnetic fluids in biomedical domain.

In vivo:

For diagnosis, as MRI contrast agents[74–78] which need special attention for the synthesis to reduce the nanoparticles toxicity to the lowest level possible. In addition to the cytotoxicity elimination, the coating aid for the surface functionalization, since the MRI contrast agents should have a specific target, which it recognized by an antibody fixed onto the particle surface.[79] These types of particles are generally used to detect the cancer cells by MRI.[73,80]

Weissleder et al[76] reported for the first time transgene expression *in vivo* by MRI. Magnetic nanoparticles were synthesized and coated with dextran and transferrin and engineered transferrin receptor (ETR) was the studied gene that its overexpression accompanied with more uptakes of nanoparticles by cells.

In vitro:

Magnetic fluids or ferrofluids are characterized by their speed response to the magnetic field and this property satisfies the exigency of separation and extraction tools makers. Early, biomolecules extraction and separation, based on the classical methods, was too difficult and long in addition to the high quantities needed of organic solvents. Nowadays, magnetic fluids provide numerous types of micro and nanostructures as and magnetic responsive solid supports to create biomolecules extraction and separation tools[81]. Initially magnetic particles were used in immunology for immunoassay as solid supports for enzymes[82] and antibodies.[83]

Therapeutic Applications

For clinical therapeutic or the using of magnetic fluids as cancer treatment by magnetic fluid hyperthermia (MFH), is the one innovative application of this ferrofluid in medicine. The cancer cell treatment by MFH is achieved through magnetic field modulated cell membrane damage. Upon exposure to the alternating external magnetic field, the magnetic nanoparticles continuously emit heat.[37,48] The therapeutic effect came from two mechanisms, the compromised integrity of the cell membrane and promoted apoptosis, in contrast to the more common magnetothermal effect, which requires a high-frequency oscillating magnetic field (52 $kA.m^{-1}$) to achieve high magnetic saturation. For practical therapeutic applications with minimized side effects, it is critically important to obtain optimum heating efficiency to reach the desired hyperthermia temperature at 41-46 °C during a few hours. It was reported the use of such magnetic nanoparticles $(Zn_{0.4}Mn_{0.6})$-Fe_2O_4 with high specific loss power in cancer cell studies achieved 84.4% HeLa cells dead after the application of the AC magnetic field for 10 min. When compare with Feridex, 13.5% of those cells were dead.[49] The advantages of using nanometer size magnetic ferrofluid for cancer cell treatment could be tumor penetration depth.

Cells and bacteria separation

This technique was used to create submicron magnetic structures that can adsorb and desorb the biomolecules, cells, bacteria and virus[71]. The idea in these types of particles is that their interaction status with the target molecules is sensitive to the environmental conditions such as pH, salinity and temperature. Superparamagnetic particles based on ferrofluids are prepared and biotinylated for specific cells sorting,[84] likewise Sestier et al[85] prepared

magnetic particles coupled with enzymes or antibodies and measured the particles electrophoretic mobility by laser-Doppler velocimetry, as a technique to follow the charge surface modifications. Bacteria also can be captured and magnetic separated using functionalized magnetic particles,[86] herein, FePt nanoparticles were prepared and the vancomycin (Van), spectrum antibiotic, was attached to the nanoparticles surface. Van can bind to the terminal peptide D-Ala-D-Ala on the cell wall of Gram-positive bacterium via hydrogen bonds. Figure 2 illustrates the bacteria mechanism separation using Van-coated magnetic nanoparticles.

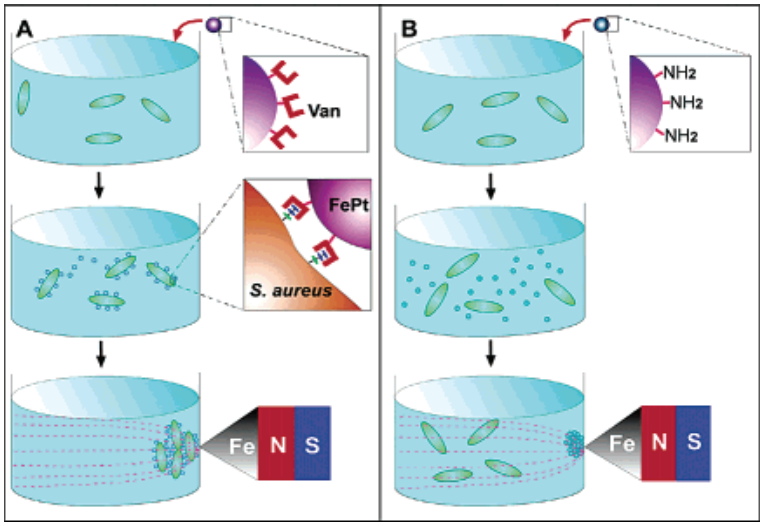

Figure 2: capture and separation of bacteria by interaction with Van surface functionalized magnetic nanoparticles.[86]

More recently, Joo et al[87] reported a sensitive method to separate and quantify the pathogen bacteria, *salmonella*, using antibody-immobilized magnetic nanoparticles and TiO_2 nanocrystals for UV detection.

Nucleic acids extraction and separation

The extraction of nucleic acids (DNA and RNA) based on magnetic particles realized for the first time by Uhlen,[88] whereas the streptavidin-biotin technology was used to prepare the magnetic beads, which were used to separate DNA and RNA. Furthermore, magnetic fluids need a surface treatment step for nucleic acids extraction[89] that the interaction between the particles and the nucleic acids is typically electrostatic thus the particles surface should have an opposite charge to the DNA and RNA molecules.

Silica nanoparticles, as separation tools, are well known that they can reversibly adsorb/desorb DNA.[90] This property of silica is used to create silica-coated magnetic particles and apply them for extraction and separation of nucleic acids.[91–98] In addition, an another treatment for magnetic particles to be suitable for nucleic acids separation is based on the

polymerization; the magnetic fluids were coated by a polymer shell and magnetic latex[45,99–102] was used to extract DNA and RNA.[103–106] Moreover, magnetic latex based on ferrofluids used also for proteins separation *in vitro*[107,108]

5. Magnetic emulsion

Magnetic emulsions are like all other normal emulsions, oil in water (o/w) or water in oil (w/o), excepting that in a magnetic emulsion the ferrofluids are used as the emulsified phase. Thus the magnetic emulsion is an organic ferrofluid in water (off/w) or an aqueous ferrofluid in oil (aff/o). This preparation was first reported by Bibette,[109] here a direct emulsion w/o was prepared using organic ferrofluid as emulsified phase and SDS as surfactant.

Montagne et al[100,110] also prepared stable oil-in-water (O/W) magnetic emulsions by the emulsification of an organic ferrofluid in a water solution containing nonionic surfactants. Following the coprecipitation methods, γ-Fe_2O_3 nanoparticle with a diameter of 7 nm was obtained and then coated with oleic acid layer dispersed in octane medium to perform a ferrofluid. This magnetic fluid was then emulsified in a water solution containing nonionic surfactants in order to obtain stable ferrofluid droplets. By controlled shearing and size sorting under a magnetic field, magnetic droplets with an average diameter of 260 ± 10 nm were obtained (Figure 3).

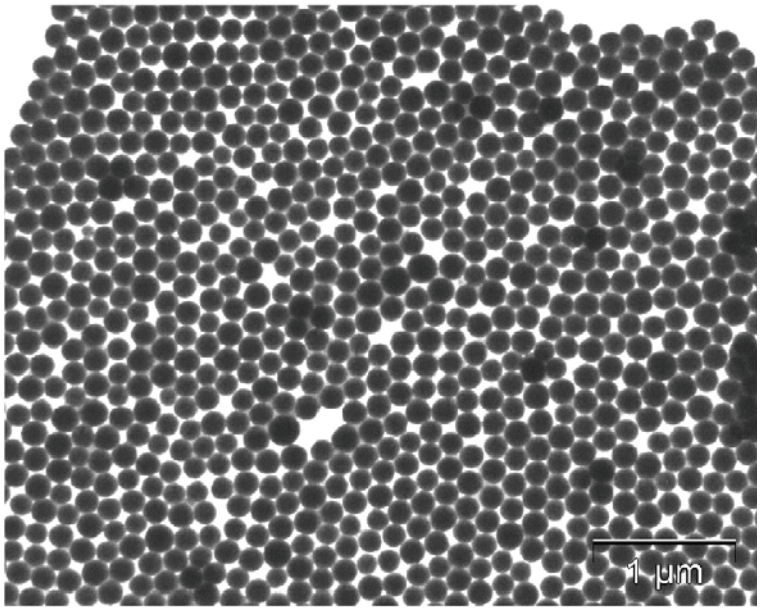

Figure 3: Transmission Electron Microscopy (TEM) image of ferrofluid droplets.[110]

Ramírez et al[111,112] successfully prepared water ferrofluid emulsions by using miniemulsion process. First of all, the hydrophobic magnetic particles with a diameter of 20 nm were obtained by a coprecipitation process of Fe^{2+} and Fe^{3+} with a molar ratio of 3:2 in ammonium

solution using oleic acid as a stabilizer. The hydrophobic magnetic particles could be easily dispersed in octane. In the next step, the hydrophobic magnetic particles in octane were reformulated to stable water-based ferrofluids. For that, a miniemulsion process was carried out to produce magnetite/octane-in-water dispersions. Stable aqueous miniemulsion with droplets consisting of the magnetite/octane dispersions were obtained by using sodium dodecyl sulfate (SDS) as surfactant. After evaporation of octane, a water-based ferrofluid consisting of oleic acid coated aggregated magnetite dispersed in water phase was obtained. The magnetic aggregates must have a surfactant double layer, the first layer is oleic acid, which provides a hydrophobicity of the particles for later encapsulation, and the second layer being SDS promotes the stabilization in water. Figure 4 shows schematic representation of the magnetic ferrofluid preparation using miniemulsion process and TEM image of magnetic aggregate.

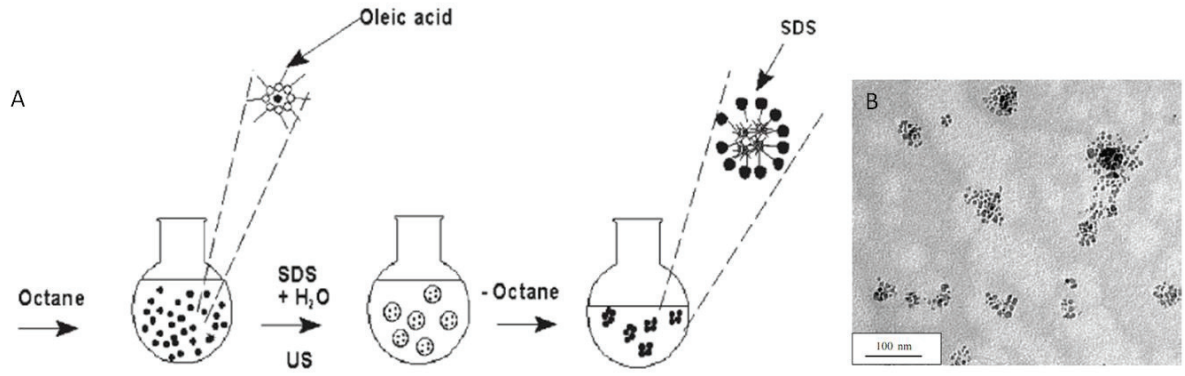

Figure 4: Schematic representation of the preparation of magnetic ferrofluid with water-based system using miniemulsion process (A), TEM image of magnetite aggregates obtained after a miniemulsion process in water (B).[111,112]

The size of these aggregates depends on both the oleic acid (introduced in the first step) and the SDS concentration and can be adjusted to be between 40 and 200 nm, Figure 4B. Similarly, Xu et al[113] prepared water-based ferrofluid by using miniemulsion technique. This miniemulsion was composed of oleic acid-magnetite nanoparticle aggregations in octane droplets stabilized by SDS with a typical hydrodynamic diameter of 123 nm.

6. Conclusion

Magnetic fluids or ferrofluids are a stable dispersion of magnetic nanoparticles in a liquid, organic or aqueous, carrier. They were born in early 1960s by grinding the magnet for very long time, more than twenty days, then these products drastically studied and developed. Due to their widespread of ferrofluids new synthesis methods were suggested for more rapid and industrial production. Chemical coprecipitation is considered as the most used method to

synthesize the ferrofluids and the obtained nanoparticles satisfied wide rang applications. With average of diameter varied from 3 to 20 nm, superparamagnetic properties and high colloidal stability in aqueous and organic carriers.

Ferrofluids are widely used in industry such as in magnetic recording media, they highly answered to the increasing requirements of big bites. Furthermore, they used in magnetic paints and inks that provided high-speed reading, treatment and archiving the information. As well as in the biomedical applications, ferrofluids fit the use in vivo for MRI contrast agents and thermotherapy. In addition they were used in vitro, especially for magnetic separation and extraction. More recently, a new form of ferrofluids is presented in literature under the form of emulsions. They called ferrofluids emulsion or magnetic emulsions, which have a significant attraction in the applications of future.

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Résumé

Les nanoparticules encapsulées par la silice : préparation et applications

Le procédé de sol-gel a été développé initialement pour préparer des particules de silice et en particulier de la silice colloïdale. Ce procédé relativement simple et en une étape a été étendu par la suite à l'encapsulation de particules inorganiques. Par conséquent, plusieurs types de particules comme les particules composites et les particules hybrides ont été préparées et utilisées dans des applications diverses et variables. Récemment, l'encapsulation sol-gel via l'émulsion inverse a été utilisée pour préparer de particules à morphologie contrôlée cœur-écorce. Les particules composites ou hybrides peuvent être élaborées par encapsulation sol-gel soit directement soit via l'émulsion inverse. Cette encapsulation a généralement pour but :

- protéger les cœurs encapsulés et maintenir une bonne stabilité chimique;
- améliorer la biocompatibilité ;
- Fonctionnalisation de surface modulable

La fonctionnalisation est la deuxième étape importante opérée après l'encapsulation pour rendre les particules applicables. Cette action peut être résumé par la fixation (principalement chimique) de groupes fonctionnels amine, carboxylique, thiol, aldéhyde… à la surface des particules, pour l'immobilisation des anticorps, des antigènes et d'autres molécules. La fonctionnalisation peut-être également introduite par greffage de polymères ou par polymérisation sur semences.

Les particules composites ou hybrides fonctionnalisées utilisées dans le biomédical sont principalement évaluées pour l'extraction et la purification des biomolécules et en particulier les acides nucléiques, comme support d'entité protéiques, dans l'imagerie biomédicale, voir dans certains applications in vivo. Enfin, nous pouvons dire que la méthode d'encapsulation par la silice est très pratique et améliore largement les propriétés des particules encapsulées.

Silica-Coated Nanoparticles: From Preparation to Applications

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Abstract

Nowadays, nanotechnology is drastically developing and significant research efforts were devoted to prepare well-defined and surface functionalized nanostructures for various applications. Therefore, silica coating is an excellent method used to product core shell nanostructures, which modify the nanoparticles surface nature.

Several kinds of nanoparticles can be modified by silica that include gold, silver, zinc, platinum, iron oxide, latex nanoparticles and other organic nanoparticles. The silica shell plays multiple roles whose are protection, functionalization, biocompatibility and colloidal stability. In this review, silica-coated nanoparticles, their preparation methods, especially, sol-gel and reverse microemulsion, properties and characterization were discussed. Finally, the bio-nanotechnology applications such as nucleic acids separation and detection, proteins and enzymes immobilization, caner therapy and bioimaging were discussed.

1. Introduction

Functionalized nanoparticles applications have received special attention nowadays, principally in biomedical domain. These applications are represented in imaging agents[1], genes delivery[2], drug delivery[3], cell labeling[4,5,6], bio-sensor designing[7] and bio-macromolecules separation[8]. And due to their unique properties like size, physical and functional surfaces, nanoparticles offer countless advantages in nanoscience. Very small particle reaches easily to its target, e.g., on or into the cell. In addition, metal nanoparticles

such as gold, silver, platinum and iron oxide have high densities that allow being facile distinguished, this property open roads for various applications. And so that, nanoparticles surface considered the key of their use as supports, carriers and detectors.

Composite nanoparticles that consist of more than one material are widely reported in literature. For this type of nanoparticles, the preparation is based on merge of two or more compounds during the synthesis. The needed final form of the composite nanoparticles is depending on the aim of their preparation. Therefore, they are represented generally in the form of nanospheres or nanocapsules and silica is extensively used in research to produce nanocapsules or core-shell nanoparticles.

Using silica to encapsulate nanoparticles is related to its properties and advantages:

(1) High protection for encapsulated nanoparticles.

(2) Silica is biocompatible that permit to reduce the toxicity of encapsulated nanoparticles.

(3) Silica surface is highly active and can be easily functionalized by many functional groups such as amine, carboxyl, vinyl and thiol.

(4) Enhance the colloidal stability.

(5) Easy and low cost production.

Silica encapsulation is applied for several inorganic and organic nanoparticles, which showed higher performance in comparison to non-modified ones. And for more performance, additional steps of synthesis were applied to functionalize the silica shell such as adsorption, grafting and in-situ polymerization.

In this review, the two most important synthesis methods used to prepare silica shell, sol-gel process[9] and reverse microemulsion[10], will be described. Then, variety of inorganic nanoparticles such as iron oxide, gold, silver, quantum dots, titanium and zinc, which used as solid cores for silica shell preparation, will be discussed. The review also will discuss some organic template, used as a core supporter for silica shell, like polystyrene latex,

poly(methacrylic acid) latex and organic pigments. Therefore, this review will give a general overview of the techniques and strategies used to characterize the core-shell nanoparticles followed by a brief discussion of their properties and some biomedical applications.

2. Sol-gel Process

Sol-gel reactions, as a method to prepare ceramic particles and inorganic glass shells, have been extensively studied for decades. From the first application of this reaction by Stöber et al[9], thousands of articles have been published describing the synthesis, properties, characterization and the applications of sol-gel products. Stöber prepared monodisperse silica particles and in a range of controllable size from 0.05μm to 2μm. Now it is well known that the Sol-Gel process is consist of two steps, the first step is the hydrolysis of a silica alkoxide and the second is a polycondensation reaction. The two steps of sol-gel reaction can be described as follows:

- Hydrolysis:

$$(OR)_3 - Si - OR + H_2O \xrightarrow{OH^- \text{ or } H^+} (OR)_3 - Si - OH + R - OH$$

R= alkyl group

- Polycondensation:

$$(OR)_3 - Si - OH + HO - Si - (OR)_3 \xrightarrow[OH^- \text{ or } H^+]{} (OR)_3 - Si - O - Si - (OR)_3 + H_2O$$

And the following reaction summarizes the complete Sol-Gel process of full-condensed silica:

$$Si(OR)_4 + 2H_2O \xrightarrow{OH^- \text{ or } H^+} SiO_2 + 4R - OH$$

The most common silica precursor, which used in sol-gel process, is tetraethyl Orthosilicate (TEOS) and this is due to two important properties of TEOS; first that it can be easily purified by using standard techniques, second that its reaction is slow and controllable[11]. As all chemical reactions, many factors influence the kinetic of the hydrolysis and condensation in the sol-gel process, which include temperature, water/silane ratio, catalyst and the nature of solvent[12]. Cihlar[13] studied the effect of pH and catalyst on the

hydrolysis and polycondensation of TEOS. It was found that the hydrolysis depends on pH of solution and not on the chemical nature of catalyst. In addition, minimum rate of catalysis was found at pH around 7. While, a high rate of hydrolysis was found by both catalysts acid and base. Finally, the condensation took place by both acid and base catalysts but it was faster by strong acids.

Sol-gel process were applied in several syntheses like as in preparation of polymer/silica hybrid materials[12] and silica-coated nanoparticles which, include silver, gold, iron oxide, zinc and titanium. Nanoparticles exhibit great properties that differ from their bulk materials. They are used in various domains especially in pharmaceutical and biomaterial synthesis. One of the most important methods of nanoparticles treatment is the encapsulation to be functionalized later. The sol-gel process is applied to prepare a silica shell onto large number of nanoparticles used for biomaterials synthesis. The silica shells added an additional property to these particles (the core). That leads to core-shell structure which, have different properties, par example, silica-coated iron oxide nanoparticles can be used as contrast agents for MRI and functionalized shell for cancer cells detection.

3. Reverse Microemulsion (RM)

Reverse microemulsion is one of important methods used to prepare core/shell nanoparticles. Therefore, this approach, water in oil emulsion, is applied to encapsulate particles by a silica shell. Generally, it is suitable to encapsulate aqueous nanoparticles.

First, RM is used to prepare nanoparticles and there are two approaches to do this. The first, by mixing two emulsions, one is containing the nanoparticle precursor and the other is containing the precipitate agent. In the second, the precursor and the precipitate agent are mixed directly in one emulsion, in which the precipitation reaction takes place. Thus, the nanoparticles (already prepared), the precursor and the precipitate agent are introduced in the droplets of RM, so the precipitate reaction will take place inside the droplet but outside the

nanoparticle forming core-shell nanoparticles. Figure 1 shows the structure of one droplet of water in oil, containing the nanoparticle, the precursor and the precipitate agent. Over time, the precursor precipitate and coat the nanoparticle in the aqueous phase forming core shell nanoparticle.

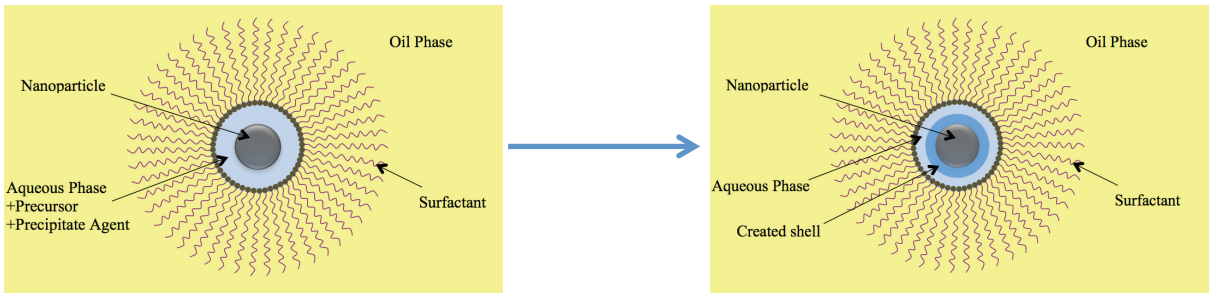

Figure 1: schematic representation of core shell nanoparticle preparation by RM.

RM is considered a very efficient coating technique that can be used without surface activation step[14], which is necessary in another coating techniques.

4. Silica-Coated Inorganic Nanoparticles

Nowadays, inorganic nanoparticles are important composition of biomaterials and bio-nanotechnology. Usually, they are presented as colloid and suspension forms that need high precautions to deal with. First and most important obstacle appears throughout the preparation of nanoparticles is the stability. Secondly, using inorganic nanoparticles for *in vivo* or *in vitro* applications must be strictly controlled. Therefore, to improve the colloidal stability and the biological applications of nanoparticles, the particles surface should be targeted.

Silica coating is a well-known method used to enhance the stability colloidal, reduce the toxicity, protect the core materials and formulate the surface for further functionalization. Iron oxide, gold, silver, quantum dots, zinc, titanium, cobalt and others are used in nanotechnology for medical and pharmaceutical applications. Thus, as an enhancement step these materials have been coated by silica shell.

4.1. Iron Oxide Nanoparticles

Iron oxide nanoparticles in general and especially magnetic nanoparticles are widely used during the last ten years to prepare colloids for bio-applications. Magnetic nanostructures, due to their remarkable properties such as superparamagnetism, high field irreversibility and high saturation field, have attracted increasing interest in various fields, especially in biomedicine and bioengineering[15] such as nucleic acids separation[16,17,18], drug delivery[19], protein and biomacromolecules adsorption[20,21,22], vaccine carrier[23], cell isolation[24] and MRI contrast agents[25,26]. However, these magnetic nanoparticles should be separated in nonmagnetic matrix to avoid aggregation, reduce contact with other surfaces in specific applications, endow them better acceptance in bio-environmental and add more functionality to these particles. Silica encapsulation is an ideal approach to provide all features required for perfect utilization of magnetic nanoparticles.

Deng et al.[15] prepared silica-coated magnetic nanoparticles via sol-gel process. They found that ethanol is good alcohol to have regular shape and morphology of silica shell. Moreover, the volume ratio of alcohol/water and the amount of catalyst (ammonia aqueous) and TEOS have important role in this synthesis. Shi et al.[27] synthesized silica-coated elliptical hematite particles by controlling the reduction time and obtained particles have two uniform layers of silica, about 55 and 30 nm. The magnetization measurements were shown, per gram of α-Fe_2O_3 for the core, with thin shell and thick shell of 1.48, 0.96 and 0.43 emu/g respectively. Narita et al.[28] used the reverse micelles to prepare silica-coated iron oxide nanoparticles. Iron oxide nanoparticles have been prepared via co-precipitation of $FeCl_3$ and $FeCl_2$ in aqueous ammonium hydroxide solution then these nanoparticles were coated with oleic acid. Secondly, iron oxide nanoparticles dispersion was added to (Igepal® CO-520) cyclohexane solution, all together were sonicated for five minutes then TEOS was added to the mixture at once and the reaction was kept at room temperature. In this work, they

controlled the thickness of silica shell via controlling the pH and reaction time, figure 2. Finally, silica-coated iron oxide nanoparticles were treated with a silane-coupling agent containing imidazolium cationic moiety to prevent their aggregation.

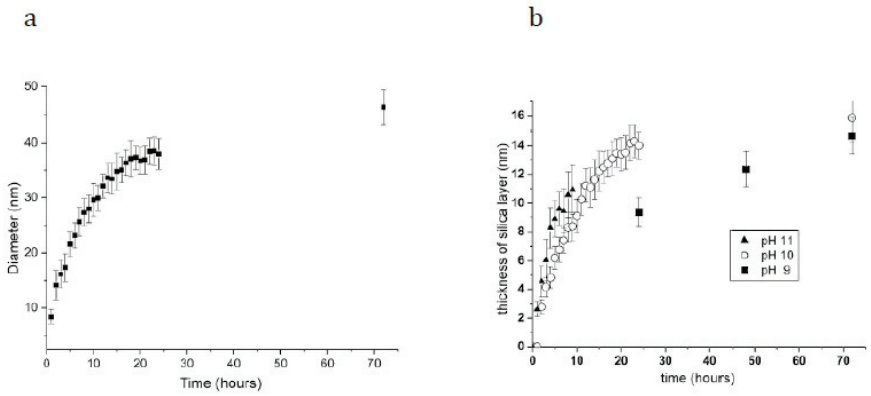

Figure 2: (a) silica-coated magnetic nanoparticles diameter evolution as a function of time and (b) silica shell thickness as a function of time.

Hong et al.[29] synthesized silica-coated Fe_3O_4 nanoparticles. Fe_3O_4 nanoparticles were prepared via co-precipitation of Fe^{3+} and Fe^{2+} with ammonium hydroxide and then these nanoparticles were reacted with sodium citrate and sol-gel reaction was applied to encapsulate these nanoparticles with silica shell. Oleic acid was grafted on the surface of silica-coated Fe_3O_4 nanoparticles and then a final layer of poly(ethylene glycol) was added to these nanoparticles. Ferreira et al.[30] prepared silica-coated magnetite nanoparticles. First, magnetite nanoparticles have been prepared via $FeCl_3$ and Na_2SO_3 precipitation in a solution of ammonium hydroxide and then these nanoparticles have been coated with silica via Stöber method using TEOS as a source of silica. Measured size is 31.5 nm for magnetite and 43.3 nm for silica-coated magnetite and the oxidation state of magnetite was conserved after silica coating. Kralj et al.[31] synthesized amino and carboxyl functionalized silica-coated magnetic nanoparticles. Precipitation from aqueous solution of Fe^{3+} and Fe^{2+} was used to synthesize maghemite nanoparticles and a citric acid solution was added to the synthesized nanoparticles to prevent their aggregation. A layer of 1 nm thickness of silica was coated the magnetic

nanoparticles via TEOS hydrolysis in an alkaline catalyst. A surface functionalization step, by amino and carboxyl groups, was followed the synthesis, figure 3. 3-(2-aminoethylamino) propylmethyldimethoxysilane (AMPS) was the amino containing compound grafted on the surface of silica-coated nanoparticles to functionalize them by amino groups. And two methods were used to add carboxyl groups using succinic anhydride (SA). 1- Amino functionalized nanoparticles were re dispersed in *N,N*-dimethylformamide (DMF) and SA dissolved in DMF were incubated together under stirring for 20h. 2- SA and AMPS were reacted to give tow reagents, (AMPS-SA)-1 and (AMPS-SA)-2 which dissolved in ethanol and incubated with silica-coated nanoparticles at pH 11 and 50°C for 5h under stirring.

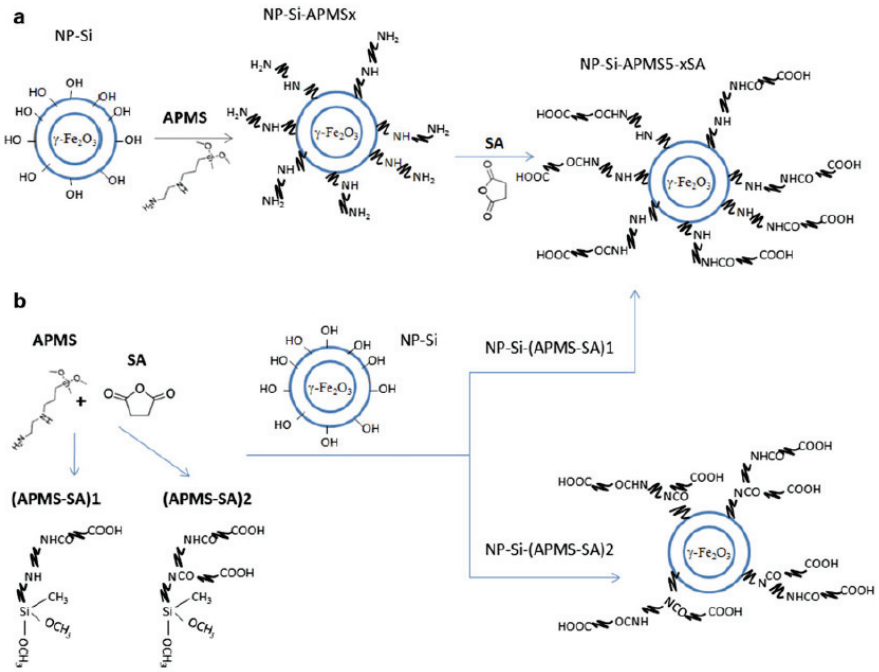

Figure 3: schematic representation of the functionalization of silica-coated magnetic nanoparticles.

Hsieh et al.[32] prepared polyaniline-coated silica/maghemite nanoparticles. γFe_2O_3 particles with 10-20 nm of diameter were prepared via reverse micelle method[33], these nanoparticles have been coated with silica shell via modified Stöber reaction. Obtained particles have diameter from 52 to 195 nm and the saturation magnetization of silica/magnetic nanoparticles decreased by increasing TEOS concentration due to the increase of silica shell thickness. Finally, in situ polymerization of aniline was applied to coat the silica/magnetic

nanoparticles. Furthermore, TEOS had an important role in the final particles morphology because at high TEOS content, a decreasing in polyaniline coating effective was observed.

4.2. Gold Nanoparticles

As iron oxide, colloidal gold nanoparticles have been widely applied in nanotechnology due to their unique specifications and properties such as quantum size, variant composition and shape-dependent optical properties[34,35]. Thus, the enhancement of colloid gold nanoparticles stability is enormously studied and many stabilization methods were proposed such as chemical stabilizers, which were applied during nanoparticles synthesis like citrate group[36], alkanthiols[37] and other sulfur legends. Furthermore, polymers were used to improve the colloidal stability of gold nanoparticles such as polypyrrole (PPY)[38] and poly(ethylene glycol) (PEG)[35,39]. Recently, silica encapsulation of nanoparticles is widely reported in literature to improve the colloidal stability of gold nanoparticles. Selvan et al.[40] synthesized silica-encapsulated gold nanoparticles using tetrakis (hydroxymethyl) phosphonium chloride (THPC) reduction and a sol-gel process via tetramethylorthosilicate (TMOS) hydrolysis without any external stabilizing agents. Ghica et al.[41] synthesized paramagnetic silica-coated gold nanoparticles via the reduction of Au (III) chloride salt. First, gold nanoparticles were prepared with diameter of 5nm. Then, AuNPs were coated by silica shell using Stöber method using modified silica precursor, which prepared by coupling 3-aminopropyltrimethoxysilane and 3-carboxy-proxyl free radical. The average size of obtained particles was 100 nm and each silica particle contains about 10 AuNPs.

Stöber method offers the possibility to encapsulate nanoparticles by controlled shell thickness using different concentrations of TEOS. As studied Liu et al[42], they synthesized and functionalized monodisperse silica-coated gold nanoparticles, figure 4. In this study, TEOS has been used to encapsulate gold nanoparticles with different silica shell thicknesses, from 35 to 55 nm, according to the used amount of TEOS. Moreover, a silane-coupling agent was

used to covalently graft carboxylic groups onto the silica shell, 1.8 x 10^4 carboxylic groups per silica/gold particle are estimated. Finally, amino-terminated oligonucleotides have been covalently conjugated with these nanoparticles that used to build probes for fast DNA colorimetric detection.

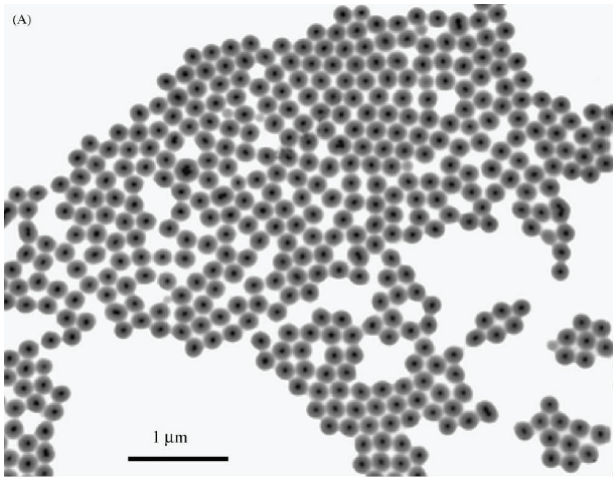

Figure 4: silica-coated gold nanoparticles.

Han et al.[14] prepared silica-coated gold/silver nanoparticles via reverse microemulsion. Gold nanoparticles were distributed in water in cyclohexane reverse microemulsion using (Igepal® CO-520) as dispersant, ammonium hydroxide as catalyst and TEOS as a source of silica. Obtained particles, after 5h of reaction, have average size of 24 nm and after total consumption of TEOS (15h) particles average size increased to 35 nm. Further, to add a functional amine group APTES was mixed with TEOS at a molar ratio of 1:15.

4.3. Silver Nanoparticles

Silver nanoparticles were widely utilized for bio-applications that they are well known as antibacterial agents and nowadays they have a particular interest in nanotechnology[43,44] and bioengineering[45]. Silica coating has been applied on silver nanoparticles, like AuNPs and magnetic NPs, to improve their stability colloidal and for additional functionality. First, silver nanoparticles were distributed in silica glass film via the sol-gel process[46], this types of

structure has a non-linear optical properties. Then, silica-coated silver nanoparticles started to be synthesized and used.

Reverse microemulsion, as well as sol-gel[47], was used to prepare silica-coated silver nanoparticles via water-in-cyclohexane emulsion[48]. RM considered being more suitable to encapsulate silver nanoparticles by silica, because AgNPs don't have strong affinity for silica as well as iron oxide NPs. Thus, the encapsulation of AgNPs by sol-gel method requires a pretreatment of NPs to promote the deposition and adhesion onto the AgNPs surface. Hagura et al[49] used the RM method, in that, the AgNPs were dispersed in cyclohexane using a nonionic surfactant: polyoxyethylene (5) nonylphenyl ether (Igepal®CO-520). Then the silica shell was prepared by hydrolysis and condensation of TEOS, herein, silica shell size was controllable by controlling the reaction time and the initial TEOS concentration. Silica-coated AgNPs applied to enhance the fluorescence properties of fluorescein isothiocyanate (FiTC), so the metallic nanoparticles reduce the self-quenching and increases the fluorescence of dye when it is in contact with it. Viger et al.[50] prepared dye-doped silica shell on Ag nanoparticles; TEOS and silane coupling agent chemically bound to FiTC were used to introduce the dye, figure 5.

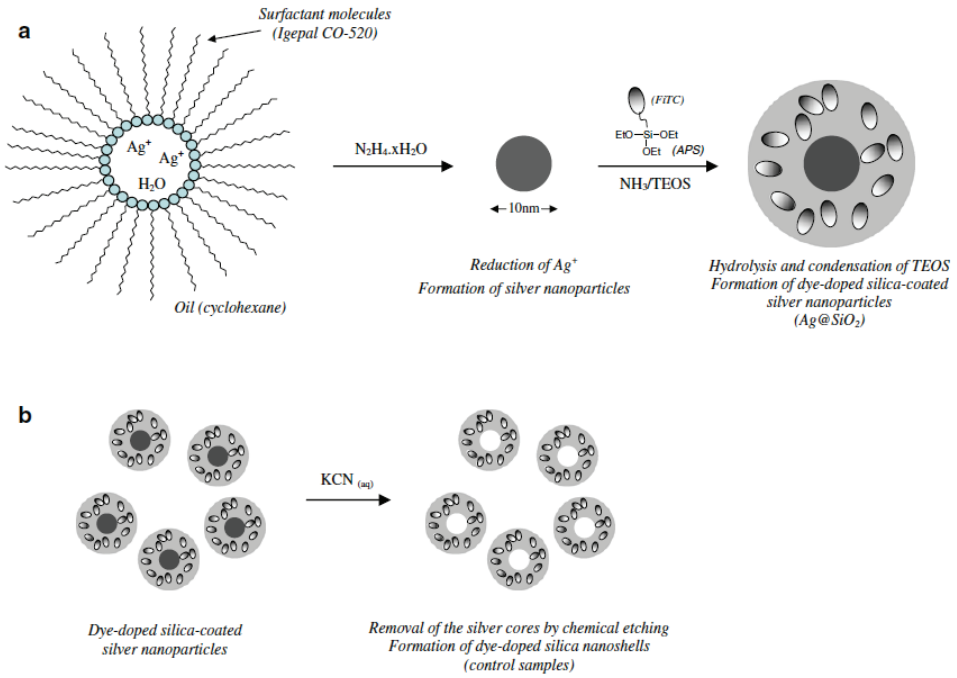

Figure 5: schematic representation of synthesis of FiTC-silica-coated silver nanoparticles via reverses microemulsion (a) and silver core removal by chemical treatment (b).

4.4. Quantum dots

Currently, quantum dots (QDs) similar to all nanoparticles are used in nanotechnology to create optical nanoscale devices. They were extensively used from 1980s for electronic and optic applications since these nanoparticles possess unique photochemical and photophysical properties, stable against photoblanching and can be excited for multicolor emission with a single light source[51]. Furthermore, the nanocomposites silica/QDs were reported in literature and many synthesis mechanisms were proposed to enhance the useful of QDs, especially in biology and diagnostic applications.

Yang et al.[52] coated quantum dots by silica shell via the reverse microemulsion method, figure 6. They prepared water (containing QDs and ammonia) in cyclohexane and n-hexane emulsion by using Triton X-100 as surfactant and then TEOS is introduced under vigorous magnetic stirring. The obtained particles have size from 45 to 109 nm.

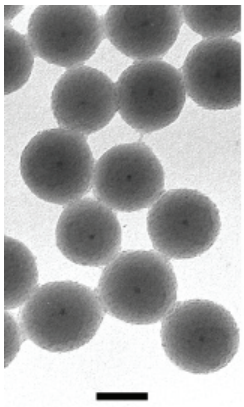

Figure 6: TEM image of silica-coated quantum dots (CdTe@SiO_2), scale corresponds to 50nm.

Finally, amino groups have been grafted on the surface of these fluorescent nanoparticles. The aim of this silica shell was to increase the photostability of QDs, improve the stability colloidal, graft amine groups on the surface and decrease the toxicity for further biomarker preparation based on fluorescent QDs. Other works also reported the QDs/silica core/shell nanoparticles as a technique to protect the QDs, improve the stability and reduce

the toxicity for biological applications[53,54,55,]. Koole et al.[56] succeeded to prepare silica shell on MPA (mercaptopropionic acid)-coated hydrophobic QDs. It was found that the hydrolyzed TEOS has a strong affinity to the QDs surface and it can replace the hydrophobic amine legends then QDs can go into the hydrophilic phase of emulsion where silica growth takes place. Wang et al.[57] prepared silica-coated ZnO nanoparticles via reverse microemulsion. Obtained particles have size from 35 to 75 nm and the silica shell thickness was between 11 and 25 nm. These kinds of particles can be applied as UV blockers in several products.

Darbandi et al.[58] used direct emulsion, cyclohexane in water and poly(ethylene glycol) nonylphenyl ether (NP-5) as surfactant to prepare highly monodisperse silica-coated single QD. It was found that at low and too high ammonia concentrations, irregular silica structures were formed. This was explained that at low ammonia concentrations the monodispersity is lost and at high ammonia concentrations the microemulsions are destabilized. All other reaction parameters are investigated and a reaction mechanism is proposed.

4.5. Zinc Nanoparticles

Zinc is another important metal used to prepare nanoparticles for nanotechnology applications. Mu et al.[59,60] prepared silica-coated ZnS\ ZnS:Mn nanoparticles using TEOS as a silica source. It has been found that the silica layer improves the antioxidation ability and the thermal stability, figure 7.

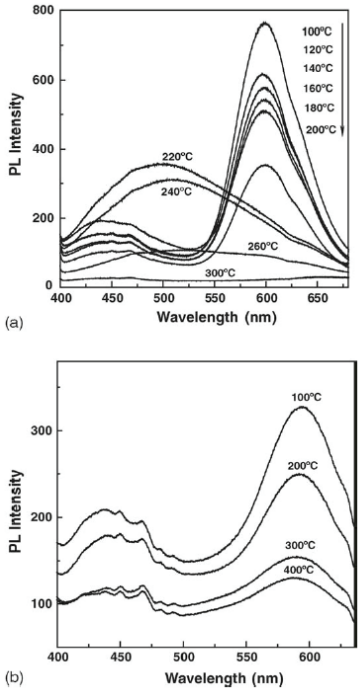

Figure 7: (a) photoluminescence spectra of non-coated ZnS and (b) of silica-coated ZnS nanoparticles annealed for 2h.

Dong et al[61] coated ZnS:Mn nanoparticles by a silica shell, they used the sol-gel in reverse emulsion technique to perform this core-shell structures, which have quenching of fluorescence intensity effect in presence of Cu^{2+} ions, figure 8. This property can be used to design Cu^{2+} detectors since the detection limit as low as $7.3x10^{-9}$ $mol.l^{-1}$.

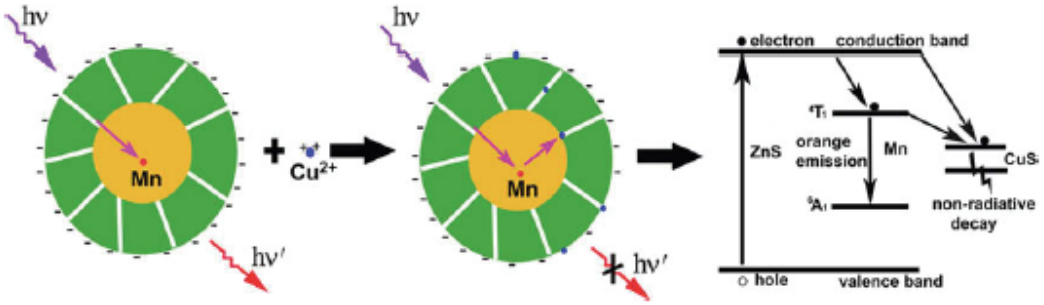

Figure 8: proposed mechanism of the Cu^{2+} quenching of fluorescence of silica-coated ZnS nanoparticles.

Hagura et al[62] prepared highly luminescent silica-coated ZnO nanoparticles dispersed in aqueous medium. Sol-gel process is used, using TEOS as a precursor of silica, to coat ZnO nanoparticles by a silica layer.

4.6. Titanium and Other Nanoparticles

TiO_2 is one of the most important compounds in skin care products[63], pigments[64]and many applications[65]. Ryan et al[66] coated titanium dioxide (TiO_2) and zirconium dioxide (ZiO_2) nanoparticles with silica using TMOS as a silica source. The silica-coated colloidal were stable to aggregation after 90 days. These structures have been used in subsurface transport experiments. El-Toni et al[67], to avoid the possible photocatalytic decomposition of cosmetic products by titanium dioxide[68], synthesized silica-coated TiO_2 nanoparticles via TEOS hydrolysis in ammonium hydroxide solution and at high temperature (120-240C°).

The cobalt also is one of important metal in nanotechnology. Bonini et al[69] prepared silica-coated cobalt-ferrite nanoparticles via reverse microemulsion and supersaturated solution. Obtained particles had radius of 5 nm and shell with thicknesses of 2.5 nm. Other particles composed of Co and Pt have been coated with silica via TEOS hydrolysis by Kobayashi et al[70]. Silica encapsulation used on other inorganic nanoparticles such as radio-labeled selenium colloids[71], CdTe nanocrystals[72], CdS nanoparticles[73], rhodium nanoparticles[74], $LaCeF_3$:Tb nanocrystals[75], CeO_2 nanoparticles[76], Pt naoparticles[77], quantum dots[78],[79], and Y_2O_3 nanoparticles[80],[81],[82].

5. Silica-Coated Organic Nanoparticles

Organic nanoparticles are newly at an important comparable with inorganic nanoparticles, developing drastically for nanotechnology applications. Usually, organic nanoparticles are made of polymers[83] such as polystyrene[84] and poly (methyl methacrylate)[85].

Hollow silica particles are produced using sol-gel process applied on polymeric nanoparticles, which are used as a solid support. First, nanoparticles are prepared by normal polymerization then encapsulated by a silica shell using sol-gel process. Finally, the polymer was removed by calcination at high temperature.

5.1. Polystyrene latex particles

Tissot et al.[86,87] prepared silica-coated latex particles via two steps. First, 3-(trimethoxysilyl) propyl methacrylate (MPS) molecules were incorporated in PS particles to present OH and OCH_3 groups at the particles surface. These groups will be the nucleation points to silica and the second step was the hydrolysis of TEOS on the PS particles surface to encapsulate them by silica shell. Finally, PS was removed by calcination step at 600 °C to have hollow silica spheres, figure 9.

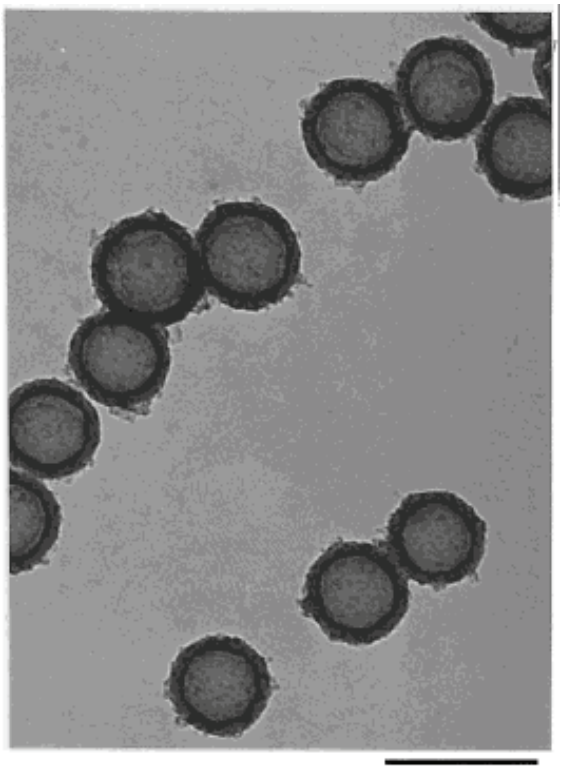

Figure 9: hollow silica nanoparticles.

Lu et al.[88] used commercial PS beads, which were encapsulated by silica via a modified Stöber method. It has been found that the silica shell, on the particles, which have positive charge, has an uniform thickness. From these core-shell particles the core was removed selectively by calcination the sample in the air at 450 °C or by dissolving PS by an organic solvent like toluene. The obtained particles have a hollow sphere shape. Graf et al.[89] proposed general method to encapsulate nanoparticles by a silica shell; they adsorbed PVP on the particles surface to stabilize the particles, which are redispersed in ethanol. This method

was applied on gold, silver, cationic and anionic polystyrene nanoparticles. While, without PVP coating anionic polystyrene particles with silica could not form uniform coating on the surface[88]. Chen et al.[90] reported a one step method to prepare monodisperse hollow silica nanoparticles. PS used as a template to build the silica sphere and the concentration of ammonium/alcohol medium and temperature have had the basic role to dissolve PS particles.

Zou et al.[91]coated poly (styrene-co-4-vinylperridine) nanoparticles with silica shell using sol-gel process and PVP as a surfactant, which is used to raise the affinity between the polymer surface and silica. Hollow silica spheres were prepared using the approach of Chen et al.[90] Cao et al.[92] coated PS particles with silica shell by using TEOS in ammonium hydroxide medium, and then CH_2Cl_2 was added to the reaction medium to remove the core.

5.2. Poly methacrylic acid latex particles

Li et al.[93] used the sol-gel process to prepare silica nanoparticles, which were used as a template to synthesize PMAA shell on it. Then, a second sol-gel process was applied to have SiO_2-PMAA-SiO_2 trilayer hybrid microspheres. Concentric hollow silica microspheres were obtained after a calcination step in which PMAA layer was removed, figure 10. The same approach has been applied to produce tetra- and penta-layer hybrid microspheres[94]. These microspheres were proposed as micro-reactors for confined reactions.

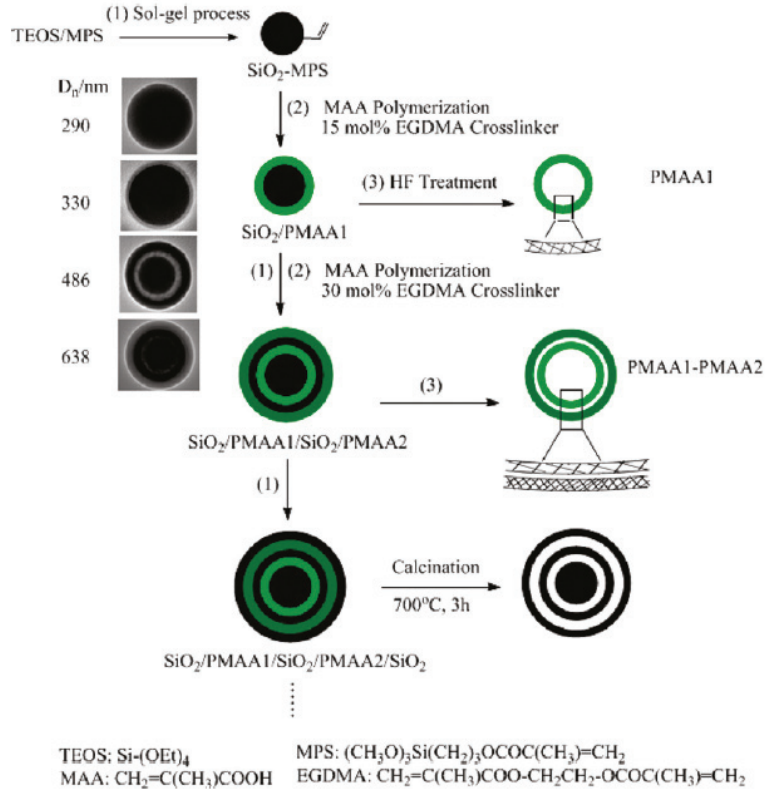

Figure 10: synthesis of hollow microsphere multi-layers of silica.

5.3. Organic pigments

The paints, inks and plastic industry are widely depending on organic pigments, which have many advantages such as color strength, photosensitivity and excellent transparency. But, some obstacles are facing the applications of organic pigments such as the poor dispersion ability, limited hiding power and poor weather durability. Yuan et al coated organic pigment particles with silica shell by two ways:

1-colloidal silica nanoparticles via layer-by-layer technique[95], figure11. Two electrolytes were deposited on the particles surface, poly (diallyldimethylammonium chloride) (PDADMAC) and poly (sodium 4-styrenesulfonate) (PSS), to have positive charge. Then, the colloidal silica nanoparticles have been adsorbed on the positive surface of pigment particles. Coating organic pigment particles by silica improved the UV scattering properties and enhanced the dispersion ability of organic pigments in water.

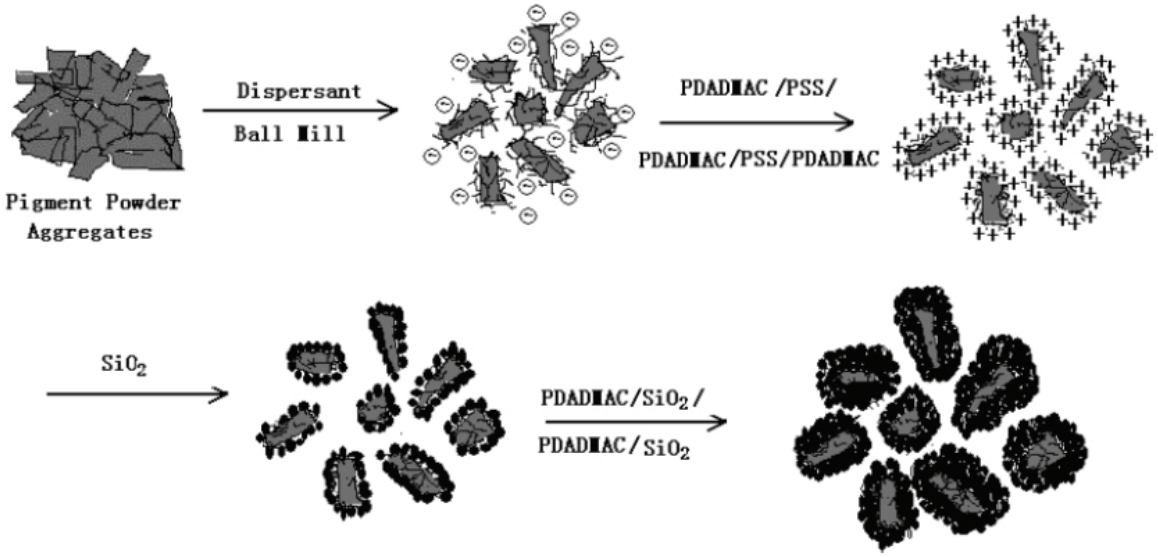

Figure 11: schematic representation of organic pigment encapsulation using silica nanoparticles[95].

2[96]*-Sol-gel process*: first, the pigment particles surface treated by PDADMAC/PSS, then a sol-gel process has been applied to coat the pigments by silica shell. Figure 12 shows the SEM images of natural and silica-coated particles pigment. Also, this coating enhanced the UV scattering properties of the pigments.

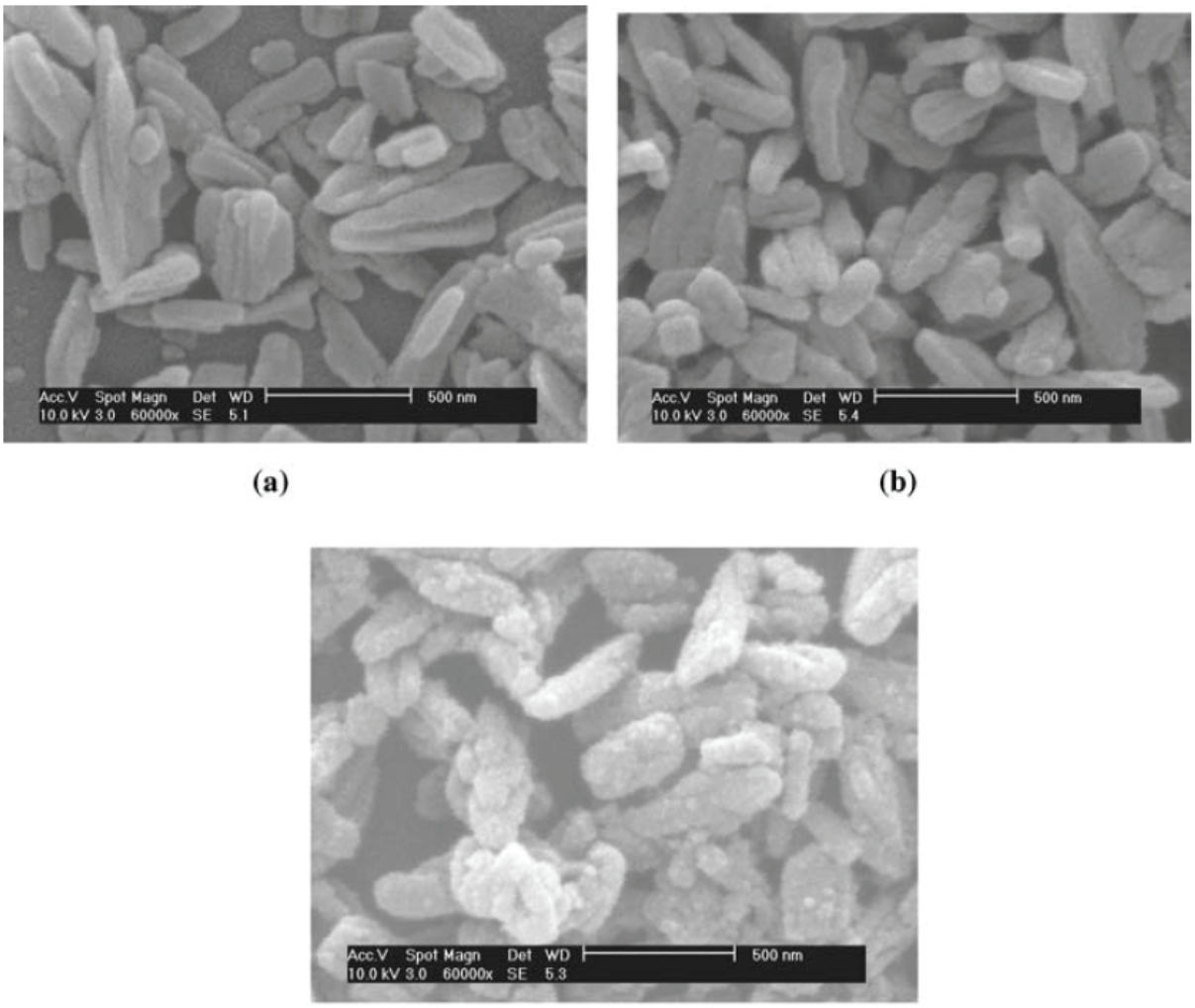

Figure12: SEM images of a) natural pigments particles and silica-coated organic pigment at different concentration of water, b) H_2O = 6.0 mol/l and c) H_2O = 7.7 mol/l[96].

6. Functionalized Silica Shell Nanoparticles

Coating by silica is the intermediate step of silica-coated nanoparticles technology, which needs one or more additional steps. Silica surface is an appropriate ground to several modifications. Normal silica surface presents –OH groups and if a silane coupling agent used during the synthesis, high number of functional groups can be added to the particles surface. That proposes numerous types of molecules that can interact with the superficial groups to modify the surface for the further application. In nanotechnology this process is called "functionalization", generally, there is two ways to functionalize the nanoparticles: (i) silane coupling agents and (ii) polymers.

6.1. Silane coupling agents

This process is a modified Stöber reaction that uses TEOS and TEOS derivatives molecules during the coating reaction. Researchers synthesized the functionalized silica-coated nanoparticles via two ways:

1-One step reaction: whereas, TEOS and silane coupling agent have been mixed and reacted simultaneously. Ashtari et al.[97] prepared amino-functionalized silica-coated iron oxide nanoparticles. N-(-ethylenamine) propylamine triethoxylsilane (EPTES) has been added with TEOS (3:5 v/v) during polymerization reaction, water in oil reverse microemulsion was used to perform these nanoparticles, (He et al.[18] used the same approach but with different silane coupling agent, AEAPS). The presence of $–NH_2$ functional groups on the particles surface was proved by fluorescamine test. Liang et al.[98] prepared amino-modified silica-coated magnetic, and silver, nanoparticles. 3-aminopropyl trimethoxy silane (APTMS) was used as a source of amino functional group in addition to TEOS as a silica precursor. Yoon et al.[99] synthesized luminescent silica-coated cobalt ferrite magnetic nanoparticles using (RITC or FITC)-modified 3-aminopropyltriethoxysilane (APS) and TEOS in ammonium hydroxide solution. Then, PEG was adsorbed on the particles surface. Therefore, obtained particles have luminescent silica shell and a superficial layer of PEG, as shown in figure 13.

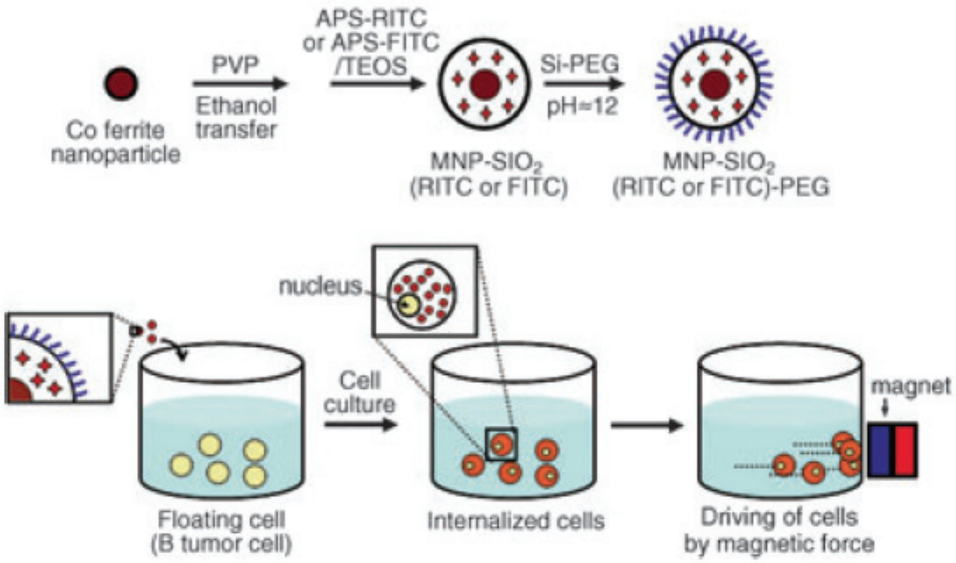

Figure 13: schematic representation of MNP-SiO_2 (RITC)-PEG synthesis procedure and cell behavior in presence of MNP-SiO_2 (RITC)-PEG and external magnetic field[99].

2-Two steps reaction: whereas, silica shell coated the particles in the first step and an additional step was recquired to functionalize the core-shell nanoparticles. Park et al[100] synthesized silica-coated magnetic nanoparticles in the first step and in the second step, amino functional groups have been added via amino-functionalized silane (APTMS, TMPEA and TMPDT). The second reaction has been done in toluene at 130°C for 7h. Sulek et al[101] used also the two steps reaction to prepare amino-functionalized silica-coated magnetic nanoparticles. Huang et al[102] synthesized silica-coated magnetic nanoparticles which were functionalized by –SH groups using MPTMS as a silane coupling agent. Bahadur et al[103] applied an irradiation by microwave to produce silica-coated silver nanoparticles. TEOS was used as silica source and without any surface-coupling agents like PVP. Functionalization step performed using Stöber process; silica-coated silver nanoparticles were mixed with APTES in ethanol/water solution for 24h at room temperature. Finally, amino-functionalized silica-coated silver nanoparticles have been treated with succinic anhydride to have carboxyl-functionalized silica-coated silver nanoparticles.

6.2. Polymers

Silica surface shows mechanical and thermal stability and presents reactive silanol groups, which facilitate the chemical modifications. Thus, coating silica with polymers promotes a great range of applications for silica nanoparticles and silica-coated nanoparticles, since this can be done via two ways.

6.2.1. Functionalization of particles by "Grafting to"

First, polymer coating has been applied on the silica particles; Bose et al[104] coated commercial mesoporous silica particles with Siloxane polymer, which was dissolved in chloroform then different amounts of silica were added to the solution to have several levels of coating. Adsorbed polymers on silica particles have been investigated by thermogravimetric and nitrogen adsorption analysis. Hayashi et al[105] prepared silica particles, which were linked with pyrenyl units on their surfaces. These units have been used to interact with polymer segments (*N,N*-dimethylaniline) that can coat the particles surfaces. Synytska et al[106] grafted carboxyl-terminated polystyrene onto silica particles. Initially, silica particles have been coated with a monolayer of 3-glycidoxypropyl trimethoxysilane (GPS), and then PS-COOH or FPS-COOH was grafted onto the surface of modified silica particles. Core-shell\silica-PS particles have been obtained and used as a model surface to study the water wetting behaviour. Yoon et al[99] prepared silica-coated Co-ferrite nanoparticles and then these particles have been coated with PEG using PEG-$Si(OMe)_3$. Polymer coating increased the biocompatibility of nanoparticles, which were used for cells separation.

This method is experimentally simple but has some significant limitations. In particular, it is difficult to achieve high grafting density because of the screening of reactive surface by already grafted polymers.

6.2.2. Functionalization of particles by "Grafting from"

This technique, called also surface initiated polymerization (SIP), depends on an initiator/precursor incorporated in the external layer during particles synthesis or grafted on the particles surfaces after particles preparation. Huang et al[107] used technique called a surface initiated atom transfer radical polymerization (SI-ATRP), on porous silica particles to coat them with a polyacrylamide thin film. 1-(trichlorosilyl)-2-[m/p-(chloromethyl) phenyl] ethane has been used as a surface initiator for polymerization and $Cu(bpy)_2Cl$ was added to control the radical population. PAAm-coated silica particles were used to separate proteins by size exclusion. Von Werne et al[108],[109] prepared polymer-nanoparticle hybrids using surface-modified silica nanoparticles and styrene/methyl methacrylate as monomers. Perruchot et al[110] synthesized polymer-grafted silica particles based on polymerization of methacrylate from modified silica surface at 20°C. First, silica particles have been treated by bromoisobutyrate-based siloxane in ethanol at 20°C. Then, methacrylate has been polymerized using CuCl:bipy catalyst system. Mori et al[111] used the same approach, functionalization of silica particles surface by inimer (initiator-monomer) then polymerize from this surface to produce hyper-branched polymer-grafted silica particles. They used 2-(2-bromopropionyloxy) ethyl acrylate (BPEA) as inimer to functionalize the silica particles and pentamethyldiethylenetriamine (PMDETA) as a monomer. Hyper-branched shells have been obtained by using both monomers, BPEA and PMDETA, during polymerization.

7. Characterization and Properties

The characterization methods used in the analysis of the chemical composition, morphology, surface and physical properties of the encapsulated nanoparticles are varied. This section will highlight on some techniques often used to study and investigation of nanoparticles. The functionality of encapsulated nanoparticles strongly depends on the surface

composition and its properties. Therefore, characterization techniques focus on the surface properties of the nanoparticles to understand the structure-application relationships.

7.1. Composition and chemical structure

Thermal gravimetric analysis (TGA) technique often used to determine the chemical composition of material by measuring weight loss as a function of temperature. Thus, this technique is useful to study the composites consist of organic and inorganic material such as silica shell on organic polymer or the opposite polymer onto silica particles. Miomandre et al[112] prepared polypyrrole-silica core-shell nanocomposites and the TGA investigations showed a drastic loss of weight, started at 210°C, in the case of homopolymer. Whereas, in the silica coated polymer case the weight loss is started at 260°C, which gave an evidence that the polymeric core is protected by an inorganic shell, figure 14. On the other hand, silica coated metallic core, silica shell showed an oxidation protector role[113,114]. Figure 15 shows that the oxidation stage of pure aluminum is faster than this in the case of silica-coated aluminum.

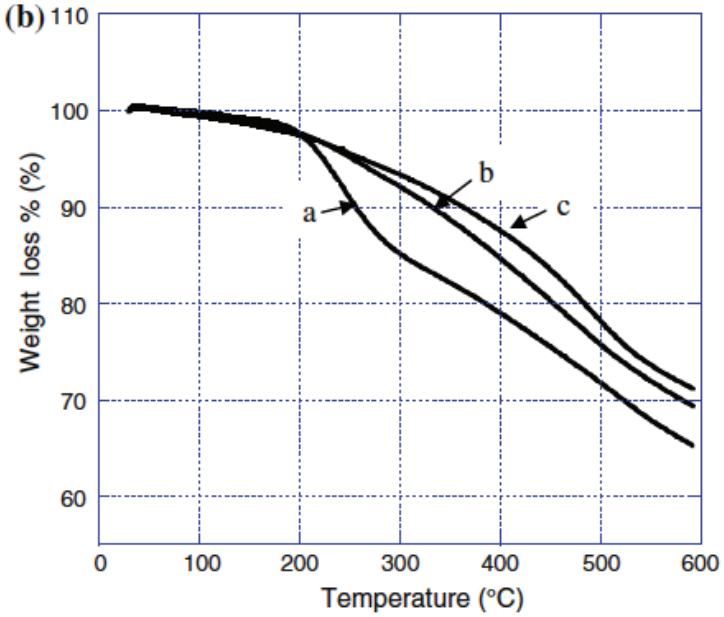

Figure 14: TGA curves of (a) homopolymer, (b) and (c) silica-coated polymer prepared by microemulsion medium using SDS as surfactant[115].

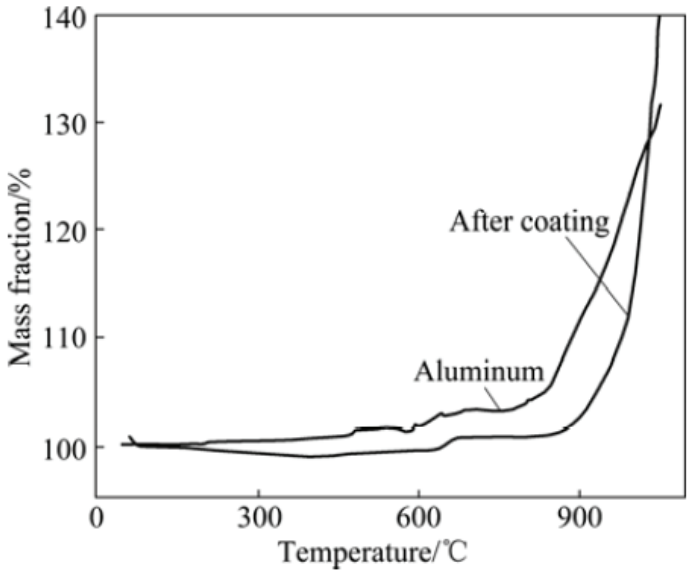

Figure 15: TGA curve of pure aluminum and silica-coated aluminium[113].

Another technique generally used to study and prove the formation of silica shell is the FT-IR, a major peak in the range of (1000-1200 cm^{-1}) that is attributed to the asymmetric stretching vibrations of Si-O-Si bonds of silica can be found in the particles. In addition, the peak at 883cm^{-1} indicates the stretching vibrations of Si-OH bonds, which means that the condensation reaction is not completed. Kralj et al[31], figure 16, used FT-IR to characterize silica coated magnetic nanoparticles and functionalized with amino and carboxyl groups. The stretching vibrations of Si-O-Si bonds, indicate the condensation of silica shell, found at (990-1130 cm^{-1}) and a second important peak at 1030 cm^{-1} corresponding to Si-O-Fe stretching vibrations representing the superficial interactions.

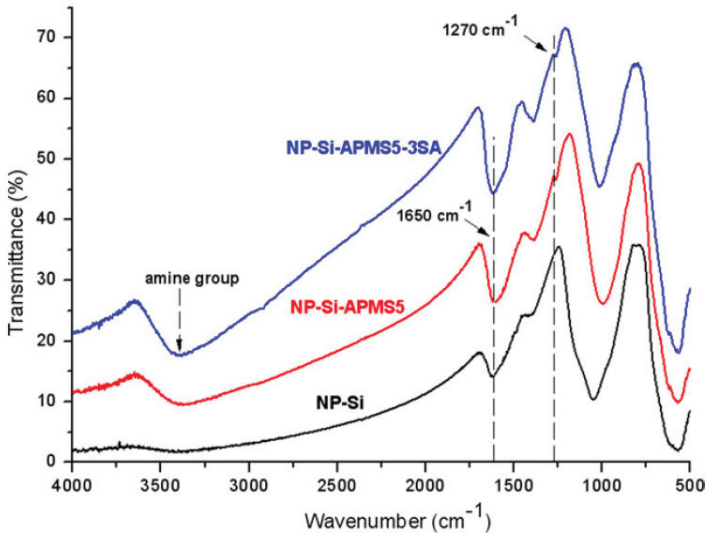

Figure 16: FT-IR spectra of silica-coated magnetic nanoparticles (NP-Si), amine functionalized NP-Si (NP-Si-AMPS) and carboxyl functionalized NP-Si (NP-Si-AMPS-SA)[31].

Employing FT-IR to study the core-shell particles supplies evidences of all bond modifications such as bonds formation[73] or double bonds disappearance. More analysis techniques were applied to characterize these particles like as X-ray diffraction (XRD)[61], X-ray photoelectron spectroscopy (XPS)[29] and UV-Vis spectroscopy, when the particles or one of the composites absorb in this range[61].

7.2. Morphology and surface properties

TEM, SEM and AFM are three microscopy techniques to study the morphology of nanoparticles. Transmission electron microscopy (TEM) is a technique based on a beam of electrons transmits ultrathin specimen giving information about the general and inner structure of the particle. Normally, all researchers and nanoparticles developers use TEM to investigate the morphology of particles. Moreover, new instruments in this field were developed such as high-resolution transmission electron microscopy (HTEM) and electron spectroscopy imaging in the transmission electron microscopy (ESI-TEM), figure 17 shows an example of TEM and HTEM images.

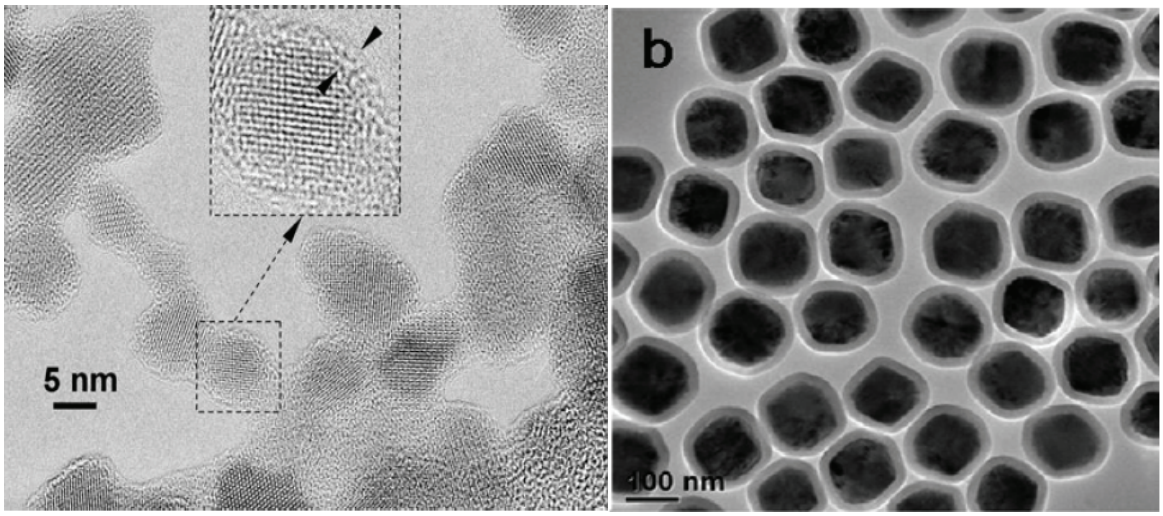

Figure 17: (a) HTEM image of silica-coated magnetic nanoparticles[31] and (b) TEM image of silica-coated iron oxide nanoparticles[116].

Scanning electron microscopy (SEM) is a useful technique to study the surface details of the particles since images 3D can be obtained from this technique as shown in figure 18.

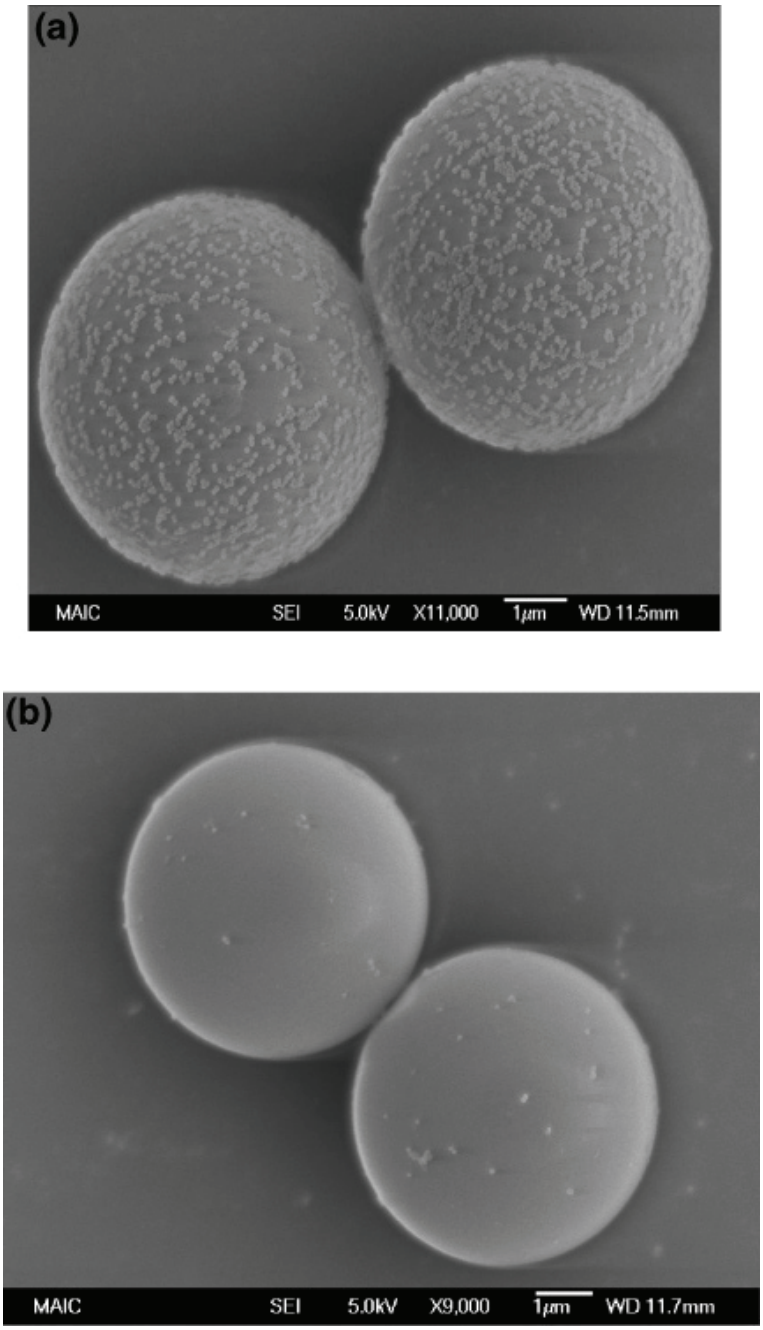

Figure 18: SEM image of streptavidin-coated silica particles (a) biotinylated NPs and (b) unbiotinylated NPs[117].

Atomic force microscopy (AFM) is the third technique of microscopy, which is providing the morphological information of the surface, figure 19. In the case of core-shell particles it is useful to measure the shell thikness[118].

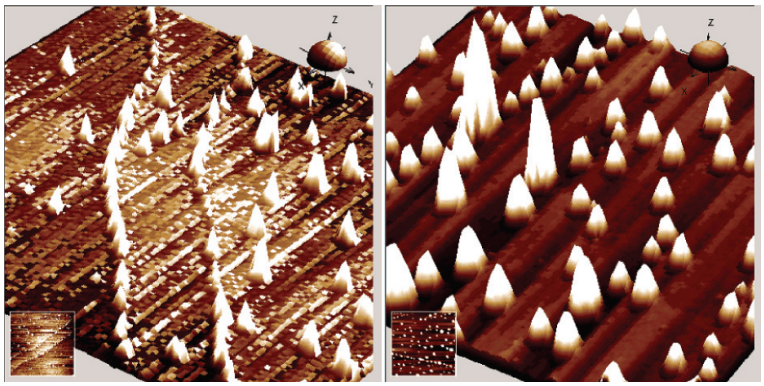

Figure 19: tree dimensional AFM image of QDs (left) and silica-coated QDs (right)[119].

7.3.Methods of size and zeta potential measurements

Dynamic light scattering (DLS) is the most commonly methods used for measuring the size distribution profile of small particles, few nanometers up to one micron, in solution (suspensions and colloids). It depends on the laser beam that hits the particle, which in its turn scatters the light in all directions. The intensity and the direction of scattered light are depending on the particle size[120].

Zeta potential is the index of the colloidal stability, it represents the potential difference between the dispersion medium and the stationary layer fluid attached to the dispersed particles. Zeta potential, as a function of pH, for silica-coated nanoparticles is essential to be determined since the silica shell has a specific zeta profile as a function of pH. Figure 20 shows curves of zeta potential as a function of pH.

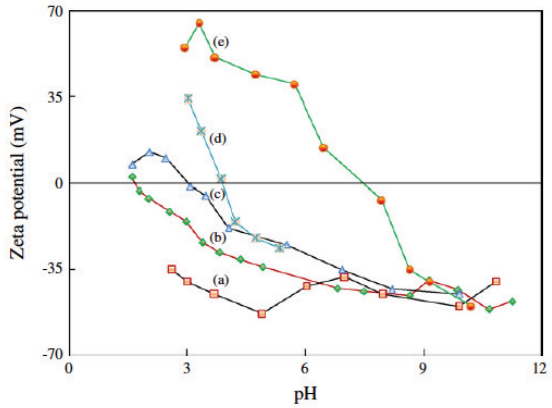

Figure 20: Zeta potential variation as a function of pH of (a) silver nanoparticles and (e) silica –coated silver nanoparticles[103].

8. Applications of silica-coated particles in life science

Silica coated particles have dual properties one is related to the core and the other is associated to silica shell. Therefore, their applications are widely expanded in several fields and especially in biomedical domain such as DNA extraction separation and detection, proteins adsorption, bioimaging, anti-bacteria, cancer therapy and cell tracker.

8.1. DNA extraction, separation and detection

Gold nanoparticles were widely used for DNA hybridization detection[121,122,123], consequently, advances in this field were developed. Silica coating is considered as an important technique for improving the functionality of nanoparticles. Cerruti et al[124] showed that silica coated gold nanoparticles have ten times lower of limit detection than non-coated gold nanoparticles, this is due to that the silica shell increased particles emissivity. The nanoparticles were irradiated by laser light at a frequency close to the surface plasmon resonance of gold and the re-emitted radiation was detected by IR camera. Furthermore, DNA hybridization detection by functionalized silica shell, which can be easily obtained due to the high reactivity of silica surface, was realized[42]. Gold is one of the best metals used to design DNA detectors but other materials are used also for this purpose like silver[98] and iron oxide[125]. DNA extraction and separation using nanoparticles is depending on the adsorption/desorption behavior of DNA molecules onto these particles in various conditions[126]. Silica coated iron oxide nanoparticles, can be separated rapidly under the action of magnetic champ, are commonly used for DNA extraction tools. Electrostatic DNA adsorption of the negative charged DNA molecule onto the silica surface positively charged[17] is the separation mechanism of DNA by silica coated magnetic nanoparticles. DNA adsorption onto the nanoparticles is not specific so the quantity extracted is maximal. Figure 21 shows the DNA detection technique using fluorescent DNA and silica NPs.

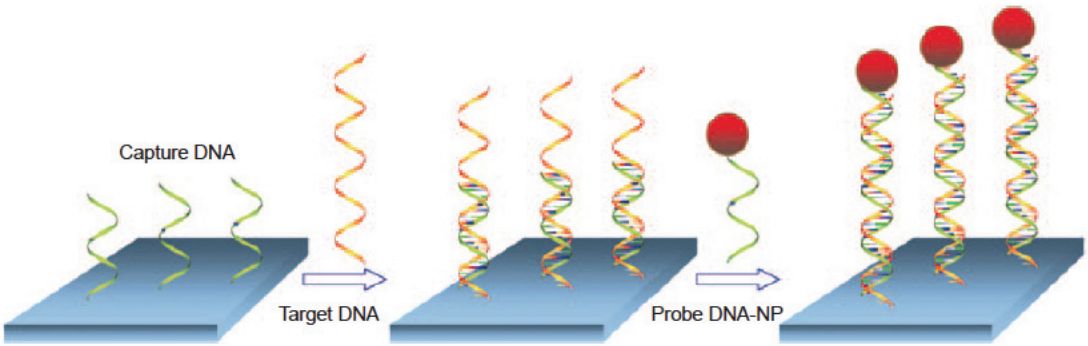

Figure 21: schematic representation of DNA detection by bioconjugated silica NPs. Known DNA are fixed on solid support and onto the surface of silica NPs, which can be detected by an imaging technique such as fluorescence. Target DNA will hybrid with capture DNA and probe DNA-NPs will hybrid with target DNA to show their position[125].

8.2. Proteins immobilization

The interaction between proteins and silica surfaces is being extensively studied for the nanotechnology, biomaterials and biotechnological applications. Proteins and enzymes adsorption onto the nanoparticles surface is an important step for these applications, however, the glutaraldehyde method is commonly used to prepare the nanoparticles surface for interaction with proteins[127]. The interaction nature between proteins and nanoparticles changes according to the aim of nanoparticles and their application. Some applications require reversible interaction (adsorption/desorption) such as chromatography[128], proteins purification[20] and proteins controlled release[22]. Others need permanent interaction like enzymes immobilization[101] for biosensors and bioanalysis. Cavaliere-Jaricot et al.[129] reported glucose oxidase (GOx) immobilized onto silica-coated quantum dots for electrochemical and indirect photoluminescence (PL) analysis of glucose. Silica shell was functionalized by amine groups, which acted as conjugation points for GOx and QDs core used to study the PL of glucose oxidation products. The nanoparticles were fixed onto a gold electrode and the oxidation of glucose onto the modified electrode was proved in the range of 2 to 25 mM glucose. The idea of PL study depends on the quenching of PL by hydrogen peroxide

produced during glucose oxidation but the sensitivity range of PL was from 0.5 to 3 mM glucose.

8.3. Bioimaging and cell tracer

Diagnosis by bioimaging techniques like MRI and fluorescence is widely used in recent years especially for cancer and cells detection[130]. MRI technique normally uses contrast agents such as iron oxide nanoparticles[131], whereas, fluorescence techniques use fluorophores[132]. Nanoparticles role in bioimaging is to provide the solid support for the contrast agents and fluorophores, since in vivo these agents should be protected from the immune system and don't show any toxicity. Silica shell offers total protection of encapsulated materials and adds the tolerance and the biocompatibility in favor of the biological systems. Photostability was remarkably observed when quantum dots and fluorescent nanoparticles were coated by silica shell[62,133], in addition, all silica coated nanoparticles showed more acceptance and longer residence in vivo than their non coated composites. Park et al[6] prepared magnetic nanoparticles doped in silica, which it is incorporated with rhodamine B isothiocyanate, MNPs@SiO_2 (RITIC). Obtained nanoparticles were biocompatible and suitable for both magnetic resonance and optical imaging and for labeling human mesenchymal stem cells (MSCs). Silica coated quantum dots nanoparticles were used to evaluate the uptake and the interaction with cells of other contrast agents like perfluorocarbons (PFCs), which have unique properties, including their low solubility in water, fats and lipids, chemical and biological stability and oxygen carrying capacity,[134] figure 22. This method can be used for screening of PFCs compounds to evaluate their uses and their targets such as macrophages and tumor cells.

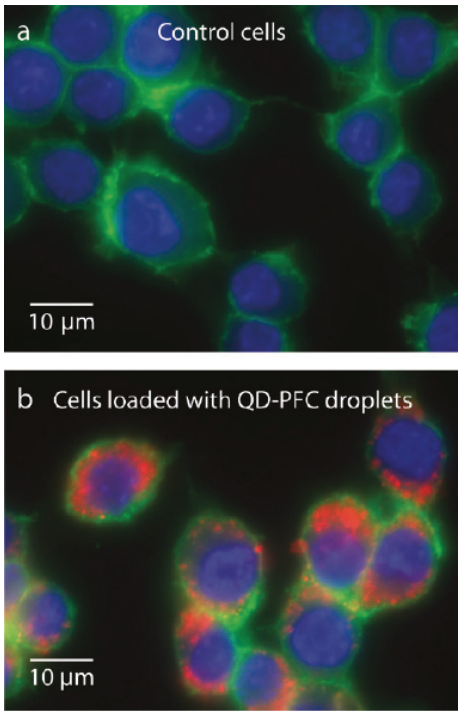

Figure 22: fluorescence microscopy images of stained cells; membrane green and nucleus blue. (a) Control cell and (b) incubated cells with fluorinated silica-coated quantum dots (orange)[134].

8.4. Cancer therapy

Novel technologies are developed recently for cancer treatment based on phototherapy[135] and hyperthermia therapy[136]. They are depending on the uptake of nanoparticles, usually made of metals, by cancer cells and enhancing these nanoparticles by an external energy source to kill the tumor cells. Tada et al.[137] reported a work on preparation of silica coated magnetic nanoparticles containing methylene blue, potential photodynamic therapy drug, into the silica shell. Magnetic core and drug were totally protected by the silica matrix and tumor cells targeting was easy and specific due to the facile functionalization of silica surface. The final result of such therapy techniques is tumor cells death via an apoptotic mechanism provoked by the enhanced nanoparticles with an external energy source. In another work silica coated manganese oxide nanoparticles toxicity was tested on the HeLa cells, which survived and a large rate were not affected, moreover, cells also survived when they were exposed to an electromagnetic field without the nanoparticles. But the combination

of both, nanoparticles and electromagnetic filed, with HeLa tumor cells killed them via an apoptosis process[138], figure 23.

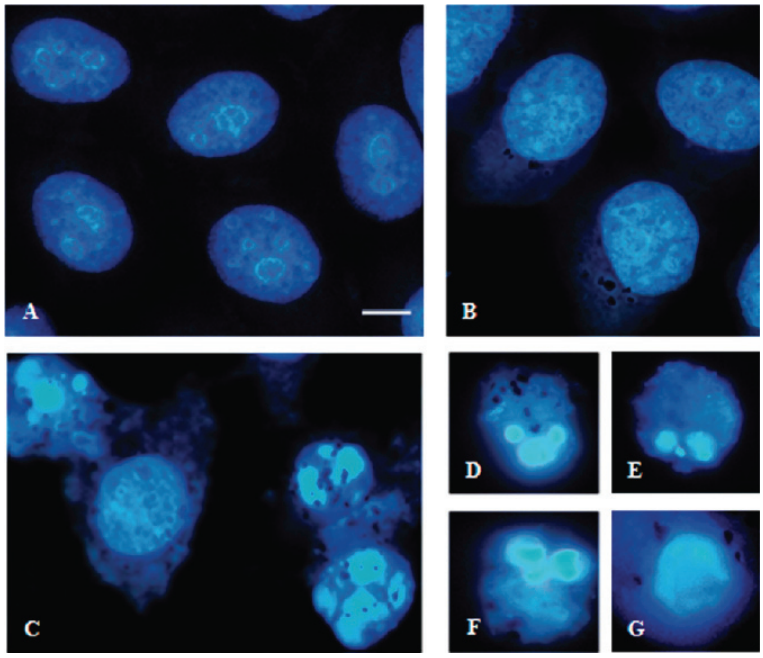

Figure 23: HeLa cells stained with Hoechst-33258: (A) HeLa cells, (B) HeLa cells + silica-coated manganese nanoparticles and (C-G) morphology deformation and apoptotic HeLa cells provoked by the nanoparticles and electromagnetic field[138].

Matrin-Saavedra et al.[139] prepared mesoporous silica coated magnetic microspheres (MMS) for hyperthermia therapy. The particles biocompatibility was investigated on three human cell types; A549, Saos-2 and HepG2, cells were treated with MMS for 24 h. An efficient uptake of MMS in all the tested cell types was observed and MMS showed high biocompatibility whereas the cells had a viability index over 0,7 when the cells were exposed of doses up to 4 $mg/ml^{-1}$for 3 days. Low frequency alternating magnetic field (AFM) was used to induce the magnetic hyperthermia and to test the ability of MMS to enhance thermal stress and cell death, M1 cells were exposed to AFM for 45 min. in the presence or absence of MMS. Medium temperature, in presence of AFM and MMS, rose to 50°C after 25 min. and stay stable to the exposure end, consequently, the cells viability was greatly decreased and release of LDH into the culture medium was observed.

9. Conclusion

Actually, nanoparticles are widely applied in all research fields and constantly more and more improvements are needed to meet the drastic increase in application number that use nanoparticles. Silica coating is one of the most important methods used to modify and improve the nanoparticles surface. Herein, the principal coating methods were cited in addition to numerous kinds of inorganic and organic nanoparticles coated by silica shell were discussed. Moreover, the powerful techniques used to characterize and study the properties of silica-coated nanoparticles were briefly explained. Finally, some biomedical applications based on silica-coated nanoparticles such as imaging, DNA detection and separation, proteins immobilization and cancer therapy were discussed.

The large number of nanoparticles could be encapsulated by silica and the variety of applications related to the properties of based core and silica shell we can understand the importance and the utility of silica encapsulation.

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Résumé

Les nanoparticules basées sur la silice pour des applications biomédicales

Les nanoparticules à écorce de silice sont largement utilisées dans le domaine biomédical car elles sont biocompatibles et leur surface biocompatible peut être bioactive. Elles ont été utilisées dans un grand nombre de domaines et des applications multiples ;

1 – extraire, purifier et détecter l'ADN pour la préparation d'échantillon ;

La surface de la silice est reconnue capable d'adsorber et désorber les molécules d'ADN en fonction de pH et de la salinité de la solution. Ces interactions contrôlables entre la silice et l'ADN ont permis de concevoir des méthodes d'extraction basées sur des particules de silice magnétiques.

2 – adsorber et immobiliser de protéines ;

Les particules de silice ont été aussi utilisées pour séparer des protéines et également pour l'immobilisation, des enzymes et des anticorps soit directement, soit après une immobilisation spécifique dans le but de développer des méthodes de détection et d'analyse enzymatique.

3 – Libération de molécules actives ;

Les particules de silice poreuses ou mésoporeuses peuvent être élaborées avec une porosité relativement contrôlée développant ainsi une grande surface spécifique capable de contenir des molécules actives principalement hydrophiles. Il est également possible de charger ces particules avec des molécules actives hydrophobes, mais pour ce faire il est nécessaire d'induire une modification de la surface interne des pores. Ces particules poreuses chargées d'actifs peuvent ainsi être utiliser la libération et le ciblage.

4 – Agent de contraste pour l'imagerie biomédicale ;

Pour améliorer la biocompatibilité et la stabilité chimique des particules magnétique et apporter une fonctionnalité de surface, l'encapsulation par une couche de silice des particules magnétiques (l'oxyde de fer) est incontournable. Les particules résultantes de ce procédé ont été utilisées comme des agents de contraste pour le diagnostic in vivo après un greffage chimique de molécules de reconnaissances pour un ciblage spécifique.

5 – Améliorer les propriétés mécaniques de produits pharmaceutiques.

Les particules de silice sont également utilisées entant qu'aditif ou excipient pour apporter une nouvelle fonctionnalité ou tout simplement pour améliorer les propriétés mécaniques d'un médicament. En effet, suite à sa qualité d'absorber l'eau la silice a été utilisée comme un expient pour améliorer les caractéristiques physiques de poudres et comprimés pharmaceutiques.

Silica-Based Nanoparticles For Biomedical Applications

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Reviews • POST SCREEN

Silica-based nanoparticles for biomedical applications

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In this short review we highlight novel uses of silica-based nanoparticles (NPs) in the biomedical sector. Silica NPs are widely used in nanotechnology because they are easy to prepare and inexpensive to produce. Their specific surface characteristics, porosity and capacity for functionalization make them good tools for biomolecule detection and separation, providing solid media for drug delivery systems and acting as contrast agent protectors. In addition, they are used as safety and biocompatible pharmaceutical additives. Here, we focus on novel techniques based on silica NPs for the most important biomedical applications.

Introduction

Considerable efforts are now being devoted to the design and fabrication of synthetic nanoscale biomaterial structures capable of functioning at molecular level in accordance to the combined rules of biology, chemistry and physics. Generally, nanoscale materials are defined as solid colloidal particles that include both nanospheres and nanocapsules. Because of their unique nano size-dependent characteristics, these materials are starting to emerge as undoubtedly the most interesting materials to shape the future of different technologies, and they will have a profound influence on almost every aspect of our lives. To meet such high expectations, researchers are trying to develop and employ a variety of nanomaterials, such as semiconductor quantum dots, carbon nanotubes, plasmonic nanoparticles (NPs), magnetic NPs and silica nanoparticles (SiNPs). In comparison to other NPs, SiNPs may appear mundane at first sight. However, from the practical viewpoint, this does not appear to be the case. In nanotechnologies, silica-based NPs have a dominant role because of their fundamental characteristics, such as size (generally from 5 to 1000 nm), unique optical properties, high specific surface area, low density, adsorption capacity, capacity for encapsulation, biocompatibility and low toxicity [1]. These features lead to SiNPs being widely utilized as an inert solid supporting or entrapping matrix [2].

Consequently, intensive research has been performed to use SiNPs in diverse biomedical applications for diagnosing and controlling diseases, identifying and correcting genetic disorders and, most importantly, increasing longevity. Thus SiNPs offer considerable advantages and have opened new avenues of biomedical research in numerous leading edge applications, such as biosensors [3], enzyme supporters [4], controlled drug release and delivery [5] and cellular uptake [6].

In view of the significance of silica-based nanomaterials for biomedical applications, as highlighted above, we review here some of the most specific milestones in ongoing research. We do not consider the environmental aspects of silica NPs. However, we highlight new widespread applications of silica-based nanomaterials in protein adsorption and separation, nucleic acid detection and purification, drug and gene delivery, imaging and pharmaceuticals.

Protein adsorption and separation

Due to their ease and speed of preparation, low cost, high specific surface area and numerous surface functionalization possibilities, SiNPs provide promising tools for specific protein adsorption and separation. The interaction between SiNPs and proteins has been

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studied extensively in the past. For example, studies have focused on conformational changes [7], the influence of SiNP size on enzyme activity [8,9] and the orientation of protein adsorption on SiNPs [10].

Ester-functionalized polypyrrole-SiNPs [11] can be bound covalently with human serum albumin (HSA) protein, and immediate flocculation is observed after the incubation of HSA functionalized NPs with anti-HSA. This suggests that we can use these NPs for visual diagnostic assays and designing biosensors. Taking another direction, Slowing *et al.* [12] succeeded in preparing mesoporous silica nanoparticles (MSNs) to transport cytochrome *c* through cell membranes. Figure 1 shows a presentation of cytochrome *c* intracellular transportation and release by MSNs. Cytochrome *c* was loaded in MSNs, which crossed the cell membrane and then released their contents in the cytoplasm. In addition, the cytochrome *c* released was subjected to an activity test, which proved that the cytochrome *c* released by MSNs can serve as an active enzyme in aqueous solution. Human cervical cancer cells (HeLa) have been selected for the intracellular delivery and release of cytochrome *c* into the cytoplasm.

Kim *et al.* [13] prepared dual mode silica-based NPs for specific binding to his-tagged proteins, isolation/purification, and site-specific protein labeling with multiple fluorophore species. Figure 2 presents the preparation and the function modes of nitrilotriacetic

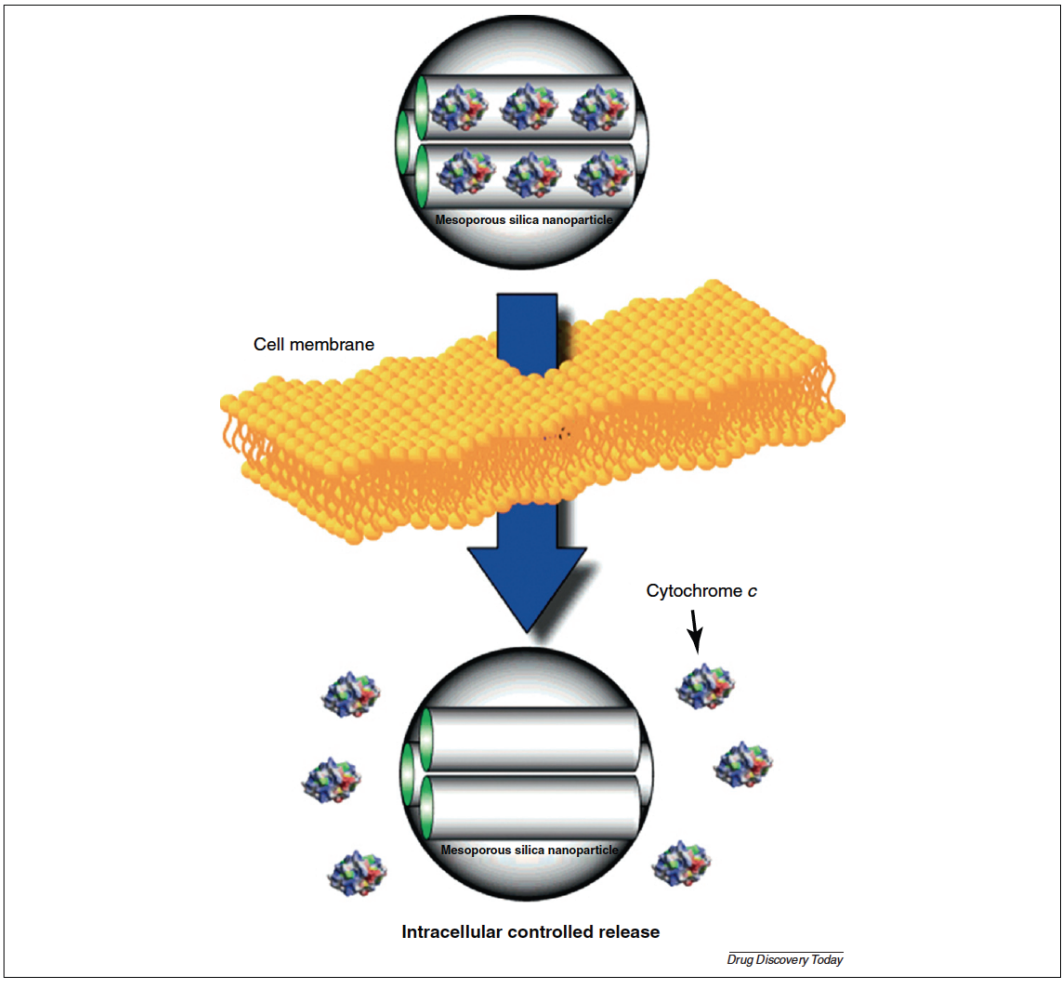

FIGURE 1

Cytochrome *c* transporting into the cytoplasm using MSNs (particle size is 265–933 nm and pores approximately 5.4 nm). Cytochrome *c* is loaded outside the cell membrane and released, under physiological conditions, into the intracellular compartment [12]. *Abbreviation:* MSNs: mesoporous silica nanoparticles.

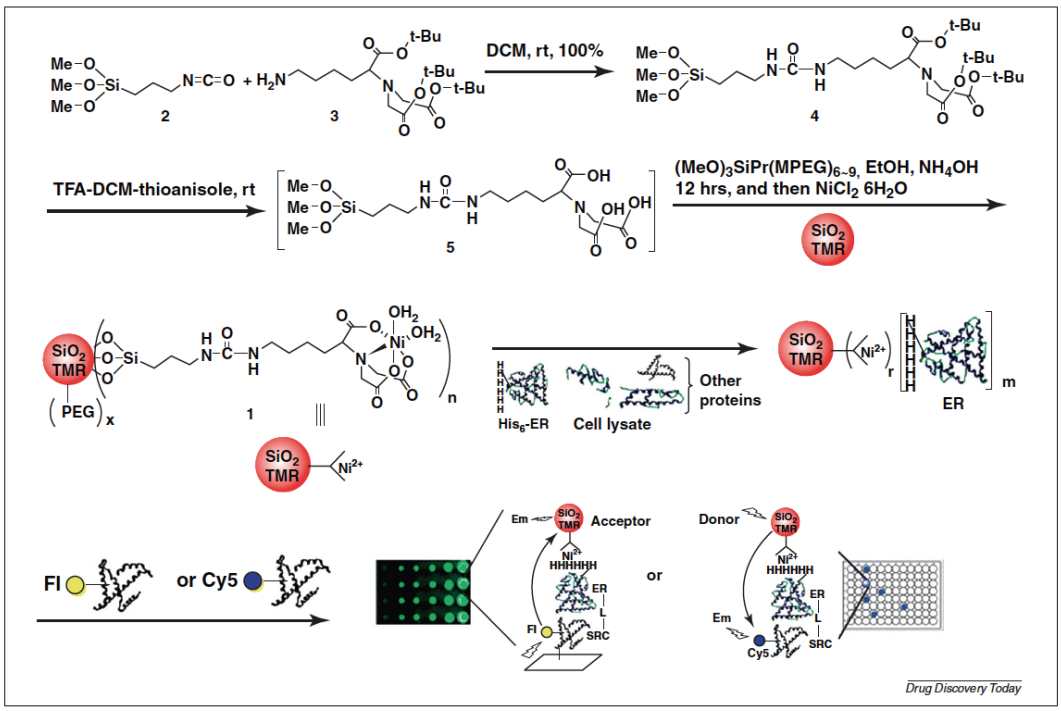

FIGURE 2

Dual-mode functional preparation of modified silica nanoparticles, 23 nm, to isolate his-tagged proteins and tagging with multiple fluorophore. Silica nanoparticles surface is modified by nickel ions Ni^{2+} for specific interaction with 6x his-tagged proteins [13]. *Abbreviation*: his: histidine.

acid (NTA)-modified dye-embedded SiNPs. The SiNPs obtained showed high specific interaction with his-tagged proteins, and approximately 30 protein units were captured per particle. A similar work using NTA-polyethylene glycol (PEG)-modified SiNPs was published [14]. His_6-GFP and his_6-biotin were specifically immobilized on prepared SiNPs. In addition, orientation, areal density and distance between immobilized proteins and solid substrate are controllable, auguring well for highly specific biosensors.

New techniques have been used to design SiNPs as solid media for protein immobilization. Shiomi *et al.* [15] covalently immobilized the hemoglobin (Hb) on SiNPs and a second layer of silica was created on the surface. Finally, after removing the Hb template, SiNPs functionalized for Hb recognition were obtained. Depending on the type of vinyl-modified SiNP, He *et al.* [16] were able to copolymerize functional and cross-linking monomers on the surface of protein imprinted NPs.

Furthermore, silica-coated iron oxide NPs have attracted increasing attention as new tools for protein binding and separation [17–19], due to their magnetic properties that provide an easy and fast method for NP separation. The combination of two materials makes it possible to enhance the properties of final products. In the case of silica coated iron oxide NPs, they have a magnetic core that can be used for fast particle separation by an external magnetic field, and a silica shell that provides greater colloidal stability, biocompatibility and a platform for fulfilling a wide range of functions. Yang *et al.* [20] reported silica-coated magnetite NPs using the reverse microemulsion technique [21]. Magnetite-containing spherical NPs were used for bioseparation, and horseradish peroxidase (HRP) was used as a protein. HRP was entrapped in the silica pores with entrapment efficiency in the range of 85–90%, thereby conserving its peroxidatic activity. In addition, it was demonstrated that the protein HRP resisted leaching from NPs for a period of more than 60 days. These biomolecular nanostructures entrapped in silica-coated magnetite NPs have been suggested for various uses such as enzyme immobilization, immunoassays, biosensors and bioseparation.

His-rich proteins were also targeted by silica-coated iron oxide NPs, and nitrilotriacetic acid/Co^{2+}-linked, silica/boron-coated magnetite NPs [22] were prepared for specific interactions with 6x His-tagged proteins. The modified NPs were approximately 200 nm in size and interacted with two protein models: C-terminal 6x his-tag and an internal 6x his-tag. Another preparation depended on the adsorption of zinc NPs on silica-coated magnetic NPs [23]. Bovine serum albumin (BSA) was used as a His-rich protein that presented reversible adsorption on zinc/silica-coated magnetic NPs. Thus these structures can be used as purification

tools for this kind of protein. Nowadays, SiNPs are becoming more specific regarding their targets. Specific detection and quantification of lysozyme by aptamer-functionalized SiNPs [24] has been achieved, with the concentration of lysozyme detected in the range of 0–22.5 μM.

Nucleic acid detection and purification

Significant information can be obtained from DNA molecules used for diagnostics, genetic investigations and therapy. DNA extraction and purification is an important step of DNA manipulation. Thus the latter has become the foundation of molecular biology, genetic therapy and genetics. The new techniques developed have increased the competence, capacity and facility for DNA molecule separation and purification.

DNA adsorption onto silica surface

In line with this direction, nanotechnology has started to emerge as one of the most important techniques applied to the field of genetics. The numerous options for preparing NPs with multifunctionalized surfaces have enhanced biomedical research through the employment of silica-based NPs for DNA detection, separation and purification. The adsorption of DNA onto the surface of SiNPs is generally controlled by three effects: weak electrostatic repulsion forces, dehydration and hydrogen bond formation [25]. For example, the interaction of DNA with silica surfaces through hydrogen bonds was studied by Raman and Fourier transform infrared (FTIR) spectroscopy [26]. Through understanding the nature of such interactions, researchers have been able to develop silica surfaces for more specific and efficient interactions. Kneuer *et al.* [27] synthesized amino-modified SiNPs, based on the modification of SiNPs with N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AEAPS) or N-(6-aminohexyl)-3-aminopropyltrimethoxysilane (AHAPS). The average size of particles was from 10 to 100 nm with a surface charge potential from +7 to +31 mV at pH 7.4. Plasmid DNA was also used to study the interaction with prepared SiNPs to form a complex protected from degradation by DNase I. Interestingly, unlike free plasmid DNA, which is totally degraded by DNase I, the addition of ten parts of SiNPs almost totally protects the plasmid DNA, with only a small fraction of supercoiled DNA being transformed into nicked circular DNA. Furthermore, although 30 parts of SiNPs completely protects the DNA, it is difficult to separate the latter at this ratio.

SiNP uses for specific DNA detection

SiNPs are also employed to design DNA biosensors through their functionalization with oligonucleotides by hybridization with target complementary DNA or RNA probes to attain variable fluorescent intensity. Hilliard *et al.* [28] reported the immobilization of oligonucleotides onto SiNPs using disulfide-coupling chemistry. To do this, 60 nm silica particles were obtained and silanized with 3-mercaptopropyltrimethoxysilane (MPTS), then oligonucleotide probes were immobilized onto the SiNPs, which were incubated for DNA hybridization. The fluorescent signal was observed at an emission wavelength of 520 nm to evaluate the efficiency of hybridization. In another work, Zhou *et al.* [29] developed new core shell nanostructures based on silica/dye-coated gold NPs, and oligonucleotide signaling probes were also immobilized. These dye-doped silica-coated gold NPs were used for labeling in microarray-based detection. Increased sensitivity with photostable signals was obtained.

In another interesting work, electrochemiluminescence DNA detection electrodes were developed based on SiNPs and gold [30] and platinum [31]. Employing SiNPs in this technology has almost tripled detection sensitivity and has also increased selectivity. 1×10^{-13} mol l^{-1} of the target DNA was detected using a $Ru(bpy)_3^{2+}$-doped silica NP DNA probe on a platinum electrode [31], while a detection limit of 1×10^{-12} mol l^{-1} was achieved with $Ru(bpy)_3^{2+}$-doped gold NPs on a gold electrode [32].

SiNPs also have an important role in building and developing a fast, cost-effective and robust isolation method for DNA extraction, purification and analysis. Nguyen *et al.* [33] studied the kinetics and conformational changes of plasmid DNA adsorption on silica in monovalent and divalent salts. They found that two kinds of electrostatic interactions should be considered: interaction between plasmid DNA and the silica surface, and interaction between subunits of the plasmid DNA that control molecule conformation. In a monovalent salt solution with Na^+ ions having low ionic strength, plasmid DNA takes a non-compact form and intramolecular electrostatic repulsion is effective. On the contrary, when increasing ionic strength, the plasmid DNA molecule takes a more compact form, which means that intramolecular electrostatic repulsion is not effective. In a divalent salt with Ca^{2+} ions that form complexes with the oxygen atoms of the phosphate groups of double-strand oligonucleotides [34,35], this interaction significantly reduces the intramolecular electrostatic repulsion of the DNA molecule, thus it adopts a highly compact form. Consequently, the attachment coefficient observed in the presence of 300 mM NaCl is 0.01–0.03, and adsorption to silica is reversible. By contrast, in the presence of 1 mM Ca^{2+} the attachment coefficient is 0.75–0.87 and adsorption is fast and irreversible.

DNA extraction by silica-coated magnetic NPs

Silica-coated magnetic NPs are commonly used to extract DNA from biological samples. Due to their fast and easy separation, magnetic NPs have become preferential tools for biomedical applications. The magnetic core of such structures endows them with separation properties while the functional shell is charged to interact with the nucleic acid molecules. Mesoporous silica and/or magnetic particles were prepared as adsorbents of DNA molecules [36], but without a functional surface these particles were not highly specific and DNA load depends on pore size. Nonporous silica-coated iron oxide NPs were prepared to isolate plasmid DNA from a bacterial cell lysate [37] and genomic DNA from plant cells [38]. This depends on the adsorption of plasmid DNA on the silica surface under a high salt concentration. Furthermore, and for more specific interaction between DNA and silica, functional groups were created onto the particle surface. Amino-functionalization is one of the applications used in this field. Kang *et al.* [39] reported the synthesis of amino-functionalized silica-coated magnetic NPs with an average particle size of 25 nm. The adsorption efficiency of these particles was four to five times higher compared to silica-coated magnetic NPs in the presence of 0.7 M NaCl. Through such efforts, human genomic DNA was successfully separated, using amino-functionalized silica-coated magnetic NPs, from saliva and blood with high efficiency and specificity.

Drugs and gene delivery

SiNPs used as carrier systems for drugs and genes have two different forms, mesoporous NPs and surface functionalized NPs.

Mesoporous SiNPs for drug delivery applications

Mesoporous SiNPs are used as carrier systems for drug delivery. The uptake and release mechanism is dependent on the drug being kept in the pores of NPs, by using additional molecules, for example, gold NPs [40], as caps to close the pores. This concept is called gatekeeping [41]. Lai *et al.* [42] reported the synthesis of mesoporous SiNPs with chemically removable CdS NP caps as carrier systems for drug delivery. Figure 3 illustrates the uptake and release mechanism, CdS NPs have the role of caps that keep the drug in the pores. Covalent interaction between the CdS NPs and the pore surface takes place after loading the drug inside the pores to ensure it stays inside them. To release the drug, a disulfide bond-reducing molecule, such as dithiothreitol (DTT) or mercaptoethanol (ME), is needed to break the covalent bond and open the pores. These kinds of systems are highly efficient for drug delivery, because they have efficient stimuli-controlled release, no additional modifications of the drug needed for loading in the pores and, what is more, they allow the transfer of a large number of drugs.

Functionalized silica nanoparticles for gene delivery applications

Functionalized silica nanoparticles (FSN) should have two different states in two different conditions for use for gene delivery. The first condition is the gene loading, which requires good affinity between the genes and NPs. In this condition, the uptake and storage of the gene molecules by the NPs and the interactive nature between them depends on electrostatic interaction. DNA molecules are negatively charged, thus researchers try to prepare positively charged NPs, such as amino groups [27]. The second condition is gene release. In this case weak interactions are preferable between the gene and FSN to assist the release of the genes from the NPs into the target.

Bharali *et al.* [43] reported *in vivo* applications of amino-surface functionalized SiNPs in gene delivery. SiNPs, plasmid DNA-binding SiNPs and free plasmid were injected into mouse brains. However, only plasmid DNA-binding SiNPs produced robust gene expression, which suggests that the gene was very well protected and transferred into the cell nuclei.

Imaging

SiNPs are used in medical imaging and its applications as contrast agents have an important auxiliary role. These are used to encapsulate contrast agent particles, such as gold [44], silver [45], iron oxide [46], organic dyes [47] and quantum dots [48]. SiNPs are generally used and applied in medical imaging because of characteristics such as higher biocompatibility, controllable size and size distribution, contrast agent protection and a large number of possible surface functions. Ow *et al.* [49] prepared highly fluorescent silica-coated fluorophore NPs with sizes ranging from 20 to 30 nm. The NPs prepared were photostable and 20 times brighter than their constituent fluorophores. The outer silica shell provides an additional choice for targeting specific cells or tissues through silica surface functionalization. Bakalova *et al.* [50] reported the intracellular localization of amino functionalized silica-shelled quantum dot micelles. These NPs demonstrated good intracellular delivery due to their small size and highly positively charged surface. In addition, due to their low cytotoxicity, NPs can be used as multifunctional tools such as contrast agents and drug/gene/protein delivery systems. Iron oxide-doped SiNPs are used for MRI cell labeling [51] and grafting has been performed on silica-coated dye NPS for constructing dual imaging probes to perform both fluorescence and MRI functions [52]. Multi-constituent nanostructures are widely used in imaging techniques, thus demonstrating the potential of multifunctional particles.

The technique of a core and multilayer shell was applied to synthesize gold coated and/or silica-coated iron oxide NPs [53] as bifunctional NPs suitable for both MRI and photothermal therapy. Lee *et al.* [54] applied this technique and reported mesoporous silica-coated dye NPs associated with iron oxide NPs (Fe_3O_4-MSN), with iron oxide as a contrast agent for MRI, the mesoporous silica shell for drug delivery and dye NPs for fluorescence imaging. These trifunctional NPs were loaded by an anticancer drug, doxorubicin (DOX), and tested in two cases: (i) *in vitro*, on B16-F10 cells, cellular uptake of NPs was confirmed by both MRI and fluorescence imaging; (ii) *in vivo*, in this case mice were used to test the capability of Fe_3O_4-MSN to accumulate at the tumor site and deliver a drug. Consequently, Fe_3O_4-MSN NPs were accumulated at the tumor site, which was verified by MRI and orange RITC fluorescence. In addition, antitumor activity at the tumor site was also observed, confirming the drug delivery function of the NPs investigated.

Pharmaceutical applications

Colloidal anhydrous silicon dioxide is generally regarded as an essentially nontoxic and nonirritant excipient in oral and topical pharmaceutical products. However, intraperitoneal and subcutaneous injections may produce local tissue reactions and/or granulomas, meaning that it cannot be administered parenterally, because silica excipients are used for several functionalities, for example, as adsorbents, anticaking agents, emulsion stabilizers, glidants, suspending agents, tablet segregates, thermal stabilizers, and viscosity increasing agents, with a percent ratio varying from 0.1 to 10 [55].

Silica has a high surface area covered with polar silanol groups, which is favorable for water adsorption. Gore and Banker [56] used these properties of silica to stabilize aspirin tablets. They studied the moisture adsorption properties of silica and its stabilizing effect for a model aspirin tablet. The experimental results demonstrated the water adsorption characteristics of silica and their dependence on surface area and pore size. In addition, 3% silica was considered as an optimum concentration for maximum stabilization. In another work, silica was added to magnesium stearate [57]. It was noticed that tablet strength was improved but it was found to induce negative effects on lubrication and did not improve disintegration. The effect of adding silica as an excipient on the bioavailability of the drug was investigated, taking amoxicillin as an example [58]. SiNPs are widely used in pharmaceutical applications as glidants. Jonat *et al.* [59] investigated the use of compacted hydrophilic and hydrophobic SiNPs as pharmaceutical excipients (glidants). They found that hydrophobic silica is the most efficient glidant as it only requires gentle mixing conditions to achieve high flowability. By contrast, hydrophilic silica strongly depends on mixing conditions. However, at low glidant

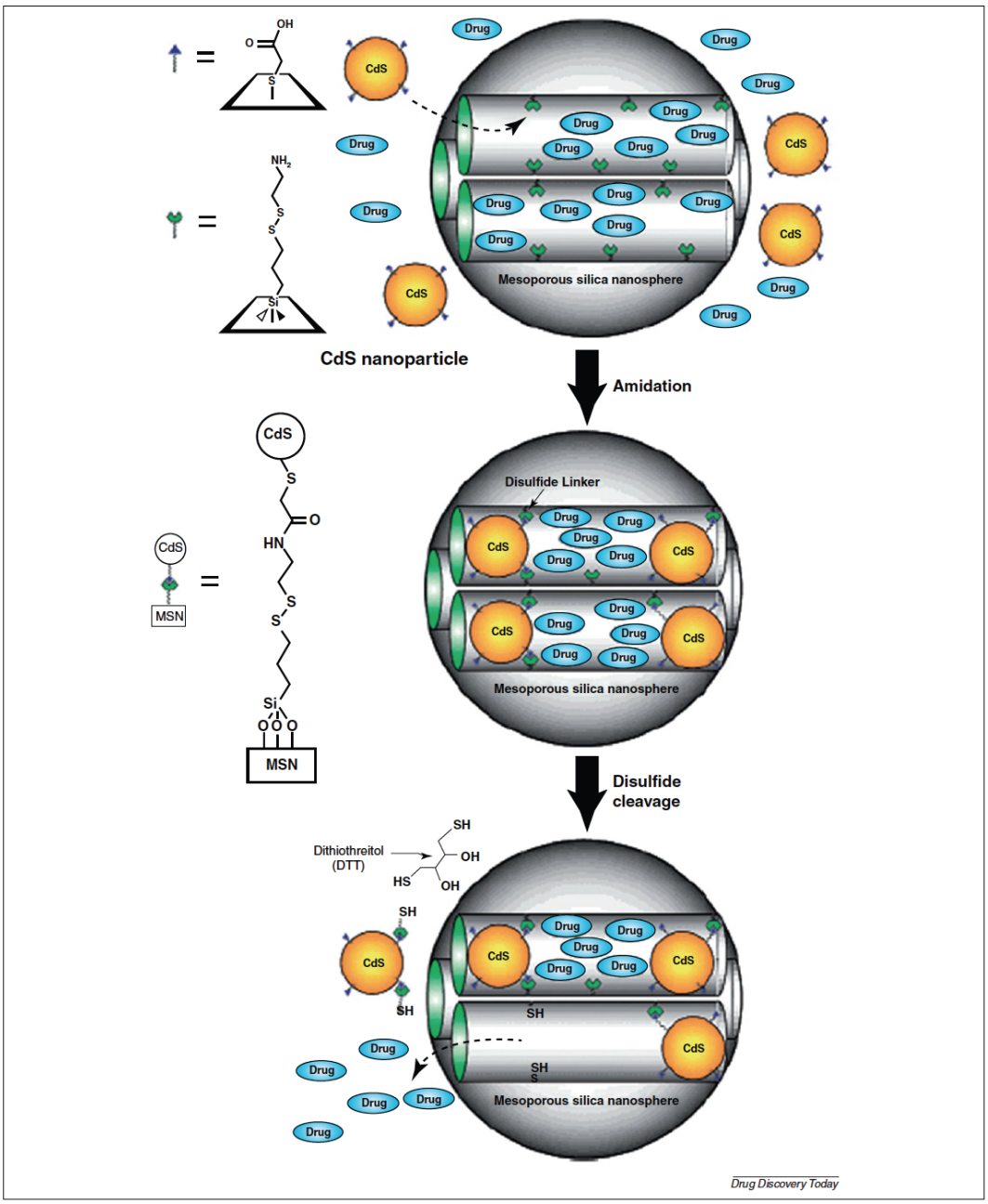

FIGURE 3

CdS nanoparticle-capped MSN-based drug delivery system. CdS (2 nm) link to MSN (200 nm) by a disulfide bond that can be chemically reduced to release the drug from MSN pores (2.3 nm) [42].

concentrations, all the silica tested maintained good flowability, even after equilibrating at high humidity levels.

Concluding remarks

SiNPs in biomedical nanotechnology have an ongoing role for designing advanced tools and systems for *in vitro* and *in vivo* applications. Interaction between silica surfaces and proteins is applied for specific protein detection and reverse interaction is used to separate proteins from biologic media. Functionalized silica NPs have successfully targeted proteins with high specificity thanks to the many options for functionalization provided by silica surfaces.

In addition, SiNPs have been widely applied in gene detection and purification, due to their specific ability to interact with nucleic acids. Using SiNPs in this field provides many advantages, such as reducing the use of organic solvents, rapid processes and low cost. Nowadays, DNA detection and extraction are useful in diagnosis and gene therapy and this should lead to new and fast techniques. SiNPs can be considered as revolutionary tools for nucleic acid treatments.

Furthermore, SiNPs used as auxiliary materials are also important. Silica shells are highly efficient for protecting imaging agents and increase their efficacy. Further uses are based on SiNPs in pharmaceutical production, in which they are used as additives to improve the mechanical properties of powders. In conclusion, it is important to highlight that due to their unique characteristics silica-based NPs are among the most important materials for understanding and promoting biomedical applications capable of having a significant impact on the health care of the future.

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III. CHAPITRE 2

ETUDES EXPERIMENTALES

Introduction:

Les études expérimentales réalisées dans le cadre de ce travail de recherche ont été scindées en deux grandes parties : (i) la préparation des émulsions magnétiques et leur encapsulation par la silice ; et (ii) l'extraction de l'ADN par adsorption sur les particules de silice magnétique élaborées.

La préparation des émulsions magnétiques commence par la préparation des ferrofluides aqueux puis organiques via la coprecipitation de $FeCl_2$ et $FeCl_3$ dans une solution aqueuse de l'hydroxyde d'ammonium. Les ferrofluides obtenus ont été émulsionnés dans une solution concentrée de SDS. L'encapsulation par la silice a été réalisée sur les gouttelettes de ferrofluides d'oxyde de fer par la technique de sol-gel en utilisant le TEOS comme un précurseur de la silice. Durant les trois étapes de la synthèse, les nanoparticules d'oxyde de fer, les émulsions magnétiques et les émulsions magnétiques encapsulées par la silice ont été caractérisées et leurs propriétés physico-chimiques ont été examinées.

La seconde étape de cette étude est relative aux interactions entre les acides nucléiques (ADN) et les particules de silice magnétique en fonction des paramètres physico-chimiques. La surface des particules de silice est couverte de groupements hydroxyle (–OH) ce qui confer à ce type de particules une charge négative dans un large domaine du pH (de 2 à 14). L'ADN est un poly-électrolyte chargé négativement suite à la présence des groupements phospho-di-ester ce qui induit des interactions répulsives en présence de la silice. Mais, en ajoutant un sel (NaCl par exemple), on peut modifier les conditions d'interaction en réduisant la porté de ces interactions via la diminution de dégrée d'hydratation des acides nucléique. Il est ainsi possible de contrôler ces interactions en changeant le pH et la salinité du milieu d'adsorption. Pour étudier l'efficacité de nos particules à adsorber les acides nucléiques, une étude systématique a été conduite en fonction de la salinité, le pH, temps d'incubation (cinétique d'adsorption, concentration en ADN etc. La désorption de l'ADN préalablement adsorbé a été réalisée et les résultats obtenus ont montré que ces particules peuvent être utilisées comme outil d'extraction et de purification rapides des acides nucléiques. Suite au caractère magnétique de ces particules de silice, la filtration et la centrifugation sont remplacées par une simple séparation magnétique en utilisant un champ magnétique induit par un aimant permanant. Un examen rapide d'extraction des acides à partir d'un lysat bactérien a été réalisé et les résultats montrent une bonne capacité de capture suite à une visualisation sur un gel d'acrylamide. Ces particules composites peuvent donc être utilisée dans les microsystèmes basés sur la microfluidique.

Résumé

Dans cette étude, la formulation des émulsions magnétiques encapsulées par une écorce de silice a été réalisée en trois étapes. Tout d'abord, un ferrofluide aqueux a été préparé et transformé par la suite en ferrofluide organique après adsorption d'acide oléique.

Le procédé utilisé pour la préparation de ferrofluide est basé sur la coprécipitation des sels ferreux et ferriques en milieu ammoniacal. Les ferrofluides synthétisés sont caractérisé avant toute utilisation dans la formulation d'émulsion magnétique. La formulation d'une émulsion magnétique submicronique en taille et présente une bonne teneur en oxyde de fer pour une séparation rapide a été réalisée er reproduite. Après une étude de caractérisation complète de l'émulsion magnétique formulée, l'encapsulation de cette dernière a été examinée en fonction de la quantité de TEOS, la nature d'agent stabilisant et également en fonction de la quantité d'émulsion semence utilisée. Les particules composites préparées au cours de ces étapes ont été caractérisées en termes de morphologie, de composition chimique et propriétés magnétiques. La tendance des résultats confirme la formation d'une écorce de silice homogène autour d'un cœur superparamagnétique. La taille des particules composites synthétisées doit est ajustée pour faciliter la séparation sous l'action d'un champ magnétique. En effet, plus la taille est petite (inferieure à 100 nm) plus la diffusion Brownien l'emporte sur la force induite par un aimant externe. Il est également indispensable d'avoir une surface des particules chargée afin d'éviter une agrégation possible imposées par la force magnétique qui force les particules à s'aligner tangentiellement aux lignes du champ.

Les particules finales obtenues sont parfaitement stables et présentent une morphologie cœur magnétique et écorce de silice, une séparation rapide sous l'action d'un champ magnétique. En ce qui concerne la taille et la polydispersité, elles restent et dépendent de l'émulsion formulée.

Silica-Coated Magnetic Emulsion for Biomedical Applications

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Abstract

The magnetic separation, extraction, purification, labeling and detection of biomolecules and cells are widely applied recently in molecular biology applications. Thus the synthesis of silica magnetic particles for biomedical applications is developing and these particles are becoming interesting as micro and nano structures. In this paper, the synthesis of silica-coated magnetic particles was performed through three steps. (i) An organic ferrofluid was prepared by the coprecipitation technique, and then, (ii) it is used to prepare the magnetic emulsion. (iii) The magnetic emulsion was coated by silica shell using the sol-gel process. The prepared particles during the three steps were characterized in term of morphology, chemical composition, magnetic properties, size, size distribution and electrokinetic properties. The results confirmed the formation of superparamagnetic core – silica shell particles. The particles diameters were found to be around 400nm and the particles respond rapidly to an external magnetic field.

1. Introduction

Over the last fifty years, magnetic colloids or ferrofluids[1–3] attracted special attention for wide range of applications such as magnetic recording tapes[4], magnetic inks and paints[5], sealing for stationary shaft[6] and more recently in biomedical fields[7–9]. In all these applications iron oxides particles were used under the colloid form as a separated nanoparticle, 5-20nm, which is suitable for these applications. Whereas, the magnetic-based separation techniques newly developed for biomagnetic materials extraction, request large particles for fast and efficient separation. In fact the large magnetic particles can be separated easier and faster than the small ones, but these particles provoke a fast sedimentation and their specific surface area is smaller. Thus, the particles size should have most attention during the synthesis and simultaneous balance between the separation speed and the sedimentation. The preparing of large magnetic particles (200-500nm) in a coprecipitation process is very difficult in term of time, energy and formulating. Therefore, the magnetic emulsion (ME) concept was developed to control the particles "droplets" size. This technique was first presented by Bibette[10], he

prepared oil-in-water emulsion using an organic ferrofluid as dispersed phase. Furthermore, Montagne et al[11,12] studied and characterized the magnetic emulsions in point of view of chemical composition, size, electrokinetic properties, stability and morphology. These studies show that magnetic emulsions are stable and the superparamagnetic behavior is conserved after the emulsification. Magnetic latex, which is magnetic emulsion coated by a polymer layer, was synthesized[13,14] via seed emulsion polymerization of styrene and DVB (divinylbenzene) initiated by KPS (potassium persulfate). The obtained particles have a core-shell form and later used as seed for polymerization to produce functional magnetic latex suitable for biomedical application[15,16].

Sol-Gel process[17] is an efficient method to prepare silica-coated particles, which are considered more chemically stable, core protected, biocompatible, easy to produce and suitable for further functionalization. Therefore, silica coating can substitute the polymerization process and avoid all problems faced in the polymerization cases such as the system complexity, time and energy consuming, expensive, using of toxic monomers and biocompatible polymers are very limited.

In this work, we report an organic ferrofluid synthesis by coprecipitation of $FeCl_3$ and $FeCl_2$ in basic medium, in which, the nanoparticles were coated with oleic acid and dispersed in octane. Secondly, the magnetic emulsion was prepared via the emulsification of organic ferrofluid in sodium dodecyl sulfate (SDS) solution. Finally, magnetic emulsion was coated by silica shell using sol-gel technique. Obtained particles were characterized by different techniques and special attention was paid for the characterization of core – shell morphology.

Experimental

1.1. Materials and reagents

Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$) and sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich and used without purification. NH_4OH 25% and ethanol 96% were purchased from VWR Prolabo and used as received. Polyvinylpyrrolidone (PVP) and tetraethyl orthosilicate (TEOS) were obtained from Fluka and oleic acid from Aldrich were used as received. Octane and cyclohexane were obtained from Acros Organics and acetone from Laurylab. Deionized water was used in all experiments.

1.2. Preparation of ferrofluids

Ferrofluid synthesis via the coprecipitation of iron (III) and (II) salts in aqueous solution of ammonium hydroxide is previously described[11]. Shortly, 10.16g iron (III) and 3.7g iron (II), with molar ratio $(Fe^{3+})/(Fe^{2+})=2$, were dissolved in 80ml water and poured in 250ml glass

reactor equipped with mechanic stirrer. The solution was stirred for 40min at 250rpm, then the speed was raised up to 500rpm and 80ml NH_4OH 25% were added drop wise to the reactor. The stirring continued for 2h to finish the precipitation of iron oxides nanoparticles. 8ml of oleic acid were added drop wise to the reactor and the stirring continued for 15min, and then dispersed in octane.

1.3. Preparation of magnetic emulsion

The ME was prepared via the emulsification of obtained ferrofluids, which were used as a dispersed phase in direct emulsion (O/W) and the continue phase was the SDS solution. According to the preliminary study 9.75ml SDS solution 12% was prepared and 0.25ml organic ferrofluid (Total Solid, TS=29.5%) was poured carefully onto the internal flacon wall, without mixing them together, then they were emulsified by ultrasound (Vibra-cell 75042, BIOBLOCK SCIENTIFIC): amplitude 60% for 3min. obtained emulsion was filtered through glass wool and kept on rotation agitation for one night. The same procedure was applied to scale up so 975ml SDS solution of 12% were prepared and 25ml ferrofluide were added as described above and emulsified by ultrasound: amplitude 90% for 7min. The emulsion was filtered and kept on rotation agitation for one night.

1.4. Preparation of silica-coated magnetic emulsion

Silica coating was performed by sol-gel process using TEOS as precursor and ammonium hydroxide as catalyst. Prepared ME (10ml) was separated by magnet and particles (droplets) were washed by deionized water then again separated and redispersed in 3ml PVP 10% and the emulsion was kept on rotation agitation for 3h. Then, 5ml of 25% NH_4OH were added to the emulsion and the agitation continued for 15min. 2ml TEOS 50% (in ethanol) were added to the emulsion and kept on rotation agitation for one night. Si-ME was separated by magnet and washed one time by deionized water and filtered through glass wool. For encapsulate one liter of ME the following procedure was performed: the ME was separated by a big magnet and washed with deionized water. The particles were redispersed in 300ml PVP 10% and transferred into a one-liter reactor equipped by mechanic stirrer and stirred for 3h at 150rpm. Then, 500ml NH_4OH were poured into the reactor. After 15min 200ml TEOS 50% (in ethanol) were added to the reactor, slowly and as several batches during 5min, so the stirring continued over night. Si-ME was separated and washed one time by deionized water and filtered trough glass wool and redispersed in 500ml water.

1.5. Size and zeta potential measurements

Dynamic light scattering (DLS) technique was used to measure and follow the evaluation of particles size from iron oxide nanoparticles to Si-ME, core-shell particles. Zetasizers (3000

HSa and Nano ZS, Malvern) were used to measure the particles size in highly diluted solution. One drop of particles was highly diluted, the number of kilo counts per second (Kcps) was adjusted between 100 and 120, and size was measured taking an average of 3 measurements. Zeta potential was measured as a function of pH in 1mM NaCl; 100µl of the cleaned particles dispersion (solids content 2.28%) were diluted with 25ml 1mM NaCl and the pHs were varied from 3 to 11 then zeta potential measured taking an average of 5 measurements.

1.6. Transmission electron microscopy

Transmission electron microscopy (TEM) images were obtained with a Phillips CM120 electron microscope (CMEABG, University of Claude Bernard Lyon I-France). One drop of highly diluted particles was placed onto a copper grid (mesh200 and covered with formvar-carbon) and dried at room temperature before TEM analysis. The analyses were performed with a field emission gun operating at 100 KV.

1.7. Thermogravimetric analysis (TGA)

Samples were dried at 70°C and obtained powders were analyzed using (TG 209 *F1*, NETZSCH), temperature was varied from 20 to 800°C with a heating rate of 10°C/min. The analyses were performed under a nitrogen atmosphere.

1.8. Infrared spectroscopy

Fourier transform infrared (FTIR) analyses were performed by FTIR spectrophotometer IR Prestige 21 (Shimadzu, Japan). Samples were cleaned and dried and the spectra were scanned over the range 4000-400cm^{-1} in the deflection mode.

1.9. Gas chromatography

Octane concentration in emulsions (droplet and solution) was measured by gas chromatography technique, GC–2010 Plus (Shimadzu, Japan). The octane concentration was investigated in the SDS solution so the emulsions were prepared and the particles were separated at different times (0 to 12 days). 100µl solution was mixed with 900µl of cyclohexane and incubated for one hour. The organic phase was separated and filtered then analyzed by GC, the octane concentration was calculated from standard calibration curve. The analyses conditions were: injection temperature 150°C, column temperature 100°C, hold time 6min, pressure 100KPa, total flow 53.8 ml/min, column flow 1ml/min, split ratio 50 and detector temperature 200°C.

1.10. Magnetization

Magnetic measurements were carried out using the Automatic Bench of Magnetic Measurements (ABMM) at CNRS-IRC Lyon laboratory. All particles were dried and

magnetization was investigated by varying the magnetic field (H) from -20000 to +20000 Oersted.

2. Results and Discussion

The coprecipitation synthesis method described above to produce organic ferrofluid leads to a highly stable colloid, oleic acid-coated iron oxide nanoparticles and well dispersed in octane. Thus, obtained organic ferrofluid was characterized and used for ME preparation.

2.1. Ferrofluid characteristics

Obtained iron oxide particles are in the nanometer scale as conformed from DLS analyses results. Figure 1 shows the size distribution of the nanoparticles, more than 70% of the nanoparticles have a diameter smaller than 20nm[18].

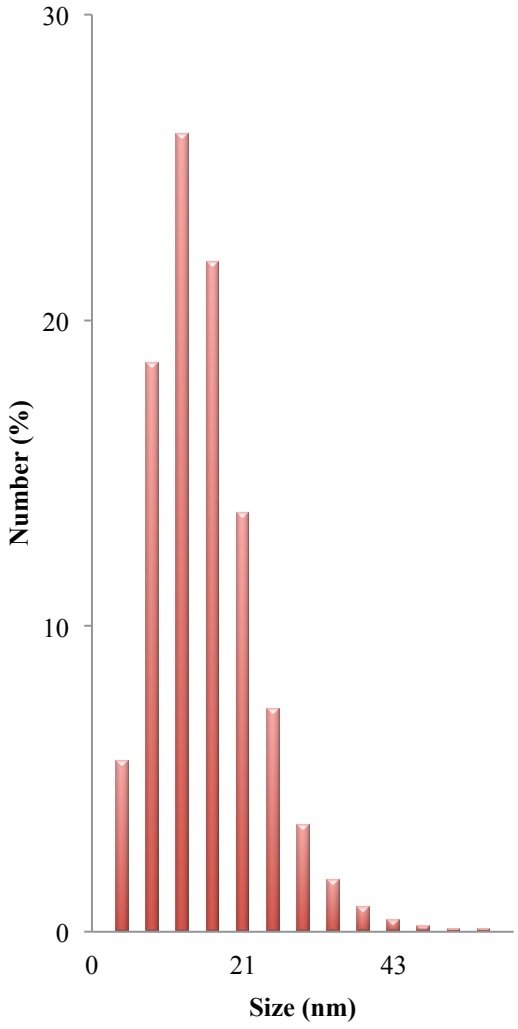


Figure 1: size distribution of iron oxide nanoparticles obtained by DLS.

In addition, TEM analysis was performed to confirm DLS results and this can be seen in figure 2 that shows the TEM images of ferrofluid nanoparticles. TEM analysis showed that the nanoparticles are smaller than 10nm, which does not correspond with DLS data. This is

due to the presence of oleic acid onto the nanoparticles surface, which may, in solution, induce the interaction between several nanoparticles to form clusters[19].

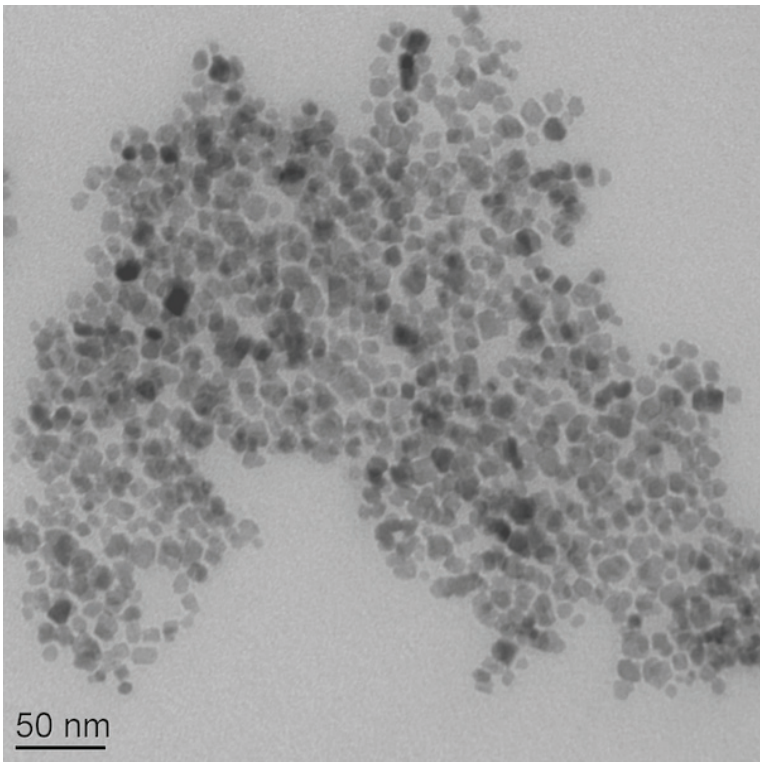


Figure 2: TEM micrograph of oleic acid-coated iron oxide nanoparticles.

The ferrofluid composition was investigated by TGA analysis that the ferrofluid was dried and the powder was heated in nitrogen atmosphere up to 800°C as shown in figure 3. From 100 to 480°C the ferrofluid lost about 31% of their weight, corresponding to the organic part of ferrofluid[20], the oleic acid coating the nanoparticles. Figure3 shows the TGA thermogram of ferrofluid in which the thermal degradation of oleic acid takes place in two temperature points, at 250°C related to free oleic acid and at 400°C related to oleic acid interacted with the nanoparticles[11]. From figure 3, the peaks at 250 and 400°C, can be concluded that the free oleic acid represents 35% and attached oleic acid represents 65%.

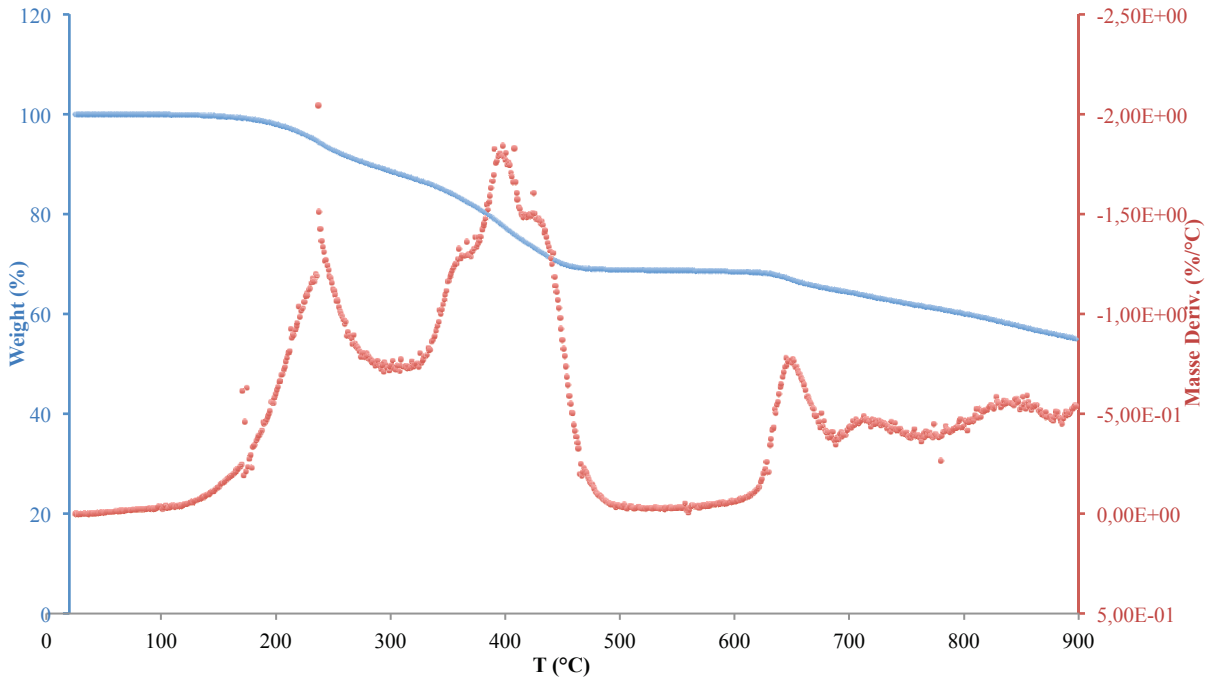


Figure 3: TGA Thermogram of organic ferrofluid, obtained under nitrogen.

FTIR technique was used to verify the interaction of oleic acid onto the iron oxide nanoparticles surface. The adsorption of oleic acid onto the iron oxide nanoparticle was investigated and proved by FTIR[21]. Figure 4 shows the spectra of pure oleic acid (blue) and iron oxide nanoparticles coated by oleic acid (red). In pure oleic acid curve two characteristic bands appeared at 1710 and 1285cm^{-1}, which were attributed to C=O and C-O stretch respectively. Whereas, in the blue curve, adsorbed oleic acid, this two bands were absent and instead of these bands two new bands are present at 1541 and 1639 cm^{-1} can be attributed to asymmetric stretch ν_{as}(COO-) and symmetric stretch ν_{sy}(COO-) respectively[11].

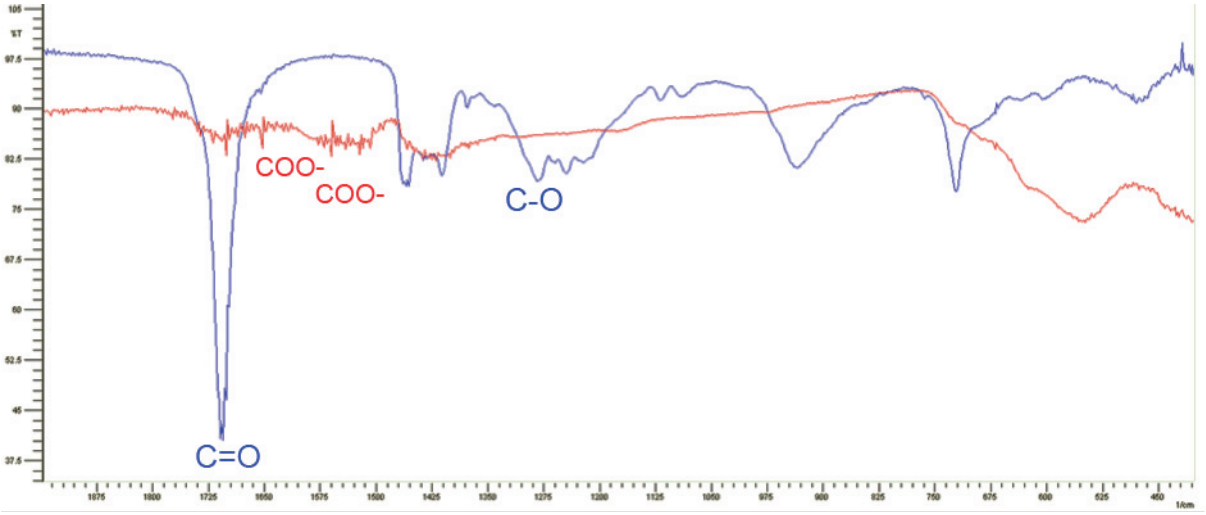


Figure 4: FTIR spectra of pure oleic acid (blue) and oleic acid-coated iron oxide nanoparticles (red).

2.2. Magnetic emulsion preparation

The magnetic emulsion is direct, oil in water, emulsion except that it contains an organic ferrofluid instead of the dispersed organic phase. This synthesis based on the emulsification of organic ferrofluid in water using SDS as surfactant. SDS was dissolved in water, 12%, and the ferrofluid was poured to the solution, which therefore was emulsified by ultrasound. The surfactant molecules stabilized the formed ferrofluid droplets and prevented the coalescence between the new droplets. First of all, droplets were 100% composed of a liquid ferrofluid, oleic acid-coated iron oxide nanoparticles dispersed in octane. Then, during storage the octane leaked outside of the droplets, which became solid particles stabilized by the surfactant and dispersed in water. Leaked octane from droplets formed microemulsion stabilized by the excess of SDS presented in the solution. Figure 5 shows the octane quantity leaked from the organic droplets toward the aqueous solution as a function of time. Octane in water emulsion is not a stable emulsion and needs high surfactant concentration because octane is hydrophobic and poorly soluble in water. Therefore, the SDS solution used in magnetic emulsion preparation was highly concentrated (12%) and this medium favor the migration of octane molecules from the large droplets to the SDS solution as micelles (microemulsion), which is more stable and has less tension than the emulsion.

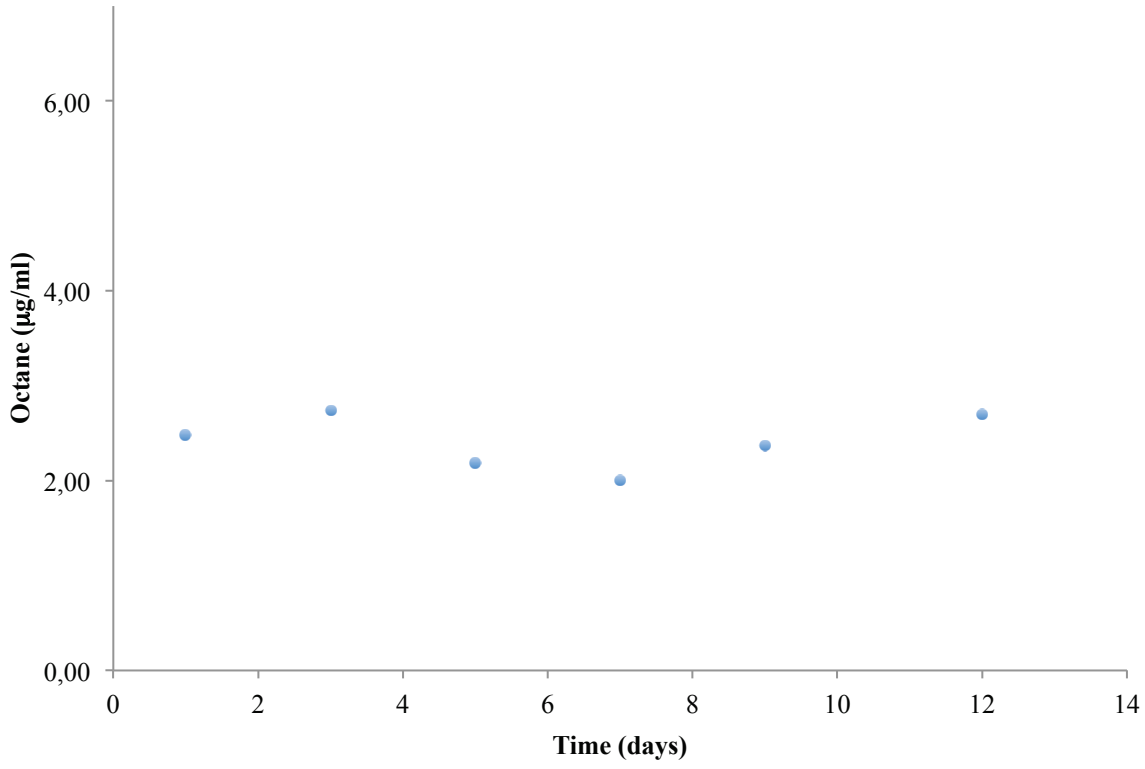


Figure 5: Octane concentration in aqueous phase of magnetic emulsion as a function of time.

TGA measurements showed that the organic ferrofluid composed of 29.5% iron oxide (oleic acid – coated) and 70.5% octane and 1ml magnetic emulsion contains 15.5µg (data from GC technique). Figure 5 shows the octane concentration in aqueous phase (continues phase of magnetic emulsion) as a function of time. Figure 5 shows that 2.5µg/ml of octane are found in aqueous phase. 2.5µg/ml represent 16% of total octane presents in magnetic emulsion.

2.3. Silica-coated magnetic emulsion (Si-ME)

Sol-gel reaction was used to prepare (core/shell) Si-ME using TEOS as a silica precursor and NH_4OH as catalyst. Silica shell more stabilized the emulsion, protected the particles and favored their use for biomedical applications. In addition, additional functionalization can be easily performed on these particles due to the silica shell, which not affect the superparamagnetism properties of the magnetic emulsion.

All sol-gel reaction parameters to synthesize core shell magnetic particles were studied and optimized such as concentration of PVP (stabilizer agent), the incubation time in the presence of PVP, NH_4OH/magnetic emulsion ratio and TEOS concentration.

2.4. Magnetic emulsion and silica-coated magnetic emulsion characterization

The emulsification procedure described above gave spherical solid particles with an average diameter of 200nm, measured by DLS and confirmed by TEM. Figure 6 shows TEM micrograph, the size and size distribution of magnetic emulsion particles.

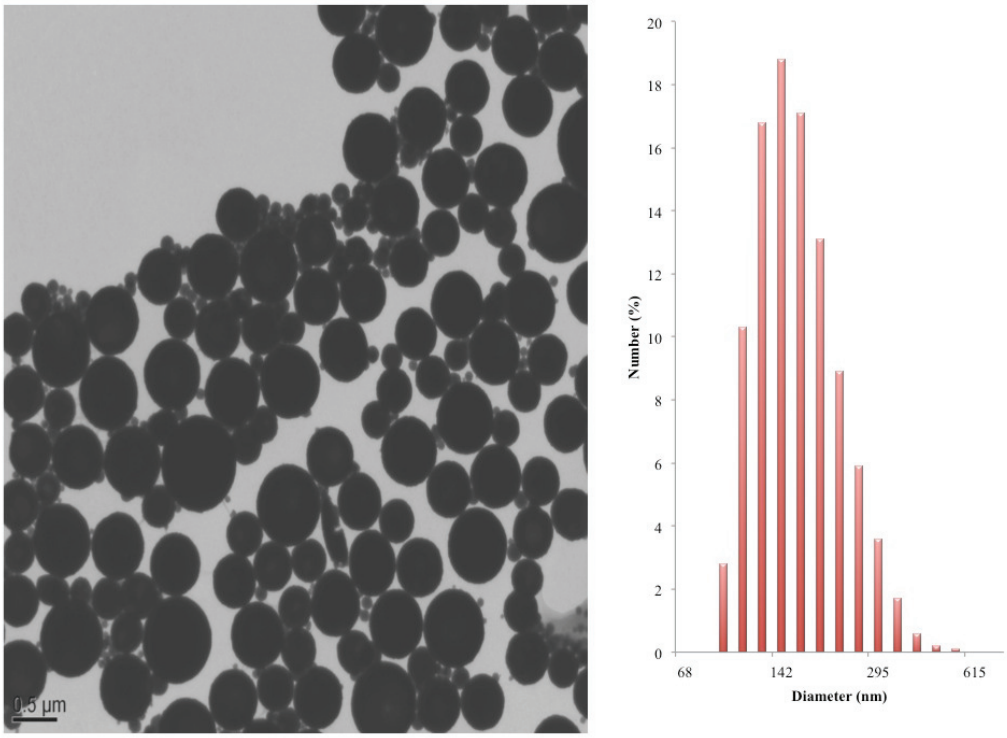


Figure 6: TEM micrograph, size and size distribution of magnetic emulsion particles.

Silica – coated magnetic emulsion was characterized by DLS and TEM also and the results show that the average particles size increased up to 400nm. This means that the silica shell is about 100nm thickness; figure 7 shows the TEM micrograph and the size distribution of silica – coated magnetic emulsion particles.

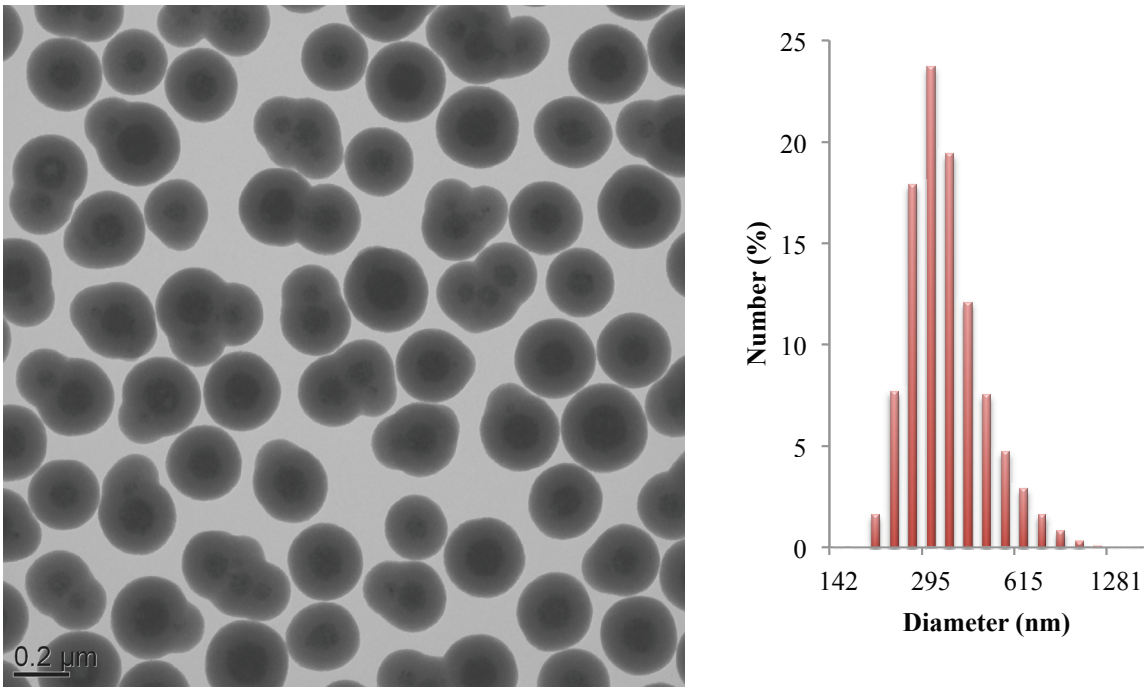


Figure 7: TEM micrographs, size and size distribution of silica – coated magnetic emulsion particles.

The zeta potential of silica particles has a specific profile as a function of pH represented by the decrease of zeta potential values with increasing the pH[22]. This is due to the presence of silanol groups (-Si–OH) onto the silica surface.

In water H-OH, OH group toward to be natural (non charged) at pH 7. At pH>7 OH is ionized and is represented as O^- and at pH<7 OH is presented as OH_2^+. In the case of silanol, the presence of Si atom near the OH group leads to weakness of interaction between O and H so the OH group could be ionized easier than the OH in water. The surface of silica-coated particles is totally coated by silanol group so the value of zeta potential of this type of particles is almost negative even in low pH, and become more negative by increasing pH due to the ionization of OH to O^-. Figure 8 represents the zeta potential of silica – coated magnetic emulsion particles as a function of pH.

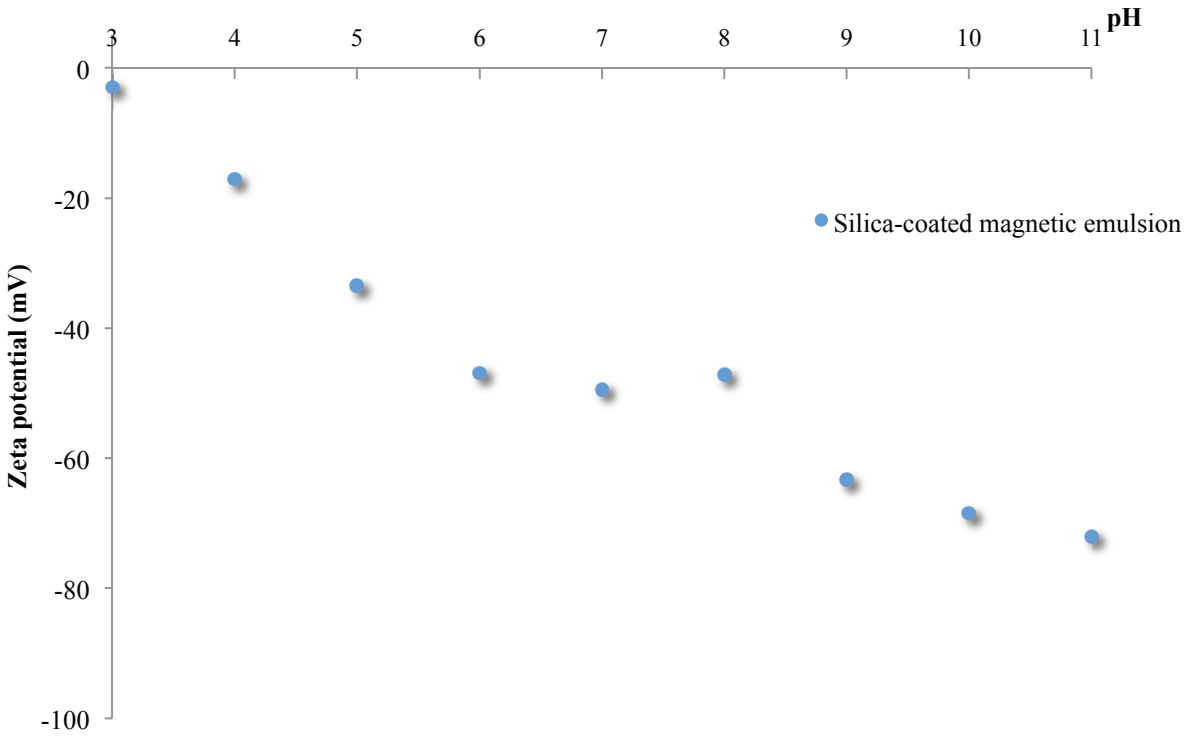
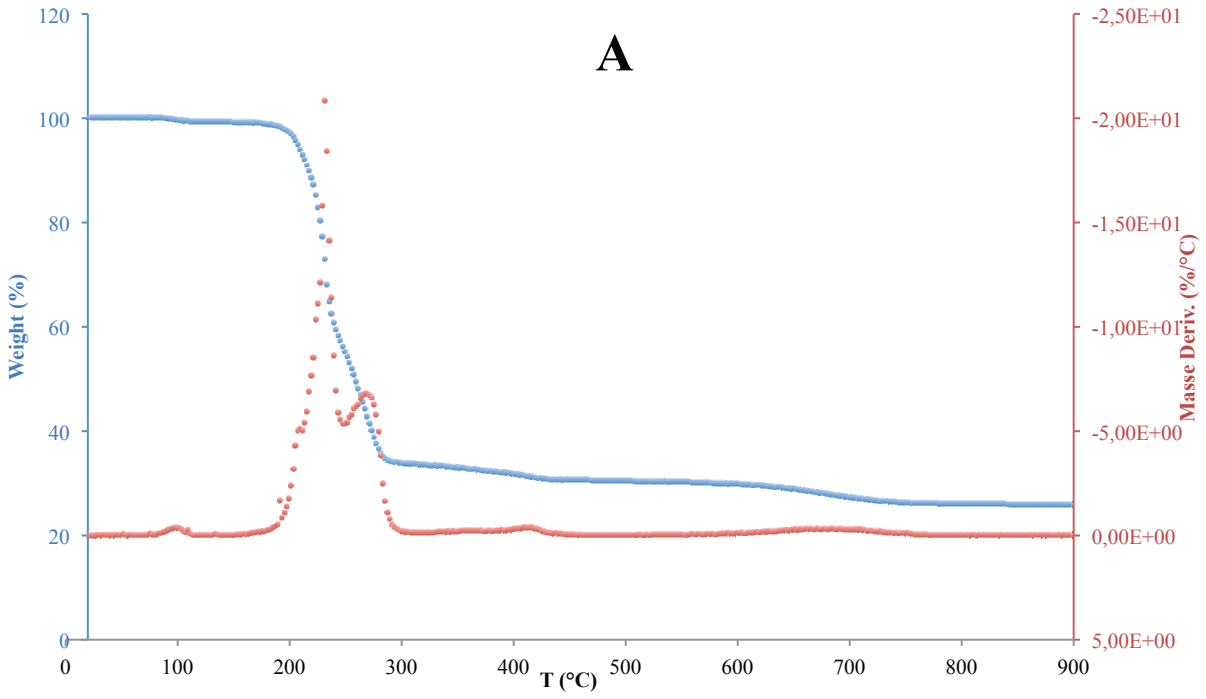


Figure 8: Zeta potential as a function of pH of Si-ME.

In addition to the structure and morphology studies the particles were characterized by TGA to investigate their composition. Figure 9 shows the TGA curves of ME and Si-ME analyzed under nitrogen atmosphere from 20 to 900°C.



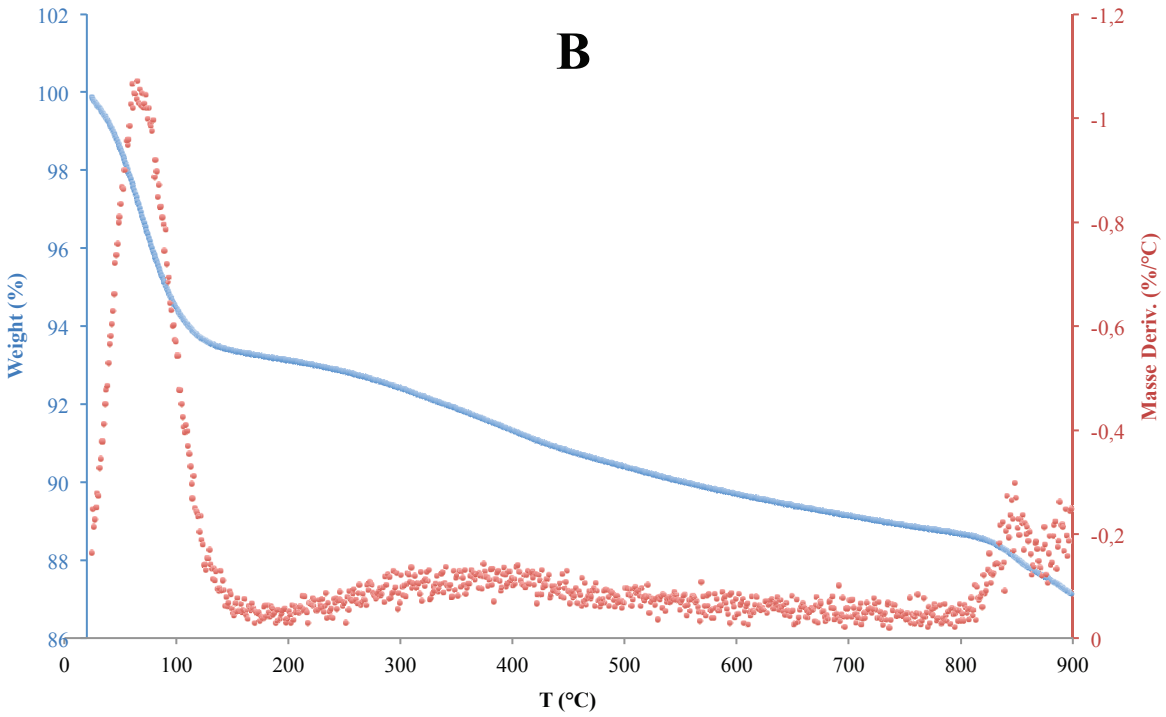


Figure 9: TGA Thermograms of magnetic emulsion (A) and silica – coated magnetic emulsion (B).

FTIR was used to characterize the particles of organic ferrofluid, silica –coated magnetic emulsion and magnetic emulsion. The figure 10 shows FTIR spectra scanned from 400 to 4000cm^{-1}. The characteristic bands of SDS[23] appear clearly in the spectrum of ME as following: 2850, 2918cm^{-1} corresponding to C-H stretch vibration, 1467cm^{-1} related to H-C-H stretch vibration, 1205 and 1248cm^{-1} corresponding to sulfate asymmetric stretch vibration and 1087, 970cm^{-1} corresponding to sulfate symmetric stretch vibration.

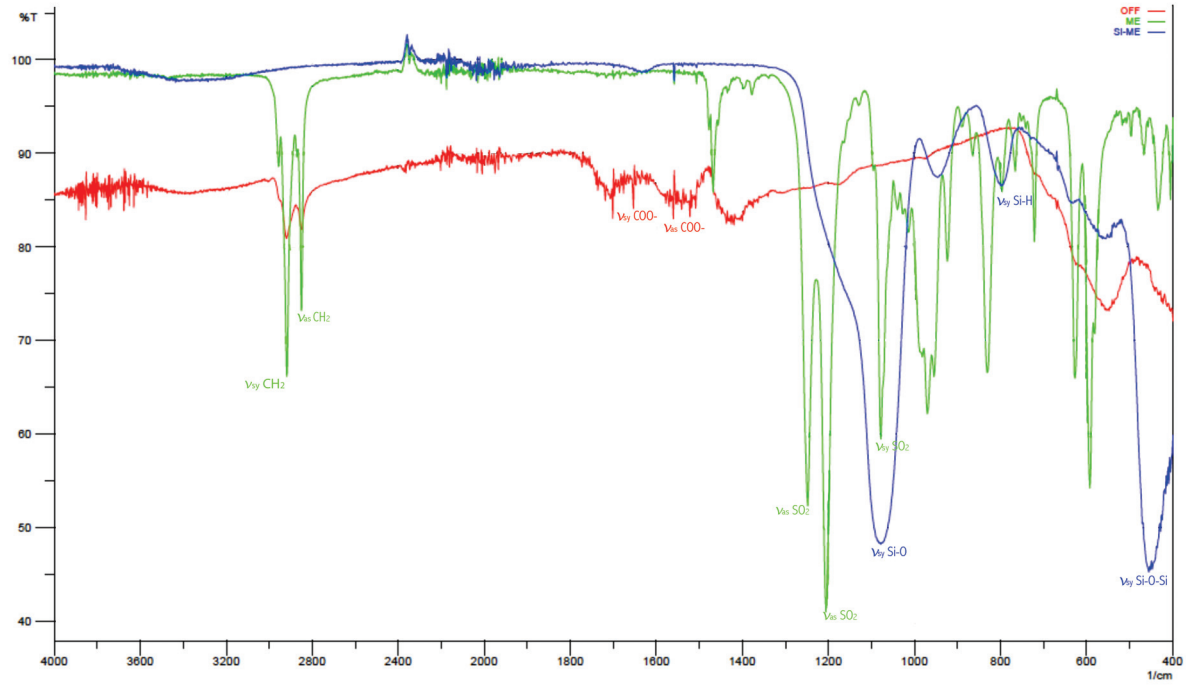


Figure 10: FTIR spectra of Organic Ferrofluid, Magnetic submicron emulsion and Silica – Coated Magnetic emulsion.

It can be seen in figure 10 that most of characteristic bands related to the SDS molecules, on the surface particles of magnetic emulsion, disappeared and were replaced by three bands at 1077, 796 and 478cm^{-1} corresponding to Si-O, Si-H and Si-O-Si symmetric stretch vibration respectively.

Ferrofluid, magnetic emulsion and silica-magnetic emulsion all were dried and exposed to a variation magnetic field from -20000 to +20000 Oersted. The magnetism measurements (figure11) show that the ferrofluid is superparamagnetic. In addition, the superparamagnetism properties are conserved in magnetic emulsion and silica – coated magnetic emulsion particles.

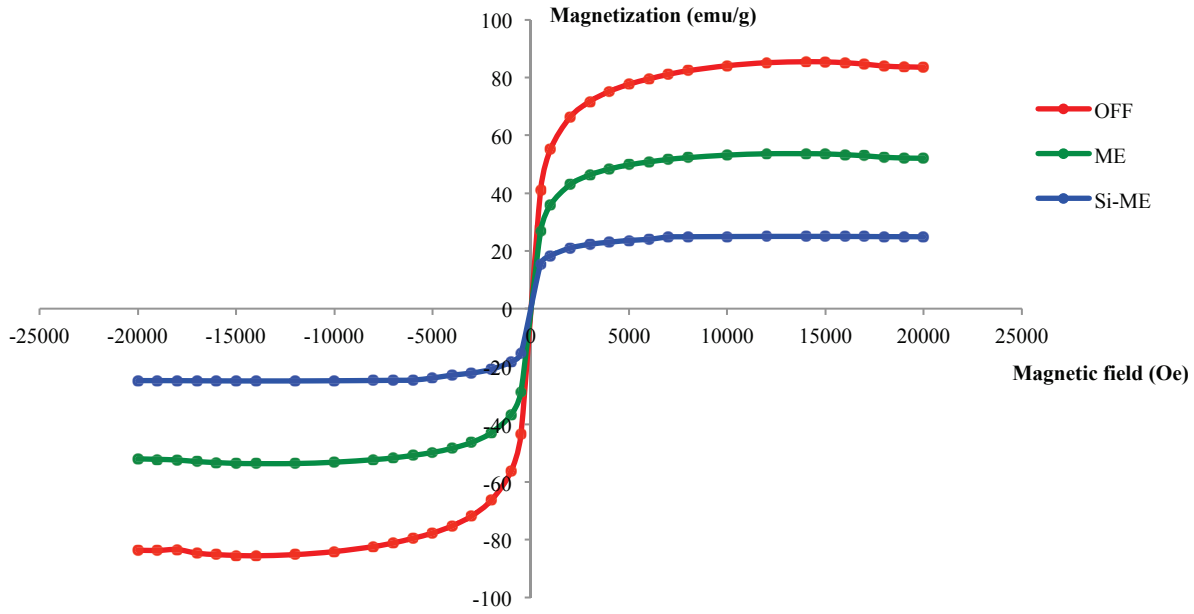


Figure 11: the variation of magnetization values as a function of magnetic field of: organic ferrofluid (OFF) •, magnetic emulsion (ME) • and silica – coated magnetic emulsion (Si-ME) •.

From figure 11 it can be seen that the magnetization was decreased about 50% between organic ferrofluid and magnetic emulsion. Moreover, in silica – coated magnetic emulsion particles the magnetization was decreased about 70% due to the thick silica shell. In addition, the three types of particles are superparamagnetic even after two steps of encapsulation.

3. Conclusion

Silica-coated magnetic emulsion particles were reported starting from the organic ferrofluids through the magnetic emulsion to the silica-coated magnetic particles. Organic ferrofluids were synthesized using the coprecipitation of $FeCl_3$ and $FeCl_2$ in aqueous solution of NH_4OH and coating by oleic acid. Resulted iron oxide particles are nanometric in size (5 to 15nm) and

superparamagnetic. Obtained organic ferrofluids were emulsified in SDS solution to prepare the magnetic emulsion. TEM, DLS, ATG, magnetization and electrokinetic data showed that the magnetic emulsion particles are negatively charged spheres of 200nm diameter, superparamagnetic and contain (maximum) 13µg/ml octane. The magnetic emulsion was undergone the sol-gel reaction to be coated by a silica shell. TEOS was used as a silica precursor, ammonium hydroxide as a catalyst and PVP as a stabilizing agent. Particles size evaluation showed that the silica shell thickness is near of 100nm and the size distribution is not so narrow, like the magnetic emulsion particles, but the encapsulation was homogenous on all particles. Encapsulated particles have negative charges that increase in negative as a function of pH. Finally, the magnetic silica particles are superparamagnetic, composed of 10% organic and 90% inorganic.

4. References

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IV. CHAPITRE 3
ETUDES EXPERIMENTALES

Résumé

L'extraction, la purification et la concentration des acides nucléiques sont de plus en plus nécessaires pour la réalisation d'un diagnostic moléculaire précoce et en particulier dans le domaine des maladies infectieuses. Le bon déroulement de ce diagnostic génique nécessite incontestablement une bonne préparation d'échantillon imposant l'extraction, la purification et la concentration des acides nucléiques avant toutes analyses. Pour répondre à cet objectif, les particules magnétiques sont largement utilisées, mais pour une intégration dans un microsystème basé sur la microfluidique, il est indispensable d'utiliser des particules magnétiques submicronique en taille.

La silice magnétique préparée dans le cadre de ce travail de recherche répond en grande partie au cahier des charges imposé par les applications bionanotechnologiques. L'adsorption des acides nucléiques sur la silice magnétique élaborée a été examinée principalement en fonction du pH, la salinité et la concentration en ADN.

En absence d'un milieu fortement salin, la quantité d'ADN adsorbée est très faible. EN revanche, l'utilisation une fore concentration en sel ou un sel bivalent ($MgCl_2$) conduit à une fore adsorption des acides nucléiques. En ce qui concerne la cinétique d'adsorption, elle est relativement rapide, un plateau d'adsorption est attend après 10 minutes d'incubation. En ce qui concerne le pH, son effet est négligeable, ceci est probablement dû aux pKa très acides des silanols et des phosphdiesters. En ce qui concerne la désorption, elle a été examinée en réalisant une adsorption dans des conditions optimales et une désorption dans un tampon TE à pH 7,8. Les résultats montrent que 90% de l'ADN adsorbé a été libéré.

DNA Separation Using Silica-Coated Magnetic Emulsion

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Abstract

Nucleic acids extraction and purification for diagnostic, research, and genes delivery and genes therapy applications is more needed nowadays. Therefore, new tools and technologies are required to replay to the increasing advances in these fields. Silica-coated magnetic particles were prepared for DNA extraction via the adsorption/desorption process. The effect of pH and $MgCl_2$ concentration on the DNA adsorption were investigated. $MgCl_2$ largely enhanced the DNA adsorption, which was optimum at pH between 9 and 10. DNA desorption was performed using TE buffer (pH 7.8). More than 90% of adsorbed DNA was released from the prepared silica magnetic particles. Finally, DNA was extracted from *E-coli* cells lysate using silica magnetic particles.

1. Introduction

Nowadays, magnetic particles are marked as powerful tools in nano and micro fields. Modified superparamagnetic iron oxide nanoparticles (SPION) are widely used in varieties of bio-applications such as MRI contrast agents[1,2], drug delivery[3,4], hyperthermia[5], proteins immobilization,[6,7] biocatalysis and bioseparation[8,9]. A variety of coating methods used to adapt and functionalize the magnetic particles surface such as polymer[10], chitosan[11], amino-dextran[12] and silica[13]. The sol-gel technique[14] is noted as an effective method to coat the small particles especially iron oxide nanoparticles[15]. Silica coating usually used for several reasons: protecting the core, increase the stability colloidal, reduce the toxicity of materials, provide an active ground for further functionalization, easy to realize and low cost production.

Nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), in addition to the proteins are the most important biological macromolecules because they are the origin of life. DNA is presented in all living species and holds the full genetic information over millions of years. The rapid development in genetic field leaded to very good understanding of the DNA molecules and their real roles in encoding, transmitting and expression of genetic information. Furthermore, diagnostics depend on the DNA investigations are widely familiarized recently.

But DNA molecules are located in the nucleus in the cell center; thus, initial extraction and purification steps are required.

Initially, phenol-chloroform extraction technique is used to extract DNA and RNA from biological samples. This technique is efficient with high yield of extracted nucleic acids but it needs time and organic reagents. Then, DNA extraction using glass and glass particles was developed[16,17]. Recently, the interaction between DNA and silica particles was studied[18,19] and results proved that silica particles are promising tools for nucleic acids extraction[20,21], purification[22] and delivery[23,24]. Developing silica particles to be used for DNA extraction is more favorable due to the low cost of raw material, easy to be synthesized, extracted faster than classical methods and no more organic solvent are needed.

Furthermore, core-shell particles were successfully prepared, which are consisted of silica shell coated an another material in its core such as gold[25,26] and magnetic particles[27]. The combination of more than one material in one particle duplicates the role of this particle, which will have the properties of each part. In the case of silica-coated magnetic particles; silica surface can be used for DNA adsorption and magnetic core can be used to separate the particles under the effect of a magnetic field.

The aim of this work is to investigate the DNA adsorption and desorption on silica-coated magnetic particles, successfully prepared via the encapsulation of magnetic emulsion by sol-gel process. The effect of pH, salt concentration and DNA concentration in solution on the adsorption and desorption were investigated. DNA extractions from bacteria by phenol-chloroform and by silica-coated magnetic particles were performed to compare the efficiency of our particles with the classical method. Obtained results suggest that silica-coated magnetic particles could be used in automated DNA extractors.

2. Experimental

2.1. Materials

Silica-coated magnetic emulsions, herring sperm DNA (purchased from Invitrogen), disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, EDTA, Tris-HCl and magnesium chloride (obtained from Sigma Aldrich), *E-coli* cells lysate (were offered by Alexandre NOIRIEL, Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, Université Lyon 1), ethanol, deionized water.

2.2. Silica-coated magnetic emulsion

The synthesis of silica-coated magnetic emulsion (Si-ME) was described in our previous work (not published). Briefly, a direct emulsion was prepared by emulsification of organic

ferrofluid, oleic acid-coated iron oxide nanoparticles dispersed in octane, in SDS solution. Then, the magnetic emulsion was coated by silica shell using the sol-gel process.

2.3. DNA adsorption procedure

Si-ME particles were washed two times by ethanol 70% and two times by deionized water. The adsorption procedure was performed in 1.5ml Eppendorf tube; the final volume of adsorption solution was 1ml. 100µl of Si-ME (2.28% solid content) were magnetically separated and the particles were dispersed in adsorption buffer then herring DNA solution 1mg/ml was added to the particles dispersion. The totally were incubated in rotation agitation for 20min at room temperature and then the particles with adsorbed DNA were separated by magnet. The DNA concentration in supernatants was measured by UV spectrophotometer (Shimadzu) at 260nm using standard calibration curve. Adsorption solution is composed of Na_2HPO_4, NaH_2PO_4, $MgCl_2$ and Si-ME. Therefore, the adsorption conditions (pH, $MgCl_2$ and DNA concentration in solution) were varied to study the effect of each one without changing the final adsorption volume of 1ml.

2.4. DNA desorption procedure

Si-ME particles with adsorbed DNA were washed with ethanol 70% and redispersed in 1ml elution buffer (10mM Tris-HCl and 1mM EDTA), which were incubated for 10min at gentle rotation agitation at room temperature. The particles were separated by magnet and the DNA in solution was measured by UV spectrophotometer at 260nm using standard calibration curve.

2.5. DNA extraction by Si-ME particles

The adsorption condition of DNA (in *E-coli* cells lysate) and Si-ME particles were adjusted to pH 9.5 and $MgCl_2$ concentration to 50mM. The Si-ME particles were incubated for 20 min with the lysate then, they were magnetically separated. The particles, in addition to the DNA adsorbed onto them, were washed by 70% ethanol. Finally, DNA molecules were eluted by adding 0.5ml of TE buffer.

3. Results and Discussion

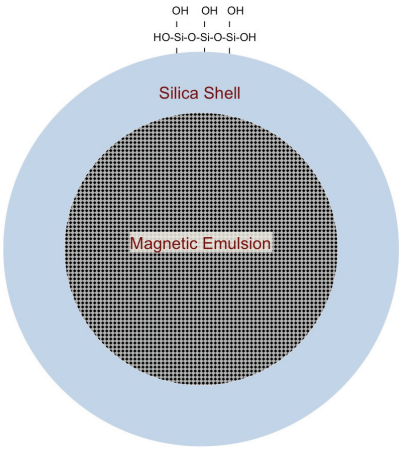
3.1. Silica-coated magnetic emulsion properties

These particles are composed of magnetic core and silica shell. The magnetic core, ~140nm of diameter, is consists of organic ferrofluid (superparamagnetic iron oxide nanoparticles coated with oleic acid and dispersed in octane). Prepared by the emulsification of organic ferrofluid in SDS solution using the ultrasound as a power source. Moreover, the sol-gel process was used to synthesize the silica shell (~50nm thickness). The particles

surface is covered by silanol groups resulted from the hydrolysis of tetraethyl orthosilicate (TEOS) according to the following reaction:

$$(C_2H_5O)_3 \equiv Si–(OC_2H_5) + H_2O \rightarrow (C_2H_5O)_3 \equiv Si–OH + C_2H_5OH$$

≡Si–OH are located on the surface whereas SiO_2, which is resulted from the polycondensation of hydrolyzed TEOS, formed the shell bulk. Zeta potential measured as a function of pH of Si-ME particles shows that the particles are negatively charged and increase, in negative, with increasing of pH. Scheme 1 shows the schematic representation of Si-ME.



Scheme 1: schematic representation of Si-ME particle

Si-ME particles, due to the presence of superparamagnetic core inside the particles, have a superparamagnetic behavior, which warrants the good function as magnetic separation tools. The figure 1 shows the variation of zeta potential (ζ) as a function of pH for the Si-ME particles, measured in 1mM NaCl. The curve presents three stages; from 3 to 6, ζ decreased rapidly with the increasing of pH due to the breaking of hydrogen bonds formed between water and silanol groups. The second stage is a plateau from 6 to 8 due to the resistance of –Si-OH groups to the ionization. Finally, the third stage as a rapid decrease of pH from 8 to 11 due to the ionization of –OH groups and the formation of $Si–O^-$.

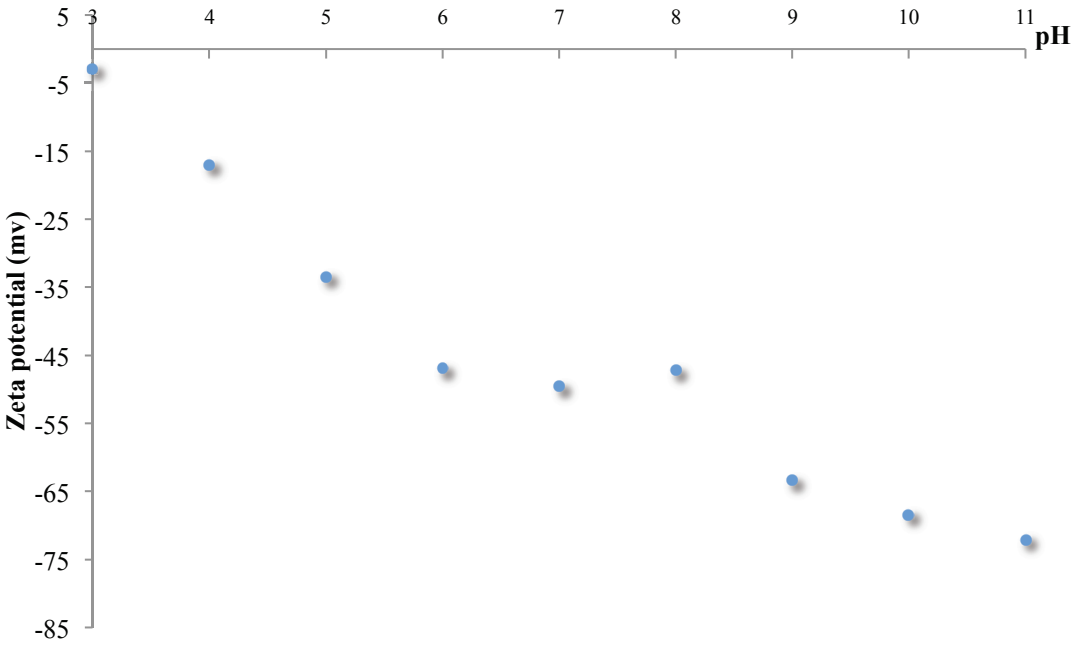


Figure 1: Zeta potential of Si-ME particles as a function of pH, in 1 mM NaCl.

3.2. DNA adsorption

DNA separation by silica particles depends on the adsorption of DNA molecules onto the particles surface at specific conditions such as pH, ionic strength, time and of course the type of particles surface.

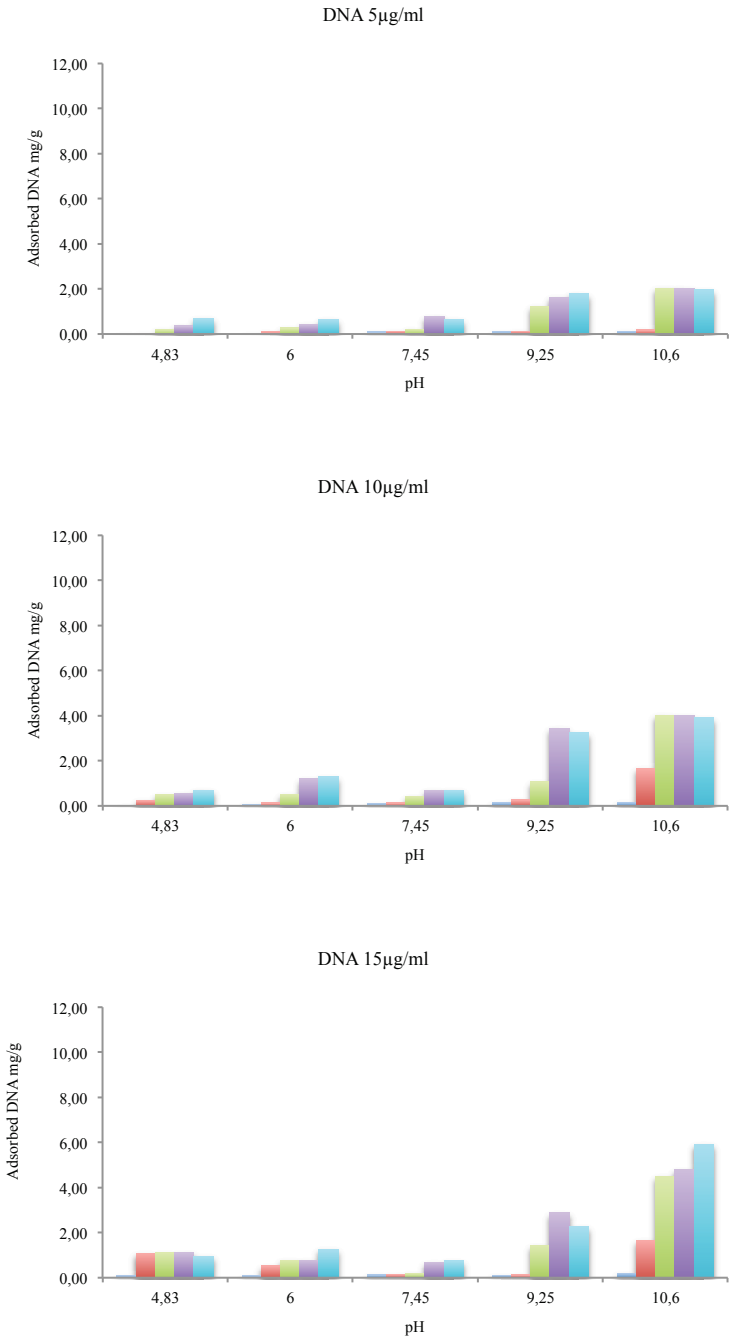
3.2.1. *The effect of pH*

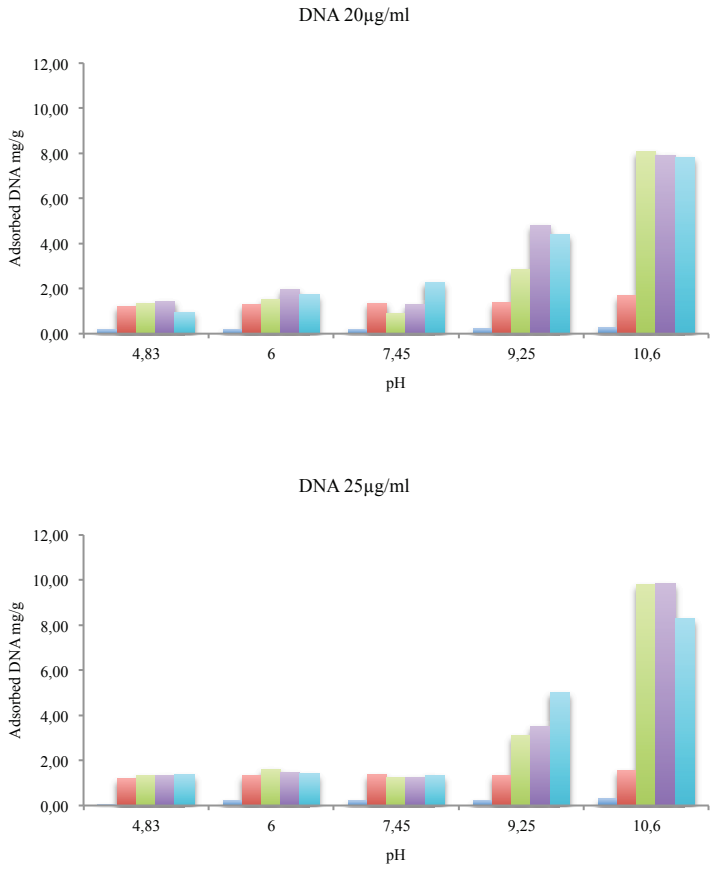
Duplex DNA molecules are strong polyelectrolyte possessing two univalent negative charges per base pair at most solution pH, due to the presence of the strong acids, phosphate diesters, on the backbone of DNA[28]. Moreover, it was found that structural stability of the duplex DNA is pH dependent and has a maximum stability near pH 7[29].

Since –OH groups coat the surface of Si-ME particles, as shown in scheme 1, they can exhibit negative or natural charge at the pH range up to 11. In these conditions the DNA adsorption onto the silica particles is decreasing with increasing pH, electrostatic repulsion because both are negatively charged. But in our case, we used magnesium chloride to reduce the electrostatic repulsion and raise the interaction efficiency between the DNA molecules and Si-ME particles. Mg^{2+} ion can make a bridge between DNA molecule and silanol group on the particles surface and this interaction is stronger in high pH than in low pH[30]. $MgCl_2$ is totally ionized in water so Mg^{2+} ions are presented in solution at any pH value. Mg^{2+} ions were chosen because they can be adsorbed easily onto the particles surface and change its charge to positive, then the adsorption of negatively charged DNA become easier and more effective. In

addition, the interaction between DNA molecules and Mg^{2+} ions is not so strong and these ions could be removed by a simple wash by EDTA.

The adsorption of DNA molecules onto the Si-ME particles was studied at five pH values; 4.8, 6, 7.4, 9.2 and 10.6. Moreover, at each pH, five $MgCl_2$ concentrations and five DNA concentrations in solution were investigated. Figure (2) shows the quantity of DNA adsorbed, mg DNA per g of Si-ME particles, by Si-ME particles as a function of pH for five equilibrium concentrations DNA and five $MgCl_2$.





■ 5mM $MgCl_2$, ■ 10mM $MgCl_2$, ■ 50mM $MgCl_2$, ■ 100mM $MgCl_2$ and ■ 500mM $MgCl_2$.

Figure 2: The effect of pH on the DNA adsorption onto the Si-ME particles with variation of the equilibrium DNA concentrations and the ionic strengths.

As can be shown in figure 2 the maximum amounts of DNA were adsorbed at pH 10.6, while a minimum adsorption was observed at pH 4.83. These results proved that the Mg^{2+} ions play as intermediate connectors between DNA molecules and silanol groups.

Then, to investigate the behavior of Si-ME toward DNA molecules in presence of 4M NaCl, as reported in most papers studying DNA adsorption onto silica surfaces. DNA adsorption onto Si-ME particles was studied as a function of pH in aqueous NaCl solution (4M). Figure 3 shows the DNA adsorbed onto Si-ME particles as a function of pH.

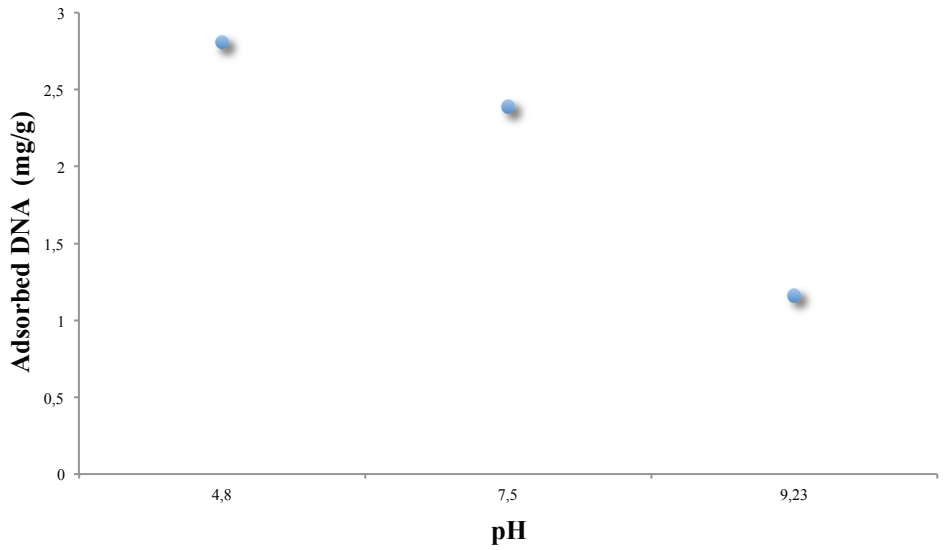


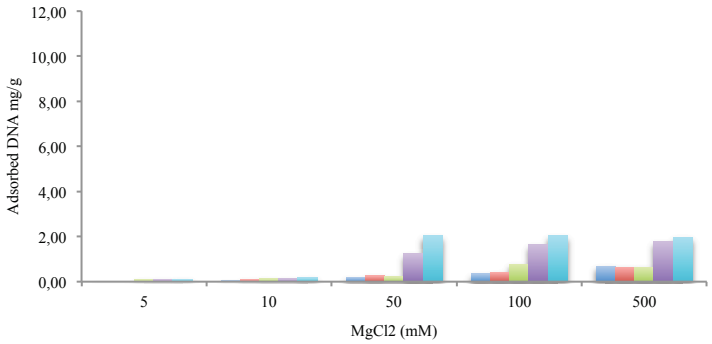
Figure 3: DNA adsorption onto Si-ME particles in presence of 4M NaCl as a function of pH.

The DNA adsorption was higher at pH 4 and decreased with increasing pH, which confirmed previous studies of DNA adsorption onto silica particles in these conditions[31,32].

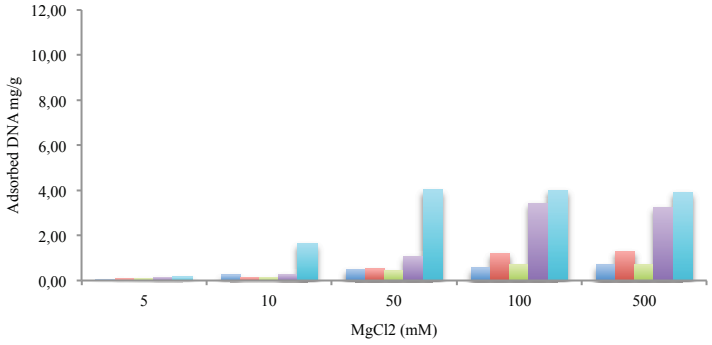
3.2.2. *The effect of ionic strength*

Si-ME particles and DNA molecules are negatively charged so the DNA adsorption onto the particles is not favorable. But, by changing the ionic strength of the reaction medium, it can modify the electrostatic interactions between them, then favorite the DNA adsorption. $MgCl_2$ is widely used in case of DNA extraction that $MgCl_2$ helps DNA relaxation[33], which rises the adsorption ability of DNA onto the particles. In addition, $MgCl_2$ forms an intermediate layer between silica surface and DNA molecules, which reduces the repulsing forces between the DNA and the particles. Furthermore, for future uses of isolated DNA such as polymerase chain reaction (PCR), $MgCl_2$ is necessary to complete this reaction so the presence of $MgCl_2$ doesn't affect the process. Herein, $MgCl_2$ was used for DNA extraction by Si-ME particles and its concentration was varied from 5 to 500mM. Figure 4 shows five histograms for five DNA equilibrium concentrations. It can be seen that the adsorbed DNA quantity is important at 50mM of $MgCl_2$ and this quantity was the maximum adsorbed DNA even with more concentrated $MgCl_2$ samples (100 and 500mM). The different colored columns represented different pH values.

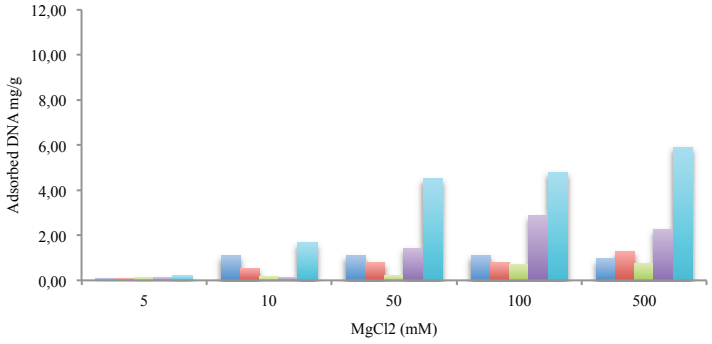
DNA 5µg/ml



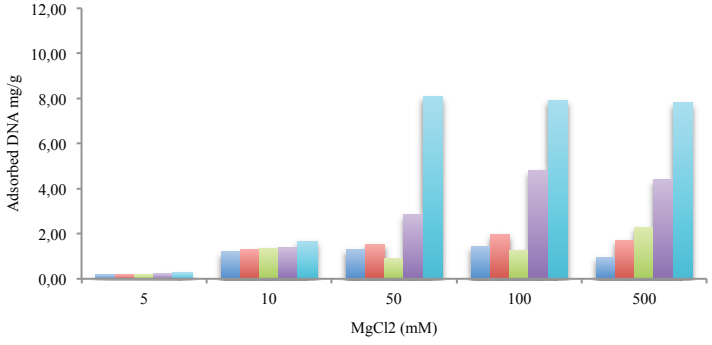
DNA 10µg/ml

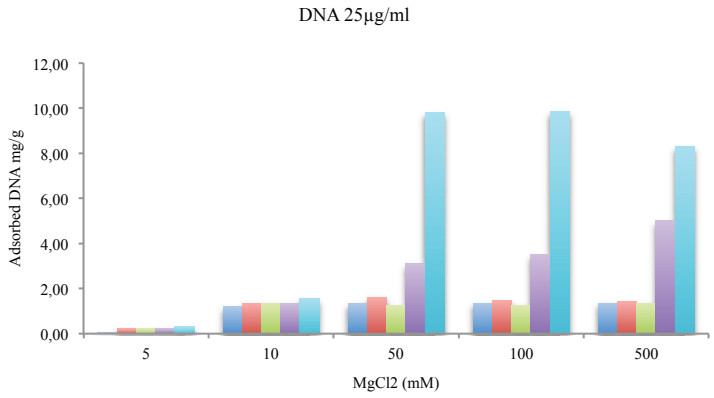


DNA 15µg/ml



DNA 20µg/ml





■ pH 4,83, ■ pH 6, ■ pH 7,45, ■ pH 9,25, ■ pH 10,6.

Figure 4: Histograms of absorbed DNA (mg/g) particles as a function of $MgCl_2$ concentrations. Experiments repeated for five equilibrium DNA concentrations at five pH values.

The saturation of particles ability to adsorb more DNA molecules may be related to that the O^- groups exposed to the surface are all saturated by Mg^{2+} ions. In addition, by comparing, in the same histogram, the adsorbed DNA quantity at different pH values it can be seen that the adsorption increased at higher pH values. Finally, according to the zeta potential measurements as a function of pH (figure 1) it is clear that the density of $-O^-$ groups increased at higher pH so there are more sites for more Mg^{2+} ions then more DNA molecules. All these observations illustrate why we have saturation of adsorption at a given pH value and augmentation at higher pH.

3.3. DNA desorption

The DNA desorption procedure was performed using TE buffer (10mM Tris-HCl, 1mM EDTA, pH 7,8). The adsorbed DNA molecules onto the Si-ME particles was washed by 70% ethanol to remove all free molecules and salt ions, then the adsorbed DNA eluted with TE buffer for 10 min at room temperature. The Si-ME particles were separated using magnet and the UV spectrophotometry, at 260nm, was used to measure DNA concentration in solution.

In absence of ionic strength and the important role of EDTA to chelate the Mg^{2+} ions, the repulsion force between DNA molecules and Si-ME particles become sufficient to disturb the interaction between them and liberate the DNA molecules. Figure 5 shows the adsorbed DNA quantities (red columns) onto the Si-ME particles and the eluted DNA (blue columns) from Si-ME particles by TE buffer. The adsorption was done at 100mM $MgCl_2$ and at pH=10.6 desorption was done as described above. Adsorption and desorption values show that more than 90% of adsorbed DNA molecules are liberated from particles following the desorption procedure.

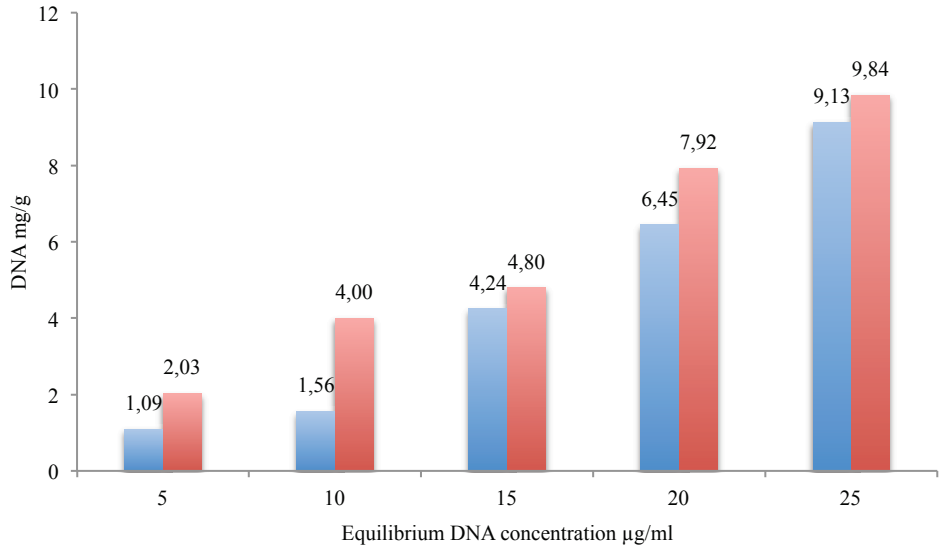


Figure 5: comparison between quantities of adsorbed DNA (red) and released DNA (blue) mg per gram of Si-ME particles.

3.4. DNA extraction from bacteria cells lysate

To study the ability of our silica magnetic particles to extract the DNA molecules from a biologic sample, we used *E-coli* cells lysate. The particles were incubated with the lysate, rotation agitation and room temperature, for 20 min at pH 9.5 and 50mM $MgCl_2$. Then, the particles, with the adsorbed DNA, were separated by a magnetic field and washed by 70% ethanol. At the end, the adsorbed DNA molecules were eluted using TE buffer as described above. The degree of purity of extracted DNA was estimated by UV spectrophotometry that the absorbance $A_{260}/A_{280} = 2$, this result shows that the extracted DNA is highly pure. Figure 6 shows the image of gel electrophoresis for DNA extracted by Si-ME particles and ordinary extraction using the kit (Macherey-Nagel "NucleoBond® Xtra Midi").

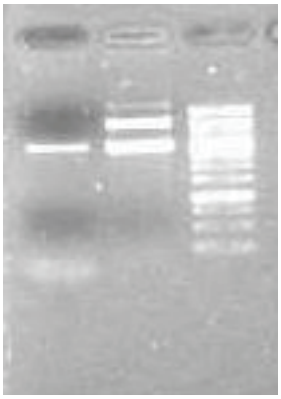


Figure 6: image of electrophoresis gel of DNA extracted using Si-ME particles (left lane) and DNA extracted by a commercial kit (middle lane), the right lane represents the molecular weight marker of DNA.

4. Conclusion

In this paper we reported the silica magnetic particles and using them as DNA extraction tools. Si-ME particles exhibited a speed responsive to an external magnetic field that permits to separate them quickly. In addition, the silica shell provided an ideal background for regulated interaction with DNA molecules. DNA adsorption process was studied in different conditions of pH, ionic strength and DNA in solution. The highest adsorption was found at high pH values, 9 and 10, due to the high density of O^- groups at these conditions. $MgCl_2$ was used to enhance the interaction between DNA molecules and silica magnetic particles, so the effect of its concentration was investigated and the best adsorption was observed starting from 50mM $MgCl_2$. Then, DNA desorption was studied using TE buffer at pH 7,8 and more than 90% of adsorbed DNA was released. Finally, an assay to extract DNA from a biologic sample was successfully performed and the obtained DNA was highly pure. These investigations of adsorption/desorption of DNA onto silica magnetic particles and DNA extraction, strongly recommend that Si-ME particles are very rapid, safety and useful tools for DNA isolation and purification.

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V. Conclusion Général, Perspectives

Les particules magnétiques sont utilisées dans des applications nanotechnologiques et plus particulièrement en bionanotechnologie. Le matériau magnétique le plus utilisé à ce jour est à base d'oxyde de fer. Dans le domaine biomédical, les nanoparticules d'oxydes de fer, ont été largement utilisées et rapidement développées. En effet, grâce à ses propriétés physico-chimiques, les nanoparticules d'oxydes de fer peuvent s'adapter et être adapter à de nombreuses applications biomédicales comme agents de contraste pour l'IRM, systèmes de libération d'actifs, traitement de tumeurs via l'hyperthermie et comme support de biomolécules pour le diagnostic in vitro. Le caractère superparamagnétique est une propriété très importante des nanoparticules d'oxydes de fer, car ces nanoparticules répondent sous l'action d'un champ magnétique et ne présentent pas d'aimantation résiduelle. En profitant de cette propriété, plusieurs applications basées sur les nanoparticules d'oxydes de fer ont été donc développées.

Dans le cadre de ce travail de recherche, des nanoparticules superparamagnétiques d'oxydes de fer ont été synthétisées via la coprécipitation de $FeCl_3$ et $FeCl_2$ en milieu basique. La surface de ces nanoparticules a été modifiée par adsorption d'acide oléique pour une meilleure dispersion dans un solvant organique (octane) pour obtenir un ferrofluide organique stable. Les nanoparticules obtenues dans l'octane sont superparamagnétiques, stables dans l'octane et ont une taille comprise entre 5 et 10 nm. Ce procédé a été réalisé à faible et à grande échelle (100 ml à 1 L).

En ce qui concerne les émulsions magnétiques (huile/eau) ont été préparées en utilisant du ferrofluide organique (dans l'octane), le SDS comme tensioactif et l'ultrason comme une source d'énergie. Ces émulsions magnétiques sont composées de gouttelettes stables de ferrofluide organique dispersées dans l'eau et stabilisées par le SDS. Le procédé optimisé permet d'avoir une taille moyenne de particules de l'ordre de 145 nm. La taille moyenne est en relation directe avec la vitesse ou la puissance des ultrasons utilisés comme dans le cas de la miniémulsion.

Après l'étape de formulation d'émulsions magnétiques stables, l'étude d'encapsulation a été conduite en examinant un grand nombre de paramètres. L'objet de cette étude été de l'obtention de particules avec un cœur magnétique et une écore de silice parfaitement homogène. Il est à noter que la présence d'une nucléation secondaire perturbe la reproductibilité et également l'encapsulation parfaite des semences magnétiques utilisées. Afin d'éviter cette nucléation perturbante, l'optimisation a été nécessaire. Le procédé

développé est très robuste, car l'encapsulation optimisé permet d'avoir une morphologie cœur-écorce reproductible. Selon les paramètres optimisés, ce procédé permet d'avoir une écorce de silice d'une épaisseur de l'ordre de 100nm. La taille moyenne des particules encapsulées dépend fortement de la taille des émulsions semences, mais une taille de 400 nm a été reproduite sans problèmes majeurs.

Les particules de silice élaborées ont été évaluées en examinant la possibilité d'adsorber des acides nucléiques. Cette adsorption a été étudiée via une étude systématique en examinant un grand nombre de paramètres ; le temps de contact, le pH et la salinité du milieu d'adsorption, la concentration initiale en acides nucléiques et également la reproductibilité d'un lot de silice à un autre. Il dégage de cette étude une tendance montrant que l'adsorption est principalement favorisée par l'ajout d'une forte force ionique favorisant ainsi la déshydratation à la fois des acides nucléiques et la surface de silice ce qui conduit à une adsorption des acides nucléiques. En ce qui concerne le pH, il semblerait que ce dernier à un effet relativement négligeable suite aux pKa très acide des deux groupements chargés engagés dans l'interaction (phosphodiester pour l'ADN et le silanol pour la silice), mai l'adsorption augmente progressivement avec le pH et en particulier à pH supérieur à pH9.

La désorption a été étudié dans des conditions fixes par l'utilisation d'un tampon TE (10 mM Tris-HCl, 1mM EDTA, pH 7,8) généralement pour le stockage et l'analyse des acides nucléiques. Sans optimisation, plus de 90% de l'ADN adsorbé a été relargé montrant ainsi, la possibilité d'utiliser ces particules pour l'adsorption, la purification et la concentration des acides nucléiques. Cependant, il reste à examiner ces particules dans des conditions réelles et par un laboratoire expert dans le domaine de la biologie moléculaire en utilisant des échantillons complexes proche de la réalité expérimentale.

Perspectives :

Ce travail de synthèse de particules de silice magnétiques a permis de montrer la faisabilité d'élaborer ce type de particules très convoités dans la préparation d'échantillons et dans les bionanotechnologies. Cette synthèse a été réalisée en débutant par la préparation de ferrofluides, formulés par la suite sous forme d'émulsions magnétiques avant encapsulation par une écorce de silice. Ce travail de recherche d'apparence simple a nécessité de conduire une étude bibliographique pointue, une bonne maitrise de la synthèse de ferrofluides aqueux et organique, des compétences en formulation, en physicochimie des émulsions directes et en physico-chimie colloïdale. En ce qui concerne le procédé sol-gel, il est relativement simple et sans difficultés majeures comme largement reporté dans la littérature. Mais, il est à noter le problème majeur qui nécessite une attention particulière est la question de la polydispersité

des émulsions magnétiques élaborées. Pour réduire cette polydispersité, il existe une solution physique qui consiste à faire un tri magnétique, ce que nous avons totalement refusé de faire lors de notre étude.

En guise de perspective, une première attention doit être portée à la formulation d'une émulsion de taille contrôlée et d'une polydispersité la plus faible possible et sans étape de fractionnement ou de tri. Ceci nécessitera de développer non seulement une nouvelle formulation, mais aussi un procédé propre afin de réduire cette polydispersité. Il est également indispensable de concevoir un système permettant de mieux contrôler la taille.

En ce qui concerne l'encapsulation, malgré la possibilité de faire une séparation magnétique pour éliminer les particules non magnétiques il est indispensable d'optimiser le procédé afin d'éviter la formation de la silice secondaire.

En ce qui concerne l'adsorption des acides nucléiques, il est important et primordial d'évaluer l'extraction des acides nucléiques en utilisant des échantillons biologiques. Ceci nécessitera d'établir des collaboration avec biologiste afin de réponse avec pertinence à la capacité d'extraire, purifier et concentrer les acides nucléiques et d'examiner par la même occasion la possibilité d'intégrer ces particules dans les microsystèmes dédiés au diagnostic biomédical in vitro.

Il sera également intéressant d'examiner la possibilité d'utiliser ces particules de silice magnétiques pour l'extraction d'autres biomolécules autres que les acides nucléiques.