



Microfluidic-based colloidal ZnO microcapsules : synthesis, structure, organization and first applications

Najla Ghifari

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Microfluidic-based colloidal ZnO microcapsules: synthesis, structure, organization and first applications

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Najla GHIFARI

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ÉCOLE DOCTORALE

Physique et ingénierie:
Electrons, Photons,
Sciences du vivant (EOBE)



Dedication

To my lovely parents Halima and Mohamed.

My sweet sisters and sister-in-law: Rajaa, Jihan, Nabila, and Malika.

My brother and brothers-in-law: Adil, Khalid, and Abdelkarim.

My niblings: Abdessamad, Idriss, Othman, Hafssa, Aya, and Ali.

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to work with him.

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Nomenclature

D_{drop} Droplets diameter

D_p Particles diameter

σ Interfacial tension

τ Time to form a droplet

t_{fill} Time to fill the main channel cross-section

w Width of the channel

Q_c Flow rates of the continuous phase

Q_d Flow rates of the dispersed phase

V_{drop} Droplets volume

Γ Surface density

μ Viscosity

$C_{surf.}$ Surfactant concentration

C_{ZnO} Concentration of ZnO precursor

$D_p(ZnO)$ Size of pure ZnO particles

$D_p(ZnO/RhB)$ Size of rhodamine B-doped ZnO particles

I_f Fluorescence intensity

N_{NP} Number of nanoparticles

N_{RhB} Overall number of rhodamine molecules in each microcapsule

$R_{drop.}$ Droplet radius

S_{drop} Surface area of each droplet

S_{NP} Overall area occupied by the nanoparticles at the droplet surface

t_{sq} Time to squeeze the emerging drop to detach it from the dispersed phase

V_{shell} Shell volume

ZnO Zinc Oxide

3D Three-dimensional

h Shell thickness

AFM Atomic Force Microscopy

Bo Bond number

Ca Capillary number

CCD Charge-coupled device

CM Confocal microscope

CNPs Charged nanoparticles

CVD Chemical Vapour Deposition

CW Continuous Wave

EDS Energy Dispersive Spectroscopy

FBAR Thin film bulk acoustic wave resonator

Fluo Fluorescein

FRAP Fluorescence Recovery After Photo-bleaching

HAADF - STEM High-angle annular dark-field scanning transmission electron
microscope

IPA Isopropyl alcohol

ITO Tin-doped Indium Oxide

MEMS Micro - Electro - Mechanical Systems

MST Microsystems Technology

n Layers number

NA Numerical aperture

Nd:YAG Neodymium-doped yttrium aluminium garnet

NPs Nanoparticles

PDMS Polydimethylsiloxane

PEB Post exposure bake

PEG Polyethylene Glycol

PVD Physical Vapour Deposition

Re Reynolds number

Rh110 Rhodamine 110

Rh6G Rhodamine 6G

RhB Rhodamine B

ROI Region of interest

SAW Surface Acoustic Wave

SEM Scanning Electron Microscopy

SFM Scanning force microscopy

Sol-Gel Solution-Gelification

SPSs Single photon sources

TCN Theory of charged nanoparticles

TCO Transparent Conducting Oxide

TEM Transmission Electron Microscope

U Velocity

UV Ultraviolet

We Weber number

WGMs Whispering gallery modes

General Introduction and Dissertation Context

This thesis work started from the combination of sol-gel and droplet-based microfluidics techniques. It focuses on the development of an original approach to generate zinc oxide (ZnO) colloidal microspheres from highly monodisperse droplets, driven by the evaporation of solvents and the meso-crystallization of dispersed polar zinc oxide nanoparticles. It covers many scientific fields at the interface of solid-state physics, soft matter physics, materials science, physical chemistry, photo-physics and photo-chemistry^[1-3].

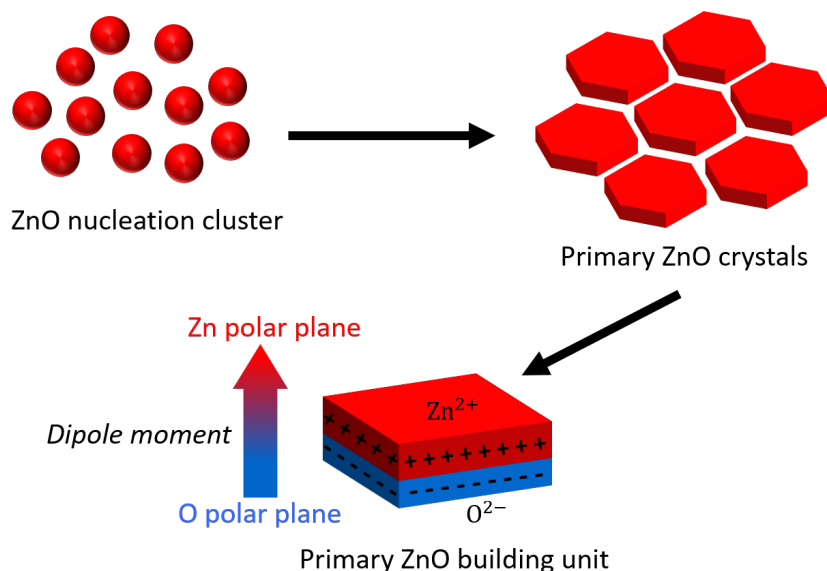
The developed original approach enables for the production of highly monodisperse microcapsules with a well-controlled size and meso-crystalline structure. Such microsystems may find applications in many technological domains, such as photovoltaics, photonics or biomedecine. We show, in particular, that our approach is efficient and promising not only for the fabrication of "smart" colloidal microspheres, but also for a better understanding the driving forces governing nanoparticles aggregation and their organisation at both nano and micro-scales during the nucleation and growth of colloidal meso-crystalline microspheres.

Mesocrystals are fascinating objects. This is a class of colloidal materials with

mesoscopically self-assembled structures, that may be found in nature or synthesized in laboratory⁴. The understanding and control of self-assembling of such materials at the nano and micro-scales is of great importance for many theoretical studies and technological applications. It is now well accepted that mesocrystals build-up via an oriented self-assembling of individual nanosized building units (nanocrystals), in a such a way that the overall orientation of the resulting larger crystals is single-crystal-like⁵⁻¹⁵. Busch and coworkers have suggested that intrinsic electric fields are responsible for the orientation of the individual primary building blocks^{16,17}. The electric charge carried by the building nanoparticles play a critical role in such a non-classical crystallization process. Hwang et al.^{18,19} developed later a theory of charged nanoparticles (TCN) in which the growth of nanostructures is basically due to the spontaneously generated charged nanoparticles (CNPs). A particular emphasis has been done during the last decades to understand the relation between crystal structure and the polarity of oxide materials, which exhibit remarkable features, such as piezo-electricity, second harmonic generation, or ferro-electricity.

Zinc oxide has been widely scrutinized due to the presence of a significant intrinsic dipole moment of its wurtzite crystal structure. The occurrence of such electric dipole moment is inherent to the tetrahedral configuration, where each type of ion Zn^{2+} or O^{2-} , has four neighbouring ions of the other type of atom, and vice versa. That is to say, the tetrahedral coordination exhibits a sequence of positively charged Zn^{2+} and negatively charged O^{2-} polar planes within the c-axis direction, thus respectively contributing to two opposite faces of polarity (0001) and (000 $\bar{1}$) perpendicular to the c-axis. The stacking along the c-axis is

non-centrosymmetric, leading to an intrinsic dipole moment and a pronounced spontaneous polarization along the c-axis²⁰⁻²⁴.



Many efforts have been devoted to synthesize microparticles with particular properties using traditional methods, such as sol-gel²⁵⁻²⁸, precipitation polymerization²⁹, etc. Nevertheless, it seems to be difficult to manufacture microparticles with very specific features and high precision control of the whole process. In fact, due to their unique properties, such microparticles play a significant role in many fields, such as the manufacture of advanced functional materials, imaging, cosmetics, biosensing, tissue engineering, drug delivery, and transport. Droplet-based microfluidics offers many advantages in producing microparticles with well-controlled size and morphology. It requires, first, the generation of highly monodisperse droplets inside the microfluidic chip, then the transport of the droplets via microchannels outside the microfluidic device, and afterwards the solidification of the droplets by solvent evaporation to form solid microparticles.

Several factors play a major role in the complex dynamic process of monodisperse droplet generation and size using microfluidic devices. In short, the geometry of the microfluidic channels, nature of the surfactants used, flow rates of the two fluids constituting the dispersed and continuous phase, wettability, interfacial tension, viscosity of the two fluids, and the adopted drying process, are of major importance.

In this work, an important part has been dedicated to the study of the effect of droplets handling parameters in microfluidic devices on their formation and on the stability of the elaborated zinc oxide microspheres. The optical and electrical properties of these microspheres have been studied both experimentally and theoretically. This work includes the synthesis, in a micrometric range from 10 μm to 30 μm , of mesoporous zinc oxide microcapsules with a thin and flexible shell. We also investigated the polar characteristics of zinc oxide nanoparticles and their self-organization. We show that the electric charges carried by the primary units of zinc oxide nanoparticles play a crucial role in the stability of the droplet interfaces (in the presence of charged molecules) and the size of the synthesized zinc oxide microcapsules. In addition, fluorescent probes were used, including rhodamine B, rhodamine 6G, and fluorescein. We conducted a study on the effect of the nature of the surfactant in the aqueous continuous phase on droplet formation and stability. Because of the presence of the electric dipole carried by zinc oxide nanoparticles, we suggest that both orientation and self-organization of zinc oxide nanoparticles in microdroplets and microparticles should be governed by a balance between electrostatic forces and interfacial forces.

We report, for the first time, the observation of self-organization of liquid mi-

crodroplets in square networks. This mainly reveals the polar aspect of zinc oxide nanoparticles on the surface of the microdroplets which tend to condense in the early stage of zinc oxide nanocrystal aggregation and microparticle formation. We have developed a model based on a dipolar field mechanism hypothesis, to explain the packing of zinc oxide nanoparticles at the droplet surfaces. The as-synthesized zinc oxide microspheres were characterized using optical microscope, scanning electron microscopy (SEM), energy dispersive X-Ray spectroscopy (EDS), transmission electron (TEM) microscopy, photoluminescence (PL), and confocal microscopy (CM).

Thesis structure

In the first chapter, we review the state of the art of zinc oxide, including its main properties and applications. An overview of the different synthesis methods, used in the literature, for the fabrication of zinc oxide nano and micro-structures is then presented and reviewed. A discussion of chemical, physical and biological techniques is provided. We put a particular emphasis on the use of sol-gel and microfluidic techniques that have been gaining popularity, and from which particularly the samples that we study in this thesis work were produced. In addition, a wide variety of morphologies of zinc oxide structures are also addressed. Then, the advantages of the microfluidics assisted sol-gel approach over conventional manufacturing methods are also described. In the second part of this chapter, we provide a comprehensive insight into microfluidics with emphasis on droplet-based microfluidics covering, its history to recent progress. First, we introduce droplet-based microfluidics as a tool for monodisperse droplets generation and describe the dynamics of the droplet formation within microfluidic systems. We then

review the commonly used microfluidic generators, including co-flow, T-junction and flow focusing geometries, and their role in controlling droplets generation. We also report important aspects of microfluidics covering its fundamental physical principles. Finally, we present microfabrication techniques for microfluidics.

In the second chapter, we provide details of all the materials and equipment employed to develop zinc oxide microparticles which is discussed in the following chapters. Similarly, the basic principles of characterization techniques used are discussed including electronic, scanning probe, and optical imaging microscopies. Then, we discuss the microfluidic device fabrication procedure used in this thesis work. While discussing this, particular attention is paid to describe the PDMS-based microfluidic devices that were manufactured and were required to obtain consistent results.

The third chapter is focused on the synthesis and the structural properties of the synthesized zinc oxide microspheres (microcapsules) and the organization of zinc oxide nanoparticles building blocks of their shell. We present the microfluidic setup used and the resulting findings. We provide a detailed description of the formation mechanism of zinc oxide implemented in our experiments. We investigate the effect of different parameters, such as the effect of surfactants, dispersed and continuous phase flow rates on droplet size. We determine the relationship between droplets size and particles size. We also study the envelope structure of the microspheres at the microscopic scale using scanning electron microscopy (SEM), and perform transmission electron microscopy (TEM) to have a better insight on the organization of the primary zinc oxide nanoparticles that constitute the building blocks of the microcapsules.

The fourth chapter is devoted to the study of the self-organization of zinc oxide nanoparticles at the microdroplet interface and through mixtures of differently labelled fluorescent microdroplets. It presents the formation of polar zinc oxide microdroplets, which might represent an interesting experimental model for the study of self-assembled materials. We develop different models regarding the interfacial organization of the nanoparticles based on zinc oxide droplets organization properties. We also present dipole-based models to corroborate the polar self-organization of zinc oxide microdroplets. We discuss the influence of charged dyes on the square organization of zinc oxide droplets in our samples. We show that charge-charge interactions dominate the forces between colloidal nanoparticles, which extend to the micro-scale. We also present experiment evidence of the selective adsorption of charged dyes at the zinc oxide droplet interface.

In the fifth chapter, we present in detail the droplet-based method to produce and control the surface properties of such highly monodisperse zinc oxide microspheres. This is achieved using a well-controlled microdroplets made up of a dispersion of zinc oxide nanoparticles or a mixture of zinc oxide nanoparticles and rhodamine B-based dyes. In this chapter, our attention is also focused on gaining insights into the shell formation mechanism process with a view to provide an original model for the description of such a mechanism, and self-organization of rhodamine B dye within the zinc oxide microspheres, as well as to present direct evidence of the shell-core structure and nanoparticles organization of zinc oxide microspheres using fluorescence confocal microscopy.

The main objective of the sixth chapter is to present two first applications of the zinc oxide microcapsules. First, we present a proof of concept for a new

approach to measure temperature in microfluidic devices with high spatial and temporal resolutions. For this purpose, we used laser-induced fluorescence (LIF) technique to induce fluorescence of highly monodisperse zinc oxide microparticles doped with suitable fluorescent dyes. In particular, we show that rhodamine B and rhodamine 6G can be used efficiently as molecular probes for measuring temperature in accurate and quick manner at the microscale of flowing zinc oxide microparticles (inside microfluidic channels). Secondly, we use the synthesized zinc oxide microcapsules for the measurement of diffusion coefficients of some dyestuffs contained within the zinc oxide microcapsules.

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Chapter 1

ZnO and Microfluidics

In this chapter, we present the state of the art of zinc oxide including its properties, applications, and synthesis methods. Then, we introduce microfluidics technology and its fundamental physical principles with a focus on droplet-based microfluidics and the commonly used microfluidic generators.

1.1 Zinc oxide: a promising semiconductor for the future

1.1.1 Zinc oxide properties and applications

Zinc oxide (ZnO) stands out as one of the most promising semiconductors that has triggered interest during the past decades, as shown by the number of the published papers for the key word ZnO according to the data collections of Web of Science that gives more than 119611 papers (May 2020). In this regard, in particular zinc oxide belongs to the class of direct and wide band gap semiconductor materials with a wide direct band gap of 3.37 eV at room temperature^{45,46}. It is a

transparent conducting oxide (TCO) that has been scrutinized for electronic materials since the 1930s⁴⁷. Moreover, ZnO has the largest exciton binding energy (60 meV) among the II-VI semiconducting materials, which is 2.4 times higher than the thermal energy at room temperature ($kT = 25.7$ meV, where k is the Boltzmann constant and T is temperature). This paves the way for strong light emission at room temperature, for example, for ZnO-based light emitting devices, as well as near-UV emission, high-temperature resistance to electronic degradation, conductivity, and transparency⁴⁸.

Zinc oxide is a binary II-VI compound semiconductor; in materials science, where the electronic configurations of zinc and oxygen are $(1s)^2(2s)^2(2p)^6(3s)^2(3p)^6 - (3d)^{10}(4s)^2$ and $(1s)^2(2s)^2(2p)^4$, respectively. Under ambient conditions, it crystallizes in a hexagonal wurtzite structure, representing its most thermodynamically stable phase, with $a = b = 0.32$ nm and $c = 0.52$ nm its lattice constants, and $\frac{c}{a}$ ratio equal to 1.633 in an ideal wurtzite structure^{49,50}. This ratio is greater than the experimentally observed values. Therefore, the existing long-distance polar interactions eventually lead to a distortion of the tetrahedral angles, hence the ratio $u = \frac{3}{8} = 0.375$ has the tendency to increase in such a way that the tetrahedral distances remain almost constant³⁰.

The crystal structure of ZnO is thus determined by the tetrahedral coordinate binding geometry, in which each zinc ion is surrounded by four oxygen ions in a tetrahedral geometry and vice versa, thus involving sp^3 hybridization⁵¹. Figure 1.1 shows a schematic representation of the structure of a unitary hexagonal cell of ZnO. This figure also indicates the most important planes including (0001), which is the basal plane that plays a crucial role in the growth of the crystal, and

two other facets (1120) and (1100).

Furthermore, ZnO is a semiconducting piezoelectric material. Due to its non-centrosymmetric hexagonal wurtzite structure and basically ionic bonding, ZnO has attractive piezoelectric properties in the [0001] direction^[52]. As a matter of fact, it has been demonstrated that ZnO exhibits the largest piezoelectric tensor of all the semiconductors with tetrahedral bonding^[49]. Besides, it has been stated that its effective piezoelectric coefficient (d_{33}) is relatively variable. For example, the effective piezoelectric coefficient ranged from 14.3 pm/V to 26.7 pm/V in the case of ZnO nanobelt, whereas it was equal to 9.93 pm/V for the ZnO bulk of the (0001) face^[53]. Because of such unique properties, ZnO has been a very adapted candidate for diverse applications, for instance, nanosensors^[54], nanoactuators^[55], thin film bulk acoustic wave resonator (FBAR) and surface acoustic wave (SAW)^[56-59].

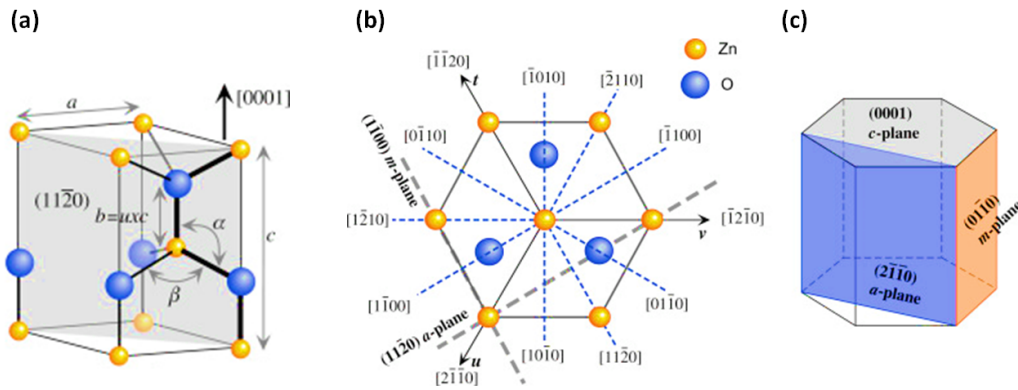


Figure 1.1 (a) Hexagonal wurtzite ZnO crystal structure, (b) Top view of the hexagonal wurtzite ZnO crystal structure showing the commonly used crystal directions on the (0001) basal plane, (c) three-dimensional (3D) vision of the hexagonal unit cell^[30].

Moreover, there is a growing interest in the use of ZnO in microelectronic devices, largely due to its semiconducting properties^[60]. Besides, its exceptional

features make it one of the most interesting materials in a variety of applications including photovoltaics^{61,62}, transparent electrodes (it can be used as a possible alternative to tin-doped indium oxide (ITO))^{63,64}, and transparent thin film transistors^{65,66}. Similarly, ZnO has generated considerable interest in the development of nanophotonic technologies and their applications⁶⁷. For example, this semiconductor material exhibits a characteristic emission spectrum including UV emission for ZnO-based quantum photonics⁶⁸, and promising ZnO-based single-photon sources (SPSs) which is of great benefit in quantum information applications⁶⁹. In addition, ZnO microspheres-based optical cavity have been extensively studied and significant resonances of whispering gallery modes (WGMs) have been observed at room temperature due to improved light confinement with high fineness and high Q factor⁷⁰. Also, thin films of ZnO have been developed to achieve both anti-reflection and passivation of the multi-crystalline silicon surface⁷¹.

Other valuable properties of ZnO incorporate the high abundance, low cost, non-toxicity and bio-compatibility, that have enabled its potential use for the development of important applications in biomedical imaging, drug delivery, gene delivery and biosensing, because of its inherent photoluminescence properties and high stability⁷²⁻⁷⁵.

Taking advantage of the useful characteristics of ZnO nanoparticles, including a wide band gap, large surface area, adaptable surface chemistry, electrostatic charge, and potential for redox cycle cascades, studies have shown that ZnO nanomaterials have the ability to selectively destroy cancer cells, which could be beneficial for cancer treatment^{76,77}. In this respect, ZnO nanoparticles also exhibit a

high degree of toxicity to bacteria and leukemic T cells^{[78][79]}. Also, ZnO nanoparticles have been shown to be a versatile nanoplatforms for various antimicrobial applications because of their interesting antibacterial properties^[80], which are recognized due to their increased specific surface area, and the dependence of the increased reactivity of the surface on the particle size. Among the most appealing features of ZnO nanomaterials is their ability to show a high efficiency and low cost that have allowed their use in the photocatalytic degradation of water pollutants^[81]. In addition, ZnO-based nanoparticles have been widely explored in the cosmetics sector, including their use in sunscreens to block ultraviolet radiation, skin care creams with inherent optical transparency and minimizing the appearance of a white layer on the skin, and so on^[82].

Application of ZnO nanoparticles		
Field of application	Example	References
Energy and electronics	Supercapacitors, solar cells, battery, photocatalysis, optoelectronic devices, gas sensors, energy conversion and storage, etc.	83-85
Biological, Biomedical and medical	Cancer imaging, anticancer, antibacterial activity, leukemic T cell treatment, drug delivery, disease detection, etc.	74,86-88
Manufacturing industry and materials	Food quality control, production of textile fabrics with specific properties: antimicrobial, self-cleaning, hydrophobic, UV protection, flame retardant, electrically conductive, heat insulating, humidity control, and high durability, etc.	89-91
Cosmetic sector	In sunscreen to protect from UV rays (UVA/UVB), helps prevent the appearance of signs of aging and improve collagen synthesis, treat various skin problems: enlarged pores, dryness, inflammation, irritation, scarring, rashes, burns, itching, and damage, etc.	82,92

Table 1.1 Overview of ZnO nanoparticles applications.

1.2 Synthesis strategies for zinc oxide fabrication

1.2.1 Nanotechnology synthesis techniques

There has been increased interest in nanotechnology mainly due to a strong revolution in the manufacture of nanomaterials with very specific features, as well as a

fascinating dependence of their properties, on the size, distribution and morphology⁹³. Such promising nanomaterials have been applied in various fields ranging from physics, chemistry, technology, to biology and medicine. There are basically two main approaches in nanotechnology for the production of nanomaterials and the fabrication of nanostructures, namely bottom-up approach (also called self-assembly approach), and top-down approach. The former nanofabrication method is based on the build-up of nanostructures from atoms and molecules having the potential to self-organize and expand so as to build nanoscale self-assemblies on the surface of the substrate⁹⁴. It may be said that the bottom-up procedure leads to a controlled production of nanostructures with more homogeneous chemical composition and with fewer defects compared to the top-down method⁹⁵. Whereas in the top-down strategy, the manufacture of nano-sized particles is largely based on cutting, milling, slicing, and shaping a bulk material into the desired product. Lithographic techniques are also employed. This approach is therefore less advantageous than the bottom-up, where well-controlled methods are widely used for the mass production of materials at lower cost. As one of the most attractive metal oxide nanomaterials, ZnO has been widely produced due to its interesting properties making its synthesis the subject of considerable studies reported by many researchers. In general, ZnO nanoparticles have been synthesized using a wide variety of techniques that can be divided into three categories: (i) physical, (ii) chemical and (iii) biological. An overview of these different strategies is shown in Figure 1.2.

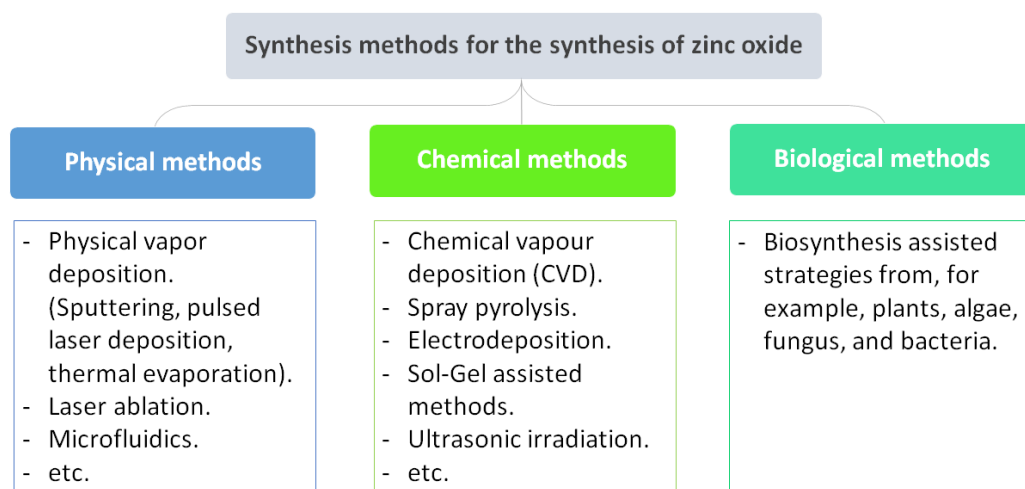


Figure 1.2 General overview of the wide variety of ZnO synthesis techniques.

1.2.2 Physical methods of ZnO nanoparticles synthesis

In physical synthesis, laser ablation^[96], pulsed laser deposition (PLD) technique^[97], physical vapour deposition (PVD)^[98], thermal evaporation^[99], and plasma method^[100], have been some of the frequently used methods employed for the generation of stable and well-defined ZnO nanostructures^[101]. In a typical laser ablation method, ZnO nanoparticles have been manufactured from bulk ZnO in a liquid by rapidly and locally heating the initial ZnO target to form nanoparticles in the medium^[102]. The synthesis medium and the experimental conditions, such as laser wavelength, fluence, ablation time, and flux density, are of primary importance for the formation of stable dispersion of nanoparticles^[103-105], and especially, for the purposeful production of ZnO semiconductor nanoparticles with specific structures and characteristics (narrow size distribution, shape, physical parameters, etc)^[106-108]. As an example of such synthesis, Svetlichnyi et al.^[105] have reported the production of ZnO nanoparticles by laser ablation using Nd:YAG laser radiation (1064 nm)

for the excitation of zinc metal targets in water and ethanol solutions. The authors observed that in ethanol solutions zinc oxidized to form dispersions of ZnO nanoparticles crystallizing in the wurtzite structure. The physical vapor deposition procedure has also been used to obtain various ZnO nanostructures; such as nanobelts, thin films, nanowires^{[98][109]}, etc, from ZnO powders. In general, PVD consists in two processes: (1) first, the evaporation of the raw material; under high temperatures, followed by atoms and/or molecules transport, and finally (2) the deposition of ZnO vapour on the substrate surface^{[110][111]}. The pressure and flow of the carrier gas as well as the temperature play an important role in controlling the final deposition step^[98]. In addition, other authors have used thermal evaporation to grow ZnO films on the surface of different substrates. It is important to note that the quality of the deposited layers as well as their properties (structure, size, etc) depended on many factors of the processing conditions such as oxygen percentage, oxidation temperature, etc^[112].

1.2.3 Chemical methods of ZnO nanoparticles synthesis

Among the commonly employed chemical techniques to produce morphologically advanced ZnO nanoparticles, we can distinguish such methods as nanoparticles generated through chemical vapour deposition (CVD)^[113], sol-gel synthesis^[114], spray pyrolysis^[115], electrodeposition^[111], and ultrasonic irradiation^{[116][117]}. Chemical vapor deposition is a process that has been generally used to manufacture ZnO semiconductor thin films^[118]. This deposition method operates under vacuum and uses precursors in the vapor phase previously generated by a precursor supply system, and deposited on the substrate surface. In general, the existence of a continuous gas flow in the reaction chamber allows the evacuation of reac-

tion unwanted products^[119]. In previous studies, it has been stated that methane may act as a reducing agent of the powder employed as a raw material to form zinc vapor. The resulting zinc vapor is subsequently reoxidized (by the oxygen supplied in the tube to the substrate)^[120]. This also helps to control the growth of ZnO nanowires and monocrystalline layers. As for the electrodeposition method, it was stated that the size, structure and morphology of ZnO nanostructures depend on the electrodeposition parameters, including electrodeposition time and potential, concentration and pH value of the electrolyte^{[121][122]}. Furthermore, Cho et al. used the spray pyrolysis technique and conventional photolithography to make ZnO-based field-effect transistors (FETs)^[123].

Sol-gel approach has been one of the most widely used chemical techniques for the synthesis of solid ZnO nanomaterials. This process is primarily used in the production of thin films, porous materials, and encapsulation; for example, of biological molecules like proteins and bioenzymes. Also known as soft chemistry, this method undergoes basically a succession of chemical reactions triggered by: (1) hydrolysis of the precursor's alkoxy groups, (2) then condensation of the hydrolyzed products occurs and their polymerization to create particles, (3) followed by expansion of particles, (4) and then the formation of networks from the clustering of particles extending through the liquid medium to form a gel. Finally, the drying process comes to provide the final ZnO product. Metal alkoxides, $M(OR)_x$ where M is a metal (Zn, in our study) and R an organic alkyl group C_nH_{2n+1} , and metal salts (like chlorides and nitrates) are the most widely used precursors. Typically, the preparation of ZnO colloidal solution takes place in an aqueous or organic medium such as alcohol. The sol-gel process also provides the possibility

to operate at much lower temperatures than conventional synthesis approaches.

1.2.4 Biological methods of ZnO nanoparticles synthesis

ZnO nanomaterials can also be yield using biosynthesis assisted techniques. For instance, by means of plant leave extracts^[124-129]. Parthasarathy et al. demonstrated the formation of ZnO nanoparticles using leaves extract of aloe vera which served as a reducing agent for the preparation, and through the addition of zinc nitrate at temperatures ranged from 60 to 80 °C^[130]. Similarly, studies by Vidya et al. also employed zinc nitrate for producing ZnO NPs with sizes varied from 30 to 35 nm. In this synthesis, authors used *Calotropis Gigantea* leaves extract^[127]. Gawade et al. reported the application of *calotropis procera* (C. P.) to synthesize spherical-shaped ZnO nanoparticles with size varied from 15 to 25 nm^[131]. Further studies reported that plants extract from other leaves such as *sapindus rarak*^[132], *ocimum basilicum* L. var. *purpurascens* benth.-lamiaceae^[129], and *nephelium lappaceum*^[128], have also been employed for ZnO NPs synthesis. Moreover, biosynthesis assisted methods of ZnO nanoparticles can also be achieved via suitable microbes like yeast, fungi, and bacteria^[133].

1.3 Nano- and microstructures of zinc oxide

Zinc oxide is unambiguously the richest of all materials as it exhibits a very large variety of structures^[134-135], thus offering a wide range of ZnO particles properties as well as their potential use for the production of functional nano- and microdevices. Many ZnO nanostructures have been produced using different synthesis strategies and under specific synthesis conditions. Figure [1.3](#) depicts an example of a great diversity of synthesized ZnO structures based on the thermal evapora-

tion of solid powders^[31]. Nanowires structures of ZnO have been reported by Zhao et al.^[136]. The synthesis was achieved using hydrothermal growth and ammonia-based etching method. ZnO structures with spherical nanocages have been produced by Gao et al.^[137]. The authors displayed that the synthesis was based on the ZnO nanocrystals self-assembly through epitaxial surface oxidation starting from solidification of the Zn liquid droplets, followed by surface oxidation and sublimation. Zinc oxide comb structures have also been reported^[138]. It is worth-

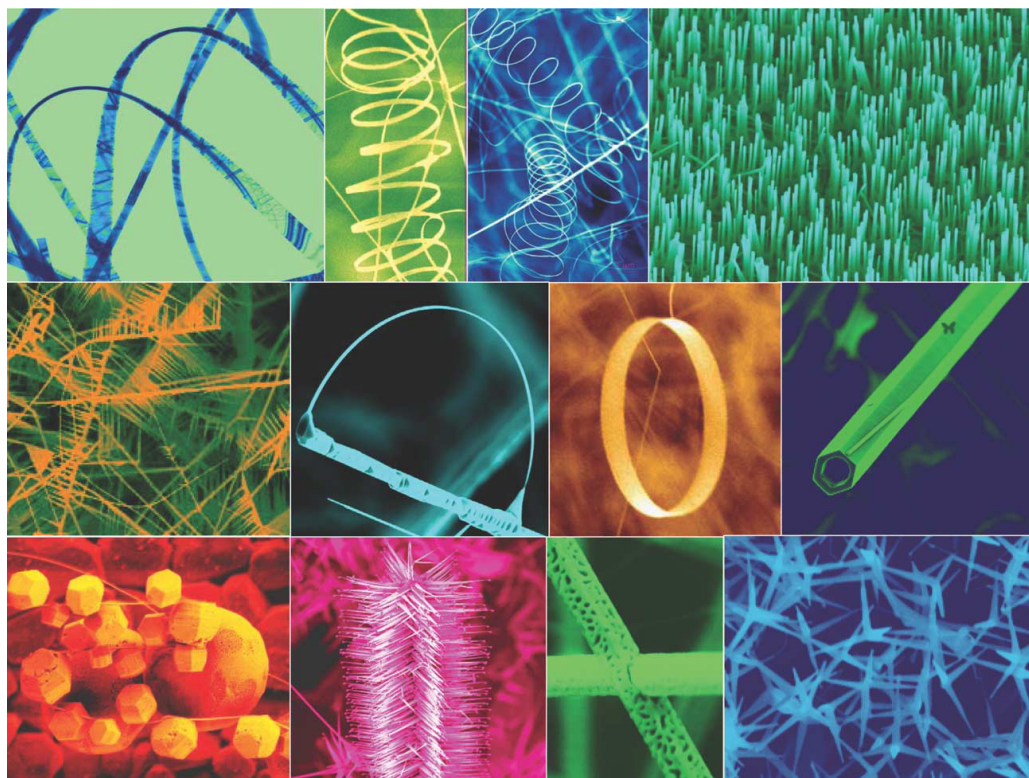


Figure 1.3 Wide variety of synthesized ZnO nanostructures. Adapted from^[31].

while to note that in this study Wang et al. stated that only the positive polar surface (0001) was active; and may have acted as an autocatalyst for the synthesis of ZnO comb-shaped structures, whereas the negative oxygen surface (000 $\bar{1}$) was inert. Therefore, they assumed that eventual growth for the manufacture of

comb-like ZnO structures could take place in the absence of external catalysts. It has also been shown that ZnO can occur in a variety of other structures including nanorings, -bows, -propellers, -springs, -belts, and -helices^{[31][135][138]}. ZnO nanotrapods^[82] and nanotubes^[139] structures have also been developed. Moreover, other zinc oxide structures such as micro-sized flowers/stars^{[140][141]}, microspheres^{[142][143]}, and microcages^[144] have been as well showing a great deal of interest.

Among the biological, chemical, and physical synthesis strategies above-mentioned for the fabrication of ZnO nanomaterials, sol-gel and microfluidics are one of the most promising and robust synthesis methods that have drawn huge attention due to the low processing pressure, temperature, and production cost, in contrast to conventional synthesis processes of producing ZnO nanoparticles. It is also worth noting that the combination of these techniques offers numerous advantages, as will be discussed further, over other synthesis procedures for fabricating micro-sized ZnO spheres, including an environmentally friendly synthesis, reliability, reproducibility, procedure performance, and technical simplicity through the use of unsophisticated and easily handled equipment. Moreover, the microfluidic-assisted approach allows the synthesis parameters to be easily adapted throughout the process, which is very useful for an accurate control of the synthesis process; at an early stage of the procedure, and thus the properties of the final particles, i.e. composition, size, and morphology, as will be discussed throughout this dissertation since we have privileged microfluidic sol-gel assisted method for fabricating our ZnO microspheres.

1.4 Microfluidics and Droplet Microfluidics: A general overview

1.4.1 The rise of microfluidics

The advent of micro- and nanotechnologies dates back several decades. This all began in 1959 when Richard Feynman launched “There’s Plenty of Room at the Bottom” at the annual American Physical Society conference at Caltech, suggesting that an enormous amount can be done in miniaturization, which could allow the study of unattainable and complicated phenomena. With these words “An Invitation to Enter a New Field of Physics” Richard Feynman opened up a new research field and was able to lead the scientific community all along to explore the nanoworld. Owing to the two challenges he had proposed, involving the construction of a tiny motor, and the possibility of fitting the entire Encyclopedia Britannica on the head of a pin^[145].

The scientific world has witnessed a significant and rapid expansion in the field of miniaturization, especially with the invention of the Scanning Tunneling Microscope (STM) by G. Binnig and H. Rohrer^[146], which allowed atomic resolution and manipulation, and paved the way for so-called nanotechnology. Thus later, in the 1980s, led to the emergence of a tremendous number of potential applications, such as micro-electro-mechanical systems (MEMS). The total size of these systems varies between 1 μm and 1 mm, besides they encompass several integrated functions (electrical, mechanical, and so on).

In addition, the acronym MEMS comes from the United States in the late 1980s, while at the same time it was also called Microsystems Technology (MST)

in Europe and Micromachines in Japan^[147]. In addition, such devices typically combine several mechanical and electrical elements in a single tiny system or integrated system to implement the desired functionality (see Figure 1.4 for some examples of MEMS devices). These systems have the ability to detect, control and actuate at the micro scale, and generate effects at the macro scale. These systems have an interdisciplinary nature that makes them present everywhere in our daily life, in automotive^[148,149], radio frequencies^[150], medical^[151], telephony^[152], aerospace^[153], etc .

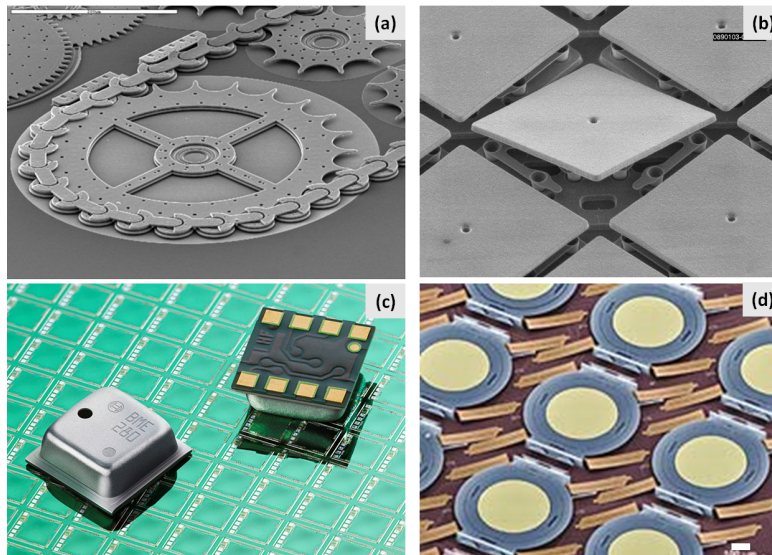


Figure 1.4 Examples of MEMS Devices. (a) a physical gear and chain. The size of the links in the chain are around $50\ \mu\text{m}$ long, less than the diameter of a human hair. Image courtesy of Sandia National Laboratories. (b) Digital micromirror device invented by Hornbeck at Texas Instruments. This device uses millions of articulated microscopic mirrors to direct light through a projection lens. (c) Bosch MEMS sensors. (d) A set of micromirrors from Lucent Technologies for routing optical light waves in the form of photons to and from one of 256 input/output optical fibres. Each mirror can be tilted in different directions to direct the light. Scale bars: $200\ \mu\text{m}$, and $100\ \mu\text{m}$.

The publications provided by^[154-162] offer excellent sources, to cite just few

examples.

Historically, in 1990s considerable progress has been made in microelectro-mechanical systems (MEMS) when it was motivated by the necessity of the miniaturization of different kind of systems (chemical, biological, and biomedical) down to submicrometric sizes. Following the trend towards miniaturization of electronic devices, these systems have employed fluid flows under more sophisticated conditions, which later gave birth to a new branch of MEMS came to be known as “microfluidics”. Since then, the field of microfluidics has been gradually set up as demonstrated thoroughly by an increased number of publications (see Figure 1.5).

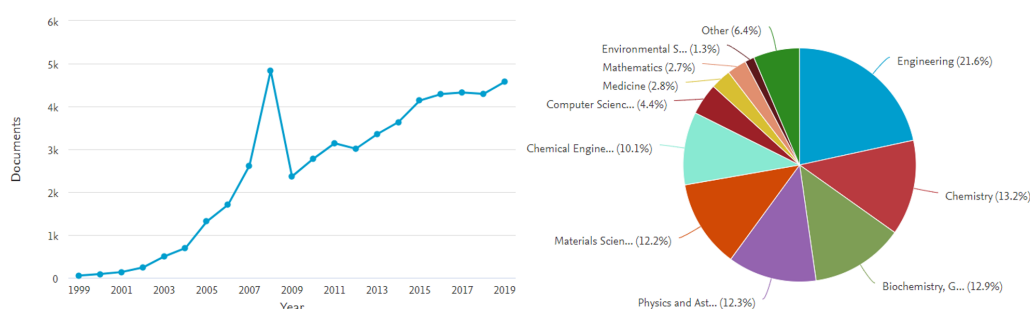


Figure 1.5 Scopus analysis of the word "microfluidics" showing real boom in this research field. published documents per year (left) and by subject area (right).

Besides, microfluidics is the science and technology dedicated to the control and manipulation of fluid flows within microsystems with integrated channels (diameters in the micrometer range ($< 1mm$)). The volumes flowing through the microchannels are typically ranging from 10^{-9} L to 10^{-18} L [157][159][163]. At this scale, the microfluidic devices have many advantages, for instance, laminar flows [164], rapid diffusion [165], rapid thermal transport [166], in addition to their safety, repeatability, efficiency and simple controllability [167]. Moreover, the large sur-

face relative to the volume of microsystems diminishes the volume of sample and reagents. Such features contribute to a reduction in cost and time needed for the performance of the reactions^[168].

1.4.2 Droplet-based microfluidic technology

The first droplet-based microfluidic experiments were conducted by Thorsen et al. in 2001. The authors demonstrated the use of a pressure-driven flow for the production of controlled aqueous droplets into a continuous oil phase^[33]. Indeed, droplet-based microfluidics presents a subcategory of microfluidics dealing with the generation and manipulation of highly monodisperse droplets of multi-phase micro-flows flowing in a carrier stream^[169]. In this emerging technique, single droplets are considered as independent micro-reactors with flexible control on size distribution (attributed to the fabrication protocol), and kHz scale rate for generation (achieved by flow rates of the different phases). These systems carry out systematically many functions for analysis, sample preparation, chemical reactions, separation, transport, detection, and so on^[163-170]. Moreover, due to the ubiquity of droplet-based microfluidics in a number of applications, numerous publications have emerged, particularly regarding some aspects including microfluidic manufacturing devices^[171-172], physics of droplets^[34,169], particle preparation^[173-174], and bio-chemical analysis^[175-177].

Droplet formation is one of the major parts of droplet-based microfluidics, it is considered as one of the first steps in its life cycle. Although two main methods are available for fabricating monodisperse droplets, including passive and active methods. The latter require the application of external forces, for instance electrical, thermal, pneumatic, acoustic and magnetic^[178-183]. Nevertheless, pas-

sive methods are the most frequently used. Different types of droplet generators have been fabricated and extensively studied^{[34][178][184]}, including co-flow^{[185][186]}, T-junction^{[187][188]} and flow-focusing^{[189][190]}, as we will discuss in more detail hereafter.

- (i) The co-flow geometry, also known as coaxial junction, is shown in Figure 1.6. It corresponds to two parallel concentric channels, where the dispersed phase fluid flows into the middle channel and continuous phase fluid flows in parallel into the other aligned channel^[32]. This geometry was first investigated experimentally and implemented in microfluidics in 2004 by Cramer et al.^[191]. The authors achieved droplet formation in two distinct regimes, dripping and jetting, by using a needle into another co-flowing immiscible fluid that led to the formation of droplets.

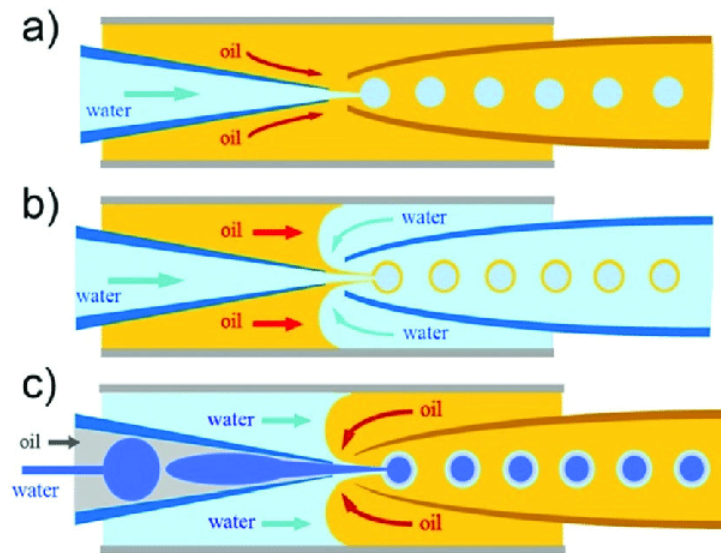


Figure 1.6 Schematic illustration of an example representing the geometry of co-flow microfluidic device for the generation of (a) single (W/O), (b) double (W/O/W) and (c) triple (W/O/W/O) emulsions^[32].

(ii) T-junction was the first geometry of microfluidic devices used to generate droplets in a periodic and controlled manner. It was first introduced by Thorsen et al.,^[33]. A typical example representing droplet production in a T-junction is demonstrated in Figure 1.7. It corresponds to two perpendicular channels, where the dispersed phase flows into the continuous phase. As a result of interactions, between the two phases, and due to the shear forces and interfacial tension at the interface of fluids^[175], the breakup of the liquid stream into droplets occurs. Therefore, droplet eventually detaches and flows within microchannels.

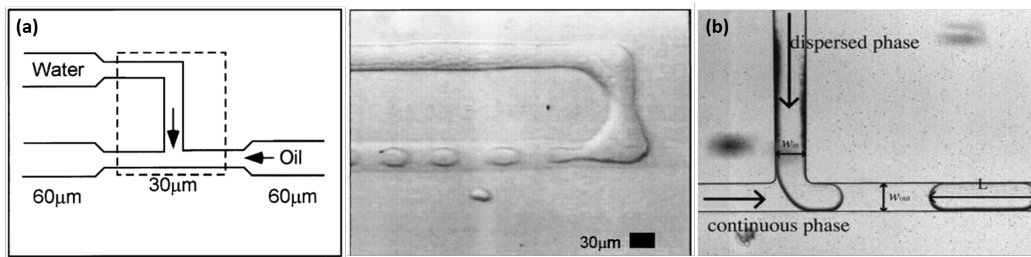


Figure 1.7 Common T-junction geometry used for the generation of droplets at 90 ° first implemented by Thorsen et al. (a)^[33], and from Baroud et al. publication^[34](b).

(iii) The flow-focusing geometry was pioneered by Ganan-Calvo et al.^[35] in order to fabricate monosized gas microbubbles in a liquid through a cylindrical geometry, it works on a so-called capillary flow-focusing where the gas is evicted of a capillary tube through a small orifice into a liquid which induces the gas bubble streaming to break into bubbles. However, it was not until 2003 that the flow focusing geometry was applied in the founding article by Anna et al.^[36] and Dreyfus et al.^[37] using an integrated channel into a plane geometry.

In this geometry, two immiscible liquids flow into three separate channels in a planar geometry, as shown in Figure 1.8. The dispersed phase liquid flows in the central channel as for the continuous phase liquid it flows into two opposite side channels. The two immiscible liquids converge toward a principal channel situated downstream of the three channels via a small orifice and get in touch. The contact between both phases results on the

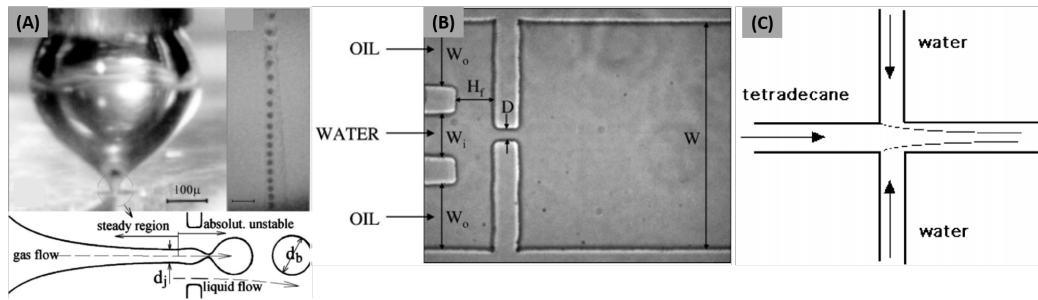


Figure 1.8 Flow-focusing geometry developed by (A) Ganan et al.^[35], (B) according to Anna et al.^[36] and Dreyfus et al.^[37].

formation of a neck which elongates and becomes thinner as the dispersed phase advances downstream. Ultimately, the dispersed phase neck breaks up into droplets^{[34][192][193]}.

Figure 1.9 shows the different typical microfluidic device geometries used to fabricate droplets, including T-junction (at different intersection angles of the continuous and dispersed phase fluids), “Head-on”, Y-shaped junction, double T-junction, “K-junction”, “V-junction”, co-flow, and flow-focusing geometries.

In the following, we will mainly focus on the geometry used during this thesis work (flow-focusing), as well as the results achieved with it.

The mechanism behind the formation of spherical monodisperse droplets is governed by forces that are generally overlooked and neglected at the macro-

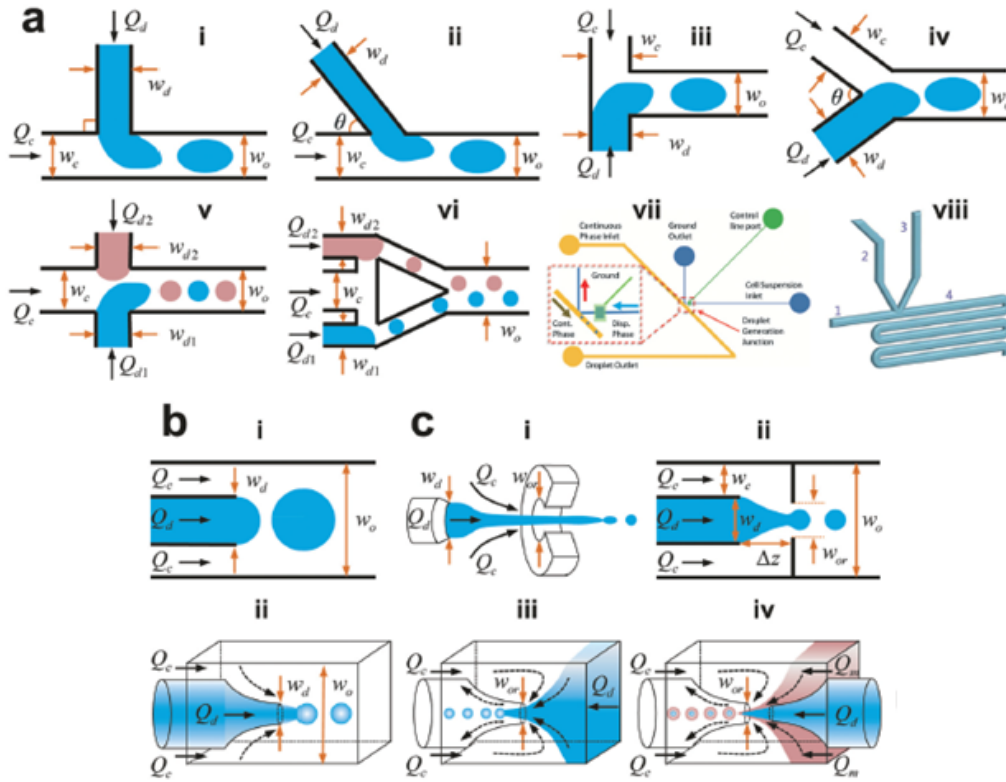


Figure 1.9 Common microfluidic device geometries for droplet generation. (a) Cross-flow: (i,ii) T-junction at different intersection angle of the continuous and dispersed phase fluids ($\theta = 90^\circ$ and $0^\circ < \theta < 90^\circ$, respectively). (iii) “Head-on” geometry ($\theta = 180^\circ$). (iv) Y-shaped junction with intersection angle of $0^\circ < \theta < 180^\circ$. Double T-junction where droplet are generated at (v) the same area, and (vi) at separated parallel T-junctions. (vii) “K-junction”. (viii) “V-junction”. (b) Co-flow: (i) Quasi-2D planar, and (ii) 3D co-flow. (c) Flow-focusing: (i) Axisymmetric, (ii) Planar, (iii) Microcapillary, (iv) Microcapillary device combining co-flow and flow-focusing geometries³⁸⁻⁴⁰.

scopic level. In the case of microfluidics, significant forces at the macroscopic scale like gravity and pressure are neglected in front of forces like capillary and interfacial tension. The interfacial tension is the property of a fluid that acts to minimize the free energy of the interface by reducing the interfacial area between two fluids. Also, the interfacial tension is important in the formation of spherical

droplets (as it is the shape of the minimum area for a given volume), as well as for droplet subsequent stability^{34,192}. Instead, the viscous forces describe how much the fluid flow throughout the microfluidic chip due to its velocity, these stresses act to stretch and extend the interface and form the droplets. In order to compare the different physical properties of liquids and describe their behavior, dimensionless numbers are very frequently used.

The bond number (Bo) describes the competition between gravitational and surface tension forces, is defined as:

$$Bo = \frac{\Delta\rho g L^2}{\sigma}$$

Where $\Delta\rho$ the mass density difference between the two fluids, g the gravity acceleration, L a characteristic length scale, and σ the interfacial tension. In the case of microfluidic systems, the Bond number (Bo) is typically small ($Bo \ll 1$). This low value indicates that gravitational forces are usually neglected at the microscopic scale.

Another characteristic number of the flow is the Reynolds number (Re) of the droplets. It illustrates the relative strength of the internal inertia forces and the viscous forces. Using the linear Stokes equation, the Reynolds number can be expressed by³³:

$$Re = \frac{\rho v L}{\mu}$$

With ρ is the mass density, v the mean velocity of the fluid, and μ the dynamic viscosity. If the inertia forces predominate this favors the turbulent flow ($Re \geq \simeq 2000$). In the opposite situation where viscous forces dominate, Re is low ($\leq \simeq$

10) promoting the very laminar flow. Basically, microfluidic systems operate at laminar flow as indicated by the low Reynolds number, due to the small channel cross-section scales and the confinement^{34,156}.

Moreover the competition between inertial forces and interfacial tension can be quantified by Weber number (We), its expression is:

$$We = \frac{\rho v^2 L}{\sigma}$$

In most applications at the microscale, the dimension scales are sufficiently small that inertia generally becomes negligible, thereby a low Weber number ($We < 1$) for microfluidics application.

Otherwise in microfluidic systems the effect of gravity can be ignored as well as the internal inertia forces as discussed directly above. Consequently, the dominant forces in the micron range are therefore those related to interfacial forces minimizing the interfacial energy between the two fluids through the formation of spherical droplets, and viscous forces acting on the dispersed phase during the deformation of the interface and the formation of the droplets. This can be denoted by the capillary number (Ca), which compares these significant forces at the microscale. Thus, this number is the most important among all dimensionless numbers, and defined as the ratio of viscous forces and interfacial forces, and is expressed by:

$$Ca = \frac{\mu v}{\sigma}$$

Where μ is generally the viscosity of the most viscous fluid in the two-phase system, v is the velocity of that phase. Besides, the capillary number varies between

Symbol	Name	Formula	Physical meaning
Bo	Bond number	$\frac{\rho g L}{\sigma}$	Gravitational/ Surface tension
Re	Reynolds number	$\frac{\rho v L}{\mu}$	Internal inertia/ Viscous
We	Weber number	$\frac{\rho v^2 L}{\sigma}$	Inertial/ Interfa- cial tension
Ca	Capillary number	$\frac{\mu v}{\sigma}$	Viscous/ Interfa- cial tension

Table 1.2 Dimensionless numbers in droplet microfluidics.

10^{-3} and 10 in most microfluidic devices³⁴.

Furthermore, the competition between interfacial tension and viscosity leads to three different flow regimes in microfluidic devices: (i) Dripping regime, where interfacial tension forces dominate viscous forces, which is indicated by low capillary number ($Ca \ll 1$). Under such a condition, one can observe the production of highly monodisperse spherical droplets. (ii) Parallel flow, occurred in the opposite situation when viscous forces dominate interfacial tension forces ($Ca \gg 1$), resulting on large deformations of droplets. (iii) Jetting regime, corresponds to the situation when capillary forces become comparable to viscous forces ($Ca = 1$). Consequently, polydisperse droplets are formed resulting on the breakup of swelling jet downstream.

Chapter 2

Materials and Methods

In this chapter, we present microfabrication techniques for microfluidics. We discuss the droplet-based microfluidics technology for particles synthesis. Afterwards, we provide details of our optofluidic detection setup. Finally, we present the experimental characterization techniques used.

2.1 Microfabrication techniques for microfluidics

2.1.1 State of the art

The fabrication of microfluidic devices can be considered as the core part of droplet microfluidics. Actually, there are several micro-manufacturing techniques such as micromachining using silicon or glass-based substrates, molding replication, modular assembly, 3D printing, and so on. Further, microfluidic device materials can be divided into three categories: (i) inorganic (silicon, glass and ceramics), (ii) polymeric (elastomers and thermoplastics), and (iii) paper. Silicon was the first material used to build microsystems for microfluidics, its use flowed nat-

urally from the silicon technologies of microelectronics that were well-developed. Indeed, silicon has its own pros and cons and is appropriate to different experimental conditions. Though the silicon is easy to manufacture and transparent to infrared, it presents a disadvantage of not being transparent to visible light, which makes typical optical detections, like fluorescence detection or fluid imaging difficult to achieve. In order to overcome the silicon opacity issue, the employment of transparent materials like glass or polymer was required. However, the fabrication techniques for silicon and glass are expensive, time-consuming, and require access to specialized facilities^[194-196]. Considerable work was alternatively performed to circumvent these disadvantages and the focus was shifted to include polymers in order to fabricate microfluidic devices. These methods are called soft-lithography. Polydimethylsiloxane (PDMS) has become undoubtedly its primary material of choice. This polymer has been providing a superior platform to support most microfluidic chips fabrications. Historically, it was pioneered by the group of G.M. Whitesides in 1990s at Harvard university^[197] and Manz et al. at Imperial College of Science in London^[198]. Furthermore, soft-lithography is a standard microfabrication technique that has led to the blow up of microfluidics in a wide variety of applications ranging from physics to chemistry and biology. The term 'lithography' refers to Greek words, that can be divided into "Litho" as stone and "Graphy" to write.

In other words, this technique makes it possible to produce a multitude of similar devices by molding on a previously fabricated master mold generally by photolithography technique. Additionally, PDMS also known as dimethylpolysiloxane or dimethicone is one of the most widely used silicone-based organic poly-

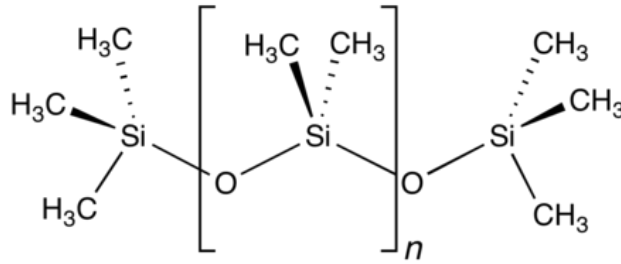


Figure 2.1 Chemical structure of polydimethylsiloxane (PDMS)

mers. Indeed, its use extends from numerous advantages coming from its intrinsic properties: (1) biocompatibility or nontoxicity^[199], (2) good optical transparency (240 nm - 1100 nm), (3) low autofluorescence^[200], (4) moldability^{[201][202]} (a sub-micron resolution through modified PDMS), (5) simplicity of sealing with a glass substrate (under easy plasma treatment to form a microfluidic device), (6) high gas permeability^{[203][204]} (for on-chip cells culture), (7) controlled PDMS thickness on a glass substrate (which enables the fabrication of stacking devices and the incorporation of micro-valves), (8) low curing temperature, (9) flexibility (young's elastic modulus of $\sim 0.5\text{-}4\text{ MPa}$ ^{[205][206]}), (10) low-cost, and (11) good mechanical properties (which could be easily tuned by changing the degree of cross-linking in the polymer, heating temperature, molar ratio of curing agent and monomer so that the bulk PDMS can be polymerized with different softness).

The chemical formula of PDMS is $\text{CH}_3[\text{Si}(\text{CH}_3)_2\text{O}]_n\text{Si}(\text{CH}_3)_3$, where n is the number of monomer repetitions (see Figure 2.1). Its siloxane bonds make it possible to obtain a flexible polymer chain with a high level of viscoelasticity depending on the size of the monomer chain. The higher the degree of cross-linking, the stiffer the sample will be. Conversely, the lower the degree of PDMS network's crosslinking, the lower its stiffness^[207].

We will describe in more detail later the microfluidic device manufacturing method adopted during this thesis work.

2.1.2 Soft micro-lithography technique

Microfluidic devices are designed and manufactured by patterning channels in PDMS using conventional soft-lithography methods²⁰⁸, which briefly refers to a group of techniques consisting on four steps: i) the fabrication of a master mold via photolithography, ii) casting PDMS prepolymer mixture in the master mold and fabrication of a PDMS-based negative replica of the template, iii) curing and separating the PDMS from the template, and iv) sealing of the PDMS stamp to a substrate.

Photolithography, known also as optical lithography, is one of the most employed microfabrication techniques, it consists of printing with light a desired pattern into a light-sensitive material to ultraviolet (UV) light. Figure 2.3 shows how master molds are fabricated with negative relief patterns of SU-8 photoresist on a silicon (Si) wafer using photolithography. The main steps of this technique are detailed as follows.

In the first step, a silicone wafer is coated with a layer of photoresist. It is important to note that there is a group of photoresists with different properties. The choice of the appropriate photoresist depends mainly on the desired film thickness (see Figure 2.2). In the present work, a silicone wafer was coated with a layer of SU8-2025 photoresist (MicroChem Corporation (Newton, MA, USA)) so as to form a uniform layer on the wafer surface. Basically, the photoresist application is carried out by spin-coating where the thickness of the deposited layer is programmed by speed, acceleration and time of rotation. The SU8-2025 photoresist

was spin-coated onto the silicon wafers through two cycles. First spun at 500 rpm for 5 to 10 seconds with acceleration of 100 rpm/second, and the second cycle depended on the desired thickness.

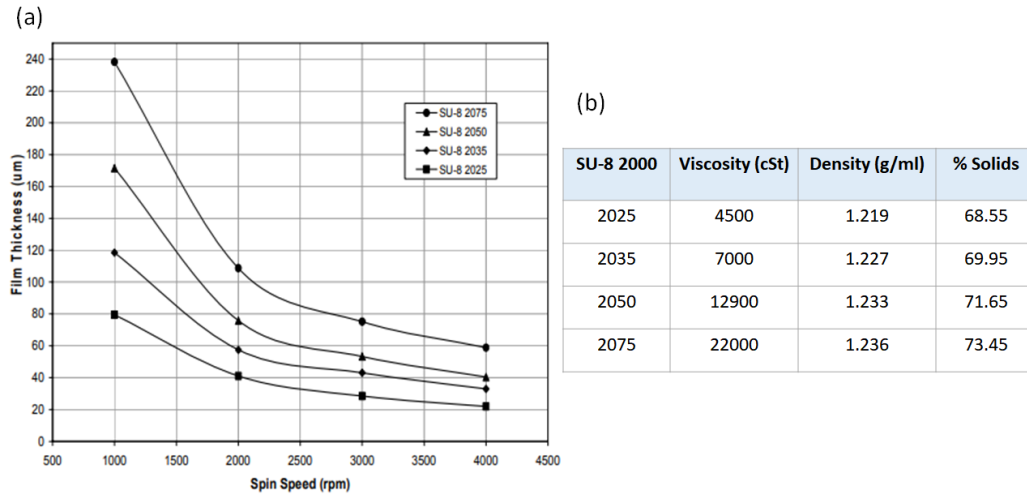


Figure 2.2 Spin Speed versus film thickness of SU-8 2000 resists (SU-8 2025, SU-8 2035, SU-8 2050 and SU-8 2075) (a) and their properties (viscosity, density, and % solids) (b).

The spin-coating process is subsequently followed by “Soft baking”. It consisted of exposing the wafer and photoresist to low temperatures (65 °C and 95 °C) by means of hot plates under ambient atmosphere. This step aims at eliminating solvents in the resist and improving its bonding, as well as its solidification. The recommended values of the parameters including soft bake times, spinning speed, baking temperature and duration can be determined from the available photoresist processing guidelines.

After photoresist coating, mask alignment is one of the most important and critical steps in the photolithography process. For the work involved in this dissertation, we used MJB4 mask aligner from SUSS MicroTec with an I-line type

lithography optic system through an exposure wavelength of 365 nm. At first, the silicon wafer is placed in the support of UV exposure machine. The photomask is then loaded into the mask holder on top of the substrate and photoresist. It must be aligned with the wafer's surface, so that the pattern can be transferred onto its surface. Once the mask has been accurately aligned with the silicon wafer, the assembly is then irradiated with UV light through the pattern on the photomask under programmed time duration, which is determined by the energy adsorption of the photoresist and the power of the lamp. Besides, photoresist is a light-sensitive polymer which can be positive or negative, depending on whether it is desirable to make the exposed regions more soluble during development process, or vice versa.

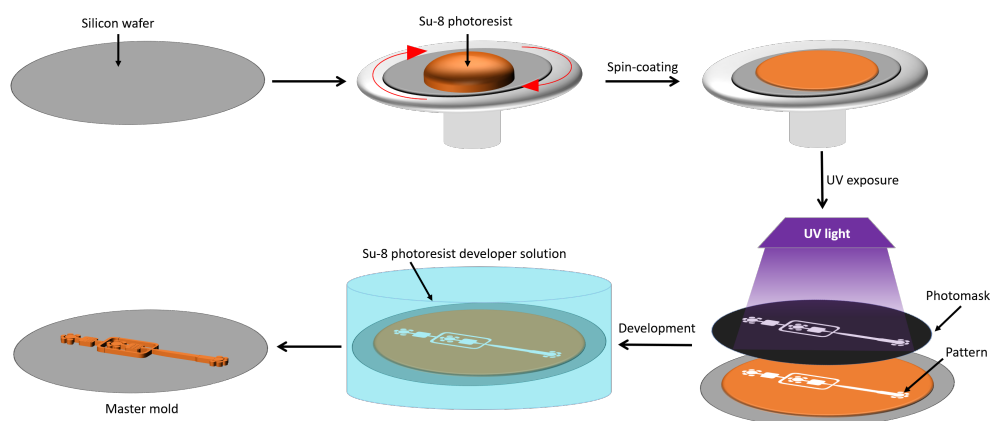


Figure 2.3 Schematic illustration of a SU-8 mold fabrication on a silicone wafer through photolithography.

As aforesaid, we used SU8-2025 photoresist which is a negative photoresist. Upon UV illumination, the uncovered zones of the photoresist polymerized. A post expose bake (PEB) was then performed in order to selectively cross-link the exposed regions of the film using two-step contact through a hot plate at 65 °C

and 95 °C. Following PEB, a development process was carried out using SU-8 developer (propylene glycol monomethyl ether acetate; MicroChem Corporation). Consequently, the exposed regions became insoluble in the developing solution, instead, the unexposed regions of the photoresist breakdown and became soluble and hence washed away, thus leaving behind the geometric pattern on the silicon substrate. Thereafter, the substrate was rinsed briefly with isopropyl alcohol (IPA), and dried with a gentle stream of air (or nitrogen). Subsequently, an optional hard bake was done between 150 and 200 °C for few minutes in a convection oven to further cross-link the material.

Through careful adjustments of the microfabrication process conditions, the master mold was successfully fabricated and served many times manufacturing microfluidic chips using PDMS technology.

The fabrication of the microfluidic devices performed in our experiments involved the employment of the previously fabricated master mold in order to produce a microstructure replica by molding the polydimethylsiloxane (PDMS). The PDMS (Sylgard 184 silicone elastomer base, Sigma-Aldrich) was first mixed with curing agent (Sylgard 184 silicone elastomer, Sigma-Aldrich) with a weight ratio of 10:1, the mixture was then degassed using a vacuum pump at room temperature and the solution was poured onto the mold and placed in the oven for polymerization at 75°C for 2 h. The PDMS stamp was then removed from the mold; and a replica of the microchannels was obtained. Inlet (to introduce the fluid(s) into the channel(s)) and outlet (to collect the product) ports were punched out using a Miltex biopsy punch with plunger of 1 mm diameter.

The PDMS block and the glass slide (50 mm x 75 mm) were eventually

cleaned and dried (with compressed air), and finally treated with oxygen plasma for 20 s to enable their bonding and sealed to make the microfluidic device. Figure 2.4 shows the schematic illustration of the PDMS molding procedure.

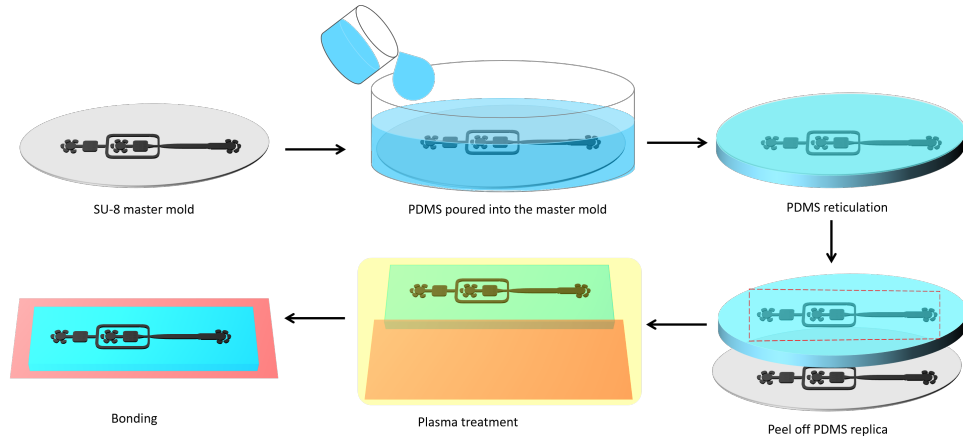


Figure 2.4 Schematic fabrication of microfluidic devices with PDMS replica molding.

2.2 Droplet microfluidics

2.2.1 Droplet-based microfluidics for particles synthesis

Droplet-based microfluidics has evolved into a powerful tool for the synthesis of monodisperse nanoparticles and microparticles with uniform sizes and morphologies²⁰⁹⁻²¹². In particular, the production of highly ordered self-assembled colloidal crystals and mesocrystals has gained recently increasing attention for many applications based on the design of integrated materials with advanced features at the nano- and micro-scales^{411,178,213}. Nevertheless, the high control and accuracy of the structure and morphology of such arrangements at the microscale is still challenging. In contrast to classical crystallization process, colloidal crystallisation is based on a particle-mediated aggregation and self-assembly of nu-

cleated nanocrystals. The formation of mesocrystals includes oriented attachment growth mechanism involving self-organization and self-assembly of particles or crystallites along a preferential crystallographic orientation giving hence rise to the formation of a crystal in an asymmetric manner^{5,20,214}.

In the present thesis work, we focus more deeply on a precise ZnO microparticles fabrication using droplet-based microfluidics. As we have detailed in Chapter [1](#), zinc oxide is of particular interest owing to its properties, including a wide band gap (3.37 eV) and a large exciton binding energy (60 meV) at room temperature. Besides, this makes it one of the most used materials in optoelectronic devices, ultraviolet photodetectors, optofluidic micro-thermometry²¹⁵, UV sensing and emissions²¹⁶⁻²¹⁸. Furthermore, it is a promising and very appealing material for a variety of other emerging applications including solar cells²¹⁹⁻²²², photocatalysis²²³⁻²²⁵ and water treatment^{218,225}. Various synthetic approaches using different chemical and physical methods²²⁶, such as electro-deposition²²⁷⁻²²⁹, sol-gel²³⁰, and hydrothermal²³¹⁻²³⁴ have been employed to fabricate several types of ZnO structures including nanorods^{227,235}, nanowires^{232,236}, micro^{231,233,234} and nanospheres^{237,238}. Nevertheless, the fabrication of well defined micrometer-sized particles is still challenging. Among all the aforementioned studies, only two reported in references^{233,234} are related to the synthesis of ZnO microparticles, which suffer from a poor control of the particle sizes and a narrow particle size distribution, because of the use of a standard hydrothermal condensation procedure.

Microfluidic methods were applied recently to synthesize ZnO nanoparticles^{169,178}. Various experimental parameters were shown to have a significant effect on the fi-

nal properties of these nanoparticles. For instance, Zukas et al. investigated the effect of reagent concentrations, reactor design, temperature and residence time on zinc oxide particle size, size distribution and morphology by using a co-flow microfluidic geometry^[218]. Baruah et al. reported the synthesis of ZnO nanospindles, nanospheres and nanosheets with different sizes by changing the microfluidic channel dimensions and the flow rate^[224]. A continuous synthesis of ZnO nanoparticles has been reported by Kang et al. using a silicon-based microfluidic system and a time pulsed mixing method^[219]. Ladanov et al. reported the synthesis of ZnO nanowires by hydrothermal growth on a silicon substrate^[239].

However, as mentioned above, these microfluidic studies were mainly devoted to the synthesis of ZnO nanoparticles with sizes ranging at the sub-micron scale. More particularly, the fabrication of zinc oxide microspheres and microcapsules with highly monodisperse and controlled size ranging in the micrometer range (and above) is still missing. Due to their particular properties particles with such features have gathered increased attention in many industrial and academic research fields, notably in bioreactors and optical biosensors^{[70][240-243]}, catalysis^[244-247], biomedical fields such as drug delivery^[248-251], and bio-pharmaceuticals^[252-253].

2.2.2 Optofluidic detection setup

Control and monitoring of droplet size and droplet size distribution are among the most relevant and challenging characteristics of droplets. They can affect their use for a highly quantitative analysis. Their determination is often challenging. Various methods have been developed in order to monitor in real-time the size and distribution of large populations of droplets.

Here we present a home built optofluidic system, which was developed in our

group during the PhD thesis of Zain Hayat (2015-2018). It enables the detection of the fluorescence of droplets at kHz rates in highly sensitive manner and in a broadband range. For this purpose, microdroplets must be doped with fluorescent dyes. The design of such a system relies on the use of laser sources, a set a dichroic mirrors, filters and a parabolic reflector (PR), as detailed in Figure 2.5.

A particular feature for this optofluidic setup, relies on the use of an off-axis parabolic reflector (PR) with a 3.2 mm hole (MPD269V-M01, Thorlabs). It enables to collect efficiently the emitted fluorescence of the droplets over a large band of wavelength (including wavelengths that are shorter than the excitation wavelength). The hole is just large enough to accept a collimated laser beam input without altering the reflection properties of the parabolic mirror. It enables the incident excitation laser beam to hit the sample without using any cut-off dichroic filter and to collect a large amount of emitted fluorescence of the sample. The excitation sources, which consisted of two CW filtered laser beams (532 nm and 488 nm), may be merged and directed together to target the sample on the microscope stage, with a non polarizing beamsplitter (BS). A half-wave plate ($\lambda/2$) and a polarizer (P) enable to modulate the intensity of the laser beam.

Imaging system consists of an Olympus IX73 inverted microscope, equipped with an integrated XY stage with flexible manual positioning that allowed us to easily track microdroplets and microparticles. The laser light is first reflected up into the microscope objective and focused in the microfluidic channel using a dichroic mirror (DM1, DiO2-R532, Semrock). Light emitted from fluorescent droplets is then captured by the microscope objective and channeled back to the detectors through the parabolic reflector and a set of lenses and a periscope. Flu-

orescence light is separated from the laser beam by notch filters (NF-488 and NF-532; Semrock) and then split into tow parts, below and above 560 nm, thanks to a single-edge dichroic beam splitter DM2 (FF562-DiO3, Semrock). Detectors consist of two photomultiplier tubes (PMT, H5784-20; Hamamatsu Photonics). The signal output from the PMTs is then analysed using a PCIe-7841R FPGA acquisition card (National Instruments) which execute a FPGA Labview program at an acquisition rate which maybe increased up to 200 kHz. Flowing droplets are identified by periodic peaks of fluorecence with a typical period of about 1 ms.

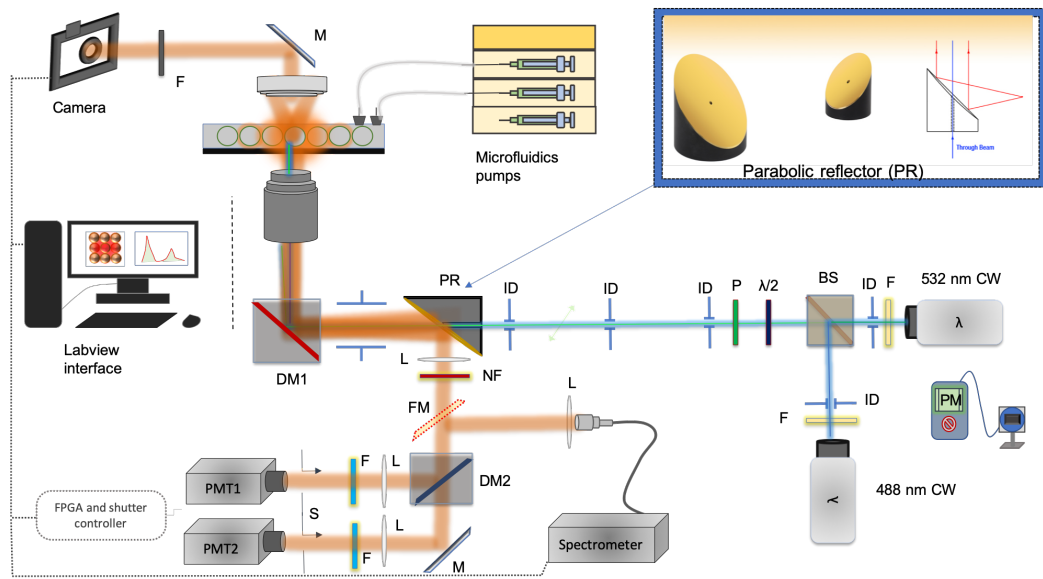


Figure 2.5 Experimental optofluidic setup for high throughput fluorescence measurements. Two CW lasers can be used simultaneously for excitation (532 nm and 488 nm) with the use of a beamsplitter (BS), a set mirrors (M), iris diaphragms (ID), a half-wave plate ($\lambda/2$), a polarizer (P) and DM1 dichroic mirror (DiO2-R532, Semrock). For the detection part, a notch filter (NF) is used to avoid pollution of recorded signals by multi-reflected excitation light. Two components of the fluorescence signals emitted by dye-doped droplets are separated with DM2 dichroic mirror (FF562-DiO3, Semrock), filtered, and collected on two different photo-multiplier tubes (PMTs).

2.3 Characterisation techniques

2.3.1 Scanning electron microscopy

Scanning Electron Microscopy (SEM) is a remarkably versatile technique that has long been used to examine and visualize the morphology of microstructure and characterize chemical composition. It involves the use of a fine electron beam produced from electrons. These are produced at the top of the column and accelerated downwards. They then pass through a combination of lenses and apertures where electromagnetic lenses are used to focus the electron beam produced on the specimen. In fact, since the 1890s when it was demonstrated that the magnetic field can deflect electrons in numerous experiments, electron microscopy was developed and the light source was replaced by a high-energy electron beam²⁵⁴⁻²⁵⁶. The electron beam then interacts with the surface of the sample deposited on a moving vacuum stage. The interaction between the electrons and the sample leads to the formation of secondary electrons of lower energy which are amplified, detected by appropriate detectors and converted into different electrical signals. This allows the topography of the scanned surface to be reconstructed and an image to be provided.

2.3.2 Transmission electron microscopy

Transmission Electron Microscope (TEM) was pioneered, in 1931, by a German electrical engineer and physicist, Max Knoll and Ernst Ruska^{257/258}. The TEM allows to perform detailed atomic and electronic structures characterization of a specimen as well as the analysis of its chemical composition²⁵⁹. The structural

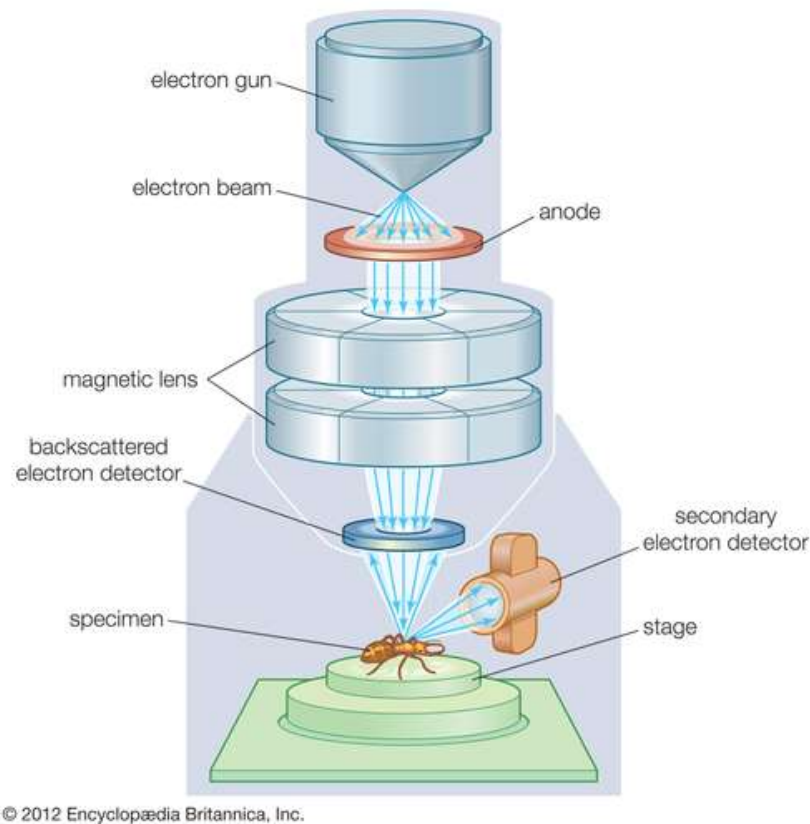


Figure 2.6 Schematic diagram of Scanning Electron Microscope (from Encyclopædia Britannica, Inc⁴¹).

characterization of the TEM consists on the use of a focused electron beam of high energy electrons, focused using electromagnetic coil, within a high vacuum. The transmitted electrons through the sample are then detected and the image is magnified using the projector lens and focused onto an imaging device^{258,260}. The TEM provides very good resolution, of better than 1 nm for typical TEM, at high magnification imaging.

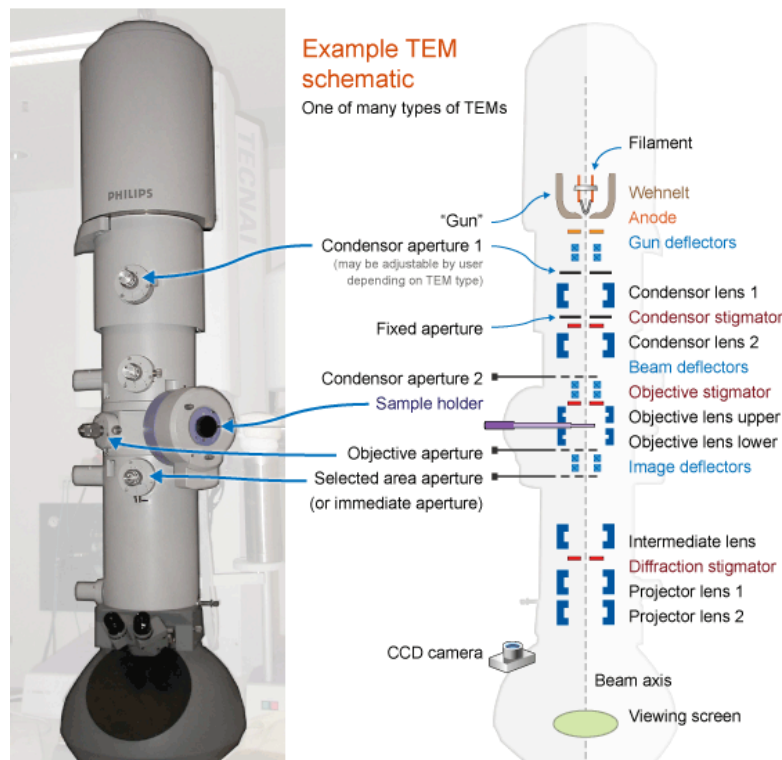


Figure 2.7 Transmission Electron Microscope schematic diagram⁴².

2.3.3 Atomic force microscopy

The atomic force microscopy (AFM), also known as the scanning force microscopy (SFM), was developed in 1986^{261,262}. It is a very high-resolution, on the order of 1 nm lateral and 0.07 nm subangstrom²⁶², technique for three-dimensional topographic imaging of a sample surface. It can be also applied to perform force spectroscopy and manipulating the sample properties like scanning probe lithography, and atomic manipulation. It involves a very sharp tip, also called needle, probe, or stylus, located at the end of a flexible cantilever made from Si or Si_3N_4 ^{43,262}. The tip is then moving across the sample surface and touching it. The tip-surface contact enables in fact precise scanning over a sample surface either by attractive or

repulsive forces causing the cantilever to deflect towards or away from the surface, respectively. Topographic information is produced using an optical system using a laser beam. It consists of sending a laser light on top of the cantilever. The incident beam is then reflected to detect cantilever deviations to or from the surface, causing slight changes in the direction of the reflected beam. These changes are then recorded on a photodetection area where the displacement can be measured by measuring the signal intensity or by triangulation²⁶².

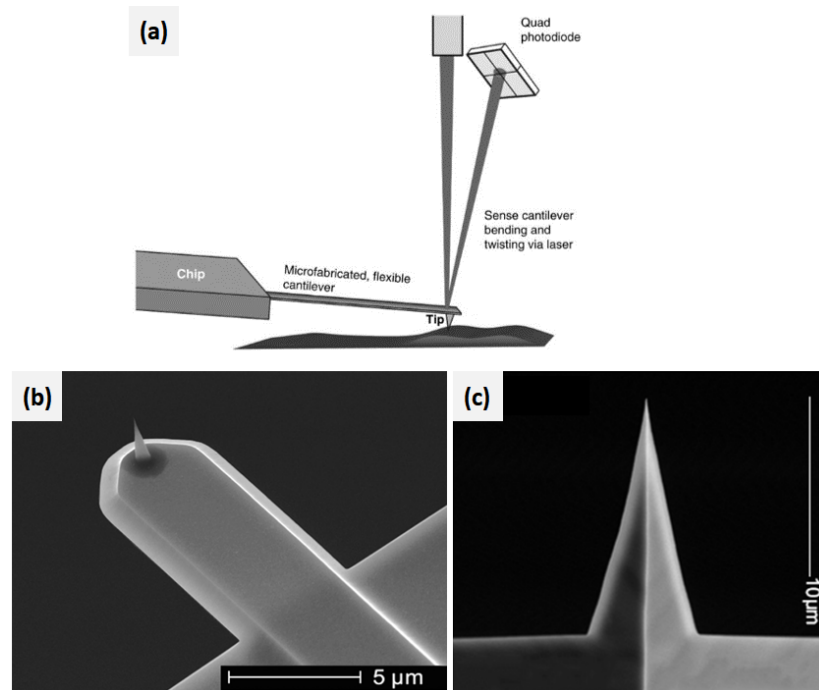


Figure 2.8 (a) Illustration of a typical atomic force microscope showing its components including tip, cantilever, laser beam, and quad photodiode⁴³. SEM images of a real cantilever and tip (b) of the atomic force microscope used during our experiments, and (c) taken from the front side.

2.3.4 Confocal microscopy

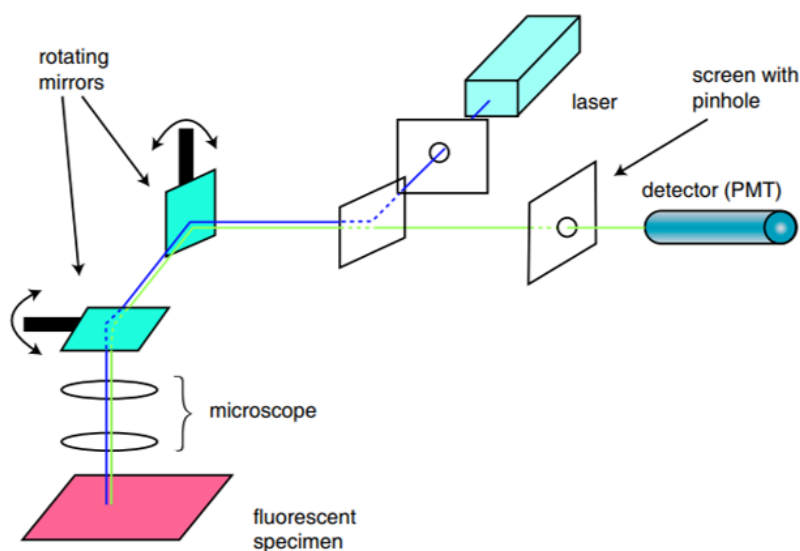


Figure 2.9 Confocal microscope basic setup. The laser light is scanned over the sample by the scanning mirrors to pass to the detector through a pinhole in its path.^[44]

The confocal microscope (CM) was invented by Marvin Minsky^[263] in 1955. It is a powerful and unique imaging technique that has been increasingly used for detection and visualization of specimens comprising fluorescent molecules. This technique allows the reconstruction of three-dimensional (3D) images with high resolution. It involves the excitation of the dyed specimen with laser light, at suitable wavelength, at a particular point of the sample. Ultimately, the laser scan across the entire sample by means of a set of horizontal and vertical, X-Y scanner, scanning mirrors earlier reflected and directed by a dichroic mirror. The resulting fluorescence of the fluorochromes, is then detected by the photomultiplier^[44] and the laser light is passed through a pinhole positioned in front of the photomultiplier in order to capture the fluorescence coming from the focal plane, thus considerably

improving the Z resolution^{44,262}.

2.3.5 Fluorescence recovery after photobleaching technique

Fluorescence Recovery After Photobleaching (FRAP) is a powerful fluorescence imaging based technique. It has been widely used to study the dynamics of local mobile fluorescent molecules at the micrometer scale^{264,265}. Basically, the sample is labeled with a fluorescent molecule. Then, the diffusion processes is analyzed to perform measurements of kinetic properties. For example, mobile fraction, diffusion coefficient, and transport rate of the fluorescently labeled molecules²⁶⁶⁻²⁶⁸. This technique will be discussed more extensively in Chapter 4.

Chapter 3

Synthesis and Structural Properties of monodisperse Zinc Oxide microspheres

In this chapter, we describe the microfluidic-based synthesis for the fabrication and real-time monitoring of uniform-sized and stable zinc oxide microdroplets. We also present the experimental setup used. We study the kinetics of these microdroplets. Next, we perform experimental investigations on the parameters controlling zinc oxide droplets formation and stability. We also examine the envelope structure of the microspheres using scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

Parts of this chapter were first published in N. Ghifari et al.^{[269](#)}.

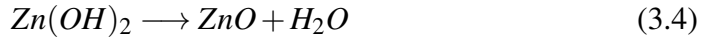
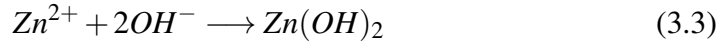
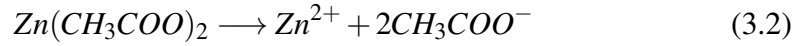
3.1 Microfluidic-assisted synthesis of zinc oxide and droplet production monitoring

3.1.1 Droplet synthesis using microfluidics

The synthesis of colloidal ZnO nanoparticles (NPs) can be performed via different types of precursors dissolved in alcohol solvents as already pointed out. In this work, zinc oxide NPs were prepared using soft-chemistry (sol-gel) as it offers a cheap and simple way with a high throughput. First, 0.6 g of zinc acetate dehydrate $Zn(CH_3COO)_2 \cdot 2H_2O$ (99.999 %, Sigma-Aldrich) was used as ZnO precursor and dissolved in 5 ml of methanol ($\geq 99.5\%$, GPR RECTAPUR ®, VWR Chemicals). Next, the solution was heated to 60°C under magnetic stirring during 1 h until the zinc acetate dehydrate was fully dissolved and the mixture turned into a homogeneous and transparent solution. These nanoparticles served as primary building units for ZnO droplets and particles.

ZnO nanoparticles grow according to a formation mechanism which includes successive chemical reactions. First, the dissolution of zinc acetate dehydrate in the presence of methanol contributes to the dehydration of zinc oxide precursor which results in the formation of anhydrous zinc acetate and water. Followed by a preliminary dissolution of the anhydrous zinc acetate to form the zinc ion and acetate. The latter will become acetic acid. It then takes place a chemical reaction between the species present (zinc and hydroxide ions) in the solution causing the precipitation of zinc hydroxide, which is ultimately converted to ZnO nanoparticles. The formation mechanism of zinc oxide nanoparticles from zinc

acetate can be summarized in these chemical reactions:



Afterwards, the resulting ZnO nanoparticles dispersion was immediately transferred into syringes and supplied to the microfluidic device using Nemesys syringe pumps (Cetoni GmbH) in order to generate highly monodisperse droplets in a controlled manner. Therefore, we used PDMS-based microfluidic flow-focusing devices fabricated by patterning channels in PDMS using conventional soft-lithography method^{[197][208]}. The microfluidic chip manufacture was detailed above.

The microfluidic device consisted of two inputs, one for the carrier oil phase and the other for the dispersed phase, a main square channel cross section of about $60 \mu m \times 60 \mu m$ and an output for the collection of the droplets in a Petri dish. The flow rates of the carrier oil (Q_c) and the dispersed phase (ZnO dispersion) (Q_d) were set using Nemesys syringe pumps (Cetoni GmbH). Hence a suitable fluorocarbon oil HFE 7500 ($C_3F_7CF(OC_2H_5)CF(CF_3)_2$) (3-ethoxy-dodecafluoro-2-trifluoromethyl- hexane, Inventec) having a density of $1.61 g/cm^3$ was used as

the carrier oil phase. Actually, this oil has the advantage of not inducing PDMS swelling and being also chemically inert. More important of our application, it is very convenient for our microparticles synthesis protocol and does not solubilize any of the ZnO dispersion solutions. The schematic of the experimental setup is shown in Figure 3.1.

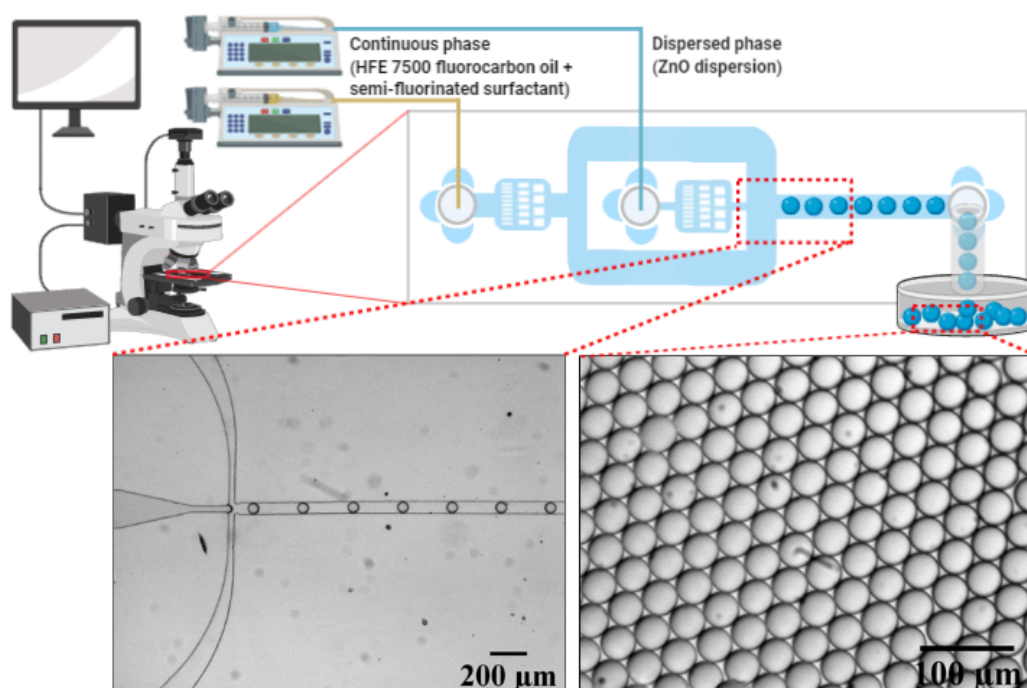


Figure 3.1 (Top) Schematic illustration of the microfluidics setup used and the flow-focusing microfluidics design for zinc oxide droplets generation. (Down) Optical micrographs of real-time generation of droplets showing stable and monodisperse zinc oxide droplets fabrication.

The dispersed phase consisted of a zinc oxide solution (dispersion), which flowed into the central microfluidic channel. This phase was squeezed by the two streams of the continuous oil phase and resulted in a breakup of zinc oxide colloidal solution into small droplets. The formation of droplets is achieved when the interfacial forces dominate the viscous forces. This is defined by the capil-

lary number ($Ca = \frac{\mu U}{\sigma} \ll 1$, where μ is the viscosity, U the velocity, and σ the interfacial tension).

Once the droplets were generated at the nozzle region of the microfluidic device, they were transported along the microfluidic channel by the carrier oil phase and moved towards the outlet. The droplets thus generated were then collected in a Petri dish (pre-filled with a solution of HFE 7500 oil) using a tube connected to the output of the microfluidic device, and kept at room temperature under atmospheric pressure. Figure 3.2 depicts the schematic illustration of the formation mechanism of the ZnO microspheres.

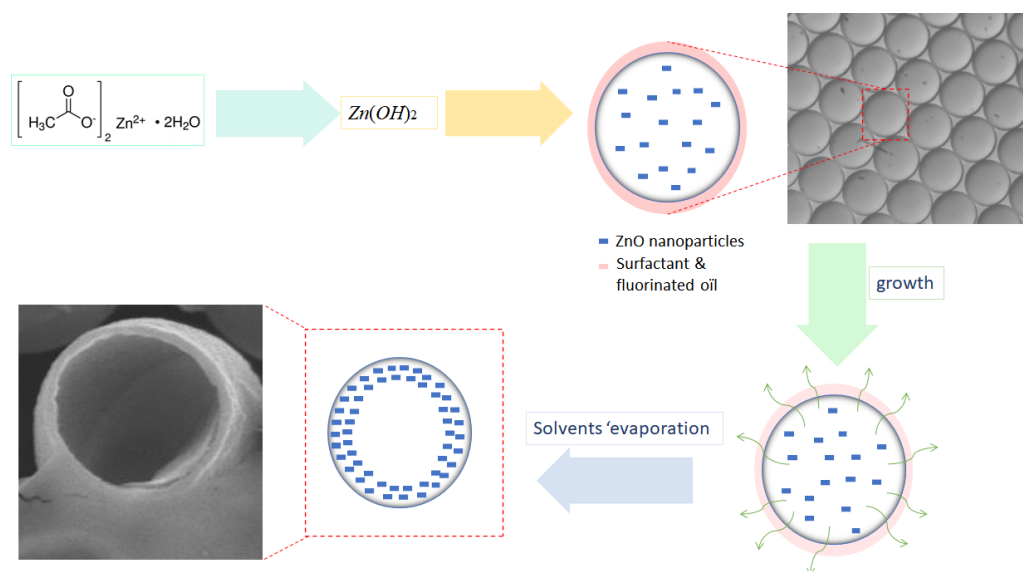


Figure 3.2 Schematic illustration of the formation mechanism of ZnO microcapsules.

3.1.2 Droplet kinetics study

We studied the kinetics of zinc oxide microcapsules formation from microdroplets having a diameter of about $48\ \mu m$. The results are shown in Figure 3.3. Figure 3.3(a) shows that the decrease of droplets size versus time follows two regimes. The first regime is relatively fast and lasted about 25 minutes, during which the droplets size dropped from $44\ \mu m$ to $21\ \mu m$ (that is approximately 1.25 times larger than the final size of the microparticles). We observe experimentally that the condensation of ZnO nanoparticles starts significantly and practically one hour after droplets production and collection in the Petri dish. It is also interesting to note that during this step, the condensation kinetics are fast and the droplets size decreased linearly with time. The second regime of droplets size reduction is much slower than the previous one. It lasted several hours during which the microparticles size decreased slowly from $21\ \mu m$ to approximately $17\ \mu m$.

Considering that the condensation of the droplets is mainly governed by the diffusion and the evaporation of the solvent of the dispersed phase, the formation of zinc oxide microspheres is based on a phase separation process, in which the dispersed zinc oxide phase is transformed into a precipitated solid membrane, due to the diffusion of the solvent within the aqueous phase and then to its evaporation at the oil/air interface.

In a previous study dedicated to droplets based formation of silica microspheres. Bchellaoui et al. developed a model to explain a similar linear decrease of droplets size versus time²¹⁰. It has the particularity to take into account the droplet radius (R_{drop}) at a given time (t), and assuming that the droplet volume rate is proportional to the droplet surface in contact with the air, due to solvent

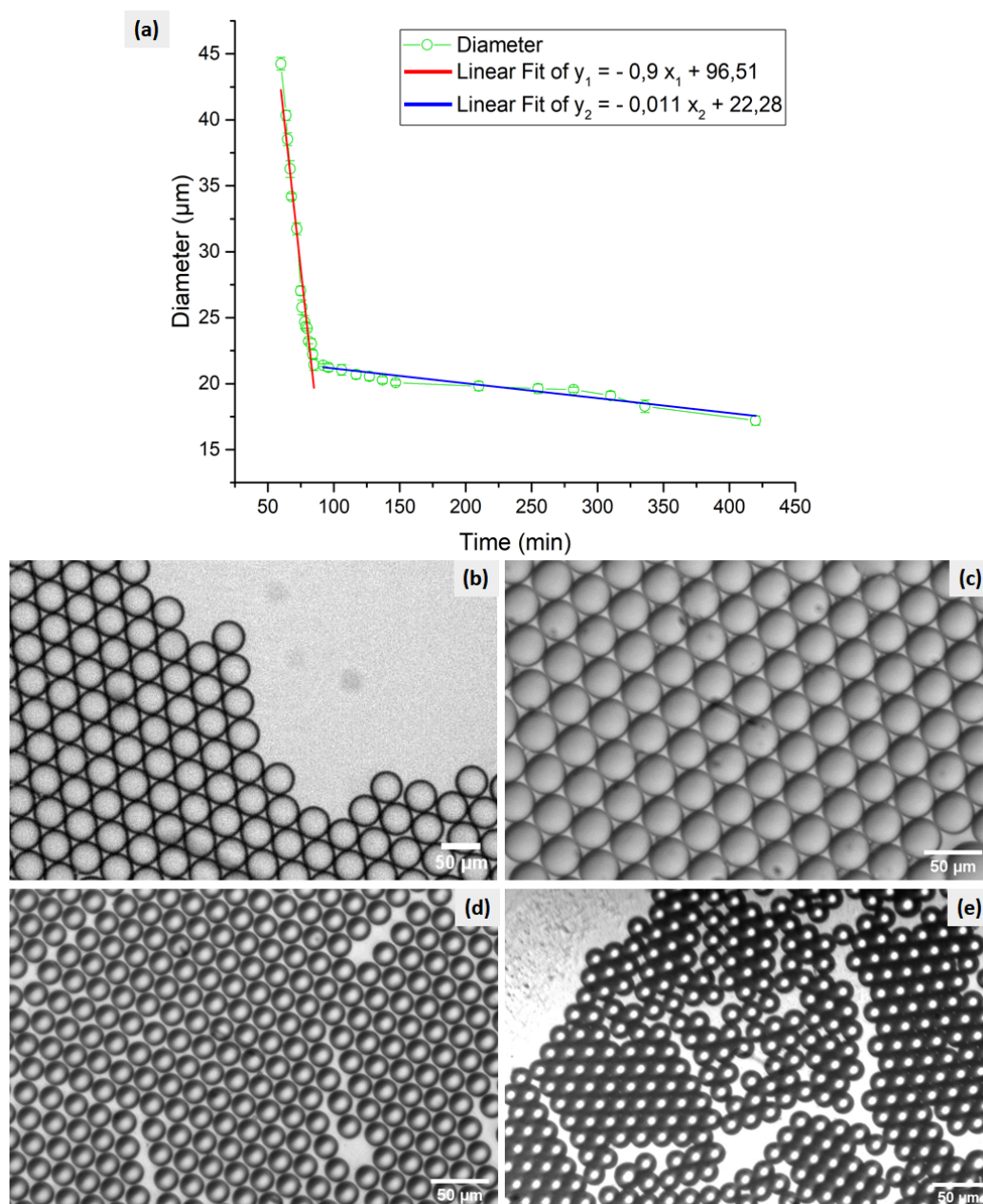


Figure 3.3 Variation in the diameter of the microdroplets of the ZnO dispersed phase as a function of time (a). Optical micrograph of a monolayer of zinc oxide microdroplets at the oil/air interface. The droplets were collected in a Petri dish as a function of time, for different droplet sizes for instance (b) 42.2 μm , (c) 63.2 μm , (d) 21.5 μm , and (e) 18.3 μm .

evaporation ($\frac{dV_{drop}}{dt}$):

$$\frac{dV_{drop}}{dt} = 4\pi R_{drop}^2 \frac{dR_{drop}}{dt} \propto -R_{drop}^2 \quad (3.5)$$

Integration of equation 3.5 leads to:

$$R_{drop}(t) = -A_1 t + A_2 \quad (3.6)$$

$$R_{drop}(t) = -B_1 t + B_2 \quad (3.7)$$

Where A_1 and A_2 are the arbitrary constants of the first step, while B_1 and B_2 are the arbitrary constants of the second step. The determined experimental values are $A_1 = -0.9$ ($\mu m/min$) and $B_1 = -0.01$ ($\mu m/min$).

It is also worth mentioning that in contrast to silica microspheres formation, the evaporation kinetics of our ZnO microspheres followed a dissimilar mechanism that resulted in two consecutive steps as we have noted. In the first step, we observed a very rapid and significant linear decrease in the droplet size of 0.9 ($\mu m/min$). While this value was equal to 0.331 ($\mu m/min$) for silica microspheres²¹⁰. We speculate that in our case the solvent diffused rapidly through the ZnO droplets and then evaporated. This behaviour can be mainly governed by the porous structure of the ZnO microspheres, leading to a reduction in the duration of the solvent evaporation process at the oil/air interface. Then, above about 80 minutes, the condensation kinetics appeared to be slower. Such a decrease can probably be attributed to the decrease in the porous structure of the system which, over time, consequently led to the formation of ZnO microspheres.

Thus, the solvent evaporation kinetics for the formation of ZnO microcapsules plays a key role and responds to several characteristics influencing the properties of the synthesized microspheres. This is particularly highlighted in Figure 3.4, where one can clearly see the influence of the solvent evaporation process. Further, the evaporation kinetics of the solvent in the surrounding fluorinated oil phase, during the formation of ZnO microcapsules, plays a critical role in obtaining highly monodisperse mesoporous ZnO microcapsules with a particular morphology. Indeed, in this experiment, the evaporation of the solvent was accelerated by exposing the droplets directly to air and microscope light. In fact, the droplets accumulated on the surface of the oil as they are less dense than the perfluorinated oil phase used in these experiments.

In contrast to the previous case, ZnO microdroplets began to condense rapidly after they reach the surface of the fluorinated oil, that was already deposited in the Petri dish. The solvent diffused very rapidly from the microdroplets of the ZnO dispersed phase into the continuous phase and evaporated at the oil/air interface. It is important to note that in this case the evaporation kinetics of the solvent was very fast, this was done in few minutes (3 minutes) or even seconds, which makes the monitoring of the variation of the droplet size as a function of time delicate. In addition, we have noticed the formation of a weak residual of ZnO within the microparticles formed, to result on a honeycomb-like structure as revealed in Figure 3.4.

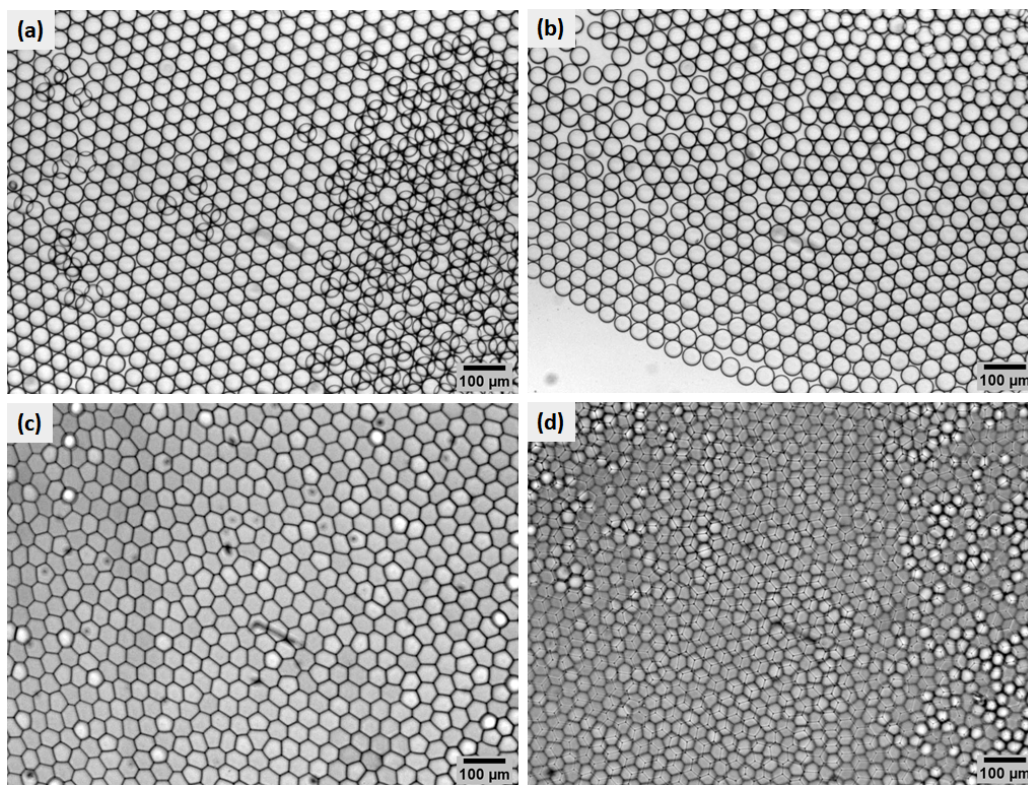


Figure 3.4 Optical micrographs of zinc oxide microdroplets collected in a Petri dish at the oil/air interface during evaporation of the solvent, (a, left) monolayer and (a, right) bilayer of zinc oxide microdroplets, (b,c) monolayer of zinc oxide microdroplets, and (d) bilayer of zinc oxide microdroplets.

3.2 Experimental investigation into the controlling parameters of ZnO droplet formation

3.2.1 Effect of surfactant on droplet formation

The term "surfactant" is an acronym of surface-active agent, also called wetting agent, meaning that the molecules act on the surface or interface of a system. Typical surfactant possess an amphiphilic molecule consisting of both hydrophilic (polar/water-loving) and a hydrophobic (non-polar/fat-loving) groups. As a re-

sult surfactant molecules have a tendency to preferentially adsorb at interfaces between immiscible phases; polar and an apolar mediums; for instance water/oil. As a matter of fact their adsorption at interfaces decreases the interfacial tension between the two mediums, which is related to the change in concentration of adsorbed molecules at the interface. This change is expressed by the Gibbs adsorption equation in terms of surface excess:

$$\Gamma = -\frac{C_{surf.}}{RT} \frac{d\gamma}{dC_{surf.}}$$

Where Γ is the surface excess (adsorption per unit area), $C_{surf.}$ is the surfactant concentration, R is the gas constant, T is the absolute temperature, and γ the surface or interfacial tension. In addition, it has been reported previously that other properties of the interface; for example flow, interactions, etc; could be also modified by surfactant^[270]. Surfactants are predominantly of use in droplet-based microfluidic technology, as they can influence and be exploited for a very good control during the manufacture of droplets in microfluidic systems. Thus the understanding of how surfactants can affect the synthesis of ZnO droplets and which role they are playing on the performance of droplet-based microfluidics synthesis in particular in terms of droplet stability, size and size distribution is of great practical importance^[271].

First, we investigated the impact of surfactants presence on ZnO droplets formation and stabilization. Therefore, two types of experiments were conducted including the formation of ZnO droplets in the presence and the absence of surfactant. In the first scenario, we synthesized ZnO droplets using a commercial non-ionic polymeric fluorinated surfactant namely dSURF (0.5% in 3MTMNovecTM

7500 oil, Fluigent). In this experiment, monosized droplets were obtained and revealed to be stable, as displayed in Figure 3.5(b).

Conversely, in the absence of any surfactant, uncontrolled merging phenomena was observed at the exit of the microfluidic channel, as depicted in Figure 3.5(a).

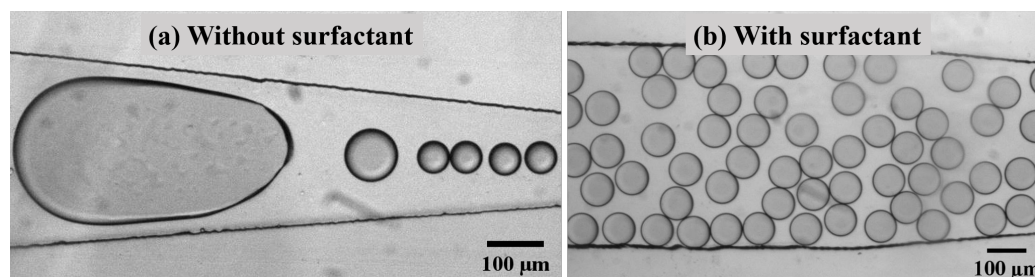


Figure 3.5 Effect of surfactant presence in zinc oxide droplets generation, production of ZnO droplets (a) without and (b) with surfactant.

It is clear that the introduction of dSURF (0.5% in 3MTMNovecTM 7500 oil) leads to the stabilization of produced droplets in the microfluidic device against coalescence. In that case, the surfactants molecules ensure that droplets do not merge, and preferably adsorb at interfaces between a polar group dissolved in water for example, and an apolar medium that prefers to be in oil or air as already discussed above. Due to this effect, the surfactant forms a thin lubrication film and surrounds the droplets. Consequently, it greatly increases their stability and thus hinders their fusing upon contact after formation, by reducing the interfacial tension, steric repulsion between surfactant molecules and the coupling of surfactants to hydrodynamic flows, which induces surfactant gradients at the droplet interface known as Marangoni effect^{270/272}.

The droplets produced in the microfluidic chip usually have a defined volume. By preventing the droplets from coalescence, one guarantees that each droplet retains its defined volume, which is the key to high precision and reproducibil-

ity of the chemical and physical phenomena that take place in the microfluidic chip. Other than stabilization, the existence of surfactant influences the droplets formation process by modifying capillary forces and interfacial viscoelasticity²⁷¹.

Such observations demonstrated evidently the crucial role of surfactant in our droplet-based microfluidic systems. It is a well-known fact that the droplets properties are defined by a very large number of parameters, for example, chip geometry, viscosities of the immiscible liquids involved, and the flow rates of both phases (dispersed and continuous). Although surfactants have been added in our microfluidic systems, it has been revealed in the following experimental studies that the situation is more complicated, as it will depend upon their nature. Since the use of unsuitable surfactants has led to the fabrication of unstable droplets, which can trigger their fusion, understanding the effect of surfactant on the performance of microfluidic devices, in particular in terms of surfactant nature, is of great practical importance, as we will detail throughout the following parts of this chapter.

3.2.2 Effect of surfactant on droplet stability

As previously mentioned, the properties of the droplets are determined by a large number of parameters. In particular, device geometry, viscosities of liquids involved, as well as their flow rates. Therefore, different experimental studies have been accomplished in this work. Beside, the situation is complicated even further if surfactants are present, whose influence will depend upon their concentration and type.

Though the addition of surfactant in the continuous oil phase is mandatory with aim of stabilizing droplets against uncontrolled merging^{34 270}. In our study,

where ZnO nanocrystals primary building blocks carry a net electric charge on their surfaces, one may expect that the electrical charge carried by surfactant molecules may play a significant role in the stability of ZnO microdroplets.

To check this hypothesis, we examined the effect of two different surfactants: (i) a commercially non-ionic polymeric fluorinated surfactant (from Fluigent), named dSURF (0.5%), and (ii) a negatively charged fluoropolymer surfactant, so-called TB-Krytox (2%w/w). Figure 3.6 shows optical micrograph of ZnO droplets and SEM image of the fabricated ZnO microspheres in the presence of TB-Krytox. It can be observed from this figure that droplets stabilized by the negatively charged surfactant (TB-Krytox) were successfully produced and collected in a Petri dish, whereas they were only stable in the early stage of condensation. Indeed, droplets became unstable as the droplets size started to reduce significantly following the solvent evaporation and the condensation process, as illustrated in Figure 3.6.

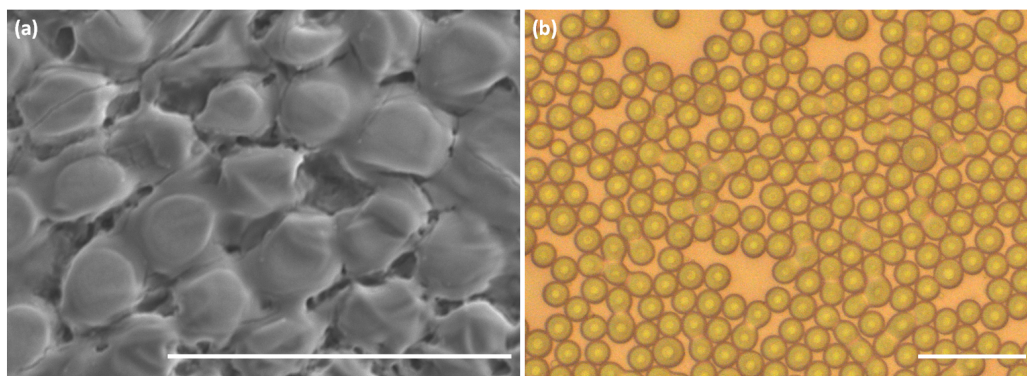


Figure 3.6 (a) SEM image of the as-prepared ZnO microspheres in the presence of TB-Krytox. (b) The corresponding optical micrograph of ZnO droplets. It demonstrates the surfactant effect on the stabilization of zinc oxide droplets and particles. Scale bars, 50 μm .

To our knowledge, the observation of a delayed merging process of condensed

zinc oxide microdroplets is reported for the first time. Interestingly, such a merging process did not occur when the non-ionic surfactant (dSURF) has been used. In this case, droplets remained stable during the whole process of production and condensation of droplets, as depicted in Figure 3.7. We would like to point out that these observations clearly demonstrates the crucial role of surface electric charges on the stability of ZnO microdroplets and microparticles, and the effect of the electrical charges carried by surfactant molecules. Basically, when droplets are not efficiently stabilized, they merge immediately after they bump into each other while flowing within the observation microchamber, or further when getting into contact with each other in the microfluidic channels. We speculate that ZnO microparticles carry a net electric charge on their surface due to the electrical charges carried by ZnO nanocrystals whose aggregation leads to the formation of such a charged surface.

These observations demonstrate the significant role of surface electric charges on the stability of ZnO microdroplets and microparticles. They also confirm that ZnO microparticles carry a net electric charge on their surface due to the electrical charges carried by ZnO nanocrystals building units. In fact, it is well known indeed that the (wurtzite) ZnO crystal lattice is constructed by a number of alternating planes stacked along the c-axis direction. As the planes consist of tetrahedrally coordinated Zn^{2+} and O^{2-} ions, the structure of ZnO nanocrystals consists of Zn^{2+} terminated (0001) face and O^{2-} terminated (000 $\bar{1}$) face. Consequently, ZnO nanocrystals possess an intrinsic dipole moment along their c-axis.

We suggest that the higher stability of ZnO microparticles in the presence of non-charged surfactant molecules is ensured by both contributions from (i) the

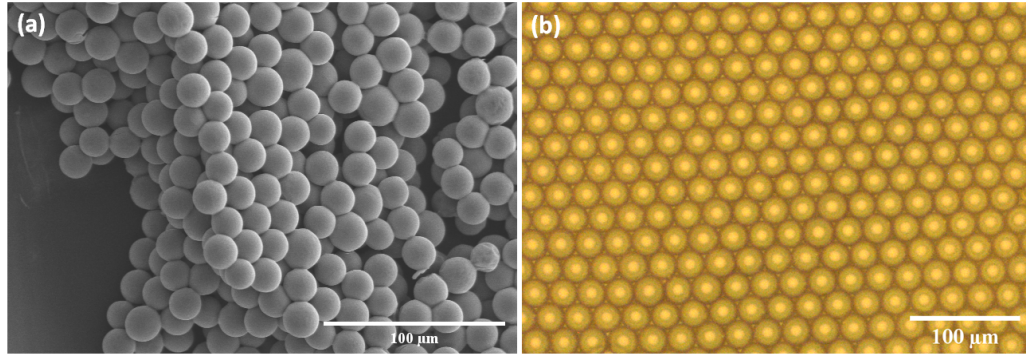


Figure 3.7 (a) SEM image of the fabricated ZnO microspheres using dSURF fluorosurfactant. (b) Optical micrograph of ZnO droplets in the presence of the same surfactant.

colloidal repulsive forces of the adsorbed surfactant layer around droplets and (ii) from the repulsive electrostatic forces between charged ZnO nanoparticles, which aggregate at the droplets interface. In contrast, when using the charged TB-krytox surfactant, electrostatic repulsion forces should be screened by the negative charge of the terminal carboxylic acid of the $TB^{(+)} - Krytox^{(-)}$ surfactant monolayer.

3.2.3 Microparticles size versus droplets size

A precise control of synthesized microparticles size can be achieved easily by changing the flow rates of the dispersed and the continuous phases, and the size of the generated droplets.

Let's first assume that interactions between nanoparticles inside microparticles are independent of the size of this system. If we consider a formation model of microparticles based on droplet condensation (driven by the solvent evaporation process and aggregation of hard nanoparticles), then the number, N_{NP} , of nanoparticles remains constant during the process, regardless the way nanoparticles organize inside microparticles. Then, the conserved number N_{NP} should scale

similarly as L_{drop}^3 and as $L_{part.}^3$, where L_{drop} and $L_{part.}$ are characteristic sizes of droplets and microparticles, respectively. This means that $\frac{D_{drop}}{D_p}$ ratio should be remain constant. Hence, if we assume that the interactions between nanoparticles are independent of the size of the microparticle, then one may expect a linear dependence between the droplet size and the microparticle size. Experimentally, we observe indeed that the size of synthesized ZnO microspheres depends almost linearly on the size of the initial droplets, as depicted in Figure 3.8. We find also that the final volume of the microparticles corresponds approximately to a third of the initial volume of the droplets. Nevertheless, we notice in the case of pure ZnO microspheres a break of the microparticles size at a droplet size, D_{drop} , around $50 \mu m$ (see Figure 3.8(a)). It is worth noting that such deviation is observed in the case of RhB doped microparticles for large microparticles, i. e. , $D_p > 20 \mu m$ (obtained from droplets with a typical size $D_{drop} > 60 \mu m$). We will discuss in more detail these interesting features in Chapter 5. Basically, in order to determine accurately the slope of the linear part of the curve, we take also into account the trivial zero value of the microparticle size at $D_{drop} = 0 \mu m$. Therefore, we find for pure ZnO microparticles and rhodamine B dye doped microparticles the following linear relationships between the size of pure ZnO microparticles and the size of microdroplets, respectively:

$$D_p(ZnO) \simeq 0.34 \times D_{drop} \quad (3.8)$$

$$D_p(ZnO/RhB) \simeq 0.33 \times D_{drop} \quad (3.9)$$

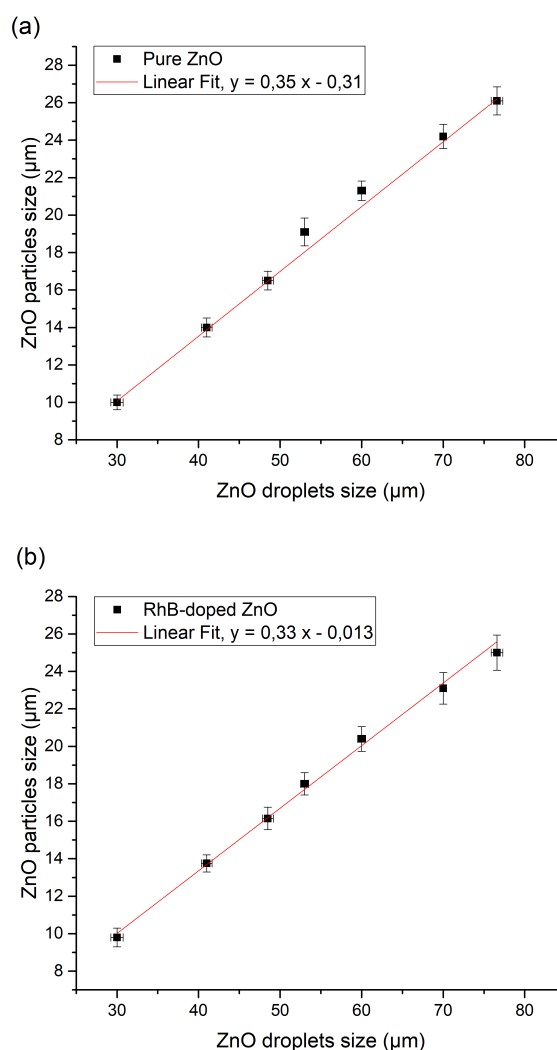


Figure 3.8 Effect of droplet diameter on the final size of zinc oxide microspheres in the case of (a) pure ZnO microdroplets and (b) rhodamine B-doped zinc oxide microdroplets.

3.2.4 Effect of flow rates on droplet size

The droplet formation mechanism was experimentally investigated and observed for a range of flow rate conditions. Particularly, we studied ZnO droplets formation in a simple flow-focusing microfluidic device with varying the flow rate of

the continuous and dispersed phases, separately.

Moreover, we studied the effect of the dispersed phase and continuous phase flow rates on the droplet size, as illustrated in Figure 3.9(a) and Figure 3.9(b). In this study, the flow rate of the dispersed phase has been changed in a large range, from $Q_d = 30 \mu L/h$ up to $Q_d = 250 \mu L/h$ for a given flow rate of the continuous phase which was equal to $Q_c = 300 \mu L/h$. After each change in the flow rate, we gave the microfluidic system the time to stabilize for at least 10 minutes. Droplet production was monitored using a CCD camera (IDS) equipped with a 10X lens for this experiment. The images were then processed and analyzed using ImageJ software. The presented values of droplets size versus flow rate is an average value of 20 measured droplets. The experimental error of the droplets size did not exceed 3%. It is observed that the size of droplets varies practically linearly with the flow rate of the dispersed phase (Q_d). We attribute such feature to the relative high viscosity of the sol. Indeed, according to the model initially developed by Garstecki et al.²⁷³:

$$\frac{L}{w} \sim \tau \times Q_d$$

Where w and τ represent the width of the channel, i. e. , the nozzle, and the time to form a droplet, respectively. Also, the time τ may be considered as the sum of the time t_{fill} it takes to the dispersed phase (sol) to fill the cross-section of the main channel and the time t_{sq} , to squeeze the emerging drop to detach it from the dispersed phase: $\tau = t_{fill} + t_{sq}$.

Since t_{fill} scales as $\frac{1}{Q_d}$ and t_{sq} scales as $\frac{1}{Q_c}$, the (normalized) size of droplets should scale as:

$$\frac{L}{w} \sim 1 + \frac{Q_d}{Q_c}$$

As discussed in detail by Garstecki et al. in ref. ²⁷³, the scaling law simplifies in the case where $Q_d \gg Q_c$ to:

$$\frac{L}{w} \sim \frac{Q_d}{Q_c}$$

Whereas for $Q_d \simeq Q_c$, the droplets size becomes practically independent of Q_c (and Q_d). These scaling laws are in very good agreement with our results, as shown in Figure 3.9(a) and Figure 3.9(b).

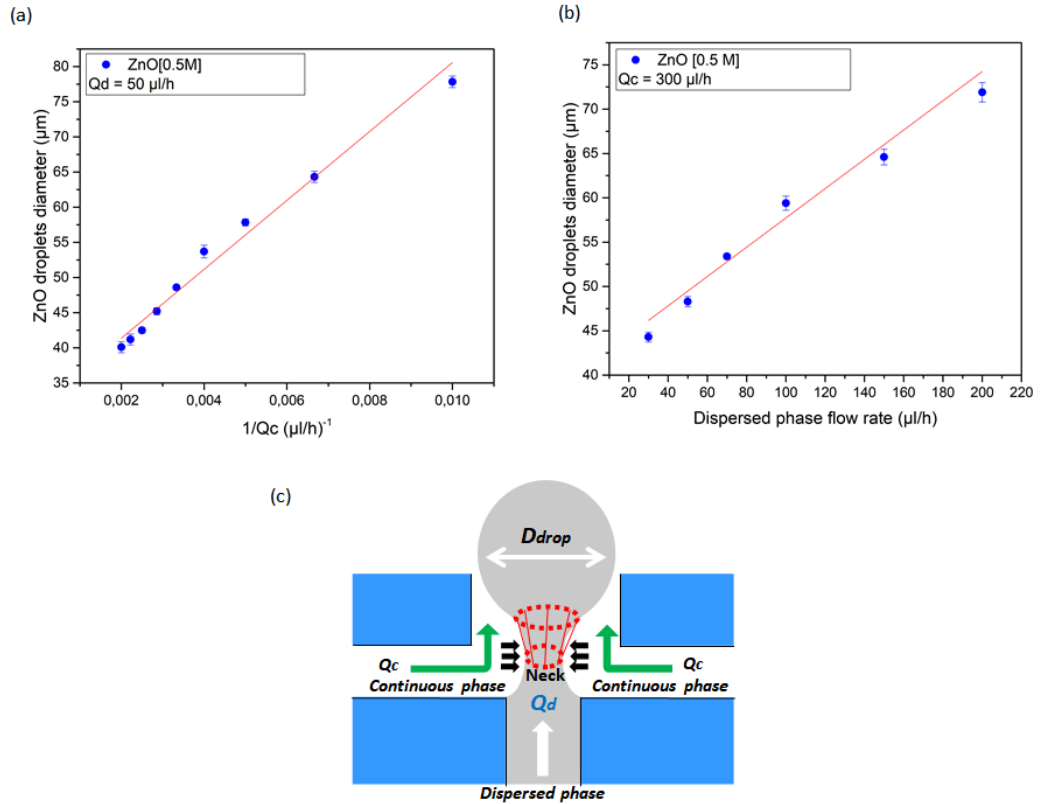


Figure 3.9 (a) Effect of the continuous phase and (b) the dispersed phase flow rates on the mean diameter of the generated droplets at a flow rate of the dispersed and continuous phases equal to $Q_d = 50$ μL/h and $Q_c = 300$ μL/h, respectively. (c) Schematic illustration of ZnO droplets generation process through the microfluidic flow-focusing system composed of a flowing zinc oxide solution (Q_d) and a carrier oil phase (Q_c).

3.3 Structural and morphological analysis

The morphological features of zinc oxide microspheres were examined and measured using Scanning Electron Microscopy (S-3400N, Hitachi) equipped with an Energy Dispersive Spectroscopy (EDS). Figure 3.10 depicts SEM images of ZnO microspheres obtained from droplets with a diameter of $48 \mu\text{m} \pm 0.5 \mu\text{m}$.

It is observed that the microparticles have a spherical shape with a mean external diameter of about $16.5 \mu\text{m} \pm 0.4 \mu\text{m}$. Energy Dispersive Spectroscopy (EDS) analysis was performed to check the chemical composition of the obtained microspheres. An oxygen peak at about 0.52 keV and Zn signals at about 1 keV and 8.6 keV were observed in the EDS spectrum (Figure 3.11(c)). An analysis of the ob-

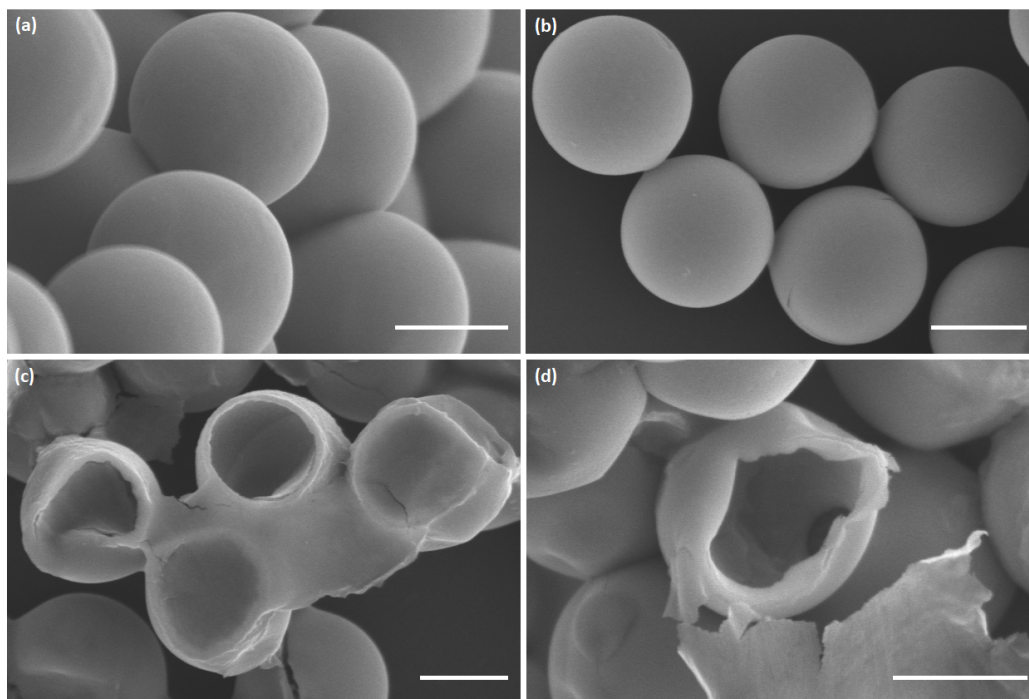


Figure 3.10 Scanning electron microscope images of $16.5 \mu\text{m}$ size zinc oxide microcapsules, shell thickness was found about $0.6 \mu\text{m}$. Scale bars, $10 \mu\text{m}$.

tained photoluminescence data shows that ZnO microspheres exhibit an emission band at 3.32 eV which may be due to the free excitons²⁷⁴⁻²⁷⁶, as shown in Figure 3.11(b).

The scanning electron microscope image demonstrates also a hollow feature of the fabricated microspheres with a mean shell thickness of about $0.6 \mu m \pm 0.1 \mu m$ (see Figure 3.10(c) and Figure 3.10(d)). Worth noting, the measured shell thickness is in good agreement with the calculated one as detailed hereafter. Within the reasonable assumption that the droplet content does not dissolve in the continuous fluorocarbon oil phase, the mass of ZnO (m_{ZnO}) of the overall ZnO nanocrystals contained in the droplet should be conserved in the microcapsule shell. Hence $m_{ZnO} = C_{ZnO}V_{drop}M_{ZnO}$, where C_{ZnO} , V_{drop} , and M_{ZnO} represent the concentration of ZnO precursor (zinc acetate), the volume of a single droplet and the ZnO molar mass, respectively. Let $A = \pi D_p^2$ be the surface area of a microcapsule of a diameter D_p , h its shell thickness and ρ its mass density. One may write then $m_{ZnO} = \rho \pi D_p^2 h$ and deduce h according to the following equation:

$$h = \frac{C_{ZnO}V_{drop}M_{ZnO}}{\pi D_p^2 \rho} \quad (3.10)$$

Considering the standard value of zinc oxide mass density, $\rho = 5.6 \text{ g/cm}^3$, the used concentration $C_{ZnO} = 0.5 \text{ M}$ of ZnO precursor and the initial microdroplet size, $D_{drop} = 48 \mu m$, one may deduce a value for the thickness of the microcapsule shell of about $0.6 \mu m$, which is in very good agreement with the measured value.

Further detailed structural and morphological analysis of the obtained ZnO microspheres was carried out by transmission electron microscopy (TEM) within the MATMECA consortium (supported by the ANR under contract number ANR-

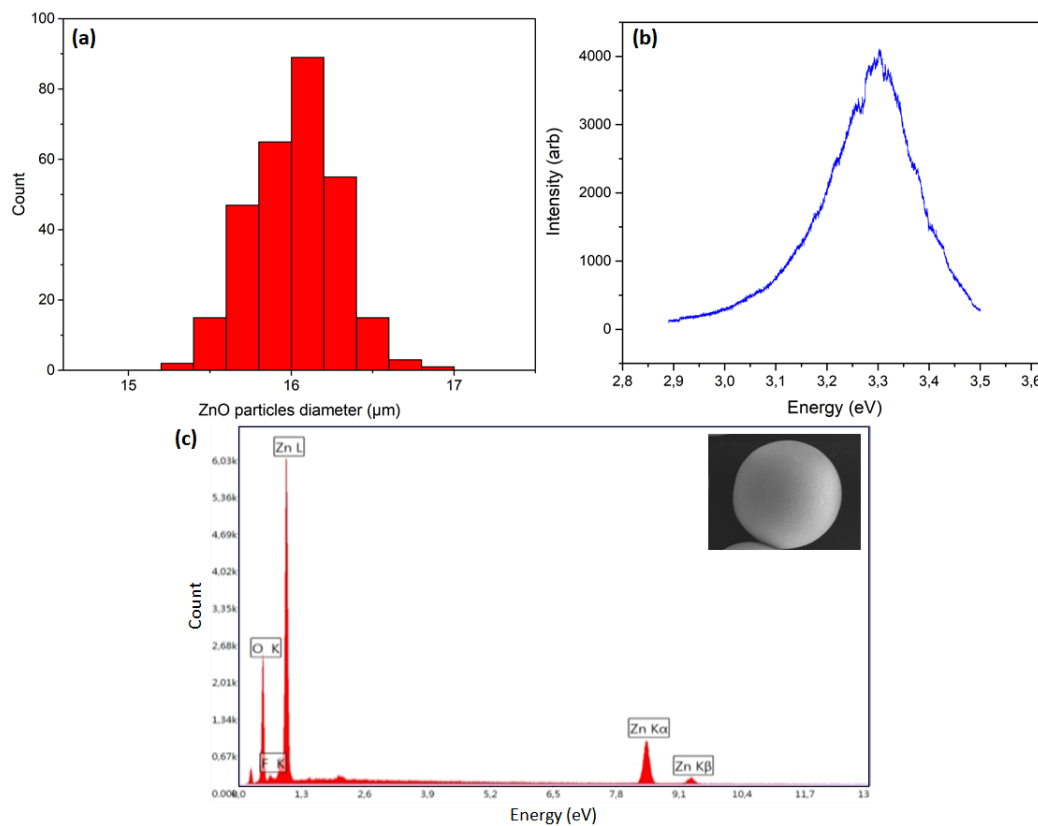


Figure 3.11 Size distribution (a), photoluminescence spectra (b), and EDS analysis (c) of ZnO microcapsules.

10-EQUIPEX-37) using S(TEM) Titan Cubed G2 60-300 microscope. Figures 3.12(a) and 3.12(b) present the low and high magnification TEM images, respectively. The low magnification TEM image indicates that the ZnO microsphere has a rounded edge, which was previously observed by Liu et al.²⁰. Further, the high magnification TEM image of an ultra-thin area of the microsphere's edge points out that the nanoparticles are structurally uniform and well-oriented as shown by the parallel lattice fringes. Besides, the corresponding TEM image exhibits a lattice spacing of 0.26 nm, which corresponds to the d-spacing of the (0001) planes²⁷⁷. The elemental mapping analysis was performed to clearly investigate

the elemental distributions of the hollow microspheres synthesized in this work. The results of high-angle annular dark-field scanning transmission electron microscope (HAADF-STEM) and elemental mapping indicate that the microspheres were composed of zinc (Zn) and oxygen (O). It can be seen that they were distributed homogeneously within the entire ZnO hollow structure, as shown in Figure 3.12.

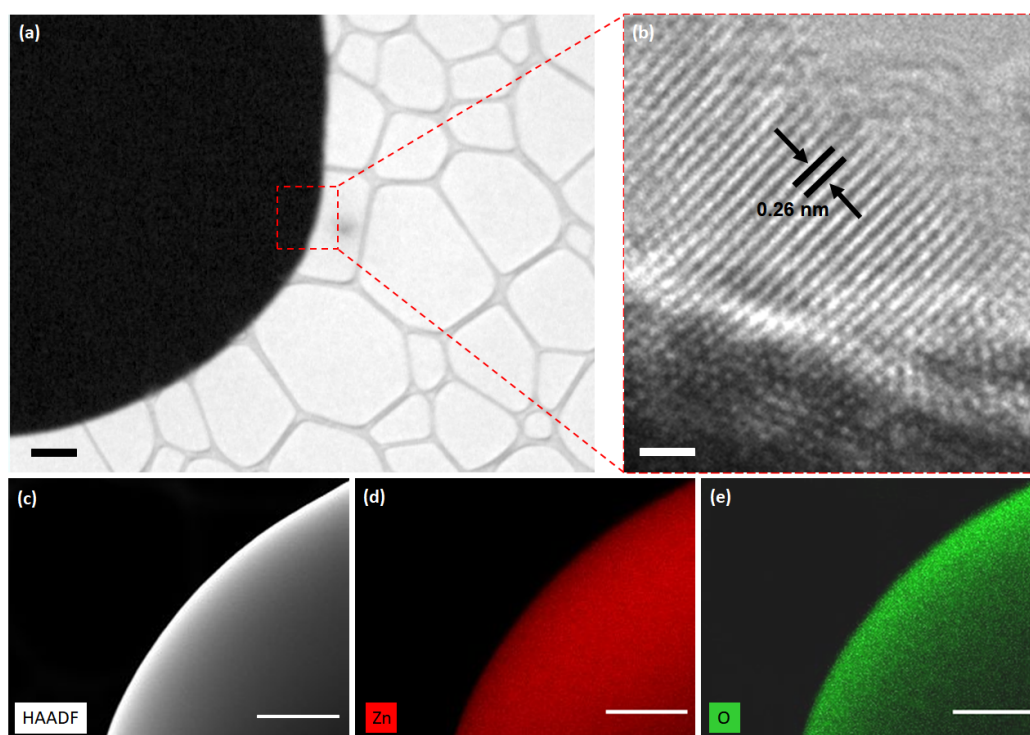


Figure 3.12 Transmission electron microscopy images of (a) a single ZnO microsphere and (b) high magnification of the edge of the microsphere showing the lattice fringes, (c) High-angle annular dark-field scanning transmission electron microscope (HAADF-STEM) image of the edge of the microsphere, (d,e) revealing the presence of oxygen and zinc on the surface of the microsphere. Scale bars, (a) $1\ \mu\text{m}$, (b) $1\ \text{nm}$, (c,d,e) $2\ \mu\text{m}$.

Our investigations strongly suggest that the formation mechanism of the microspheres is based on a non-classical crystallization pathway. In contrast to the

classical crystallization, where the crystal is formed by clusters from building units such as atoms, ions or molecules. The crystal growth mechanism behind the formation of our microspheres is in accordance with a particle-mediated process involving mesoscopic transformation of self-assembled, metastable or amorphous precursor particles inside the droplet. Indeed, the primary zinc oxide nanoparticles formed in the early stages of the reaction reorganize within the droplet. Afterwards, the evaporation of the solvent gives rise to significant internal forces going from the inside to the outside of the droplet, thus promoting agglomeration of the aggregates on the contour of the microsphere.

3.4 Investigation into porous and soft ZnO microcapsules

To give further insight onto the hollow and porous features of zinc oxide microspheres, we investigated their swelling in the presence of a polar solvent such as water. Figure 3.13 presents a schematic illustration and optical micrographs of ZnO microparticles before and after the addition of water. The penetration of water molecules inside the microcapsules induces an increase of their volume by a factor 2, followed by the rupture of their shell (membrane), as illustrated in Figure 3.13, under the effect of osmotic pressure. This experiment gives another confirmation of the porous feature of the synthesized zinc oxide microspheres.

Figure 3.14 shows high resolution SEM images of the microparticles surface, which gives a better view of the porosity of the microcapsule shell and enabled us to give an approximate size of ZnO nanoparticles building blocks, that is approximately 100 nm.

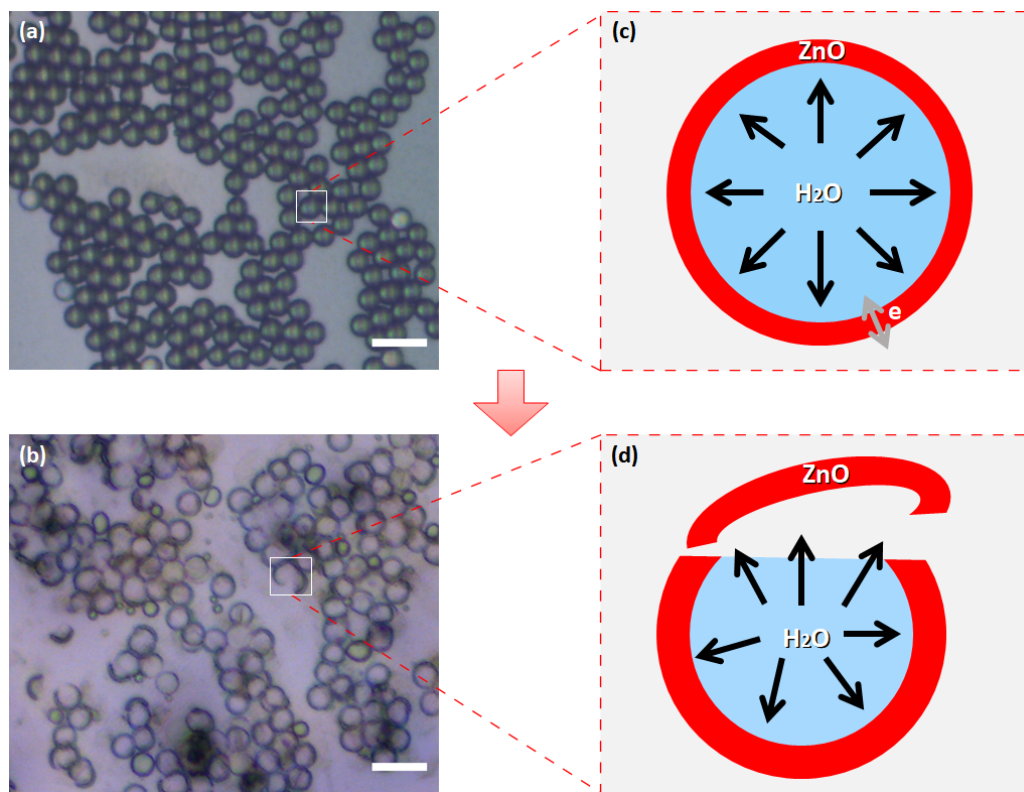


Figure 3.13 Optical micrograph of ZnO microparticles (10 μm) and a schematic illustration before (a,c) and after they are swollen (13 μm) with water (b,d), emphasizing the porous and hollow features of zinc oxide microparticles. Scale bars, 30 μm .

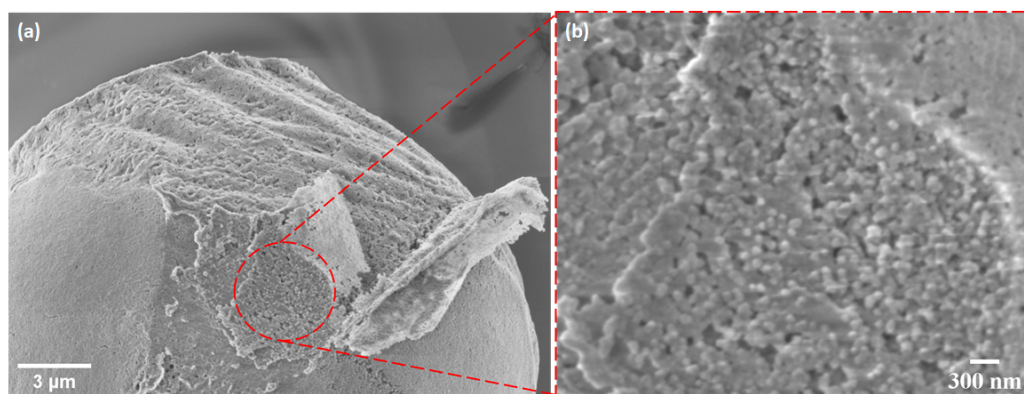


Figure 3.14 (a) Low and (b) high-magnification SEM images of a zinc oxide microsphere surface demonstrating the porous nanosized zinc oxide particles.

ZnO microcapsules ($16.5 \mu\text{m} \pm 0.4 \mu\text{m}$) were deposited on a thin carbon film of the TEM grid. Noteworthy, TEM image of a single pure ZnO microsphere shows that the microsphere flattens after few seconds of observation under the effect of the incident electron beam of 200 kV, to form a thin ZnO square, as shown in Figure 3.15(a). Such a behavior may be explained by the hollow feature of the microspheres. On the other hand, ZnO microsphere does not flatten in the presence of a charged dye (rhodamine B), but it deforms after a few seconds of observation, as shown in Figure 3.15(b).

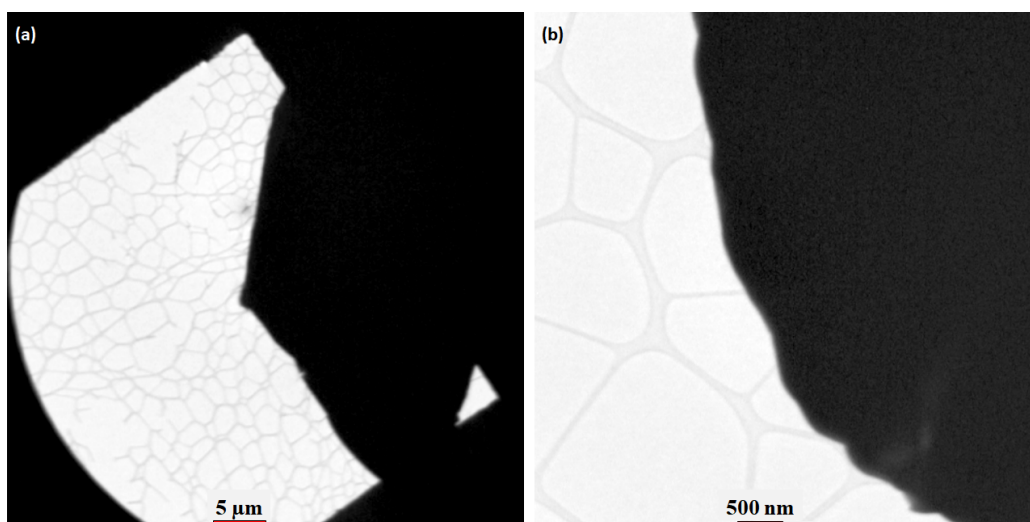


Figure 3.15 TEM images of a single pure ZnO microsphere revealing that the microsphere flattens after couple minutes of observation under the effect of the incident electron beam of 200 kV in the case of pure ZnO (a) and in the case of doped ZnO microsphere with a charged dye (rhodamine B) (b).

Chapter 4

Organization of ZnO nanoparticles at the microdroplet interface in the presence of fluorescent dyes

This chapter is devoted to the investigation of the polarity aspect of zinc oxide nanoparticles and their self-organization at the microdroplet interface. We present experimental evidences of the preferential adsorption of charged fluorescent dyes at the zinc oxide droplets interface and establish different models to support our findings.

4.1 Effect of the presence of fluorescent dyes and ions on droplet size

4.1.1 Introduction

Because of the presence of electrical charges that are carried by ZnO nanoparticles building blocks, we investigated the effect of the addition of electrically charged dyes molecules, such as rhodamine B (RhB), on the size of ZnO droplets for a large range of dispersed phase flow rates (Q_d) and continuous phase flow rates (Q_c). Indeed, we investigated the effect of two additional xanthene dyes: rhodamine 6G (Rh6G) and fluorescein (Fluo), see Figure 4.1. Noteworthy, zinc oxide droplets were stabilized in these experiments using the non-ionic dSURF surfactant (0.5 %).

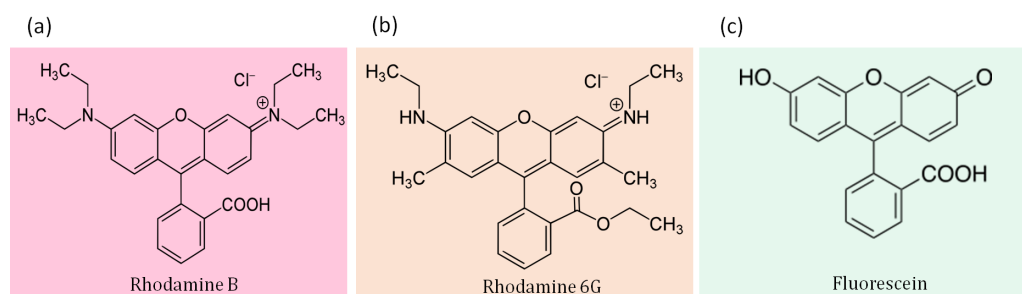


Figure 4.1 Chemical structures of the dyes used in this study, rhodamine B (a), rhodamine 6G (b), and fluorescein (c).

Prior to ZnO droplets generation, ZnO nanoparticles dispersion was prepared as described previously²⁶⁹, and then doped with a solution of rhodamine 6G and/or fluorescein at 0.1 mM, 0.01 mM, and/or rhodamine B ($\geq 95\%$, (HPLC), Sigma-Aldrich) at 0.1 mM, 0.01 mM, and 0.002 mM. This enabled the investi-

gation of the effect of a very small concentration of a charged dye on the droplet formation behavior. To obtain the required concentration in the experiments, the dyes were dissolved in distilled water. The concentration of ZnO nanoparticles was fixed throughout the experiment at 0.5 M. ZnO droplets were fabricated by varying the continuous phase flow rate (from 100 $\mu\text{l}/h$ to 450 $\mu\text{l}/h$) or the dispersed phase (from 20 $\mu\text{l}/h$ to 200 $\mu\text{l}/h$). The droplet formation was considered to be monodispersed (the average droplet diameter was measured from approximately 20 droplets). The size of ZnO droplet obtained in these experiments ranged from 40 μm to 90 μm , depending on the experimental conditions, as reported in Figure 4.4 and Figure 4.2.

ZnO droplet size and size distribution were monitored using our home-built optofluidic fluorescence detection setup. In this experiment, ZnO droplets were excited using a 532 nm CW laser while flowing in a 60 μm x 60 μm microfluidic channel, and the fluorescence was detected in the range 550 nm - 700 nm.

4.1.2 Effect of the continuous phase flow rate

Figure 4.2 shows the variation in the average diameter of zinc oxide droplets in function of the continuous phase flow rate in the case of doped zinc oxide with rhodamine B (Figure 4.2(a)), rhodamine 6G (Figure 4.2(b)), and fluorescein (Figure 4.2(c)). The flow rate of the dispersed phase was kept constant at 50 $\mu\text{l}/h$ while varying the continuous phase flow rate from 100 $\mu\text{l}/h$ to 450 $\mu\text{l}/h$. It should be noted from the results shown in Figure 4.2 that the average diameter of ZnO droplets gradually decreased by increasing the continuous phase flow rate. The same trend was evident for all the experiments. When the flow rate of the dispersed phase was constant in the microfluidic system and the continuous phase

flow rate was increased, the droplets generated were smaller since the velocity of the continuous phase was also increased.

To cite an example, at 50 $\mu\text{l}/\text{h}$ dispersed phase flow rate the 150 $\mu\text{l}/\text{h}$ and 300 $\mu\text{l}/\text{h}$ continuous phase flow rate generated droplets of 64.3 μm and 48.6 μm , respectively. The droplet diameter decreased as the continuous phase flow rate increased. Besides, when ZnO dispersion phase flow rate was remained constant (at $Q_d = 50 \mu\text{l}/\text{h}$) and for the same continuous phase flow rate value ($Q_c = 300 \mu\text{l}/\text{h}$, for example) the droplets were larger when a solution of a charged dye was added to the ZnO nanoparticles dispersion. For example, rhodamine B (0.1 mM)-doped ZnO solution generated bigger droplets diameter ($D_{drop} = 65.3 \mu\text{l}/\text{h}$) than pure ZnO solution ($D_{drop} = 48.6 \mu\text{l}/\text{h}$) at the same ZnO dispersion phase flow rate and continuous flow rate values.

We believe that such a significant increase in the ZnO droplets diameter in the presence of rhodamine B-doped, rhodamine 6G-doped, and fluorescein-doped ZnO droplets; for the same flow rates values of both phases was clearly affected by increasing electrical charges in the solution even though in the presence of only a small amount of the dye in the droplets ($[\text{RhB}] = 0.002 \text{ mM}$).

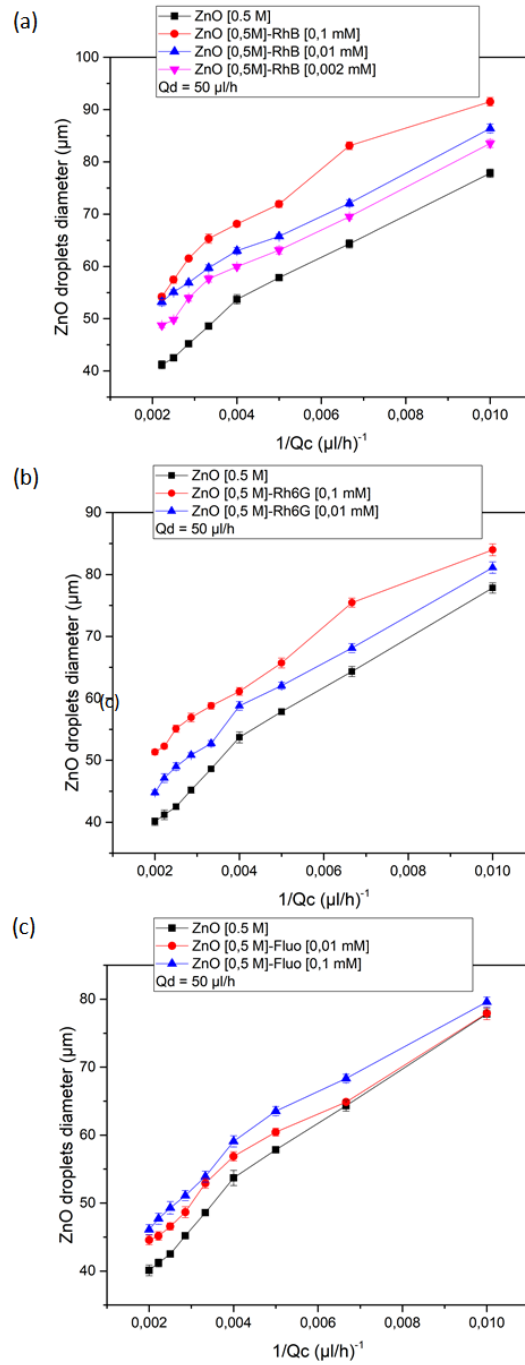


Figure 4.2 Effect of the continuous phase flow rate on droplet size at a constant flow rate of the dispersed phase ($Q_d = 50 \mu\text{l/h}$), in the presence of different concentrations of RhB (a), Rh6G (b), and fluorescein (c).

Moreover, it is clear from the results shown in Figure 4.2 that the droplets diameter is smaller when the continuous phase flow rate is high. Because this leads to the increase of shear stresses exerted on the interface of the filament of ZnO dispersion sol. On the other hand, when the flow rate of the continuous phase is lower there is sufficient time for a larger internal flow of the ZnO dispersion solution and less pressure upstream, and thus fill a larger volume resulting on the formation of bigger droplets^[191 278 279]. We confirmed these findings by real-time fluorescence experiments of ZnO droplet size and size distribution, as indicated in Figure 4.3.

Figure 4.3 illustrates real-time fluorescence intensity recording from zinc oxide droplets flowing in the microfluidic channel at a constant dispersed phase flow rate. Rhodamine B, at a concentration equal to 0.1 mM, was used as a fluorescent probe for the detection of droplet generation and content. Indeed, the laser spot was well placed in the microfluidic channel to detect in real-time the passage of each droplet, which is described by a single fluorescence pulse on the graph.

Taking into account the large selection of the continuous phase flow rates, 100 $\mu\text{l/h}$, 200 $\mu\text{l/h}$, 300 $\mu\text{l/h}$, and 450 $\mu\text{l/h}$, we can plainly see that the continuous phase flow rate changes the droplet frequency as well as the signal integrity. In addition, the results show a well-defined and high controlled droplet size and size distribution acquisition, which gives a valuable insight into the effect of the continuous phase flow rate on ZnO droplet size and size distribution. As previously stated, the size of the generated droplets depends on different parameters, such as the geometry of the microfluidic device, the flow rate of the continuous phase and the dispersed phase, as we will further point out throughout this thesis work.

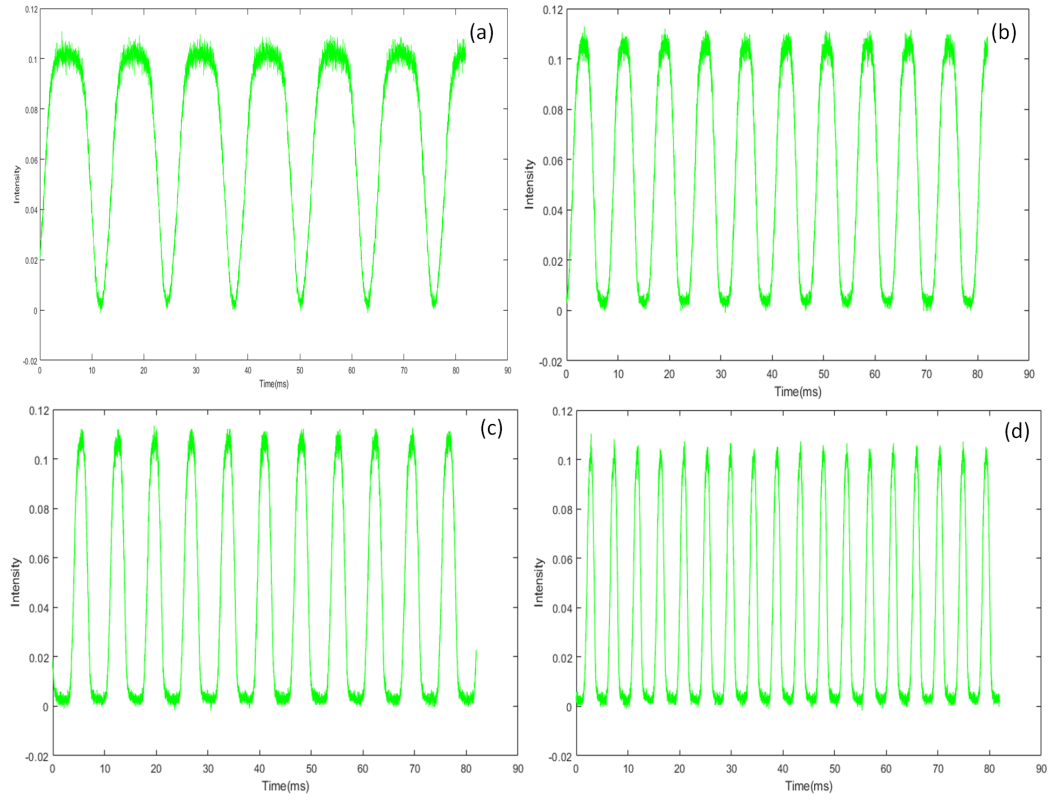


Figure 4.3 Fluorescence intensity versus time recorded from ZnO microdroplets doped with rhodamine B [0.1 mM] at 50 $\mu\text{l}/\text{h}$ of the dispersed phase flow rate (Q_d) over a wide range of the continuous phase flow rate (Q_c), (a) $Q_c = 100 \mu\text{l}/\text{h}$, (b) $Q_c = 200 \mu\text{l}/\text{h}$, (c) $Q_c = 300 \mu\text{l}/\text{h}$, and (d) $Q_c = 450 \mu\text{l}/\text{h}$.

4.1.3 Effect of the dispersed phase flow rate

Alternatively, at a fixed continuous phase flow rate ($Q_c = 300 \mu\text{l}/\text{h}$) and over a wide range of dispersed phase flow rates ($20 \mu\text{l}/\text{h} \leq Q_d \leq 200 \mu\text{l}/\text{h}$), the average diameter of ZnO droplets were found to be gradually increasing by the increase of ZnO dispersion phase flow rate in all cases, as evidenced in Figure 4.4(a), Figure 4.4(b), and Figure 4.4(c). The ZnO droplets produced were about 44.3 μm , at flow rates $Q_d = 30 \mu\text{l}/\text{h}$ and $Q_c = 300 \mu\text{l}/\text{h}$, of the continuous phase and

dispersed phase, respectively. However, under the same continuous phase flow rate and at bigger flow rate of dispersed phase ($Q_d = 100 \mu l/h$, for instance) ZnO droplets diameter was typically bigger ($D_{drop} = 59.4 \mu m$). From the graphs shown in Figure 4.4 the correlation between the ZnO dispersion phase flow rate and the average diameter of the droplets indicates that the flow rate of the dispersed phase significantly affected the diameter of the generated droplets.

These results are consistent with other previous studies that have reported a dependence of droplet size on the dispersed phase flow rate [193,280,281]. According to other researchers, droplets diameter increases as the velocity of the dispersed phase increases [193,282]. These investigations revealed that droplet size increases at least slightly with increasing the dispersed phase flow rate. Furthermore, the influence of some specific parameters were also examined such as liquid viscosities and interfacial tension on the droplet formation mechanism [281,283,287]. Moreover, they reported the existence of a large continuous phase flow threshold value above which droplet size remains constant regardless of the dispersed phase flow rate.

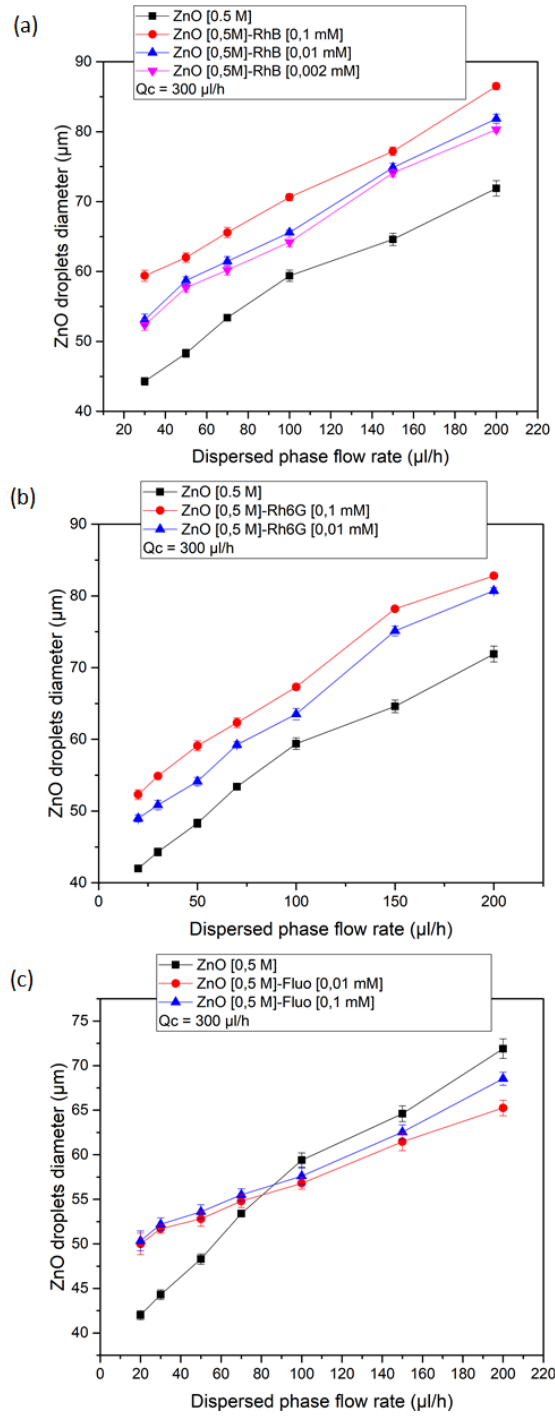


Figure 4.4 Effect of the dispersed phase flow rate on droplet size at a constant flow rate of the continuous phase ($Q_c = 300 \mu\text{l/h}$), in the presence of different concentrations of RhB (a), Rh6G (b), and Fluo (c).

Overall, ZnO droplets size increases with increasing the dispersed phase flow rate because the shear stresses exerted on the emerging droplet interface are relatively low so that the droplet can detach significantly from the dispersed phase jet at the orifice of the flow focusing device. As a result, the size of the generated droplets is larger and the generation frequency is important as long as the force required by the droplet to break the dispersed phase flow is reduced. Moreover, for a given continuous phase flow rate the higher the flow rate of the ZnO dispersion phase the higher the droplet frequency, as demonstrated from fluorescence intensity real-time recorder from ZnO droplets doped with rhodamine B solution [0.1 mM]. The droplets were excited by a continuous wave (CW) laser at 532 nm while flowing in the microchannel (see Figure 4.5).

Interestingly, we noticed that increasing the concentration of rhodamine B, rhodamine 6G, and fluorescein solutions, the average droplets diameter barely increased for a fixed liquids flow rates in all experiments. For example, one finds that ZnO droplets diameter increased from $53.4\ \mu\text{m}$ (black curve, Figure 4.4(a)) to $61.46\ \mu\text{m}$ (blue curve, Figure 4.4(a)) and $65.58\ \mu\text{m}$ (red curve, Figure 4.4(a)) as the ZnO dispersion phase was doped with rhodamine B at 0.01 mM and 0.1 mM, respectively. For instance, fixing the flow rates of the dispersed phase at $50\ \mu\text{l/h}$ and the continuous phase at $300\ \mu\text{l/h}$, we analyzed the droplets diameters varying the concentration of the dyes. It can be seen that the droplets diameter is larger in the case of doped droplets with the dyes. At intermediate concentrations, the droplet diameter is larger than pure ZnO droplets. Even though at very low dye concentration ($[\text{rhodamine B}] = 0.002\ \text{mM}$), the effect of the rhodamine B is still noticeable and droplets are slightly bigger than pure ZnO droplets.

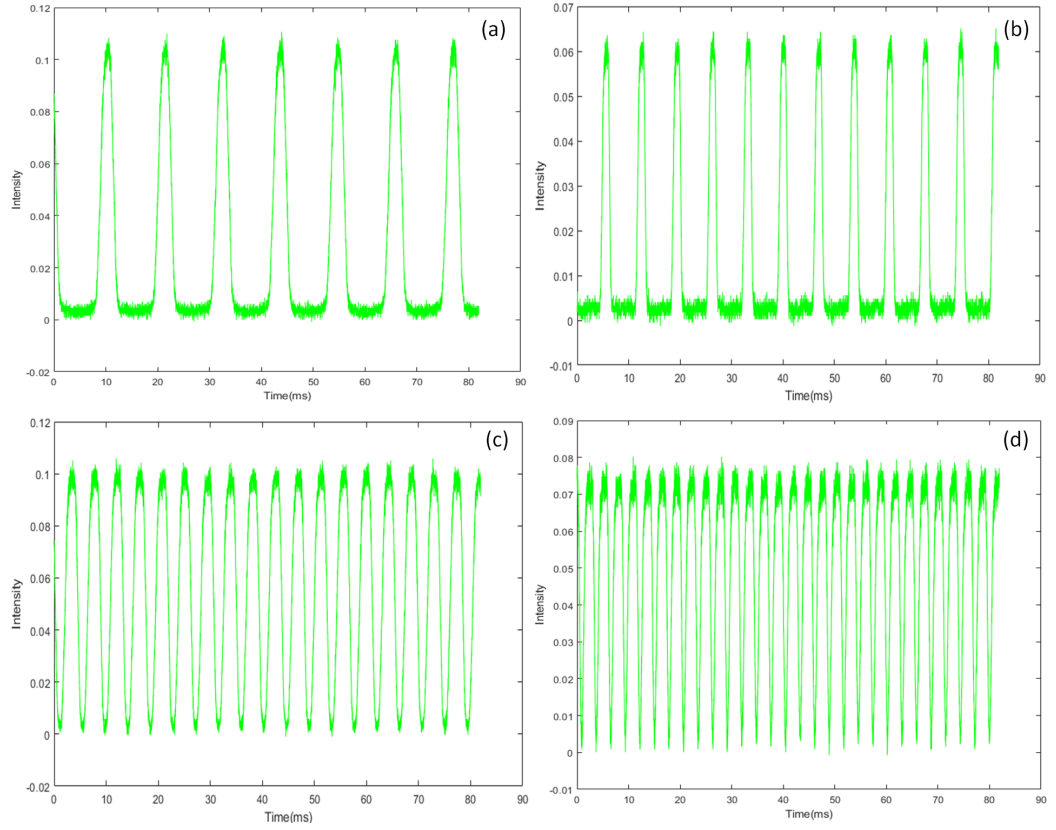


Figure 4.5 Fluorescence intensity versus time recorded from ZnO microdroplets doped with rhodamine B [0.1 mM] at 300 $\mu\text{l/h}$ of the continuous phase flow rate (Q_c) over a wide range of the dispersed phase flow rate (Q_d) (a) $Q_d = 20 \mu\text{l/h}$, (b) $Q_d = 50 \mu\text{l/h}$, (c) $Q_d = 100 \mu\text{l/h}$, and (d) $Q_d = 200 \mu\text{l/h}$.

From the current study, we found that for the same experimental conditions, (at fixed dispersed or continuous phase flow rate and for the same condition), ZnO droplets have bigger sizes when the fluorescent dyes were used. In addition, the particle charge effect becomes slightly significant at high concentration of the dyes. It is quite clear that the resulting ZnO droplets diameter, however, is influenced by dynamical forces depending on the flow rates of the dispersed phase (Q_d) and the continuous phase (Q_c) within the microfluidic devices. Indeed, we attribute the effect of the dyes presence to the electric charges carried

by their molecules. Therefore, to check the dependence of ZnO droplets diameter on electrical charges we conducted the following experiments in the presence of potassium chloride (BioXtra, $\geq 99.0\%$, Sigma-Aldrich).

Exploiting the aforementioned results, we prepared a mixture of different labelled ZnO droplets in the presence of potassium chloride (*KCl*), which allowed to perform experiments in mediums entirely solvated with K^+ and Cl^- ions. Besides, we increased the interactions complexity by investigating ZnO nanoparticles suspension that consisted of several types of solutions carrying out diverse charges, in particular rhodamine B, rhodamine 6G, and fluorescein at various concentrations.

We present our observations in Figure 4.6. In fact, this figure shows how the continuous phase flow rate affects ZnO droplets average size. While Figure 4.7(a) and Figure 4.7(b) display the impact of the dispersed phase flow rate on ZnO mean droplets size. From these findings, we found that; in all our experiments, the addition of *KCl* actually increases the average droplets size of ZnO produced in the microfluidic system. We believe that under such conditions the electrical charges of ZnO nanoparticles are significantly higher and also the long-range repulsive forces²⁸⁸⁻²⁹⁰. In addition, we would also like to point out that we have noticed a decrease in droplets size in the case where the dyes were used separately. On the other hand, when several dyes have been mixed, the droplets size was larger as the electrostatic interactions range was important. As one would expect, we confirmed that the electrostatic interactions within ZnO microdroplets can be tuned by charged particles addition in the medium.

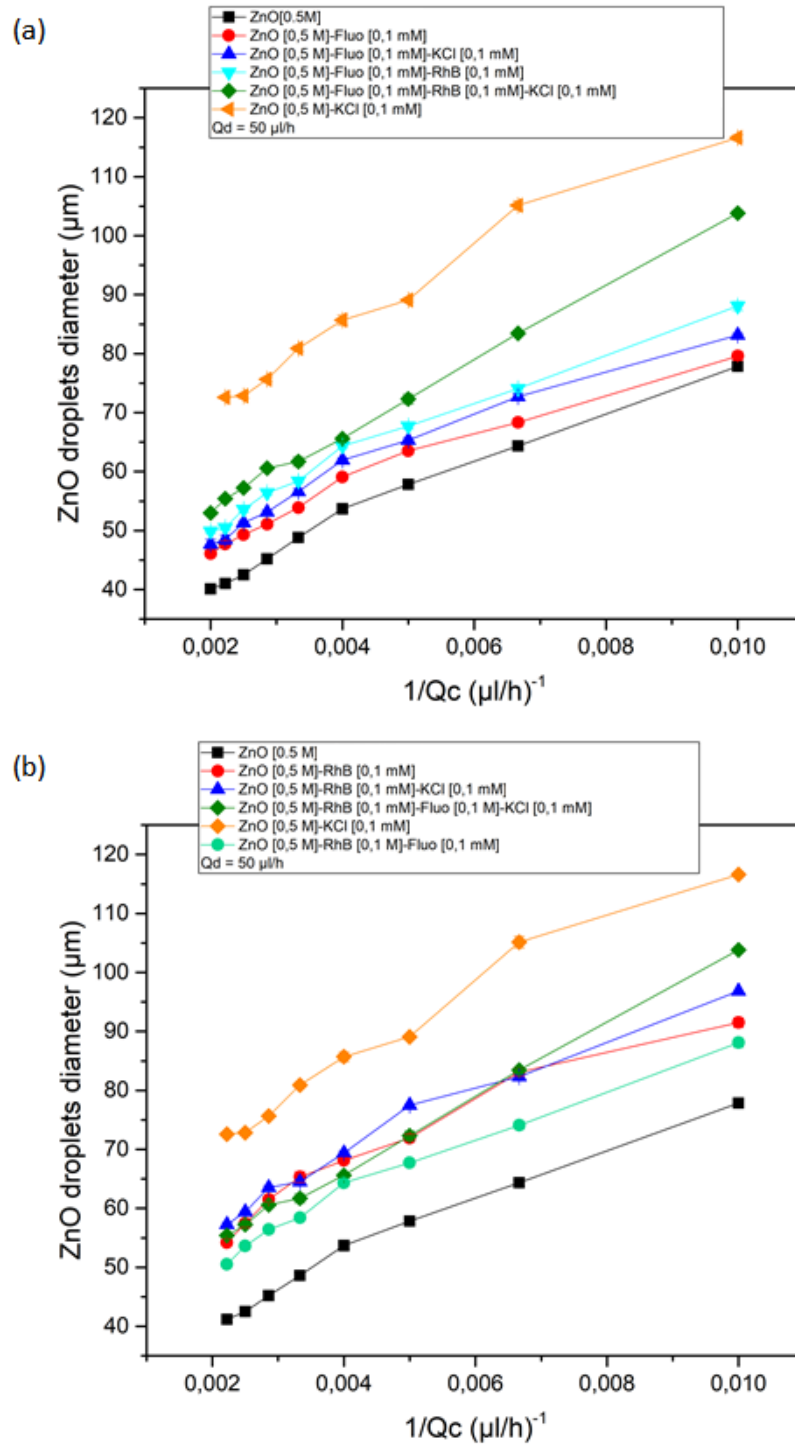


Figure 4.6 The effect of KCl solution on droplets diameter versus continuous phase flow rate for a given dispersed phase flow rate equal to 50 $\mu\text{l/h}$.

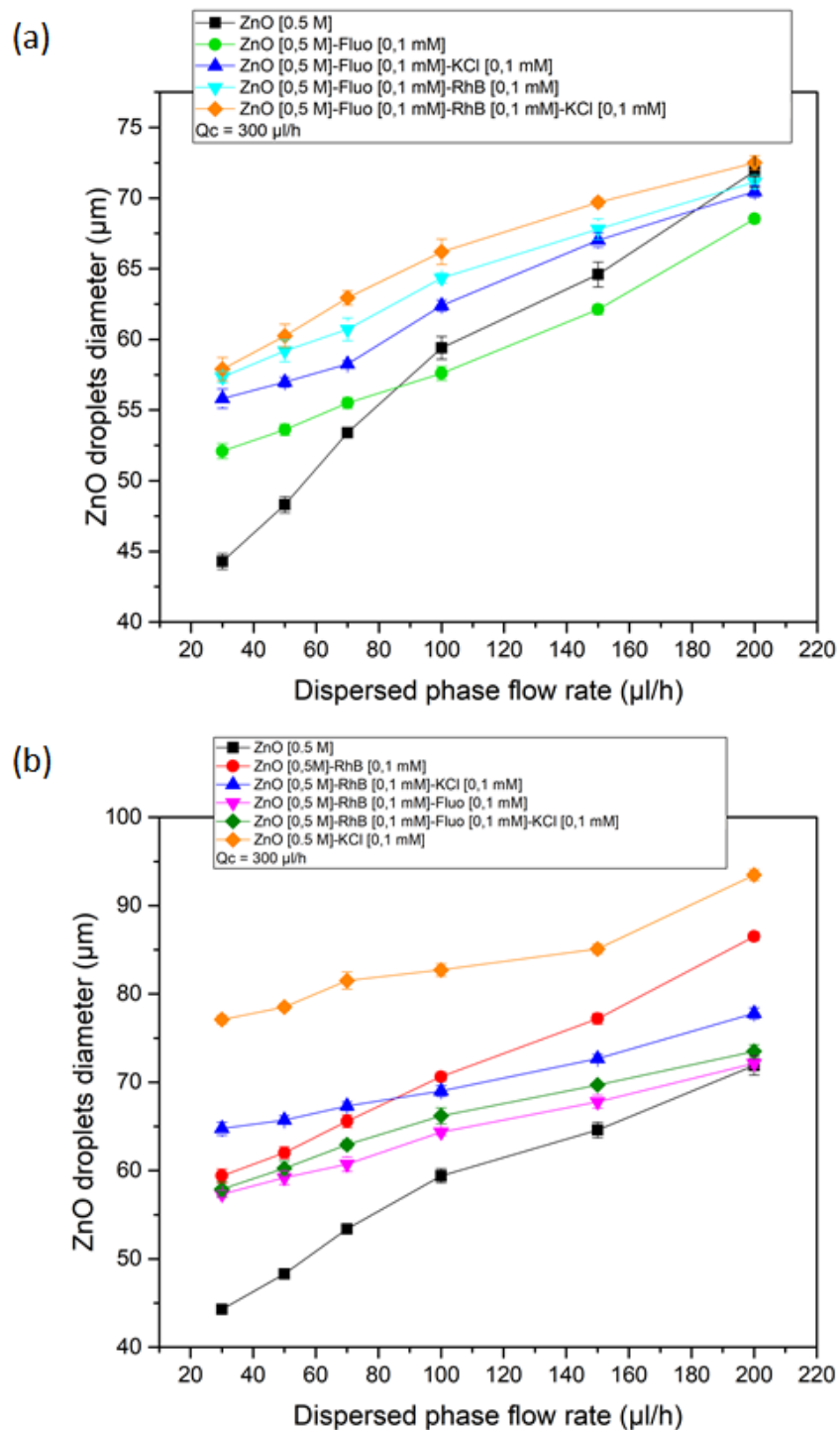


Figure 4.7 The effect of KCl solution on droplets diameter versus dispersed phase flow rate for a given continuous phase flow rate of $300 \mu\text{l/h}$.

4.2 Experimental evidence of polar ZnO microdroplets formation

In order to better understand the effect of the presence of a permanent and a strong electric dipole carried by ZnO nanoparticles on their organization and orientation in the condensing droplets, and later in the shell of the synthesized ZnO microcapsules, we produced two types of ZnO microdroplets. In the first set of experiments, we fabricated pure zinc oxide monodisperse droplets (0.5 M). In the second set of experiments, we doped the dispersed phase (ZnO nanoparticles suspension) with rhodamine B molecules, at a concentration of 0.1 mM. Then, we collected the two types of ZnO droplets in a Petri dish, which was previously pre-filled with HFE 7500 oil, and analyzed their organization at the oil/air interface, using optical microscopy. Moreover, it is useful to keep in mind that we used the non-ionic dSURF surfactant for stabilizing droplets, in order to avoid strong charge-charge interactions between surfactant molecules and ZnO nanoparticles, which would force the nanoparticles to adopt a specific orientation at the droplet interface. Also, at the used basic pH (8.2), rhodamine B molecules are present in their zwitterionic form, as shown in Figure 4.8.

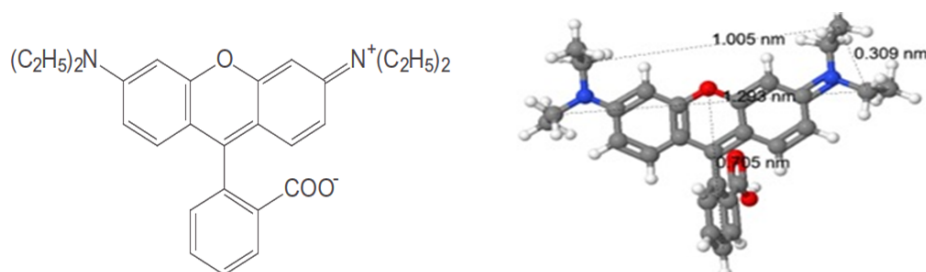


Figure 4.8 Zwitterionic form of Rhodamine B at a basic pH.

Figure 4.9 shows optical microscopy images of the organization of ZnO droplets at the oil/air interface (in the Petri dish) in both experiments. Interestingly, these images reveal that despite using a small ratio of doping rhodamine B by respects to *Zn* atoms (this concentration refers to the initially used zinc acetate concentration), $\frac{[RhB]}{[Zn]} = 2.10^{-4}$, the two types of droplets organize in two different patterns: dye-doped droplets organize in an unexpected square pattern. Whereas pure ZnO droplets organize in a standard close packing hexagonal pattern, as can be seen in Figure 4.9(a). In the latter case, we assume that the droplets carry a priori a neutral global surface charge. Accordingly, the ZnO droplets act in such a way as to minimize their total surface area in order to reduce the free energy of the interface. This therefore corresponds to an organization of droplets in a hexagonal network, where each drop is surrounded by six others.

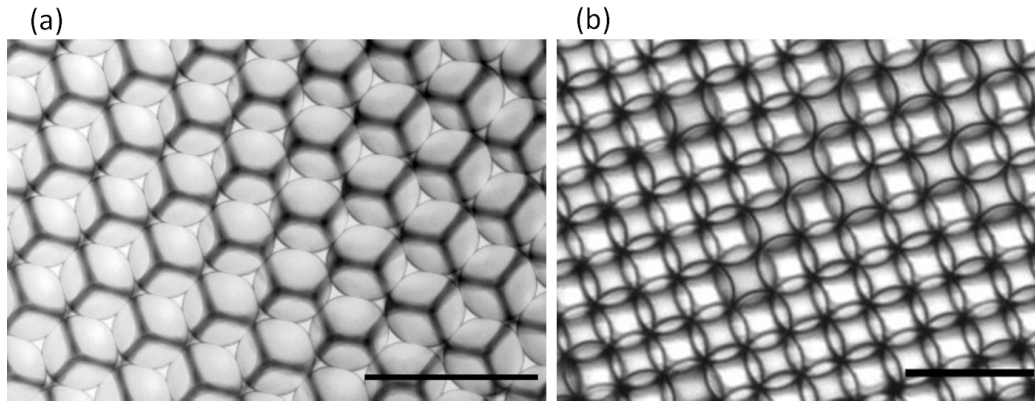


Figure 4.9 Optical microscopy images of ZnO droplets collected after their generation at the oil/air interface showing a long range hexagonal order (a) and doped droplets with rhodamine B dye displaying a square organization (b). This organisation highlights the existence of a spontaneous electrical polarization within these droplets. Scale bars, 100 μm .

The observed transition from a hexagonal organization to a square organiza-

tion of microdroplets at the microscale reveals a change of the organization of ZnO nanoparticles at the nanoscale. This may indicate in turn a change in the balance between the main interactions that govern the organization of ZnO nanoparticles at the droplets interfaces (droplet/oil and droplet/air). More precisely, strong electric interactions between ZnO nanoparticles would favor electrically stable anti-parallel or tail to head orientations, whereas strong interfacial interactions with the surrounding media (oil and air) would favor electrically unstable parallel orientation of the electrical dipole of ZnO nanoparticles. Consequently, the screening of electric interactions by adding ions or charged molecules may lead to a shift in such a balance and hence in nanoparticles orientation and droplets organization.

4.3 Experimental evidence of the adsorption of rhodamine dye molecules at the droplet interface

4.3.1 Analysis of rhodamine B fluorescence intensity in flowing droplets

Taking account of the observed effect on the organization of ZnO droplet by the addition of a very small number of rhodamine B molecules by regards to the total amount of ZnO units in each droplet (one RhB molecule for 5000 ZnO units), it can be inferred that rhodamine B molecules should adsorb preferentially at the droplet interface where a thin layer of ZnO nanoparticles start to self-organize. We suggest that such adsorption of rhodamine B molecules is favored by the presence of surfactant molecules at the droplet interface, enhanced by the hydrophilic

PEG block of dSURF molecules, as reported by some authors²⁹¹. In order, to verify this assumption, we carried fluorescence detection experiments of rhodamine molecules in flowing dSURF stabilized droplets, using our real-time detection optofluidic setup shown in Figure 2.5.

Figure 4.10(a) shows a detailed fluorescence intensity peak of one of the flowing ZnO droplets, which were doped with a rhodamine B dye solution (0.1 mM). It shows that the droplet fluorescence peak has an asymmetric shape, with a sharp increase of the fluorescence intensity at the back by regards to the slope of the increase of fluorescence at the front. Also, a quick comparison of the area under the fluorescence peak in region A and B of the observed shape of the fluorescence peak, indicates clearly that rhodamine B dye density is higher at the back of the droplet than at its front.

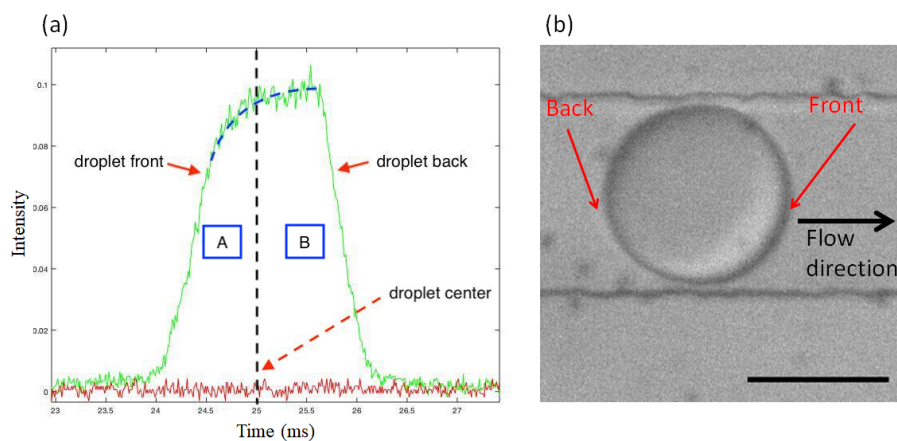


Figure 4.10 Shape of the fluorescence intensity signal versus time emitted from RhB-doped ZnO microdroplets in the presence of dSURF surfactant (a). The corresponding optical microscopic image of the microdroplet flowing inside the microfluidic channel (b). Scale bar, 50 μm .

The size of the droplets was about 62 μm and the size (width) of the microflu-

idic channel was approximately $70\ \mu\text{m}$, as shown in Figure 4.10(b). From this figure, it can be noted that the shape of the droplets remains perfectly circular as the droplets move along the microfluidic channel, without any deformation. Therefore, one may expect that the fluorescence intensity peaks issued from such flowing circular droplets would have been of a symmetric Gaussian shape, unlike what we observe. Indeed, the intensity of the fluorescence should increase as the droplet flows through the channel and passes in front of the laser spot (which remains still during detection while droplet is moving), it reaches its maximum intensity when the laser spot footprint and the droplet reach their maximal overlapping. Nevertheless, the observed shape of the recorded fluorescence peaks is not Gaussian, nor symmetric.

4.3.2 Tip streaming effect

To explain the strongly asymmetric shape of fluorescence peaks, one should take account of the so-called tip-streaming effect, which corresponds to the accumulation of droplet stabilizing surfactant molecules at the rear of the droplets when flowing in a microchannel^{292,293}.

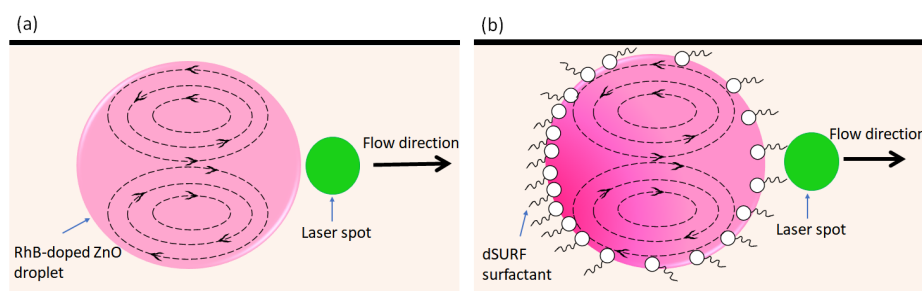


Figure 4.11 Schematic illustration of tip-streaming effect in RhB-doped droplet carried by a flow of HFE oil: (a) without surfactant and in the presence of surfactant (b).

Because of the parabolic profile of the flow velocity in a Hagen-Poiseuille regime, the droplet is submitted to a velocity gradient (through it) with a maximum velocity in the central part of the droplet and a minimal one at the microchannel wall region. As a matter of fact, the induced re-circulation flow brings surfactant molecules from the front of the droplet to its back, creating therefore a surface density gradient of surfactant molecules, which engenders on the surface during this movement, an asymmetrical distribution of the dSURF surfactant and its accumulation at the back of the droplet, as depicted in Figure 4.11.

This effect has been observed by other authors. For instance, Hayat et al. reported the accumulation of rhodamine B (positively charged) at the back of the droplets in the presence of the krytox surfactant, which is negatively charged²⁹³. In addition, Baret et al. studied the accumulation of fluorescent surfactant molecules (in aqueous solution) named Krytox-PEO at the back of droplets circulating in a flow composed of a perfluorinated carrier oil²⁹².

On the other hand, in our study, where we used a surfactant (dSURF) which is neither charged nor fluorescent, if there was no preference for the adsorption of rhodamine B molecules with the surfactant, we should not see any effect whether it is at the front or at the back of the droplets, and the signal would be more or less symmetrical. But if more rhodamine B is placed at the back of the droplets, the fluorescence signal of the rhodamine B will be stronger at their back. This is the proof that there is indeed a preferential adsorption of rhodamine b molecules due to the non-ionic dSURF surfactant. Additionally, these results present a direct evidence of the rhodamine B interaction with the surfactant molecules (especially the hydrophilic part that is in contact with the droplets interface).

4.4 Different models for the organization of ZnO nanoparticles at the droplet interface

As far as we know, the organization in a square array of genuine liquid microdroplets is reported here for the first time. Such organization may be explained by two models of ZnO/RhB nanoparticles organization at the droplets interface, leading either to droplets with opposite charged surfaces or polar droplets, as we discuss in the following.

Model 1

The formation of two types of microdroplets with opposite surface charge: a positively charged surface, which would be induced by a self-organization of ZnO nanoparticles exhibiting all their Zn^{2+} planes outwards by regards to the droplet interface (a1 model), and a second type of droplets with a negative surface charge due to the organization of ZnO nanoparticles with an opposite orientation (b1 model), as shown in Figure 4.12(a1) and Figure 4.12(b1), respectively.

We may list also two additional possible orientations of ZnO nanoparticles (though they lead to droplets with non charged surfaces). They consist of either an anti-parallel radial orientation (c1 model , Figure 4.12.c1) or a planar 'tail to head' orientation (d1 model, Figure 4.12.d1). They are both favored by attractive electric forces between ZnO nanoparticles. It is noteworthy that these two last orientations are more stable, than the previous (radial) parallel orientations, if one considers only electrical interactions between ZnO nanoparticles.

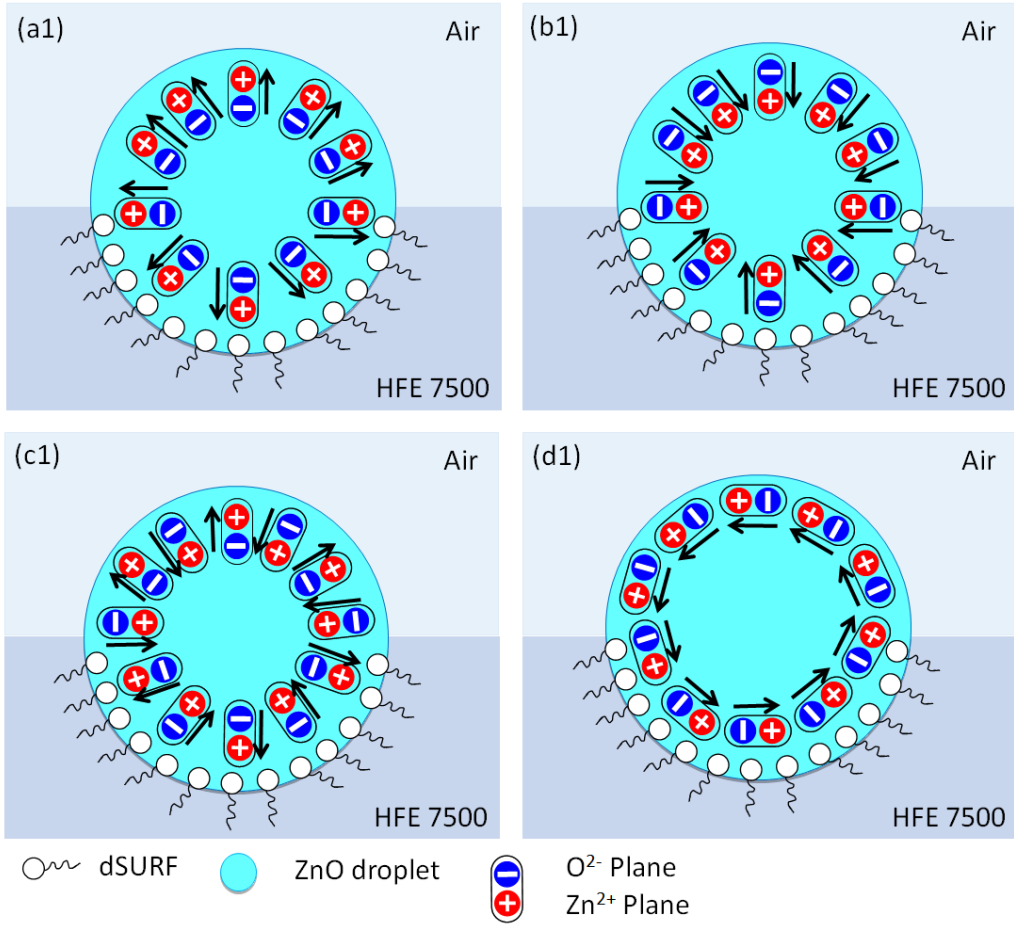


Figure 4.12 Organization models for ZnO nanoparticles at the droplet interface: The coexistence of both organizations (a1) and (b1) models may explain the observed square lattice of droplets, unlike the non polar organization of (c1) and (d1) models.

Model 2

The formation of polar microdroplets with a net electrical dipole induced by a polar ordering of ZnO nanoparticles, as sketched in Figure 4.13. We assume for this model that interfacial forces dominate electrical forces acting between ZnO nanoparticles at the droplet/air interface, whereas due to the presence of dSURF

surfactant at the droplet/oil interface, dipolar electrical interaction between ZnO nanoparticles located at this interface dominate interfacial forces.

Within this model, two types of ZnO nanoparticles organizations may be considered at the droplet/air interface. They both consist on a parallel radial organization with either O^{2-} or Zn^{2+} planes are oriented outwards (by regards to the droplet interface), as sketched in Figure 4.13(a2) and Figure 4.13(b2), respectively.

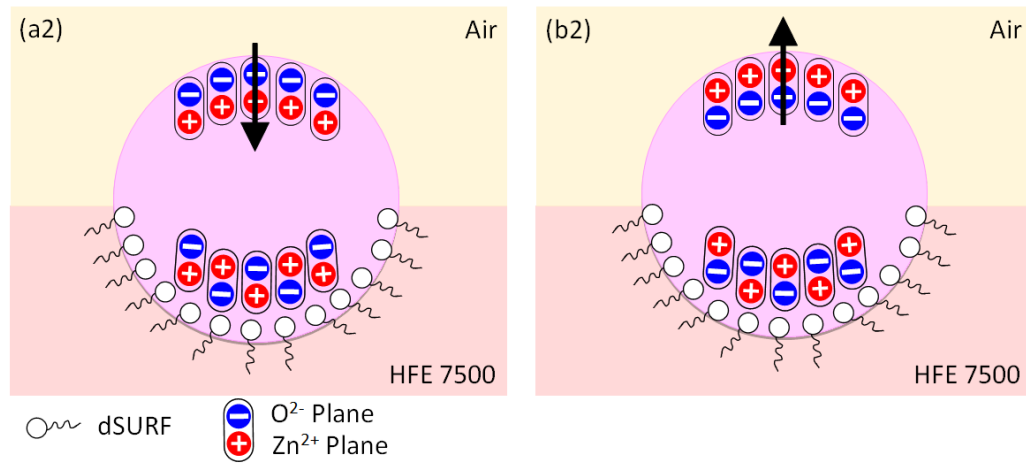


Figure 4.13 Schematic illustration of the asymmetric organization of ZnO nanoparticles which may lead to the formation of polar microdroplets which is compatible with a square organization of microdroplets at the oil/air interface.

4.5 Suggested models for droplets square organization

Actually, except 'c1' and 'd1' models, the previously suggested models are compatible with the observed square self-organization of RhB doped ZnO micro-

droplets. Nevertheless, additional parameters should be taken into account in order they match with the desired organization, as we discuss in the following.

For the first two models, 'a1 model' and 'b1 model', which lead to droplets with a positive surface charge and a negative one, respectively, it is mandatory that the two types of organizations coexist, in order to have both attractive and repulsive electrostatic interactions, between nearest droplets, to give rise to a square organization, as shown in Figure 4.14(a). However, as discussed previously, such organizations are not favored by regards to dipolar interactions between nearest ZnO nanoparticles located at the droplet interface. Therefore, one should consider that interfacial tensions between the droplets and its surrounding phases (oil and air) should be strong enough to stabilize such an organization.

Nevertheless, if one should assume that interfacial tensions should be minimized for one type of orientation (let's say 'a1 model'), it cannot be minimized for the opposite orientation (that is 'b1 model'). This argument demonstrates unambiguously that interfacial tensions should not be taken into account (alone) to explain the formation of a square lattice of ZnO droplets which would be governed by charge-charge interaction between nearest droplets, as sketched in Figure 4.14(a). More precisely, if interfacial tensions were strong enough, they would lead to one type of orientation, either 'a1 model' or 'b1 model', which would lead to hexagonal close packing of the droplets, as sketched in Figure 4.14(b).

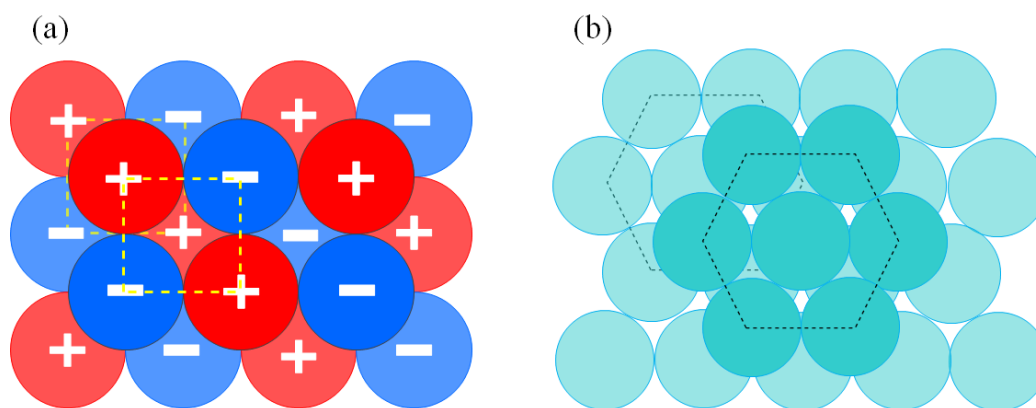


Figure 4.14 Schematic illustration of ZnO microdroplets organizations in a square (a) and hexagonal (b) lattices.

We speculate that, because of the role played by dSURF surfactant molecules at the droplet/oil interface, the interfacial tension of this interface should be much lower than the interfacial tension of the droplet/air interface. A weak interfacial tension would favor an anti-parallel orientation of nearest ZnO nanoparticles, whereas a strong interfacial tension would favor a polar orientation of ZnO nanoparticles, as depicted by 'a2' and 'b2' dipolar models. We assume that in Figure [4.14](#), the '+' sign can indicate either a positive surface charge or an upwardly oriented electric dipole, and the '-' sign can show either a negative surface charge or a downward-facing electric dipole.

4.5.1 Suggested role of rhodamine B molecules in the formation of square organization of ZnO droplets

As experimentally observed, the addition of rhodamine B molecules to ZnO nanoparticles suspension plays an important role in the organization of the droplets at the

oil/air interface. We suggest that the addition of such charged molecules leads to the screening of dipole-dipole interactions between ZnO nanoparticles and the weakening of repulsive electrical forces between two neighboring parallel electrical dipoles. In a such a manner, interfacial forces may take over electric forces and induce a transition from an anti-parallel orientation of ZnO nanoparticles to a parallel orientation, at either droplet/air interface and/or droplet/oil interface.

Nevertheless, because of the presence of dSURF surfactant molecules at the droplet/oil interface, interfacial forces at this interface are presumably too weak by regards to dipole-dipole forces to be able to induce a parallel orientation of ZnO nanoparticles at the droplet/oil interface (even in the presence of rhodamine B molecules). Therefore, we suggest that the ordering of ZnO nanoparticles is most likely as the ones depicted by 'a2 model' or 'b2 model'.

4.6 *Top-down* hierarchical organization of ZnO nanoparticles at the droplets interface

As discussed above, the ability of surface tension to lead to the formation of electrical dipoles at the droplet/air interface is quite interesting. One may ask therefore the following questions:

- What would happen when two neighboring droplets, with both their electrical dipoles oriented initially upwards, rotate in order to set their dipoles anti-parallel and to maximize their dipole-dipole interactions?
- Do ZnO nanoparticles, that are initially oriented parallel at the droplet/air

interface, change the orientation of their dipoles, to make them anti-parallel to each other once they come at the droplet/oil interface?

If the answer to the second question is yes, the organization of the droplets in a square lattice would not be observed. Therefore, one should admit that the shift of oriented parallel ZnO nanoparticles from droplet/air interface to droplet/oil interface should not induce a change to anti-parallel orientation, as shown in Figure 4.15. The same reasoning may be held for the anti-parallel orientation of ZnO nanoparticles initially located at the oil/droplet interface, which should not change their orientation when brought to the droplet/air interface. The locking of initially adopted orientation enables in turn to organize droplets with their overall electrical dipole anti-parallel, contributing therefore to create a long-range dipole-dipole attractive interaction between droplets. Such long range dipole-dipole interaction should become the driving force, that govern the observed square organization.

Also, one may reconsider now 'a1 model' and 'd1 model', which lead respectively to positive and negative surface charges. In this case, the stronger (+)/(-) charges interactions (by respect to dipole-dipole interactions) between opposite charged droplets may be responsible for the formation and stability of two types of opposite surface charged droplets (and neglecting therefore the effect of interfacial tension). In such a way, macroscopic electrostatic interactions would act to induce the observed square organization of the ZnO microdroplets, which is consistent with the experimental finding shown in Figure 4.9.

As a main conclusion for this section, we have shown that the organization of ZnO nanoparticles at the nanoscopic scale (at the droplet interface) is mainly

governed by long range droplet-droplet interactions which take place at the microscopic scale. That is to say, ZnO nanoparticles and droplets organizations follow an unusual top to bottom ordering hierarchical scheme.

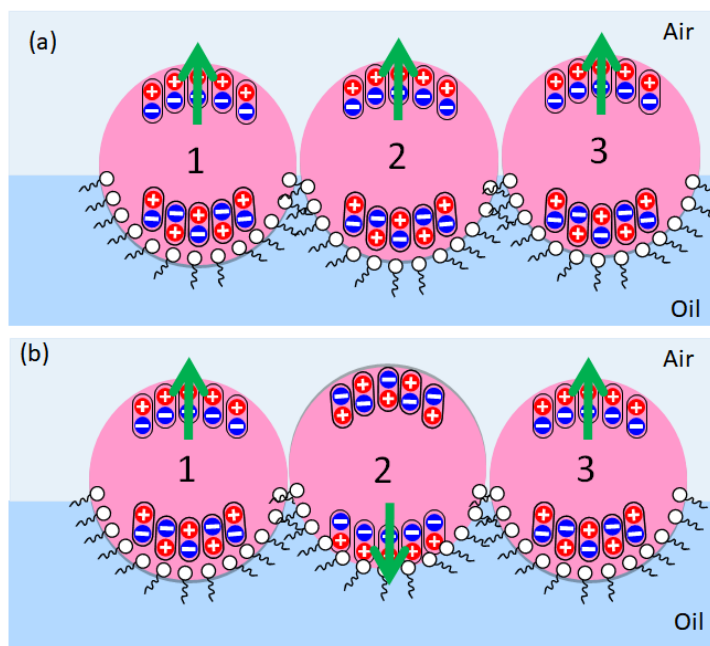


Figure 4.15 Schematic illustration of dipolar interactions between polar ZnO droplets (see text for details).

Chapter 5

Confocal analysis, structural properties and formation mechanism of ZnO microcapsules and their shell

To better understand the aggregation process of primary ZnO nanoparticles leading to the formation of ZnO microcapsules, we dope the primary ZnO nanoparticles dispersion with a solution of different dyes such as rhodamine B (RhB) at a 0.1 mM concentration. We focused in the previous chapter on the interfacial organization of such nanoparticles based on ZnO droplets organization properties. In this chapter, we use confocal microscopy to analyze the organization and the resulting outstanding electrical and optical properties of the synthesized ZnO microcapsules.

5.1 New insights into the organization of ZnO nanoparticles using confocal microscopy

5.1.1 Direct evidence of the shell-core structure of ZnO microparticles

Figure 5.1 shows fluorescence and white field confocal images of doped ZnO microspheres with different sizes. More precisely, images (a-b) corresponds to microparticles with a size of about $12.4 \pm 0.3 \mu m$ (sample 1). They show circular fluorescent regions surrounded by a narrow dark region, whose width is found to be about $1 \mu m$. The size of the initial droplets was about $30 \mu m$. Images (c-d) correspond to another sample of RhB-doped ZnO microcapsules made of droplets with a diameter equal to $48.5 \pm 0.3 \mu m$ (sample 2). The size of the circular fluorescent region is found in this case equal to $16.8 \mu m$. They are surrounded by a dark tiny region whose width is about $1.3 \mu m$. The comparison of white-field images with the fluorescence images indicates clearly that ZnO microspheres are in close contact and form a perfect hexagonal array. Therefore, one may conclude that rhodamine B dye is excluded from the microcapsules shell, and only the inner part of the microcapsules is fluorescent. These images confirm unambiguously that the doped ZnO microspheres are made of non fluorescent shell, whose thickness is about half of the width of the dark strip separating two close fluorescent regions, i. e. , $0.5 \mu m$, and $0.65 \mu m$, for microcapsules of sample 1 and sample 2, respectively. It is interesting to note that these values are very close to the previously measured ones for the shell thickness of microcapsules made from $48 \mu m$

droplets (see Section 3.3).

It is note worthy that microparticles for all samples should be re-suspended in HFE oil, in order to facilitate their homogeneous deposition and distribution on the microscope plate. Therefore, microparticles absorb oil, swell and their size increases. This is the reason why, the measured sizes of the microparticles are higher than those deduced from SEM analysis.

Another important remark concerns the full-like feature of the microcapsules when they are analyzed using fluorescence confocal microscopy. Indeed, we expect to observe a hollow structure as this imaging technique performs, for a given Z position, a X-Y scan in order to construct, pixel by pixel, the image of the sample. As mentioned above, since microcapsules are filled with oil during the confocal analysis and also because of the observed (small) solubility of rhodamine B dye in HFE oil, which is actually enhanced by the presence of the surfactant. Thus, the microcapsules appear like full fluorescent microspheres and not as hollow.

Figure 5.1(e-f) corresponds to another sample of doped microcapsules with a measured size equal to $19.9\ \mu m$ (sample 3), which were left several hours on the microscope plate before recording confocal images (in order to let HFE oil evaporates). It is known indeed that HFE 7500 has low vapor pressure of about 0.4 atm at normal temperature and pressure. This is why, microcapsules condense completely at the air/oil interface within few hours at normal temperature and pressure. Figure 5.1(e) shows clearly that once microcapsules have expelled their confined oil, we can see clearly their hollow feature.

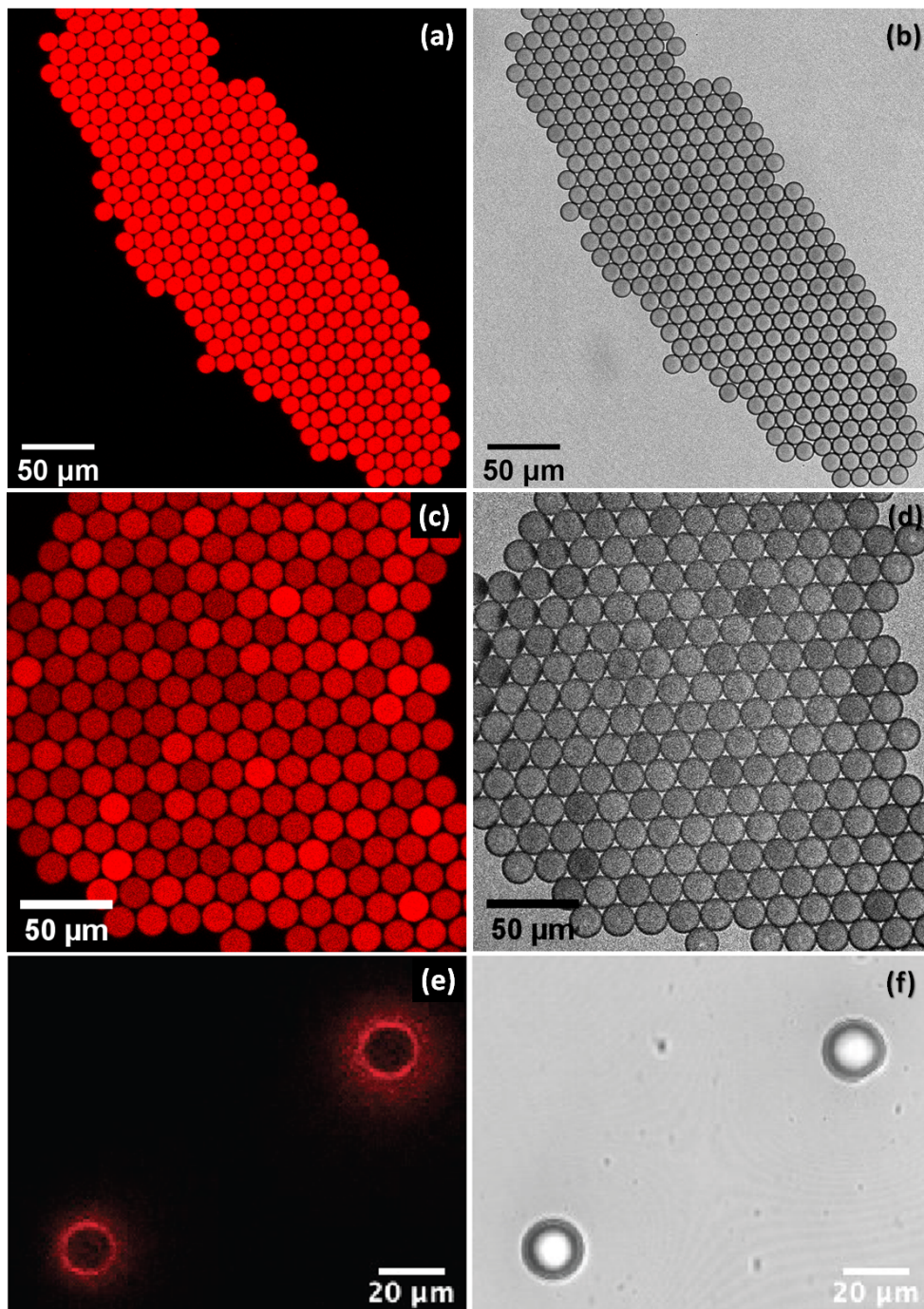


Figure 5.1 (a, c, e) Confocal fluorescence and (b, d, f) the corresponding white field microscopy images of 12.4 μm , 16.8 μm , and 19.9 μm zinc oxide microspheres (where we can see clearly the capsular structure of the microparticles), respectively.

5.1.2 Qualitative analysis of the electrical charges distribution on the surface of the microcapsule shell

Another interesting feature related to the distribution of the surface electrical charges of ZnO microcapsules may be inferred from the analysis of fluorescence images. Figure 5.2(a) shows another confocal fluorescence microscopy image of RhB doped microcapsules, obtained from droplets with a typical size of $46\ \mu\text{m}$, where we can see a noticeable discrepancy in the fluorescence intensity of the different microcapsules. This phenomena can be also observed with doped microcapsules shown in Figure 5.1(c) but is not with microcapsules shown in Figure 5.1(a), which were obtained from droplets with $30\ \mu\text{m}$ diameter. Figure 5.2(c) and Figure 5.2(d), which correspond to standard optical microscopy images of sample 2 and sample 3, respectively, show also a difference in the staining of microcapsules with rhodamine B, from one microcapsule to another.

Figure 5.2(b) shows a plot of the normalized intensity distribution of microcapsules fluorescence obtained from the analysis of the fluorescence intensity of approximately 222 microcapsules. We can see clearly two types of microcapsules, one type that we label P_l (where "l" stands for low), where its normalized fluorescence intensity is centered around 0.6 and a second type that we label P_h (where "h" stands for high), which fluorescence intensity is centered around 0.7.

It is worth noting that all droplets and microcapsules contain the same concentration of rhodamine B dye, $[RhB] = 0.1\ \text{mM}$. In order to understand the observed discrepancy in the fluorescence intensity, it is important to keep in mind that microcapsules are filled with HFE oil. Actually, two effects may therefore explain the observed phenomena:

1. It may originate from a difference in the final concentration of RhB molecules in the microcapsules, due for instance to a hypothetical difference in the porosity of the microcapsules.
2. It may originate from a difference of the interaction between rhodamine B molecules and the internal surface of the microcapsule shell which may vary from one microcapsule to another.

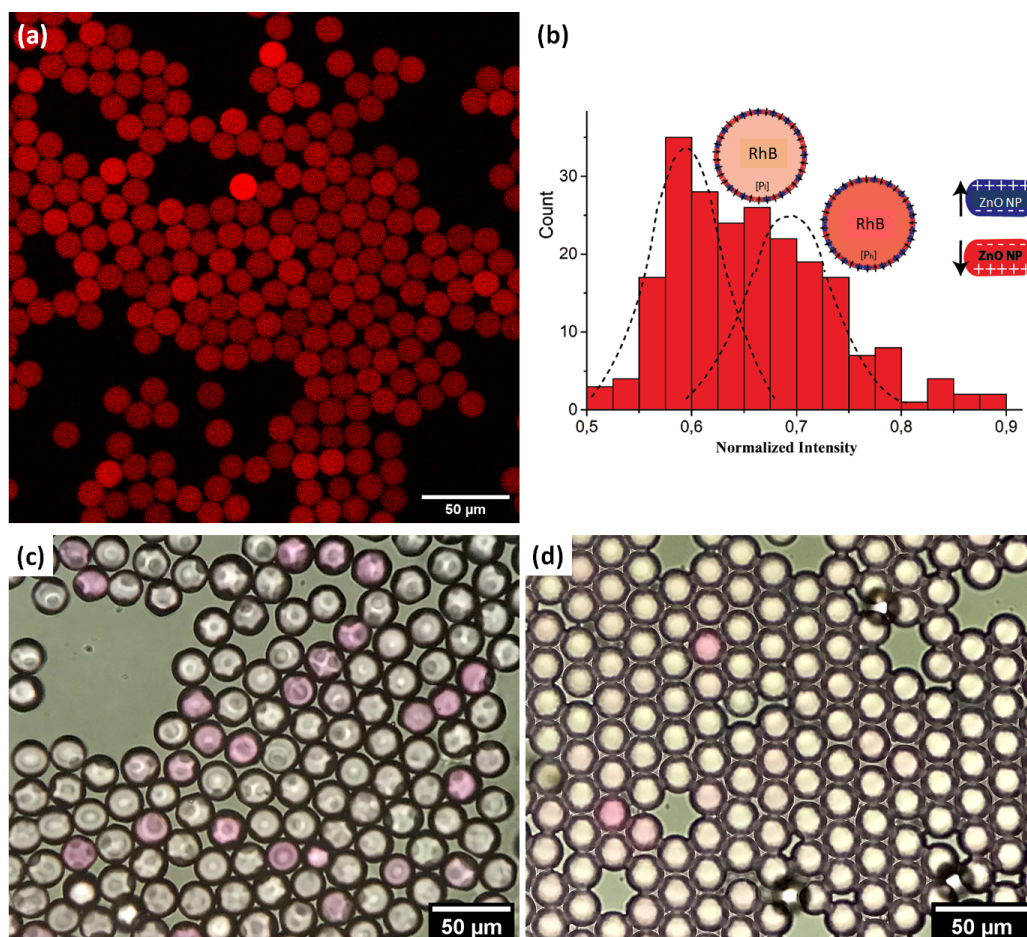


Figure 5.2 (a) Confocal fluorescence and (b) the corresponding distribution of normalized fluorescence intensity; (c, d) optical microscopy images showing a non homogeneous distribution of rhodamine B dye in different microcapsules (average microcapsules size: 24 μm (c) and 20 μm (d)).

We argue in the following that the second hypothesis is more likely to be the correct one. Indeed, as discussed previously, rhodamine B molecules may undergo strong electrical interaction with charged ZnO nanoparticles. Indeed, as mentioned in section (4.2), at the used basic pH (8.2), rhodamine B molecules are present in their zwitterionic form. Therefore, the negatively charged acetate group of RhB molecules may adsorb electrically on the positively charged surface Zn^{2+} planes of ZnO nanoparticles. Moreover, from a chemical point of view, zinc acetate complexes are known to be stable chemical species. Also, we initially used zinc acetate as a reactant to synthesise ZnO nanoparticles.

Therefore, we suggest that depending on the orientation of ZnO nanoparticles at the inner surface of the microcapsules shell, RhB molecules would adsorb more or less strongly on such surface. The higher will be the density of nanoparticles with their Zn^{2+} planes oriented inward, the higher will be the number of adsorbed rhodamine B molecules on the inner surface of the shell and the lower will be the number of rhodamine B molecules transported by the inward flux of HFE oil from outside the microcapsules.

Also, we observe in Figure 5.2(b) two types of microcapsules, a first with a higher fluorescence intensity and a second with a lower one. This result shows that two types of ZnO nanoparticles orientation coexist at the inner surface of the microcapsules. Moreover, we can estimate roughly the proportion of each population by taking into account the number of counts of each population, as shown in Figure 5.2(b). We find, $P_l \simeq 50\%$ and $P_h \simeq 50\%$.

Therefore, we may conclude that approximately 50 % of ZnO nanoparticles should orient their Zn^{2+} planes towards the inner part of the microcapsules (as

such orientation adsorb strongly RhB molecules at the shell interface impeding their diffusion and transport inside the microcapsule by the incoming flux of oil). Also, assuming that the orientation of ZnO nanoparticles is conserved along (across) the microcapsule shell, we may conclude that the outer surface of the microcapsule is mainly positively charged.

We may deduce that initially, the inner surface of the microcapsules shell (of samples 2 and 3) should be predominantly negatively charged, whereas the outer surface of microcapsules should be predominantly positively charged.

5.1.3 Organization of rhodamine B dye in ZnO microcapsules

Let's first calculate the average number of rhodamine molecules in each microcapsule and the surface that they would occupy on the microcapsule shell. As shown in the previous chapter, rhodamine molecules adsorb preferentially at the droplets interface, and since RhB molecules are in minority by regards to ZnO "units" in the droplet, it would be interesting to understand how RhB molecules organize around the inner surface of the shell.

Let N_{RhB} be the overall number of rhodamine molecules in each microcapsule, and let's assume that N_{RhB} remains constant during the condensation process of microdroplets. Then N_{RhB} may be calculated simply as follows:

$$N_{RhB} = N_A[RhB]V_{drop} \simeq 3.5 \cdot 10^9 \text{ molecules/droplet}$$

Where N_A stands for Avogadro number and $V_{drop} = 5.8 \times 10^4 \mu m^3$ corresponds to the volume of each droplet (which size is equal to $48 \mu m$). Taking account of ZnO microparticles size, $R_{\mu part.} \simeq 8 \mu m$, the area of their inner surface is equal to:

$S_{\mu part.} = 4\pi R_{\mu part.}^2 \simeq 8.10^8 \text{ nm}^2$. Considering a maximum area that would occupy each rhodamine B molecule, assumed to be flat on the surface, $\sim 1 \text{ nm}^2/\text{molecule}$ (see Figure 4.8) and the calculated overall number of rhodamine molecules in each microcapsule, $N_{RhB} \simeq 35.10^8$, one may conclude that the overall rhodamine B molecules would organise approximately in four stacked layers on the surface of ZnO microcapsules ($35.10^8 \text{ molecules} / 8.10^8 \text{ nm}^2$). It is interesting to compare this result to the initial value of the surface density of RhB molecules at the droplet interface (before condensation). Taking account of the droplet size, $R_{drop.} = 24 \mu m$, and the preferential adsorption of rhodamine molecules at the droplet interface, one finds that the surface density of RhB molecules at the early stage of condensation should be about 7.10^9 nm^2 . From this value, one may conclude that RhB molecules should form less than a single monolayer at the droplet interface.

Also, we recall that ZnO nanoparticles primary building blocks carry an intrinsic dipole moment along their c-axis and carry therefore opposite electrical charges on their two opposite surfaces. Therefore, one may expect that rhodamine B molecules would adsorb strongly on the surface of ZnO nanoparticles. We speculate that ZnO nanoparticles should adsorb also at the droplet interface, instead remaining dispersed within the droplet. The building of the microcapsule shell should occur by aggregation and self-organization of such nanoparticles as the solvent evaporates and the size of the droplet decreases.

It is note worthy to compare this result to the reported literature. Colfen et al. [45] reported in previous studies related to oriented aggregation-mediated growth of primary nanoparticles in the presence of soluble organic molecules, such as polymers and surfactants, one would expect that rhodamine B molecules serve as

intercalating bridges between aggregated ZnO nanoparticles during the formation process of the microcapsule shell (meso-crystallization). Their exclusion from the formed shell indicates that attractive interaction between ZnO nanoparticles are strong enough to lead to the expelling of the dye from the shell and its adsorption on its inner surface, see Figure 5.3.

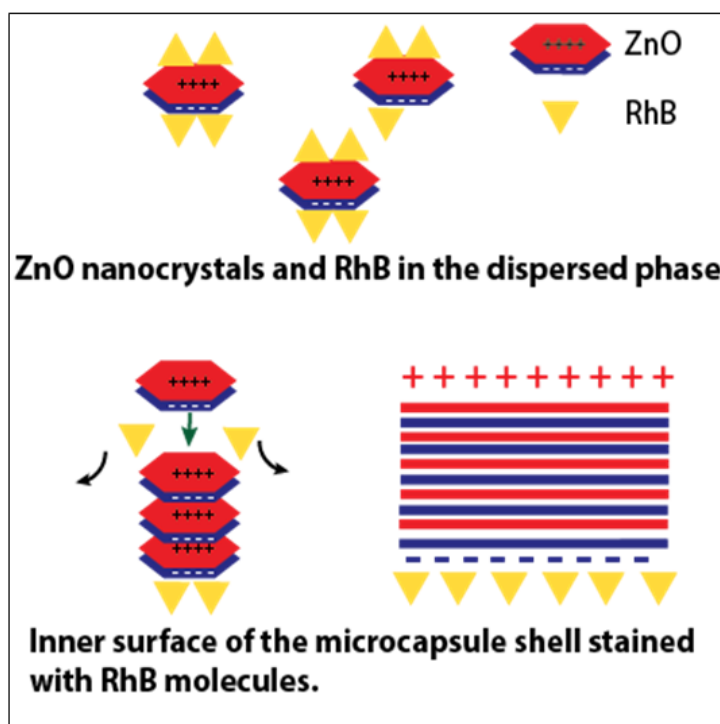


Figure 5.3 Adsorption of RhB molecules on ZnO nanoparticles at the droplet interface and in the microcapsule shell.

5.2 Shell thickness versus microparticle size

The determination of the shell thickness, h , is straightforward and is based on the initial droplet size (volume), V_{drop} , the used concentration of ZnO, C_{ZnO} (zinc acetate), and the final size (radius) of ZnO microspheres, R_p , as detailed in the following.

Let's first assume that interactions between ZnO nanoparticles are independent of the microparticle size. Within the frame of this assumption, the thickness of the shell should vary linearly versus the droplet size. The value of the slope coefficient should depend on the mass density of ZnO microspheres and therefore on the interactions between nanoparticles inside the microspheres. Since the total mass of all confined nanoparticles, m , scales as R_{drop}^3 and/or R_p^3 , the thickness of the microcapsule shell, h , should scale as R_p (or R_{drop}), that is $h_p \propto R_p$. More precisely, for a given microcapsule with a radius R_p and mass density equal to ρ , the shell thickness h may be deduced directly from the volume of the shell, $V_{shell} = 4\pi R_p^2 \times h$. Therefore, one can write:

$$h = \frac{m}{4\pi R_p^2 \rho} = K_1(\rho) \left(\frac{R_{drop}^3}{R_p^2} \right) \quad (5.1)$$

Where $m = C_{ZnO} V_{drop} M_{ZnO}$ is the total mass of zinc oxide in a single droplet, ρ is the mass density of zinc oxide, and $K_1(\rho) = \frac{C_{ZnO} M_{ZnO}}{3\rho}$ is a constant which depends on the mass density (ρ) of zinc oxide (as the concentration was maintained constant for all experiments). If we take $\rho = 5.6 \text{ (g/cm}^3\text{)}$, then $K(\rho) = 2.65 \times 10^{-3}$.

5.2.1 Case of pure ZnO microcapsules

Figure 5.4(top) shows measured values (black curve) and calculated values (red curve) of ZnO microparticles size, D_p , versus droplet size, D_{drop} . As we can notice, microparticles size follow approximately a linear dependence versus the droplet size, with a slope equal approximately to 0.34, in a very good agreement with equation 3.8.

Nevertheless, it is interesting to note that three values of D_p (obtained for D_{drop} equal to $53 \mu m$, $60 \mu m$ and $70 \mu m$) deviate noticeably from this linear feature. Such deviation is larger than the statistical size distribution deviation. This result will be discussed in detail later.

Considering the value of the slope $k = \frac{D_p}{D_{drop}} = 0.34$ of the linear dependence of the size of pure ZnO microparticles (D_p) versus the size of droplets (D_{drop}), as discussed in Chapter 3 (Section 3.2.3), we can rewrite equation 5.1 as:

$$h_{ZnO} = 12.7 \times K_1(\rho) D_p \quad (5.2)$$

Or

$$h_{ZnO}(5.6) = 3.4 \times 10^{-2} D_p. \quad (5.3)$$

We considered for the last equation the standard value of $\rho = 5.6 (g/cm^3)$.

Figure 5.5 shows the observed dependence of the shell thickness, $h(5.6)$, versus the microparticle size, in the case of pure ZnO microcapsules. It is worth noting to remind that the reported values are derived from the measured values of the microcapsule sizes, R_p , and the assumed constant value for the mass density of the shell which is taken in this case equal to the standard value $5.6 (g/cm^3)$. We find for instance a shell thickness of about $0.56 \pm 0.04 \mu m$ for $48.5 \mu m$ droplets, which compares very well, in this case, with the shell thickness value measured directly using scanning electron microscopy, as illustrated in Figure 3.10. Nevertheless, despite a roughly linear dependence of h versus D_p , as expected from equation 5.3, we notice also a break at $D_p \simeq 17 \mu m$. Interestingly, this break coincides with the observed break in the linear dependence of particle size versus

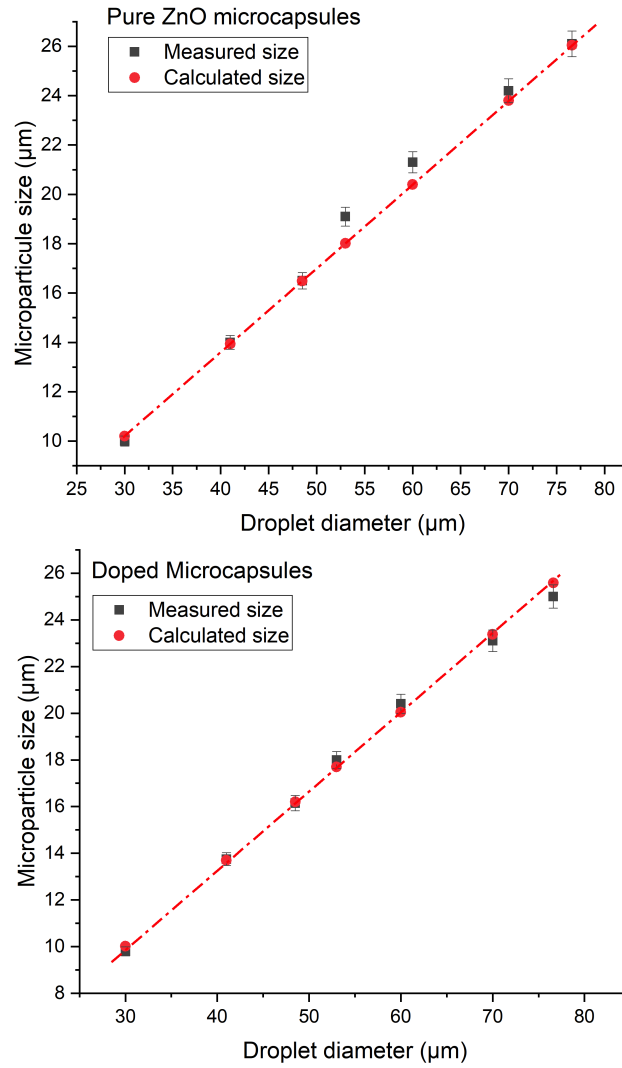


Figure 5.4 Variation of the size of pure ZnO microparticles (top) and RhB-doped microcapsules (down) versus droplet size: black curve corresponds to measured values of D_p and red curve corresponds to calculated values according to eq. 3.8 (for a) and eq. 3.9 (for b).

droplet size, at a droplet size of about $50 \mu m$, as shown in Figure 3.8.

We suggest that the observed transition in the shell thickness vs. the particle size is related to the observed deviation from the linear dependence of the microparticle size versus the droplet size, as observed in Figure 3.8.

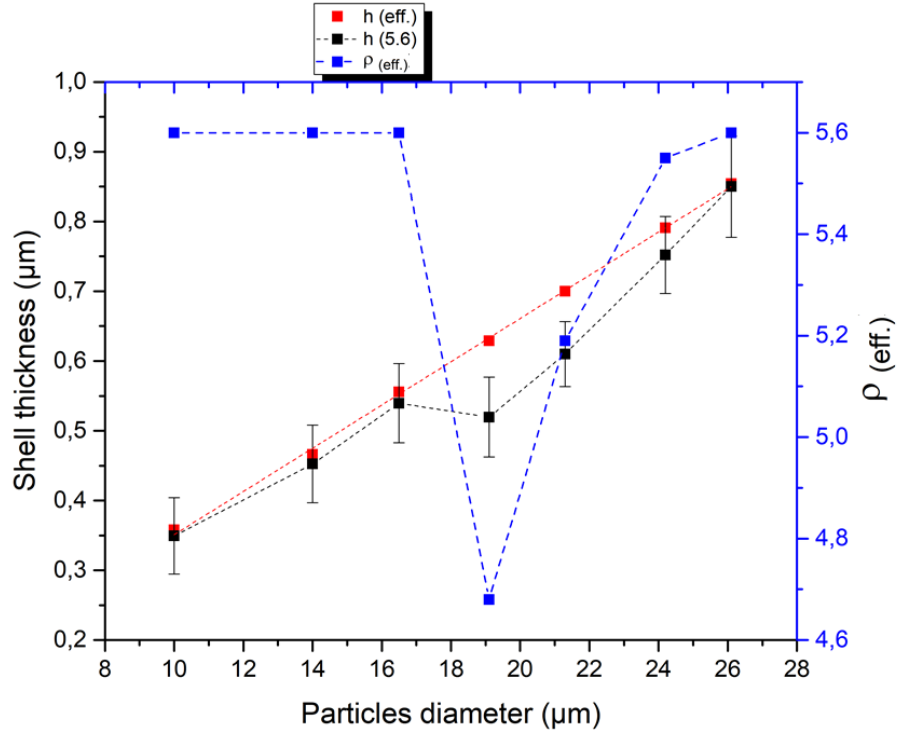


Figure 5.5 Calculated shell thickness and effective mass density of the microcapsules vs. particle size.

Indeed, according to equation (3.8), we expect the microparticle size to be of about $18 \mu m$ when starting from a droplet with a diameter $D_{drop} = 53 \mu m$. This value is noticeably smaller than the measured one, that is $19.1 \pm 0.7 \mu m$.

Therefore, one can deduce an excess size of about $1.1 \mu m$ (by respects to the expected value when the mass density of the shell is set equal to $5.6 g/cm^3$).

The same arguing may be put forward for the shells of the microcapsules that are derived from droplets with diameters equal to $60 \mu m$ and $70 \mu m$. For these shell thicknesses, values appear to be smaller than those which could be derived from equation 5.3

If we consider again the example of $D_{drop} = 53 \mu m$, we should find an excess

value for the shell thickness by regards to the reference value, calculated using equation (5.3), by about:

$$\Delta h^* = 0.13 \mu m \quad (5.4)$$

5.2.2 Case of dye-doped ZnO microcapsules

For RhB-doped ZnO microparticles, we observe a different behavior from the one exhibited by pure ZnO microparticles. Indeed, the plot of D_p versus D_{drop} , shown in Figure 5.4 (bottom), exhibits a very good linear dependence of microparticles size versus droplets size over practically the whole investigated range of droplet sizes, from $30 \mu m$ to $60 \mu m$. Considering again the value of the slope $k^* = \frac{D_p}{D_{drop}} = 0.33$ of the linear dependence of the size of microparticles versus the size of droplets, we can rewrite equation 5.1 as:

$$h_{ZnO/RhB} = 9.2 \times K_1(\rho) R_{drop} = 27.9 \times K_1(\rho) R_p \quad (5.5)$$

Considering again $\rho = 5.6 \text{ g/cm}^3$ and $K(\rho) = 2.65 \times 10^{-3}$, we can deduce the following linear relation between the shell thickness value and RhB-doped microparticle size:

$$h_{ZnO/RhB}(5.6) = 3.7 \times 10^{-2} D_p \quad (5.6)$$

Figure 5.6 shows the variation of the shell thickness h versus microparticle size as deduced from experimental values of D_p and D_{drop} according to equation 3.10. For small microparticles, we notice a very good agreement of the variation of h vs. D_p with the predicted linear feature predicted by equation 5.6. Such agreement does not hold for large microparticles, particularly for $D_p = 23.1 \mu m$

and $D_p = 25 \mu m$ (which correspond to droplet sizes equal to $70 \mu m$ and $76.6 \mu m$, respectively).

Basically, the observed deviation cannot be explained by a decrease of the mass density of the microcapsules shell, as we did previously for pure ZnO microcapsules. Indeed, in order to match h values for $D_p = 23.1 \mu m$ and $D_p = 25 \mu m$ microcapsules, one should assume a mass density value for the shell which should be equal to $6.25 g/cm^3$ and $6.5 g/cm^3$, respectively. These values are noticeably larger than the standard value ($5.6 g/cm^3$). Nevertheless, it is also worth noting that Valour et al.²⁹⁴ reported recently values of about $6.2 g/cm^3$ from an experimental study dedicated to the determination of the mass densities of ZnO synthesized under different harsh experimental conditions (very high temperatures) and using BET technique.

Therefore, in our study, one may put forward that the addition of rhodamine B molecules may enhance a close packing of ZnO nanoparticles, by either screening the repulsive interactions between ZnO nanoparticles or by establishing specific interactions between Zn^{2+} and the negatively charged acetate group (CH_3CO^{2-}) of RhB molecules, which may lead to a higher value of the mass density of ZnO. Nevertheless, as we have used a similar ratio of RhB molecules (by respects to ZnO units) for all samples. Therefore such argument should be rejected. Rather, we suggest another mechanism in the next section, which enables to explain the observed deviation of the shell thickness from the expected linear regime.

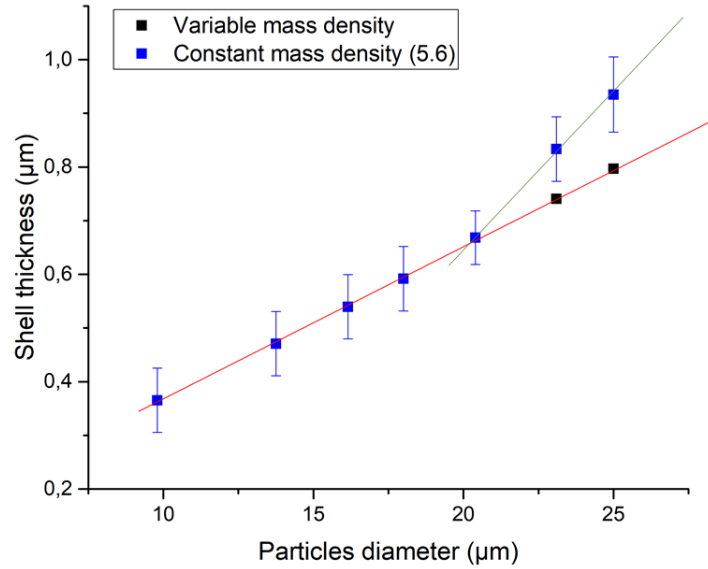


Figure 5.6 Calculated shell thickness vs. particle size of rhodamine B doped microcapsules; blue curve: deduced values from particle size and droplet size, two linear regimes for the shell formation can be deduced, one for small droplets and the second for large ones.

5.3 Experimental evidence of a layered organization of the microcapsule shell

Figure 5.7 shows interesting confocal images of rhodamine doped microparticles, which were obtained from droplets with a diameter equal to $48.5 \mu m$. It is worth noting that Figure 5.7(left) and Figure 5.7(right) correspond to images of the same ROI using a 20X objective in either fluorescence mode (FM) or white field mode (WFM), respectively.

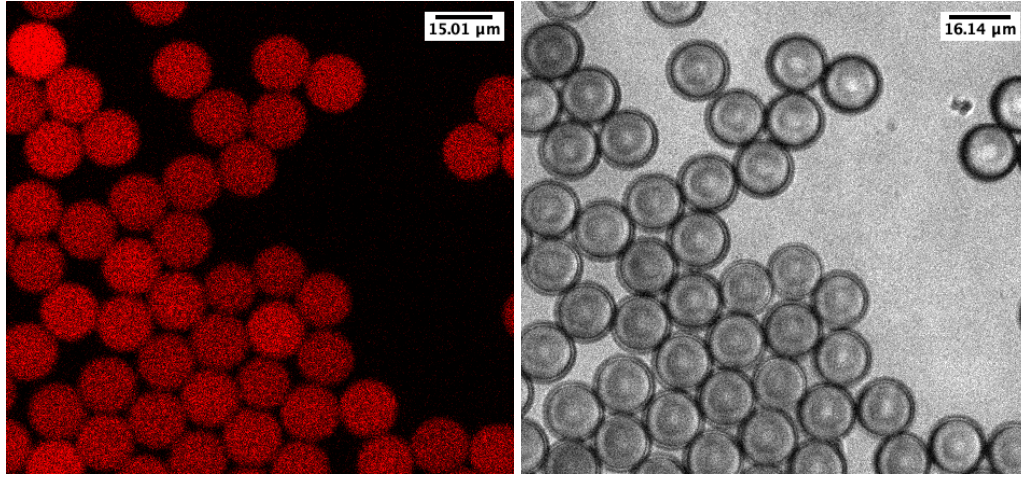


Figure 5.7 Confocal images of RhB-doped ZnO microcapsules with a mean size equal to $16.14 \mu m$ (derived from droplets with a size equal to $48.5 \mu m$) imaged with 20X objective. Comparison of the inner diameter, measured from the fluorescence image part (left) and the external diameter, measured from white field image (right), shows clearly the structure. Despite the relatively low resolution of these images, we find a good agreement between the measured value ($0.56 \mu m$) of the microcapsule shell thickness and the calculated value ($0.57 \mu m$) using eq. [3.10](#).

We can see clearly from the WFM image that the microspheres possess a capsule-like structure. Because of the relatively low resolution of this images (X20 objective), a rough estimation of the thickness of the shell can be deduced from the comparison between the $16.1 \mu m$ value found for the inner diameter of the microcapsules, as measured from the FM image, and the $15 \mu m$ value outer diameter as measured from the WFM image. Indeed, as previously argued in section [\(5.1.3\)](#), rhodamine dye molecules adsorb only on the inner surface of the microcapsule shell. We can deduce a shell thickness value equal to $\frac{D_{out} - D_{int}}{2} = 0.56 \mu m$, which is in a very good agreement with the calculated value ($0.57 \mu m$) from equation [\(3.10\)](#).

A more accurate measure of the shell thickness can be deduced from the

analysis of Figure 5.8, which shows interesting confocal images of two isolated ZnO rhodamine doped microparticles, and obtained from droplets with a diameter equal to $60\ \mu m$ and imaged using a 40X objective. In this case, microcapsules exhibit circular apertures with a size equal to $12\ \mu m$ (microcapsule on the left) or $10\ \mu m$ (microcapsule on the right). It is important to remind that microparticles were re-suspended in HFE oil prior to confocal microscopy analysis. Therefore, they absorb HFE oil, swell and eventually the shell breaks under the effect of osmotic pressure, as shown in Figure 5.8.

We used LAS X software of the SP8 LIGHTNING confocal microscope to observe and analyze the previous samples in more detail. Three-dimensional (3D) visualization and analysis allow us to understand the topology of three-dimensional images, and thus confirm the existence of a hollow structure within the synthesized ZnO microspheres, as evidenced in Figure 5.9.

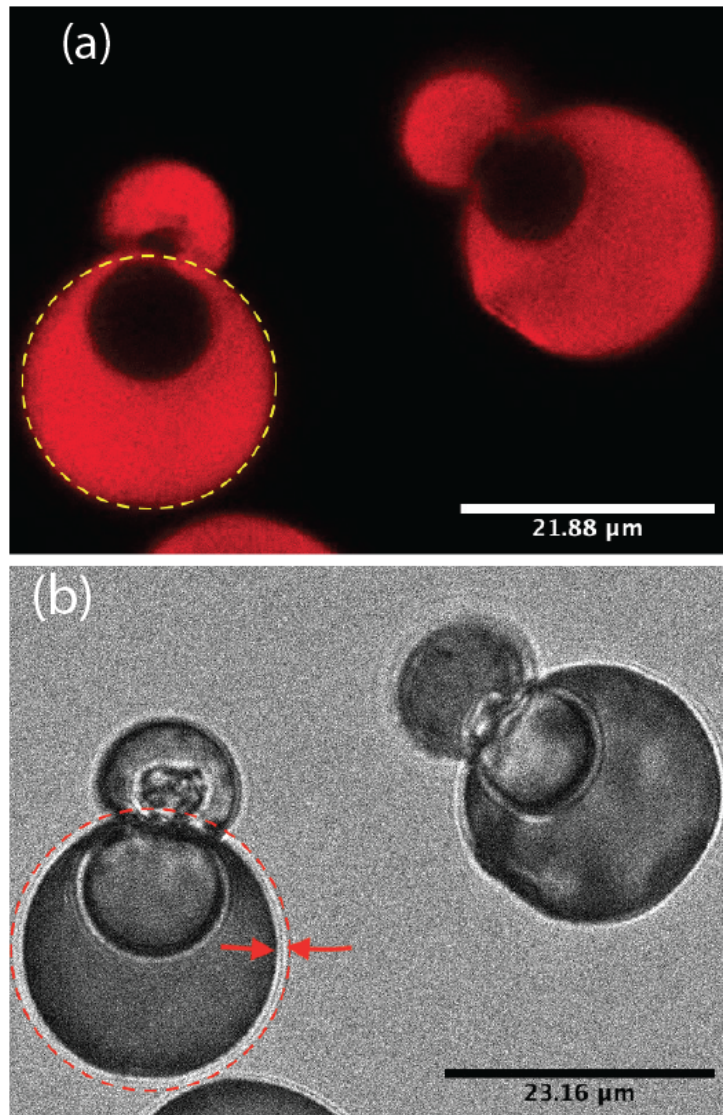


Figure 5.8 Confocal images showing the rupture of the microcapsules under the effect of osmotic pressure. These images allows to determine the thickness of the microcapsule shell, which is found in this case equal to $0.64 \mu\text{m}$, in a very good agreement with the calculated value using eq. 3.10.

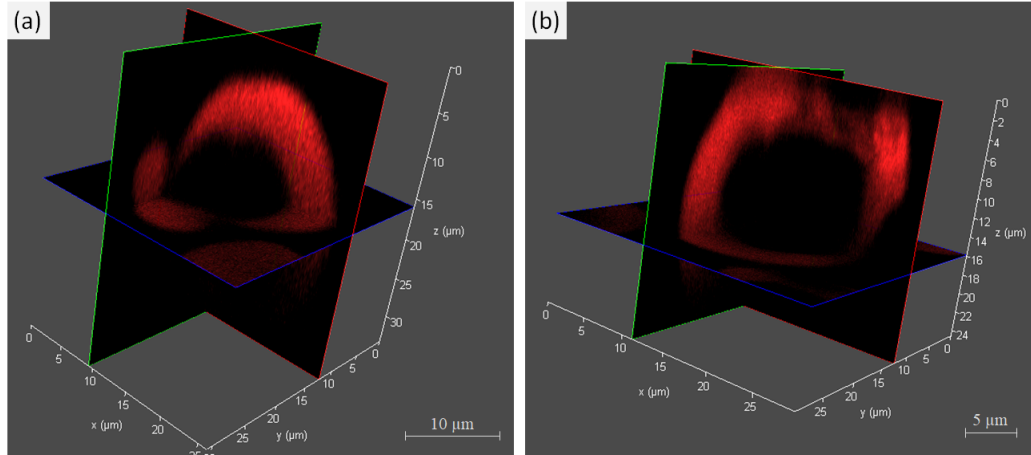


Figure 5.9 Confocal 3D images showing the hollow feature of the synthesized ZnO microspheres doped with rhodamine B from different samples.

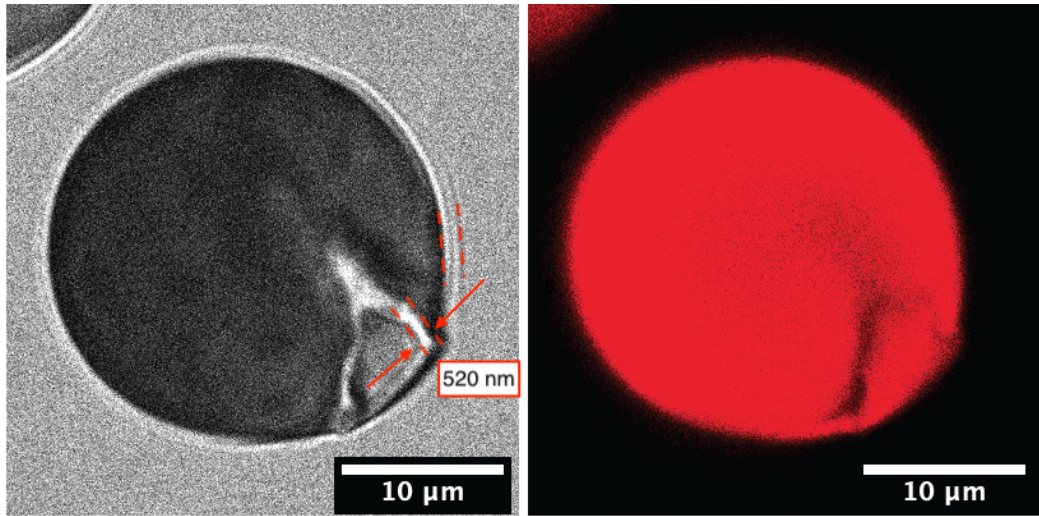


Figure 5.10 Confocal images enabling for an accurate measure of the shell thickness, which is found in this case approximately equal to $0.52 \mu m$.

In this case, we notice that rhodamine dye molecules adsorb indeed on the inner surface of the microcapsule shell only, as the interior of the open microcapsules appears dark. If we subtract the diameter $D_{int} = 21.88 \mu m$ of the circular fluorescent region (which corresponds to the inner diameter of the micro-

capsule) from the outer diameter $D_{out} = 23.16 \mu m$ of the microcapsule measured from the white field image, we can deduce a shell thickness value equal to $\frac{D_{out} - D_{int}}{2} = 0.64 \mu m$, which is in a very good agreement with the calculated value ($0.67 \mu m$) from equation (3.10).

A second important feature of the shell structure may be inferred from a careful analysis of Figure 5.8 and Figure 5.11. Indeed, the good resolution of these images enables to see clearly that the shell consists of three layers: one dark layer surround with two bright layers. Basically, this feature was also visible in the microcapsules shown in Figure 5.7, though not very clearly. Performing a Gaussian fitting of the plot profile shown in Figure 5.11, enables to deduce approximately the thickness of the different layers: $\simeq 0.25 \mu m$ for the bright regions and $\simeq 0.15 \mu m$ for the dark region.

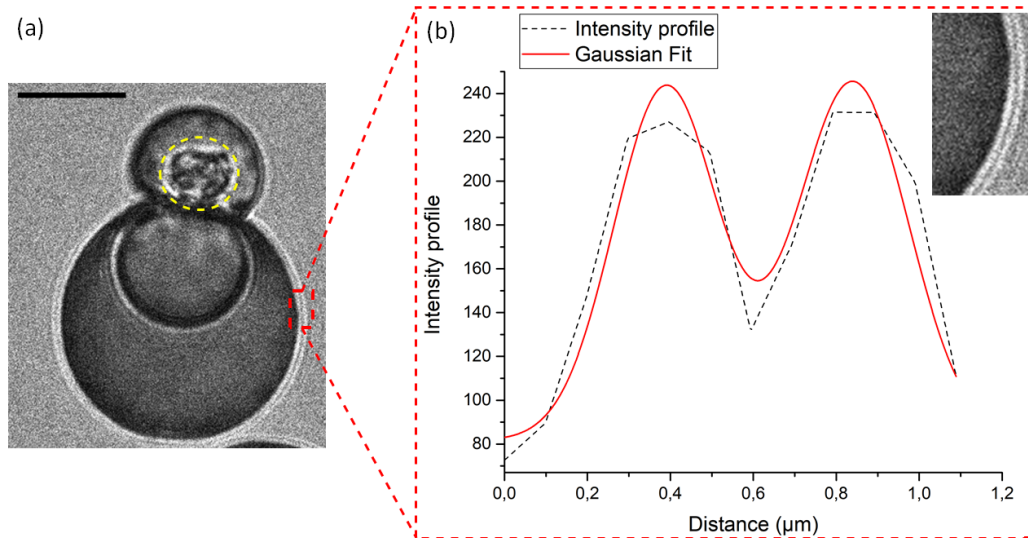


Figure 5.11 Details of the microcapsule shell observed using white field mode of confocal microscopy. Scale bar, $10 \mu m$.

5.4 Suggested mechanism for the shell and nanoparticles organization

We present in this section, an original model for the mechanism of the microcapsules shell formation. This model allows, in particular, to explain the observed dependence of the microcapsules size and their shell thickness versus the droplets size, discussed in see section [3.2.3](#).

5.4.1 General questions about the microcapsule formation

The following questions should be addressed:

- What are the main factors that determine the final size of the microparticles? In a shell nut, does the microparticle stop reducing its size after the solvent has been fully evaporated or because nanoparticles reach a minimal interaction distance, regardless of the presence or not of the solvent? Does the core of the microparticle still contains solvent even after the final size of the microparticle has been reached because of a hypothetical sealing of the shell (i. e. , solvent proofing)?
- How nanoparticles organize initially in the droplet and at its interface? Does their type of organization depends on the droplet size and the microparticle size?
- What kind of physical forces are behind the transition observed in the obtained values of shell thickness versus microparticles size?

Let's first address briefly the first questions.

We have shown in Chapter 3, Section 3.4, that the shell of the microcapsule is porous and that condensed microcapsules (once they have reached their final size) can absorb a solvent like water, or oil, and swell, and eventually break under the effect of osmotic pressure, as shown in Figure 3.13. Therefore, because of the shown porosity of the shell at its final state, we can exclude the presence of the solvent after microparticles have reached their final size.

We hence can postulate that the final size of the microcapsules is mainly determined by the close packing and the organization of nanoparticles in the shell.

Let's now address in more detail the remaining questions.

5.4.2 Nanoparticles surface density versus droplet size

We suggest from the above discussed results that the observed transition in the pure ZnO microparticle size versus droplet size is related to the surface density, Γ , of ZnO nanoparticles at the droplet interface. We need therefore to better understand how ZnO nanoparticles organize at the droplet interface. Let's first give a rough estimation of Γ (for a given droplet size).

For sake of simplicity, we will assimilate nanoparticles to nanosized cubes with a typical size of about 100 nm (as argued in section 3.4) and a standard mass density equal to 5.6 g/cm^3 . Therefore, the mass of a single nanoparticle would be about $m_{NP} = 5.6 \times 10^{-15} \text{ g}$. Considering now the mass of a single ZnO "unit", that is $1.34 \times 10^{-22} \text{ g}$, and the used concentration of ZnO, $C_{ZnO} = 0.5 \text{ M}$, one can estimate easily the number N_{NP} of nanoparticles, which are contained in a droplet.

Let's for instance consider the case of microparticles obtained from droplets with a diameter of $D_{drop} = 48 \text{ }\mu\text{m}$. We therefore deduce $N_{NP} = 4.62 \times 10^5$ nanoparticles and an overall area S_{NP} occupied by all N_{NP} nanoparticles at the droplet surface,

that is: $S_{NP} = 0.01(nm^2) \times 4.62 \times 10^5 \simeq 4620 \mu m^2$. Since the surface area of each droplet is equal to $S_{drop} \simeq 7240 \mu m^2$, all N_{NP} nanoparticles may fit at the droplet interface without overlapping. In fact, only a fraction of the droplet surface area would be occupied by ZnO nanoparticles (in this example): $\Gamma = \frac{S_{NP}}{S_{drop}} \simeq 0.64$.

Figure 5.12 shows calculated values of Γ_{NP} versus droplets size when varying from $D_{drop} = 30 \mu m$ to $D_{drop} = 76 \mu m$ (at a constant concentration of ZnO equal to $C_{ZnO} = 0.5 M$). As we may notice, nanoparticles surface density changes linearly in the investigated droplets size range, from 0.4 to 1, approximately.

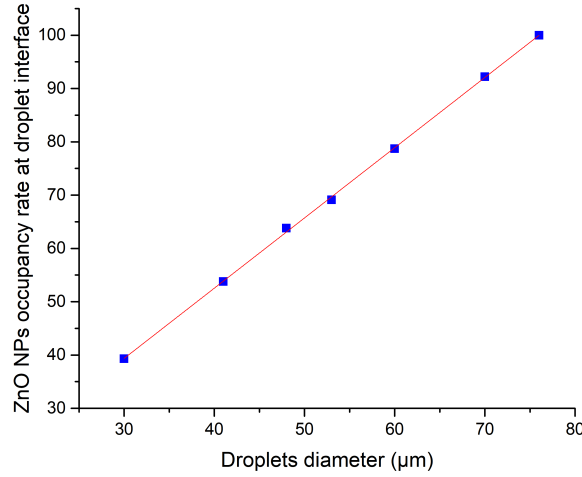


Figure 5.12 ZnO nanoparticles occupancy rate at the droplet interface.

The observed linear dependence of Γ_{NP} versus D_{drop} may be understood easily using dimensional analysis argument. Indeed, since the overall amount of nanoparticles, N_{NP} , scales as L^3 , where L is a typical size of the droplet, e. g., diameter, and the surface area occupied by the N_{NP} nanoparticles at this interface, $S_{NP} \simeq N_{NP} \times (100 nm)^2$, scales also as L^3 , one finds that Γ should scale as L (D_{drop}).

5.4.3 Initial orientation and organization of nanoparticles at the droplet interface during the condensation process

Once the droplet has been formed and brought at the oil/air interface in the Petri dish, nanoparticles start to organize at the droplet interface with either their electric dipoles oriented normal or planar by regards to the droplet interface. For both organizations, they may adopt mainly either a parallel or anti-parallel orientations. Nevertheless, we can fully exclude anti-parallel orientations for both normal and planar organizations, as they would lead to attractive interactions and the formation of full microspheres instead of microcapsules, as experimentally observed. Therefore, we will assume that nanoparticles keep their electric dipoles mainly oriented parallel, in either normal or planar organizations, which would prevent the microcapsule shell from collapsing (due to repulsive electric interaction between nanoparticles within the shell).

Also, in the case of a normal organization of nanoparticles, the exact orientation (up or down) should depend on which surface of the nanoparticle presents effectively the lowest interfacial energy with the surrounding media (air and oil). The majority suggested parallel orientation of nanoparticles, though electrically unstable, should be stabilized by interfacial forces at a nanoscopic scale and electrically at a microscopic scale, as discussed in the previous chapter, in section (4.4). Also, we expect from our results discussed in section (5.1.2), that the main orientation of ZnO nanoparticles should be with their electric dipole oriented outward (from the inner side to the external side of the shell).

5.4.4 Layering process at the droplet interface

We showed in section (5.3) that the shell of microcapsules built from $60\ \mu m$ droplets exhibits a layered structure. We suggest that such a layering process occurs also for other microparticles. During the condensation process of the droplet and the reduction of its size, the distance between ZnO nanoparticles decreases, their repulsive interaction increases and an upper layer starts to build up. It is worth noting that in this scenario, the mass density of the layer should be smaller than the standard value of the mass density of ZnO, $5.6\ g/cm^3$, as the second layer starts to form before nanoparticles come into close contact.

Starting from an initial surface density Γ_0 (for a given droplet size), as the droplet surface area starts to decrease subsequently to the evaporation process of the solvent, Γ starts to increase and nanoparticles interact more strongly. Eventually, at some critical value, Γ_c , of the surface density, nanoparticles would start to build up an upper layer. The critical surface density value, Γ_c , should be correlated directly to the a balance and the strength of involved interactions. As the solvent evaporation process continues, the droplet surface area decreases more and more and nanoparticles start to build up additional layers until the microcapsule reaches its final size. Considering the final surface area, S_p , of the microparticle and the overall surface S_{NP} that can occupy all N_{NP} nanoparticles, if put together side by side, we may define n_l as the number of built layers as follows:

$$n = \frac{S_{NP}}{S_p} \quad (5.7)$$

It is worth noting that according to this definition, we assume that all nanopar-

ticles are fully packed in the layers, which means that the surface density of such nanoparticles in each layer is equal to 1 ($\Gamma = 1$) and therefore the mass density of such layers should be very close to the standard value for ZnO. Nevertheless, because of the repulsive dipolar forces, we may consider that the formation of an upper layer starts before nanoparticles come into close contact, and hence the mass density of the layer should be smaller than the standard value of ZnO. Therefore, the number n of stacked layers as defined previously should be considered as the minimum number of layer that we could expect.

Moreover, if we assume a layer thickness to be approximately equal to the typical size of a nanoparticle, i. e. , 100 nm. Therefore, we may deduce from the calculated number of built layers, n , a mean thickness, h_l of the shell versus the initial droplet size or the microcapsule size, as shown in Figure 5.13.

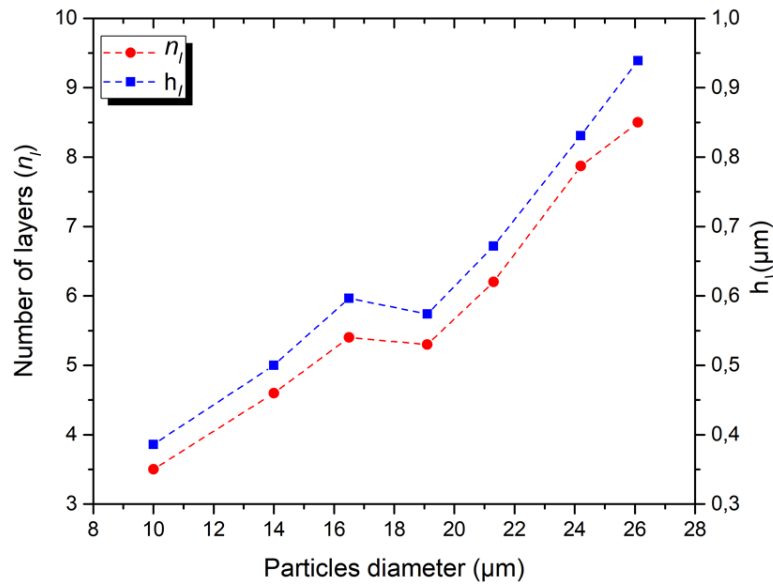


Figure 5.13 The number of built layers and calculated microparticles shell thickness, h_l , vs. particles size.

5.4.5 Physical origin of the observed transition

Let us first discuss the case of microparticles derived from droplets with a diameter equal to $D_{drop} = 76 \mu m$. Figure 5.12 shows that the initial surface density of nanoparticles for such a droplet is practically equal to $\Gamma_0 = 1$. A larger droplet would have led to an initial surface density larger than unity, which would indicate that more than a single layer of nanoparticles build from the beginning, before the condensation process of microdroplets starts. Also, because of the existence of repulsive forces between parallel nanoparticles, the building of an upper layer may occur even at smaller droplet sizes than $D_{drop} = 76 \mu m$.

We suggest that the observed transition at $D_{drop} \simeq 50 \mu m$ in the particles size and shell thickness corresponds actually to a transition between a configuration where nanoparticles organize initially as a single monolayer at the droplet interface and a configuration where they organize initially in more than a single layer. The transition would occur at a critical surface density approximately equal to $\Gamma_c \simeq 0.65$, that is approximately 65 % of the droplet surface which would be covered by nanoparticles (see Figure 5.12).

Actually, before discussing into more details the layering process of the shell, let's first remark that another scenario could be considered. Indeed, we may consider that before the formation of the upper layer and below some critical distance between nanoparticles, repulsive electrical forces may become strong enough to force nanoparticles to reorient their electric dipoles and to undergo a more stable anti-parallel organization. In this case, one should consider that the stability and cohesion of the microcapsule should rely on elastic forces that would prevent the shell from collapsing. In this case, the mass density of the layer should be

practically equal to the standard mass density of ZnO.

We will consider two different mechanisms, depending on the droplet and the microparticles sizes.

In the case of small droplets or small microparticles, e. g. , $D_{drop} = 30 \mu m$ and $D_p = 10 \mu m$, the initial nanoparticles surface density Γ_0 is relatively small ($\Gamma_0 \simeq 0.39$, see Figure 5.12). Therefore the $N_{NP} = 1.1 \times 10^5$ nanoparticles (see section 5.4.2 for calculation) may organize initially in a single monolayer with their electric dipoles predominantly oriented parallel, then at $\Gamma = \Gamma_c \simeq 0.65$, a second layer starts to build where electric dipoles keep their parallel orientation. According to the definition of the number of layers, n , given in equation (5.7), we find for $10 \mu m$ microcapsules $n_l \simeq 3.5$ layers. As discussed above, this number of layers should be regarded as the minimum number of layers that we should take into account. Let's consider, for instance, that the effective number of layers is equal to $n_{eff} = 4$ and let's the mass density $\rho(4)$ of the shell in this case. The effective area $S'(NP)$ that would be occupied by all $N_{NP} = 1.1 \times 10^5$ nanoparticles with such mass density is equal to $S'(NP) = 4 \times S_p \simeq 1256 \mu m^2$, where $S_p = 314 \mu m^2$ is the surface area of the microcapsule. Therefore, the density of nanoparticles per unit surface is approximately equal to $\frac{N_{NP}}{S'(NP)} \simeq 88$ nanoparticles/ μm^2 . Considering the typical size of nanoparticles normal to the droplet interface ($0.1 \mu m$), we may deduce a value of $\rho'_{eff} = 4.9 g/cm^3$ for the mass density of a 4 layers shell.

Let's consider now the case of large microparticles such as with $D_p = 19.1 \mu m$. We have in this case:

$N_{NP} \simeq 6.2 \times 10^5$, $S(NP) \simeq 6200 \mu m^2$, $S_p \simeq 1146 \mu m^2$, $\Gamma_0 \simeq 0.69$. The minimum

number of layers in this case should be approximately equal to 5.3 layers, which is practically the same value as for the microparticle with $D_p = 16.6 \mu m$ ($D_{drop} = 48.5 \mu m$). If we consider, for instance, that the effective number of layers is again equal to $n_{eff} = 6$, the effective area $S'(NP)$ that would be occupied par all nanoparticles with this effective number of layers would be equal to $S'(NP) = 6 \times S_p \simeq 6876 \mu m^2$. Therefore, the density of nanoparticles per unit surface is found again approximately equal to $\frac{N_{NP}}{S'(NP)} \simeq 89$ nanoparticles/ μm^2 , and therefore, the mass density value of the six layers shell would be practically the same as for the previous analyzed microparticles, i. e. , $\rho'_{eff} = 5.0 g/cm^3$ for the 6 layers shell.

Calculation for microparticles with other sizes are reported in table 5.1 (below).

$D_{part.}(\mu m)$	$D_{drop}(\mu m)$	N_{NP}	Γ	n	n_{eff}	$h(\mu m)$	$\rho'_{eff}(g/cm^3)$
30	10	$1.12 \cdot 10^5$	0.39	3.58	4	0.39	4.9
41	14	$2.87 \cdot 10^5$	0.54	4.66	5	0.50	5.2
48.5	16.5	$4.75 \cdot 10^5$	0.64	5.56	6	0.59	5.0
53	19.1	$6.20 \cdot 10^5$	0.7	5.41	6	0.58	5.0
60	21.3	$8.99 \cdot 10^5$	0.79	6.31	7	0.67	5.0
70	24.2	$1.43 \cdot 10^6$	0.93	7.77	9	0.83	4.8
76	26.1	$1.83 \cdot 10^6$	1	8.54	10	0.92	4.7

Table 5.1 Summary of the results obtained for different particle sizes.

In the case of small droplets (with a diameter below $48 \mu m$), initial nanoparticles surface density Γ_0 is relatively small and nanoparticles, which are initially quite far away from each other, organise at the droplet interface with their elec-

trical dipoles oriented parallel as discussed previously. Basically, because of the absence of specific interactions between nanoparticles and their surrounding media (oil and air), we believe that nanoparticles may organize at the droplet interface in multilayered polar clusters, whose thickness should depend on the initial surface density of nanoparticles. Their exact electrical polarisation would depend on the local orientation of nanoparticles. Nevertheless, the overall orientation of such clusters should be parallel in order to maintain repulsive forces and to prevent the microcapsule structure from collapsing.

Upon droplet shrinking, nanoparticles or nanoclusters get closer and closer. The process stops and the microparticle reaches its final size once the nanoclusters reach their minimal distance. The thickness of the shell should be determined by the initial thickness of nano-clusters: 4 layers for droplets with a diameter equal to $30\ \mu m$ and $41\ \mu m$, and 6 layers for droplets with a diameter equal to $48.5\ \mu m$.

In the case of large droplets (with a diameter above $53\ \mu m$), the initial nanoparticles surface density Γ_0 is quite large and therefore nanoparticles interact strongly since the start. Upon droplet shrinking, dipolar repulsive interaction between nanoparticles become strong enough to force nano-clusters to adopt an anti-parallel orientation just before undergoing the formation of a second layer of nano-clusters. Therefore, the final thickness of the shell would be twice (or more) the thickness of the initial nano-clusters: 3 layers for droplets with a diameter equal to $53\ \mu m$ and $60\ \mu m$, and 4 or 5 layers for droplets with a diameter equal to $70\ \mu m$ and $76\ \mu m$. The final value of the thickness of the shell will depending on the exact orientation of the dipole moment of the nano-clusters in the upper layer. Basically,

one would expect that the upper nano-clusters would orient their electrical dipoles parallel by regards to those of the lower layer in order to undergo attractive interaction between the two layers of the shell. Nevertheless, as shown in Figure 5.8 and Figure 5.11, we can see clearly two layers of about $0.25\ \mu m$ separated by a distance of about $0.15\ \mu m$.

Based on this result and on the observed deviation of the microparticle size from the linear dependence versus droplet size, shown in Figure 5.4 and discussed in more detail above, we suggest that the electrical dipole moments of the nanoclusters (or nanoparticles) in the upper layer undergo an anti-parallel orientation by regards to those of the lower layer. The distance between the two layers is directly related to the repulsive force between the two stacked layers of nanoparticles. An accurate measure of such a distance may give an interesting insight on the balance between electrical forces and elastic forces acting on the layers of the shell.

Chapter 6

First applications of ZnO microcapsules

In this chapter, we present two first applications of the fabricated dye-doped ZnO microcapsules. We show the proof of concept of a new optofluidic method for high throughput fluorescence-based thermometry. We demonstrate that this will enable temperature measurement inside optofluidic microsystems at the millisecond (ms) time scale. We use zinc oxide microparticles as temperature probes in the presence of some fluorescent dyes including rhodamine B or/and rhodamine 6G. We also present the application of ZnO microcapsules to measure the diffusion coefficients of rhodamine B and fluorescein dyes inside these microsystems.

The content of this chapter was first published in N. Ghifari et al. [215](#).

6.1 Microthermometry

6.1.1 Motivation

Microfluidics have evolved greatly in recent years as a versatile tool for many applications. The so-called Lab-On-a-Chip (LOCs) are now commonly used for the development of a wide variety of applications at the interface of various fields, such as engineering sciences and biology. As we discussed earlier in this thesis work, these miniaturized systems integrate different functionalities, which allow to perform complex processes at a micrometric scale, in contrast to standard systems which require larger quantity of reagents, time and energy. In particular, the use of droplet-based microfluidics has proven to be very efficient and powerful for many applications in biological and medical technologies^[295].

In biology, for instance, we mention here the achievement of the polymerase chain reaction, or digital polymerase chain reaction (dPCR) based on droplet microfluidics^[296-298]. It is important to specify at this point that the amplification of the DNA fragment requires three thermalization steps, first at 95 °C, then at 54 °C, and finally at 75 °C, repeated for many cycles. Furthermore, the main obstacles which need to be overcome when developing attractive microfluidic PCR systems are the challenge of measuring accuracy and controlling the temperature in the microfluidic channels^[299]. The concept would therefore be to realize an integrated digital PCR microchip, where the entire PCR thermo-cycling process can be performed.

In the context of ensuring good thermal control in microsystems and microdevices for example, methods based on micro-electro-mechanical systems (MEMS)

technology have proven to be effective for measuring temperature at the micrometer scale^{300,301}. However, several limitations have been identified while using these methods, including complex manufacturing, and expensive cost. Methods involving the use of fluorochromes have proved to overcome many of these limitations³⁰¹⁻³¹². One example is the laser-induced fluorescence (LIF) technique which generally involves the use of specific fluorescent dyes³¹³. It should be noted that many fluorophores allow the study of fluorescence intensity as a function of temperature. Hence, the use of such fluorescent molecules as specific probes is feasible for both spatial and temporal temperature measurement in a very sensitive and localized manner.

Many different fluorescent probes have been used in the field of fluorescence imaging, as well as for the development of new probes with very specific properties. Rhodamine B (RhB) and rhodamine 6G (Rh6G) are widely used as engineering fluorescent dyes and have very good spectroscopic properties. In particular, they have very high fluorescence quantum yields, e.g. 0.95 for rhodamine 6G³¹⁴. On the other hand, rhodamine B's fluorescence quantum efficiency depends on its environment and varies from 0.49 to 0.687. In addition, these dyes have a remarkably high photostability^{315,316}. Rhodamine B is considered one of the most powerful molecular probes and therefore can generally be used as temperature-sensitive marker for micro-scale temperature measurement^{308,317,317,318}.

Indeed, the molar absorptivity and concentration of the fluorescent dye used, the intensity of the incident light and the quantum efficiency of the fluorescence have a significant influence on the fluorescence intensity of the dye. The expression of fluorescence intensity, I_f (per unit volume), can be derived simply from

Beer-Lambert's law³¹⁹⁻³²¹, and is defined according to the following linear relationship:

$$I_f \propto \Phi I_0 \epsilon C \quad (6.1)$$

where Φ , I_0 , ϵ and C are the fluorescence quantum yield, incident illumination intensity, molar absorptivity and dye concentration, respectively.

Furthermore, studies have revealed a decrease in the fluorescence quantum yield (Φ) and fluorescence lifetime of rhodamine B when temperature increases. It is worth noting that at high temperatures significant non-radiative processes can occur³²². These are related to thermal agitation including vibrations, collisions with solvent molecules, and intramolecular rotations³²³.

It should be emphasized that the choice of the temperature-sensitive fluorophore is an important consideration for all experiments based on temperature-controlled features. In this context, we list here the use of two fluorescent molecules having different behaviors which motivated the setting up of this study. For example, it has been shown that the fluorescence intensity of rhodamine 6G increases linearly in response to an increase of the temperature³⁰³. As for Rhodamine 110 (Rh110), it has been reported in the literature that the fluorescence is not affected by temperature.

The stability and increase in fluorescence intensity as a function of temperature may reflect the development of complexes (dimers, trimer, etc.), which exhibit higher quantum efficiency. As a result, they are better protected against intermolecular collisions. This leads to an enhancement or stability of fluorescence intensity depending on temperature.

It should be noted that the equation [6.1](#) also demonstrates that both non-uniform illumination (I_0) and non-uniform dye concentration (C) can have a significant influence on the temperature measurement, which could lead to some measurement errors and uncertainties.

For the specific case where this is mainly related to fluctuations in laser intensity, normalizing the fluorescence intensity using, for example, a temperature-insensitive reference dye may eventually help to correct the fluctuation in laser irradiation intensity. Previous studies have shown, for instance, that rhodamine 110 has an absorption spectrum which is not very sensitive to temperature and can therefore act as a reference temperature probe. On the other hand, it has been found that it is more complicated to correct possible errors arising from a fluctuation in the concentration of the dye within the microfluidic device.

While uncertainty caused by a fluctuation of the laser illumination can be eliminated relatively easily by normalizing fluorescence intensity using a reference temperature-insensitive dye, such as rhodamine 110, those which are caused by a fluctuation of the dye concentration in the microfluidic device are much more difficult to handle. Indeed, several processes can be at the origin of concentration fluctuations, such as adsorption of dye molecules on the channel walls^{[304](#)}, trapping of molecules inside the porous substrate of the microfluidic device (e. g. , PDMS) or a non homogeneous distribution of the flow advected dye molecules along the microchannel, caused by the well-known Taylor dispersion phenomena^{[324](#)[325](#)}. The existence of all these processes may result in a local depletion or enrichment of the dye in the microfluidic device.

One should emphasize also another potential and important source of uncer-

tainty on the measure of temperature in microfluidic systems, that is the heat dissipation within a tiny volume illuminated by a focused laser beam. To our knowledge, such an effect has been often neglected in the literature. In order to minimize the increase of temperature caused by the heating of the incident laser beam, one should either use a highly sensitive detection setup enabling to minimize the illumination power and/or increase the efficiency of the heat dissipation in the illuminated area of the sample by using a suitable material with a high thermal conductivity.

Other important factors may be responsible for fluctuations in dye concentration and subsequently lead to local enrichment or depletion of the substance used in the microfluidic device. Such factors may be due both to the phenomenon of adsorption of used molecules on the channel walls³⁰⁴, a trapping of dye inside the porous substrate of the polydimethylsiloxane-based microfluidic device, for example, or a non-homogeneous molecular distribution of flux advected dye along the microchannel, caused by the well-known Taylor phenomena³²⁴⁻³²⁵.

Indeed, besides its high chemical stability and photo-stability, zinc oxide exhibits a high thermal conductivity of about 100 to 150 W/m.K (depending on ZnO structural properties)³²⁶⁻³²⁸. This value is for instance two orders of magnitude higher than the thermal conductivity of water, i. e. , 0.65 W/m.K.

6.1.2 Experimental procedure

Rhodamine doped ZnO microcapsules as temperature probes

In order to better understand the effect of temperature on the intensity of fluorescence in doped ZnO microparticles, we used three types of fluorescent nanoparticles: one type was doped with rhodamine B, a second type was doped with

rhodamine 6G and the third type was doped with a mixture of rhodamine B and rhodamine 6G. The later mixture was intended to be used as a reference for fluorescence intensity calibration and to take account of fluctuations in incident illumination intensity. Indeed, the fluorescence intensity of rhodamine B decreases linearly with increasing temperature at a ratio of about $-2\ \%/^{\circ}\text{C}$ whereas the fluorescence intensity of rhodamine 6G increases linearly with increasing temperature at a rate of about $+2\ \%/^{\circ}\text{C}$ [303 310 329 330], as shown on Figure 6.1.

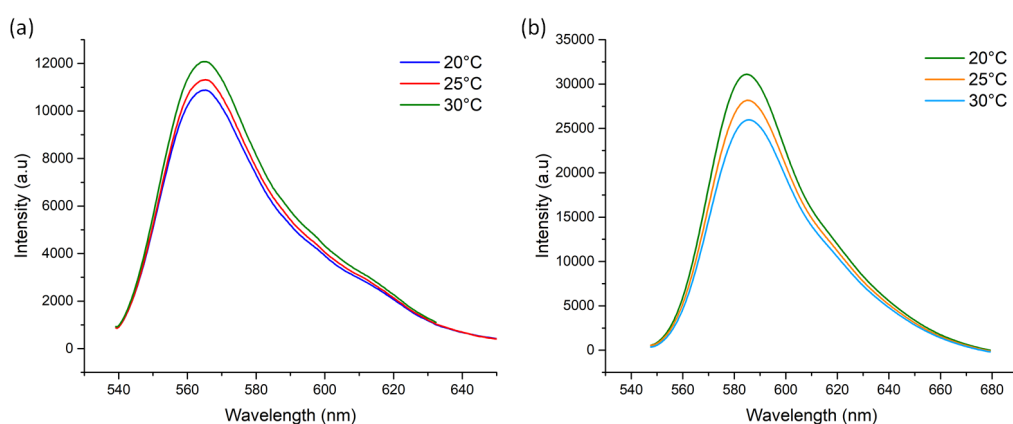


Figure 6.1 Fluorescence spectra of Rhodamine 6G (a) and Rhodamine B (b) as a function of temperature.

Since temperature has an opposite effect on the intensity of fluorescence in rhodamine B- and rhodamine 6G-doped ZnO nanoparticles, we prepared solutions of rhodamine B-doped ZnO nanoparticles, rhodamine 6G-doped ZnO and a mixture of the two dyes. A detailed description of the synthesis procedure is given below and schematized in Figure 6.2. Dispersed phase and continuous phase flow rates were set for all experiments in this study to produce stable droplets with similar shape and single droplet size equal to $55\ \mu\text{m}$.

Another PDMS-based microfluidic device has also been designed to provide

an additional function not available in the previously used microfluidic system. Indeed, this device allows the subsequent injection of preformed ZnO microparticles into the microfluidic channels that has narrowed rectangular channel ($22\ \mu\text{m} \times 38\ \mu\text{m}$, $w \times h$). The previously formed ZnO microparticles were dispersed in a HFE 7500 3M fluorinated oil solution and injected into the microfluidic device via a main inlet with a flow rate of approximately $Q = 150\ \mu\text{L}/h$. Therefore an average flow velocity of approximately $v_{constr.} = \frac{Q}{w \times h} \simeq 5\ \text{cm}/s$ can be estimated. In addition, three other inlets were designed to deliver the carrier oil, which offers the added benefit of avoiding undesired aggregation and/or sedimentation phenomena, potentially leading to clogging of the microfluidic channels.

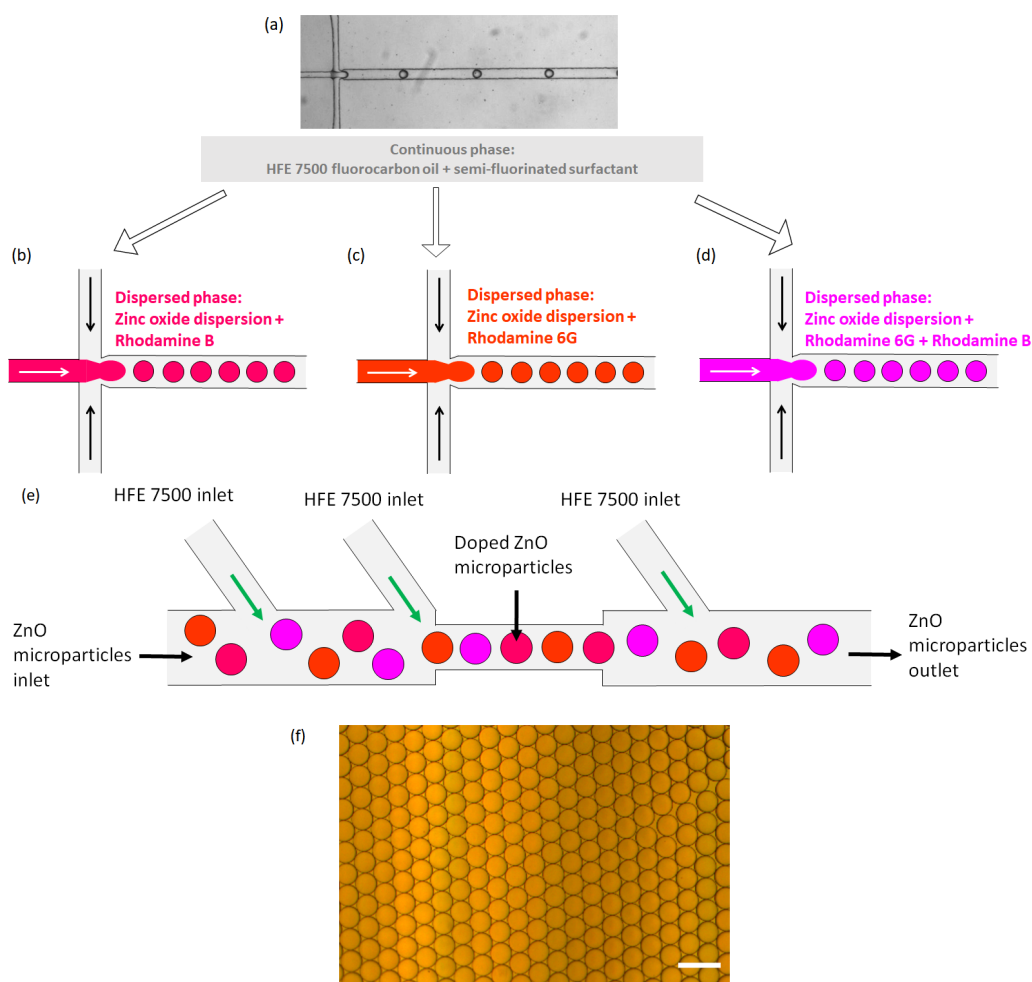


Figure 6.2 (a) Flow-focusing microfluidic geometry for zinc oxide droplet formation. Schematic illustration of the different case studies used for the generation of (b) rhodamine B-doped ZnO droplets, (c) rhodamine 6G-doped ZnO droplets, and (d) rhodamine B/rhodamine 6G-doped ZnO droplets. (e) Schematic illustration of the used microfluidic design for fluorescence analysis of doped ZnO microparticles. (f) Optical micrograph of stable and monodisperse zinc oxide droplets generated through the flow-focusing microfluidic device, Scale bar, 100 μm .

Fluorocarbon oil (HFE 7500) mixed with the commercial surfactant dSURF (Fluigent) 0.2 % (w/w) were used as the continuous phase. It is worth noting for the present study that the fluorocarbon oil used exhibits a thermal diffusivity of

approximately $\kappa = 3.6 \cdot 10^{-8} \text{ m}^2/\text{s}$.

Calibration and temperature correction

Temperature control of the entire microfluidic device was performed in all experiments using TOKAI HIT Thermo Plate (Japan) Upper incubator with standard heating stage. This apparatus is equipped with two heating plates allowing to control the temperature with an accuracy of 0.1°C on the surface of the heating plate with respect to the set temperature. This heater has been designed for use with the Olympus IX73 inverted microscope in our study. The microfluidic device was placed in the heating chamber on the dish holder located between the upper and lower heaters. The measurement of fluorescence intensity of the different samples of doped ZnO microparticles was performed in real time over a wide temperature range from 20°C to 50°C . For each temperature setting, the fluorescence spectra were recorded after a time delay of approximately 10 minutes to ensure temperature stability throughout the chamber. A large inlet channel length (about 10 cm) was used to maintain a uniform temperature distribution of the dispersion of ZnO microparticles and the fluorocarbon carrier oil.

Taking account of the used overall flow rate, $Q \simeq 150 \mu\text{L}/\text{h}$ and the inner diameter of the tubing, $D_{tub.} = 0.56 \text{ mm}$, one may deduce a flow velocity in the tubing of about $v_{flow} = \frac{Q}{\pi(D_{tub.}/2)^2} \simeq 4 \text{ cm}/\text{min}$. This means that it should take less than 3 minutes for the incoming fluid and microparticles to flow from the entry of the heating system until the inlet of the microfluidic channel, where fluorescence intensity is measured. Then the temperature dependence of the fluorescence intensity was analyzed from the recorded data.

6.1.3 Fluorescence detection and measurements

Fluorescence measurements of rhodamine B-doped ZnO particles

The experimental results of the temperature dependence of fluorescence intensity are shown in Figure 6.3(a). In these experiments, fluorescence intensity was detected and examined for rhodamine B-doped ZnO microparticles flowing in the HFE 7500 carrier oil within the microfluidic channel at $150 \mu\text{L}/\text{h}$. The emission spectra of the individual microparticles were recorded. The temperature dependence of fluorescence intensity was obtained for four different temperatures, 20, 30, 40, and 50°C , as the particles passed under laser irradiation in the narrowing zone of the microfluidic channel. As shown in this Figure, the intensity of the fluorescence spectrum of rhodamine B-doped microparticles decreases with increasing temperature. Thus, the fluorescence spectrum undergoes significant changes as the temperature of the microparticles studied changes. As explained above, such temperature rise leads to significant vibration and rotational energies as a consequence of high thermal excitation.

Figure 6.3(b) shows the peak intensity of the fluorescence of ZnO microparticles, flowing in the microfluidic channel, as a function of time. A high magnification of the fluorescence intensity peak of the ZnO microparticles doped with rhodamine B shows that this peak has an asymmetrical shape with rearward elongation of the droplet. This indicates that the ZnO droplet interfaces are deformable. As already shown above using experimental results in optical and scanning electron microscopy, these microparticles exhibit a porous structure and are consisting of a thin membrane whose thickness has been experimentally and theoretically estimated to be $0.7 \mu\text{m}$ ²⁶⁹. Moreover, numerous studies have been carried out

to investigate the deformation of the droplets inside the microfluidic channels. Cerdeira et al. attributed droplet deformation to several factors, namely the geometry of the microfluidic system used, the physical properties of the dispersed phase, and the flow conditions³³¹. Another study showed that microcapsules can easily deform under the effect of shear flow in the microfluidic channel^{332 333}.

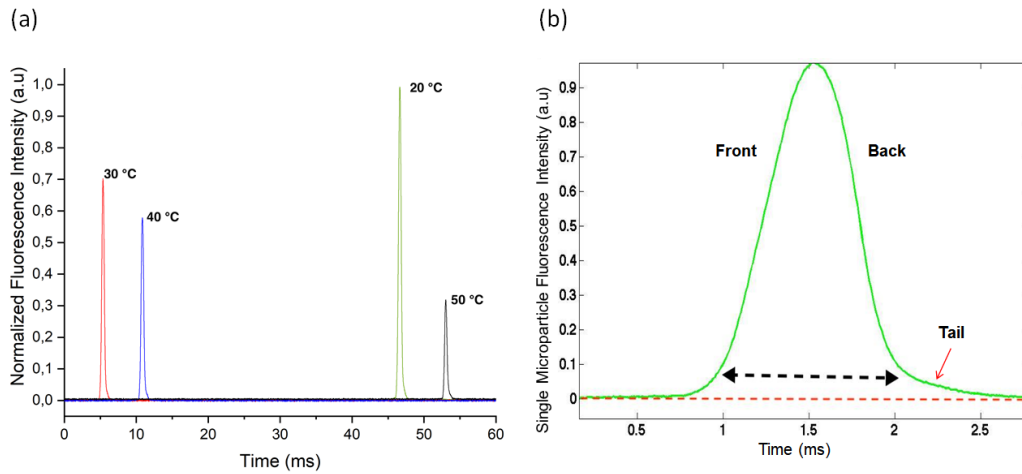


Figure 6.3 (a) Fluorescence intensity peaks within a wide temperature range from °C 20 to 50 °C. These peaks were recorded from confined rhodamine B dye in flowing ZnO microcapsules having a size of 17.8 μm along a 22 μm wide micro-channel constriction area. (b) High-magnification of the fluorescence intensity peak vs. time of doped ZnO microcapsules with rhodamine B. The red arrow shows the back of the drop at the interface with the continuous phase.

It is important to note that we have reliably and reproducibly based our experiments on operating in a controlled microparticle environment. Indeed, for each experiment, and for each temperature value, the fluorescence intensity as a function of temperature was analyzed for about twenty rhodamine B-doped ZnO microparticles with high monodispersity and stability. In addition, the fluorescence intensity was normalized by taking the fluorescence intensity at 20°C as a reference ($I_{20^\circ\text{C}} = 1$). Then the mean values of the fluorescence intensity with

the standard deviation as a function of temperature were plotted. The results are shown in Figure 6.4(a). This Figure clearly shows that the temperature increase leads to a decrease in the fluorescence intensity of rhodamine B-doped ZnO microparticles with a rate of about $(-2.2 \pm 0.1)\%/^{\circ}\text{C}$. This value is in very good agreement with the literature^{301-303,310-312}.

On the other hand, the results of the comparison of ZnO effect on the response of the normalized intensity of rhodamine B fluorescence as a function of temperature are presented in Figure 6.4(b). It can be seen that the normalized intensity of rhodamine B fluorescence decreases both for rhodamine B-doped ZnO microparticles with a concentration of 0.5 mM (shown as squares (red curve) in this figure) and for rhodamine B-doped ZnO droplets of 50 μm (shown as circles (blue curve) in this figure). We recorded the following slopes $(-2.2 \pm 0.1)\%/^{\circ}\text{C}$ and $(-1.9 \pm 0.2)\%/^{\circ}\text{C}$, for rhodamine B-doped ZnO microparticles and rhodamine B-doped ZnO microdroplets, respectively. In both cases our findings are in accordance with previous studies.

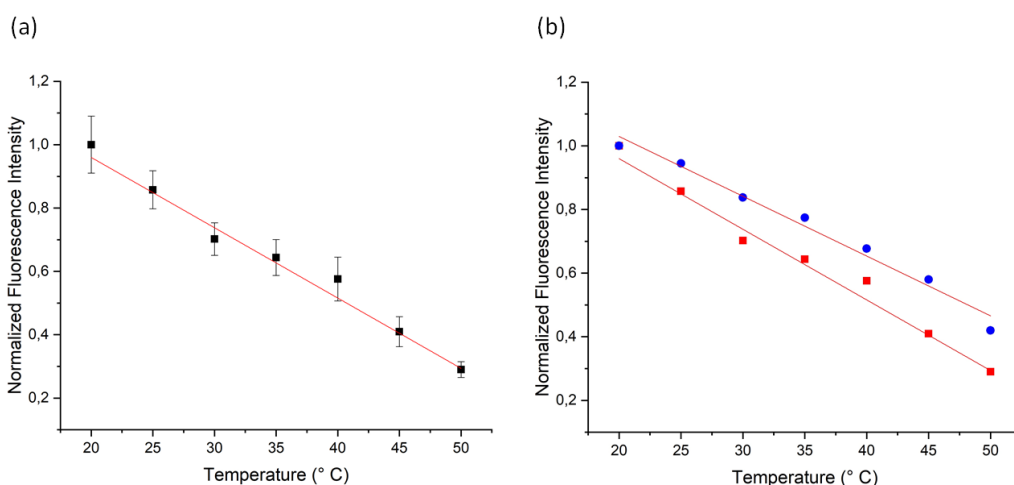


Figure 6.4 (a) Plot of the linear decrease of the normalized fluorescence intensity of rhodamine B-doped ZnO microparticles vs. temperature. (b) Comparison of the change of fluorescence of rhodamine B vs. temperature in flowing RhB-doped ZnO microparticles (squares, red curve) and flowing RhB-doped ZnO microdroplets (circles, blue curve).

Fluorescence measurements of rhodamine 6G-doped ZnO particles

Figure 6.5(a) shows the response of fluorescence intensity to temperature increase of rhodamine 6G-doped ZnO microparticles. As in most cases, rhodamine 6G exhibits an opposite response compared to rhodamine B as the temperature increases. We have noticed an increase in the fluorescence intensity spectrum of rhodamine 6G-doped ZnO microparticles, although the fluorescence intensity spectrum surprisingly became less important as we approached 40 °C where it recorded its minimum fluorescence intensity, as reported in Figure 6.5(a). As far as we know, this is the first time that such behavior of rhodamine 6G-doped ZnO microparticles has been reported.

In addition, in order to understand this behavior, we studied the pure fluorescence intensity as a function of temperature of rhodamine 6G droplets having

the same concentration ($[\text{Rh6G}] = 0.5 \text{ mM}$) and an average size equal to $50 \mu\text{m}$. Figure 6.5(b) shows the experimental results. As was established in the typical case of rhodamine 6G, we observed an increase in droplet fluorescence intensity spectrum with increasing temperature. Contrary to our previous observations, no minimum intensity was recorded in these experiments. Nevertheless, in our study, we obtained a lower slope (around $0.5 \text{ }^\circ\text{C}^{-1}$) than that reported in the literature ($1.9 \text{ }^\circ\text{C}^{-1}$)³⁰³. On the other hand, as we have seen above, when temperature increases molecular collisions in solution and the amplitude of internal molecular vibrations also increase. Therefore, a better extinction of the fluorescence is recorded when the non-radiative relaxation of the excited state is enhanced³²³.

Furthermore, from the comparison of the temperature dependence of fluorescence intensity in Figures 6.5(a) and 6.5(b) we speculate that ZnO nanoparticles induce significant changes in the rhodamine 6G fluorescence intensity spectrum along with temperature changes.

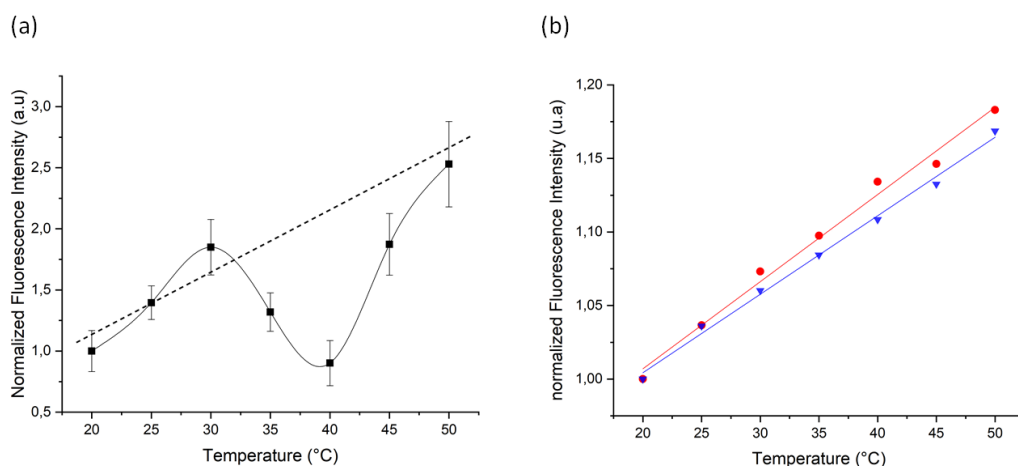


Figure 6.5 Change of the relative intensity of the fluorescence of rhodamine 6G vs. temperature in (a) ZnO microparticles and (b) in water droplet (blue curve) and in ethanol droplet (red curve).

Fluorescence measurements of rhodamine B/rhodamine 6G -doped ZnO particles

Figure 6.6 shows the fluorescence intensity versus temperature of ZnO microparticles which were doped with a mixture of rhodamine B ($29 \mu M$) and rhodamine 6G ($37 \mu M$) dyes. Because of the non linear variation of the fluorescence intensity of rhodamine 6G in ZnO microparticles, it was difficult to predict the concentration ratio of the two dyes which would lead to a constant fluorescence intensity versus temperature. Nevertheless, after several attempts, we found that the ratio $\frac{[RhB]}{[Rh6G]} = \frac{29 \mu M}{37 \mu M}$ gives a good reference for which the fluorescence intensity remains practically constant versus temperature, as shown in Figure 6.6.

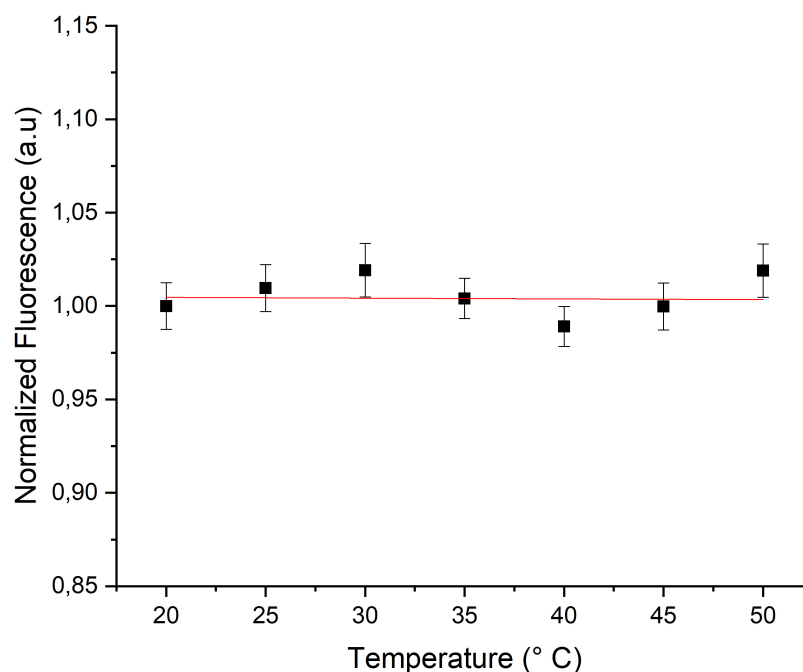


Figure 6.6 Relative intensity of the fluorescence of rhodamine B and rhodamine 6G mixture vs. temperature in ZnO microparticles.

6.1.4 Optical and electronic characterization on doped-ZnO particles

In addition, the morphology of doped ZnO microparticles synthesized according to the three scenarios presented above was studied by scanning electron and optical microscopes. Figure 6.7(a) shows a spherical ZnO microparticle doped with the fluorescent rhodamine B dye. We find that the rhodamine B nanoparticles clearly show a uniform distribution on the shell of the ZnO microsphere, with a surface having a smooth appearance. In fact, this shell essentially corresponds to the thin porous envelope, as we presented in more detail in Chapter 3 (which essentially corresponds to a thin-shell microcapsule with a thickness of about 0.7 μm).

On the other hand, we noticed that the use of rhodamine 6G led to a non-homogeneous distribution of dye within the shell of the microcapsule marked by a difference in the fluorescence intensity of the ZnO microsphere, as illustrated in Figure 6.7(b). Due to this effect, we noticed the formation of some regions on the surface of the ZnO microsphere with different degrees of luminescence and also with a pale pink color. In addition, closer observation of the scanning electron microscopy images of the ZnO microspheres reveals a porous structure of the membrane. The average pore size of ZnO pores estimated from scanning electron microscopy images was approximately 100 nm.

We speculate that such results indicate that the incorporation of dye molecules into the porous structure of ZnO leads to a difference in the fluorescence on the surface of the microspheres, which is in good agreement with the results presented above. These changes also demonstrate that the nature of the dye used, i.e., its

electrical charges, can affect the light-matter interaction within these samples.

Furthermore, we would like to point out that adding rhodamine molecules to the ZnO nanoparticles prior to droplet generation results in increased adsorption of the dye molecules to the surface of the ZnO microspheres. Moreover, we have noticed that the final droplet size obtained is different in the two synthesis cases even though we designed droplets with a single initial size equivalent to $55\ \mu\text{m}$. For example, under identical experimental conditions, i.e. same ZnO precursor concentration (0.25 M) and same initial droplet size ($55\ \mu\text{m}$). We obtained droplets having sizes of $20\ \mu\text{m}$ and $17.8\ \mu\text{m}$ for rhodamine 6G-doped ZnO microspheres and rhodamine B-doped ZnO microspheres, respectively.

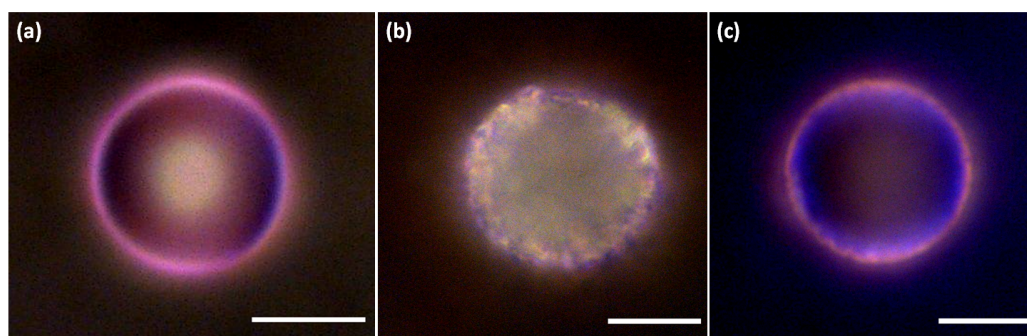


Figure 6.7 Optical microscopy images of doped ZnO microspheres with rhodamine B (a), rhodamine 6G (b) and rhodamine B/ rhodamine 6G mixture. Scale bars, $10\ \mu\text{m}$.

It can be seen that the interaction of dye nanoparticles with ZnO is driven by two different mechanisms. In this context, it can be suggested that the interaction of the rhodamine molecules employed can be triggered by interactions between their electric charges and those carried by the ZnO nanocrystal building units.

The chemical structures of rhodamine B and rhodamine 6G are depicted in Figure [6.8](#). In contrast to rhodamine 6G, which can only carry a positive elec-

tric charge, rhodamine B is a molecule that can adopt two main forms depending on the pH range of the medium. Indeed, under acidic pH conditions it occurs in the cationic form (RhB^+). On the other hand, in basic media this dye appears in the zwitterionic form after deprotonation, where the rhodamine B carries both a positive and a negative charge ($RhB^{+/-}$). Therefore, all of these elements can contribute to electrostatic attraction or repulsion between the molecules of rhodamine B or rhodamine 6G and the charged surfaces of ZnO nanocrystals. This may possibly promote a high diffusion mechanism of rhodamine 6G within the porous structure of the ZnO microcapsule shell compared to the mechanism that might occur with rhodamine B molecules in the zwitterionic form.

Since both dyes have been extensively used in the field of imaging. They have also attracted a lot of attention for various ranges of applications, and are the main representatives of the xanthene family owing to their good spectroscopic properties. In addition, further studies are needed to correlate the adsorption of rhodamine 6G fluorescent molecules in the membrane pores. Also, the investigation at temperatures close to 40°C of minimal fluorescence intensity in order to better explore the electrostatic interactions between rhodamine B, rhodamine 6G and ZnO nanocrystals at short and long distances.

6.1.5 Perspectives

We envisage to consider in a future study the use of rhodamine 110 for the construction of such reference. Also, we plan to flow and detect simultaneously the two types of microparticles (rhodamine B as a measure probe and rhodamine 110 as reference probe) in the microchannel in order to make the normalization of the fluorescence intensity signal straightforward. For this purpose, the use of two

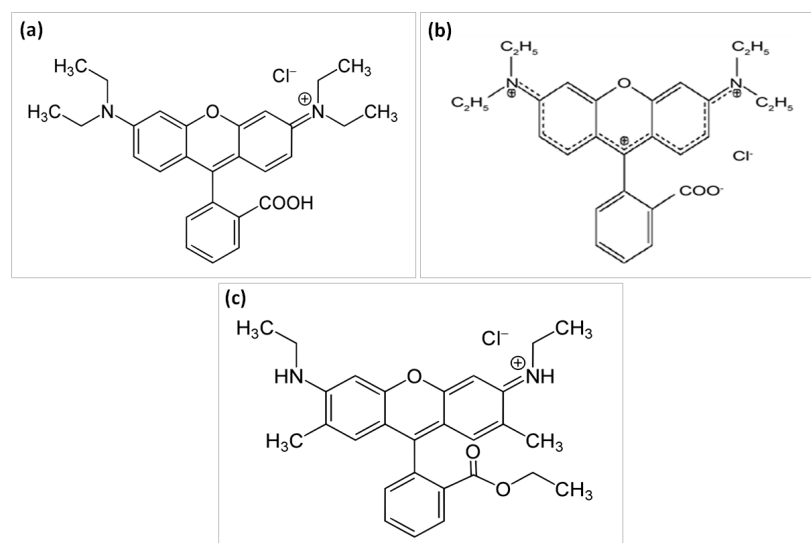


Figure 6.8 Chemical structures of the used dyes. (a) Rhodamine B, (b) zwitterionic form of Rhodamine B (presence of both a positive and negative charges). (c) Rhodamine 6G can carry only a positive charge or no charge at all.

different concentrations for the two types of microparticles will allow for the discrimination between the two types of particles while flowing at high throughput in the microchannel, as illustrated in Figure 6.9.

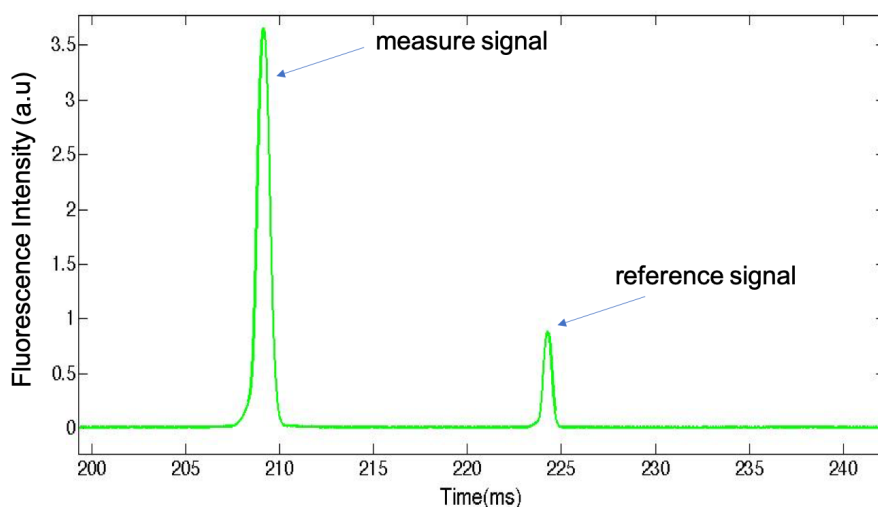


Figure 6.9 Different microparticles doped with two different dyes may be discriminated by the value of their fluorescence intensity signals. Such concentration based coding method will be applied in the future for the simultaneous recording of the fluorescence intensity signals from RhB doped microparticles (measure probes) and Rh110 doped microparticles (reference probes).

6.2 Diffusion phenomena and micromanipulation

Since we are able to control the size of the ZnO microcapsules and their contents. These particles can be used to measure, for example, the diffusion coefficients of molecules such as dyestuffs. We present, in the following, a first application which has allowed us to measure the diffusion coefficients of some dyes including rhodamine B and fluorescein contained in the ZnO microcapsules.

6.2.1 Uptake, distribution and diffusivity of charged fluorophores in ZnO microspheres

We performed additional confocal experiments, in particular fluorescence recovery after photobleaching (FRAP), in order to acquire more information on the mechanisms regulating diffusion, fusion, and charge of molecules in uniform rhodamine B- or fluorescein-doped ZnO composite microspheres

Fluorescence recovery after photobleaching of ZnO microspheres

In this work, confocal imaging FRAP experiments were carried out using Leica TCS SP8 confocal laser scanning microscope, equipped with 40X and 63X oil immersion objectives with a numerical aperture (NA) of 1.25. Throughout the bleaching events, all experiments were conducted by Leica FRAP wizard software using the zoom-in bleach mode. Fluorescence recovery after photobleaching was performed using a circular area to photobleach the inner part of the microspheres and to measure the diffusion coefficients of the fluorescent dyes within ZnO microspheres. The laser was monitored at 100 % of its power and for each fluorophore unique photobleaching wavelengths were used. After bleaching experiments, fluorescence recovery was operated at low laser intensity and at 2.58 s intervals. To conduct FRAP experiments using rhodamine B and fluorescein, experiments were performed at least 24 h after droplets generation within the microfluidic system.

Figure 6.10 represents examples of images recorded during fluorescence recovery after photobleaching experiment on rhodamine B-doped ZnO microspheres. Figure 6.10(a), Figure 6.10(b), and Figure 6.10(c) show intensities as a function of

different times (the pre-bleach, bleach and post-bleach at 85 s after photobleaching), and Figure 6.10(d) depicts the corresponding recovery curve.

As illustrated, there are basically three imaging phases, as we detail hereafter:

1. Pre-bleach: The acquisition settings are tuned to save a sequence of images at low laser power. The pre-bleach sequence is important as it serves as a reference during the FRAP experiment (see Figure 6.10(a)).
2. Photo-bleach: A region of interest (ROI, a circular area in this experiment) is locally irradiated with a short pulse of intense laser power to photo-bleach the fluorescent molecules within the selectively illuminated area. It is interesting to note that such irradiation leads to a substantial decrease in the average intensity of fluorescence as a function of time according to an exponential decay law³³⁴:

$$I(t) = I_0 e^{-Kt}$$

Where I_0 represents the initial fluorescence intensity, and K the bleaching rate constant of the fluorophore. This light exposure also causes irreversible chemical modifications of the fluorophore molecules within the selective ROI, making it non-fluorescent, as shown in Figure 6.10(b).

3. Post-bleach: It employs a time-lapse acquisition with setting similar to the pre-bleach, but longer-lasting. The gradient of fluorescence intensity leads to a diffusion of fluorophores from the local unbleached surroundings into the bleached area, whereas photobleached labeled components diffuse out over time. The recovery of the fluorescence intensity throughout the post-bleach phase allows to gain insight into the mobility of the molecules³³⁵,

diffusion³³⁶, binding³³⁷, as well as their redistribution ability after photobleaching, as illustrated in Figures 6.10(c) and 6.10(d).

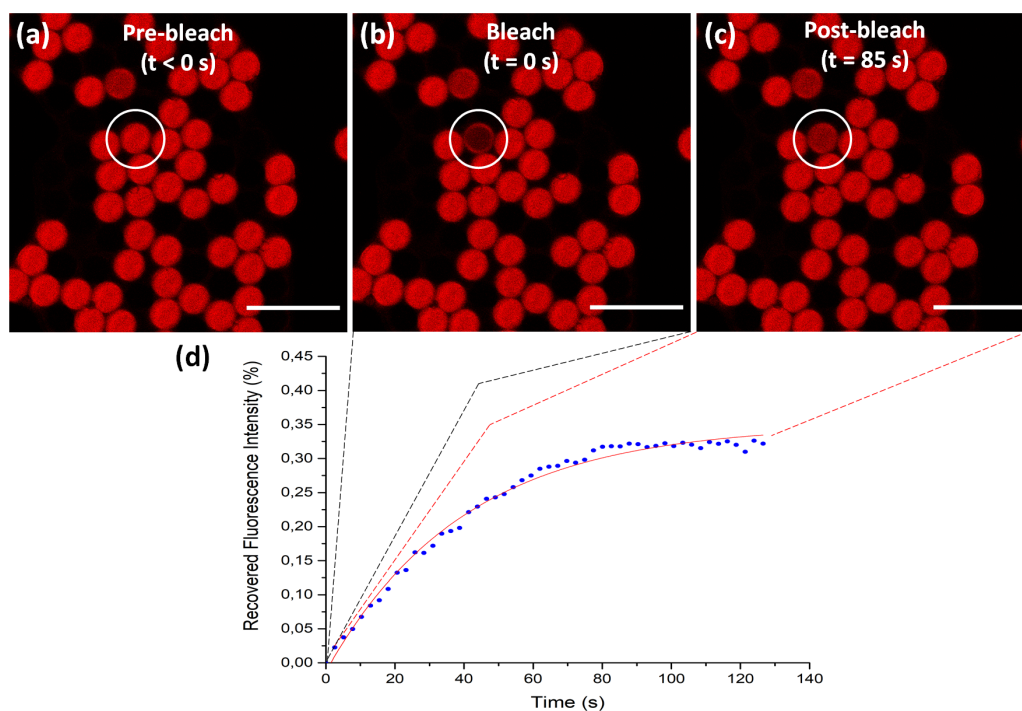


Figure 6.10 Confocal images recorded during a FRAP experiment on ZnO microspheres; uniformly labelled with rhodamine B, (a) taken before photobleaching, (b) immediately after photobleaching the spherical inner part of the microsphere, with short pulse of intense laser light, showing that the selective ROI is no longer fluorescent, (c) was recorded during the recovery after photobleaching, and (d) the recovery curve that demonstrates an increase in fluorescence intensity as the bleached dye diffuses and the new unbleached dye move into the ROI. The white circles represent the selected microsphere for photobleaching. Scale bars 50 μm .

6.2.2 Experimental measurements of diffusion coefficients of dye molecules

Furthermore, to establish quantitative information on the dynamics of the fluorophore molecules mobility at the micrometer scale, and whether the neighbouring particles have an influence on their diffusion and mobility, we have carried out the following FRAP experiments. In a separate set of experiments, we generated two colored ZnO droplets. We synthesized independently ZnO microspheres with $16.5\ \mu\text{m}$ diameter doped with rhodamine B (0.1 mM) and fluorescein (0.1 mM).

It is worth mentioning that the concentrations of rhodamine B and fluorescein were a significant consideration for FRAP experiments. They have been chosen so that ideally they were small enough not to significantly influence the signal saturation of the ZnO microspheres during the fluorescence imaging based acquisitions process (the pre- and post-bleach experiments), but also to be rapidly and permanently bleached throughout photobleaching of the targeted ROI. By the same token, they had to remain stable enough to undergo the slightest photobleaching and the fluorescence signal had to be large enough to be detected in all experiments.

We uniformly photobleached each type of microparticle throughout the inner part of the microsphere considering two categories of microparticles: surrounded and isolated ZnO microspheres. Next, the fluorescence intensity of the considered area after photobleaching as a function of time as well as their behaviour were investigated.

A comparison of FRAP results is presented in Figure [6.11](#), and measured as a function of time using confocal laser scanning microscopy. This figure shows

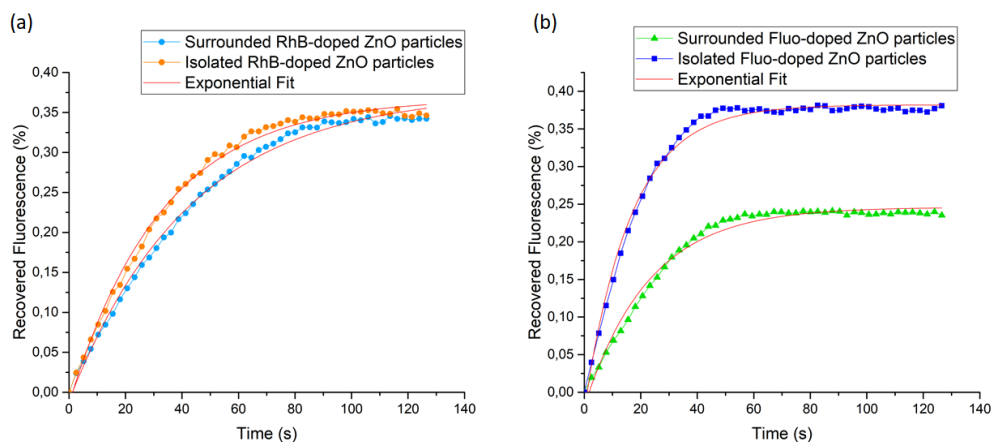


Figure 6.11 Comparison of fluorescence recovery after photobleaching graphs of surrounded and isolated (a) rhodamine B-doped ZnO microspheres (b) and fluorescein-doped ZnO microspheres.

the fluorescence recovery after the loss of fluorescence in individual used fluo-rophores microspheres due to laser illumination.

The characterization of the dynamics of rhodamine B and fluorescein molecules from these experiments becomes quite straightforward by considering the half-time of recovery ($t_{1/2}$), fractions of the mobile and immobile molecules in the bleached area, as they provide quantitative information on the dynamics of the molecules during the experiment and can be used to compare the performed experiments. Knowing the region of interest, $ROI = 112.67 \mu m^2$, for the bleaching beam that was kept the same for all experiments, and the the half-time of recovery ($t_{1/2}$), the diffusion constant of dye molecules can be easily determined to get a glimpse of the molecular dynamics of rhodamine B as well as fluorescein. It is given by the ratio between the region of interest (ROI) and the half-time of recovery ($t_{1/2}$): $D = \frac{ROI}{t_{1/2}}$. Where ($t_{1/2}$) can be determined from the FRAP graph, and corresponds to the time from the bleach to the timepoint where the fluorescence

intensity reaches the half ($I_{1/2}$) of the final recovered intensity (I_E).

Figure 6.11(a, blue graph) illustrates the average fluorescence intensity recovered of surrounded rhodamine B-doped ZnO microspheres. In this case, the half-time of recovery of surrounded particles was $t_{1/2-RhB-surr} = 29.1$ s. The average diffusion constant of rhodamine B exhibited a value of: $D_{RhB-surr} = 3.9 \cdot 10^{-8}$ cm^2/s . Meanwhile, isolated particles curve indicates slightly faster diffusion with an average diffusion constant of $D_{RhB-iso} = 4.6 \cdot 10^{-8}$ cm^2/s and a half-time of recovery equal to $t_{1/2-RhB-iso} = 24.7$ s and for practically the same fluorescence recovery of 34.2 % and 35.1 %, for surrounded and isolated particles, respectively, as can be seen in Figure 6.11(a).

These results provide very interesting evidence that there are two different diffusion behaviors in ZnO microspheres. In the case of RhB-doped ZnO microspheres, the dye molecules are not dependent on the environment of the studied microsphere. It is important to note that in this case the RhB molecules cannot pass the microcapsule envelope barrier and are therefore well confined within the microsphere. Consequently, rhodamine B molecules remain attached to the microsphere, and therefore there is no exchange between the microsphere and its neighboring molecules after photobleaching.

Besides, it can be seen that in the case of rhodamine B there is no transport of fluorescent molecules from the surrounding (external) environment. Therefore, the diffusion only takes place from the inside of the microsphere. Moreover, as the diffusion coefficient of rhodamine B is two order of magnitude times smaller than the literature value of rhodamine B in water ($D = 3.6 \cdot 10^{-6}$ cm^2/s)³³⁸, we may conclude therefore that rhodamine B molecules should be strongly attracted to the

surface of ZnO microspheres, presumably because of their two (+ and -) electrical charges.

On the other hand, the behavior is different in the case of fluorescein. One can clearly notice that the diffusion occurs outside the microsphere as the intensity of the recovered fluorescence is much higher than in the case of isolated microspheres. It can be seen that in this case the diffusion of dye molecules depends strongly on the environment of the photo-bleached microcapsule. In the case of isolated microcapsules, more fluorescence is recovered than in the case of surrounded microcapsules since the fluorescein molecules can thus move freely inside the microsphere without being hindered by the surrounding microspheres.

Another interesting information can be mentioned from the comparison of the diffusion time of the two used dyes. One can see that fluorescein molecules diffuse much faster than rhodamine B ones. One explanation for this phenomena could be the electric charges carried by the dye molecules and the ZnO nanoparticles. In contrast to the strong interactions between rhodamine B molecules and ZnO nanoparticles. The interactions between ZnO nanoparticles and fluorescein molecules should be less important.

In addition, taking account of the surface area value of the used ROI ($S_{ROI} = 112 \mu m^2$) and assimilating roughly the photobleached volume V_{PB} under the ROI with the volume of a cylinder whose base and height are equal to S_{ROI} and the diameter D_p of the microcapsule, respectively, we can estimate the expected fluorescence recovery after photobleaching by the ratio $\frac{V_p - V_{PB}}{V_p} \simeq 0.21$.

Basically, because of the demonstrated non permeability of the microcapsule shell to RhB molecules, we could use the measured $\simeq 0.35$ value for the recov-

ered fluorescence ratio as an accurate measure for the ratio of the photobleached quantity of rhodamine molecules (provided that the illuminated volume of the microcapsule is accurately determined).

It is interesting to note that, that despite the rough estimation of the exact value of the photobleached number of dye molecules and the real illuminated volume (because of the focused laser beam geometry), we find that this ratio is of the same order than the observed ratio for both rhodamine B and fluorescein, that is $\simeq 0.35$. Also, in the case of fluorescein, one can clearly see that the environment of the studied microsphere plays an important role in the diffusion. Fluorescein molecules can cross the microsphere barrier. The fact that only 23 % of the fluorescein is recovered (in the case of the surrounded particles) shows that there is a strong concentration gradient between the inside and outside of the microsphere.

However, for Fluo-doped ZnO microspheres, the intensity of fluorescence appears to be strongly dependent on the environment of the bleached particle, as shown by the recovery curves in Figure 6.11(b). From these curves the mean dynamics of the mobile molecules of the Fluo-doped ZnO microspheres can be easily extracted. It has been estimated to vary approximately from 18.71 s to 25.8 s for isolated and surrounded particles, respectively. One may also conclude that the fluorescein diffusion constant were $D_{Fluo-surr} = 6 \cdot 10^{-8} \text{ cm}^2/\text{s}$ and $D_{Fluo-iso} = 8.4 \cdot 10^{-8} \text{ cm}^2/\text{s}$ for isolated and surrounded microspheres, respectively.

Further studies are required to find out more about the forces acting between these electrically charged particles. It is important to note that the strong dependence of the diffusion constant on the environment of the photobleached areas might be caused by the high level of interactions within the encompassed particles.

It could be assumed that such microspheres interact strongly with their environment allowing the molecules therefore to be more trapped in such configuration, and preventing them from more freedom, flexibility, and rapidity of movement. Whereas, in the case of single particles, the high fluorescence recovery could be

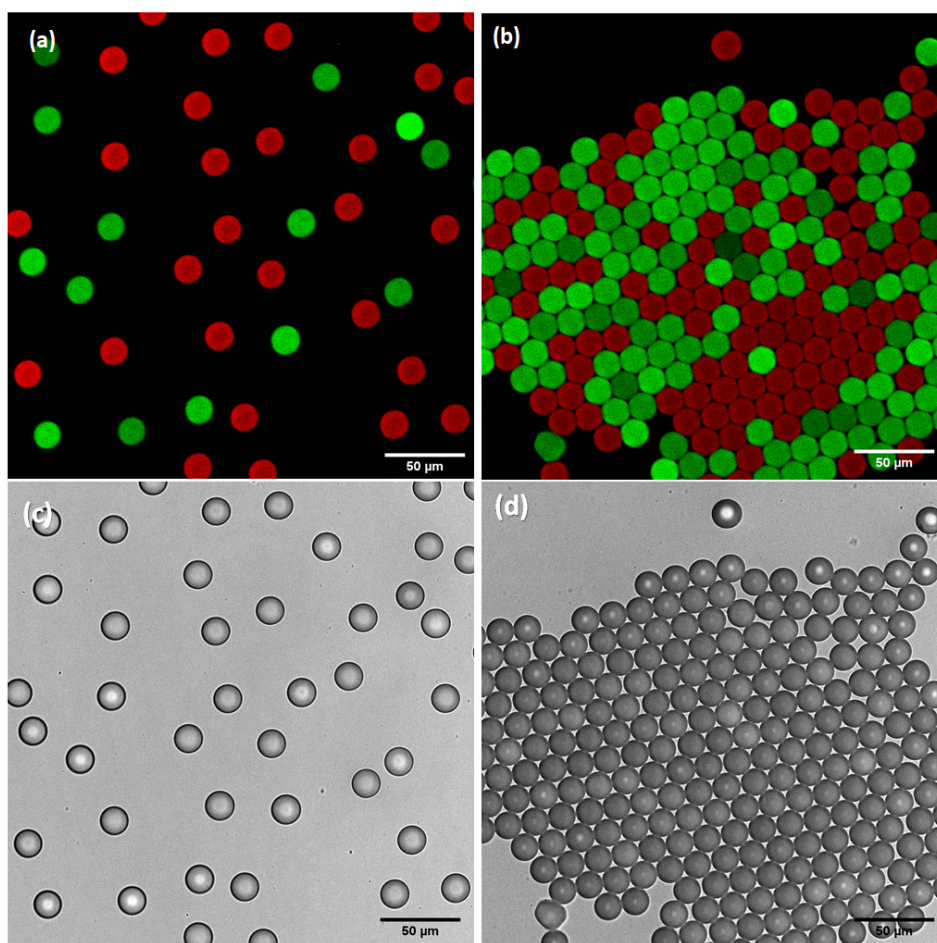


Figure 6.12 Confocal microscopy of single ZnO microspheres (a) fluorescence, (c) bright field, and encompassed ZnO microspheres (b) fluorescence, (d) bright field micrographs.

due to a lack of interactions (attractive/repulsive forces) with the enclosed particles, and thus much more freedom of the mobile fraction for moving around within the bleached area. This is particularly highlighted in Figure [6.12](#). This fig-

ure shows that the dispersed microspheres on the surface of a Petri dish which are not in contact with each other have kept their spherical shape. On the other hand, the surrounded microspheres have a spreading appearance in order to ensure a bond with the surrounding microspheres. These particles are subject to attractive forces which lead to the deformation of their spherical shape. This yields consistent results for the FRAP experiments as we used both types of assembly for our study to analyze the mobility of rhodamine B and fluorescein.

Appendix A

Optical micromanipulation of ZnO microcapsules

An interesting phenomenon was observed when we developed ZnO (0.5 M) droplets doped with rhodamine B (0.1 mM) to conduct photobleaching experiments after about 6 h of production. In this work, we generated a region of interest in the form of a disc in the center of the particles (16.5 μm in diameter) for performing FRAP experiments. These experiments were conducted by exposing this circular geometry to a high intensity laser beam. We then monitored the behaviour of fluorescent rhodamine B molecules confined within the ZnO microspheres.

The results are shown in Figure [A.1](#). At first glance, this seems very surprising, as we recorded a fluorescence recovery phase 18 times faster (fluorescence recovery at 3.9 s) than that obtained in the case of ZnO microspheres under the same experimental conditions, such as the medium where the microparticle under study is located (isolated microparticles in this experiment), the concentration of rhodamine B dye and the concentration of ZnO, as well as the bleaching protocol.

This indicates that, in this case, the dye is much less trapped inside the microsphere and therefore diffusion is less inhibited in the presence of the rhodamine B molecules.

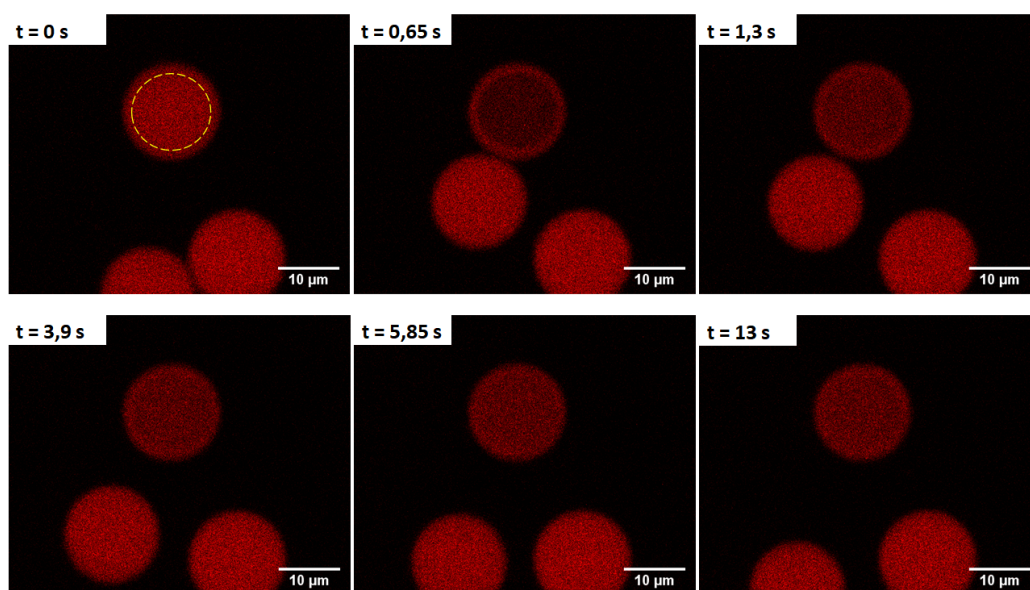


Figure A.1 Fluorescence images of the FRAP results on a ZnO (0.5 M) microsphere doped with rhodamine b (0.1 mM) before and after photobleaching at different time intervals of 0.65 s, 1.3 s, 3.9 s, 5.85 s, and 13 s. The dotted yellow circle indicates the circular ROI that was created in the centre of the microsphere to conduct the experiment.

We consider that the exposure of the region of interest to the laser beam during the bleaching process leads, obviously, to a phenomenon of diffusion of the unbleached fluorophore molecules towards the area of interest and of the bleached molecules outside, which explains the restoration of fluorescence in the bleached area. Thus, it is important to note that we have observed a significant interaction and mobility between the microspheres on a larger scale, as shown in Figure [A.1](#). We attribute these interactions to the transformations induced in the microsphere, notably the modification of its surface charge due to the laser exposure during the

FRAP experiments.

Therefore, to illustrate this, we proposed the model shown in Figure [A.2](#). To elucidate these results we assume that the microsphere (1) was positively charged before photobleaching, as was the microsphere (3) at the bottom left. However, it should be noted that microsphere (2) was negatively charged and therefore attracted by the other two positively charged microspheres. Photobleaching may be the cause of a change in the surface charge of the microsphere under study, hence the observation of a strong interaction between the oppositely charged microspheres (1) and (3) and repulsion between the microspheres that have become similarly charged, i.e. microspheres (2) and (3). Then, the phenomenon of repulsion between the microsphere (1) and (2), would probably be related to a rebalancing and redistribution of charges after diffusion between the two microspheres which contributed to the change in the charge of the microsphere (3) which became negatively charged.

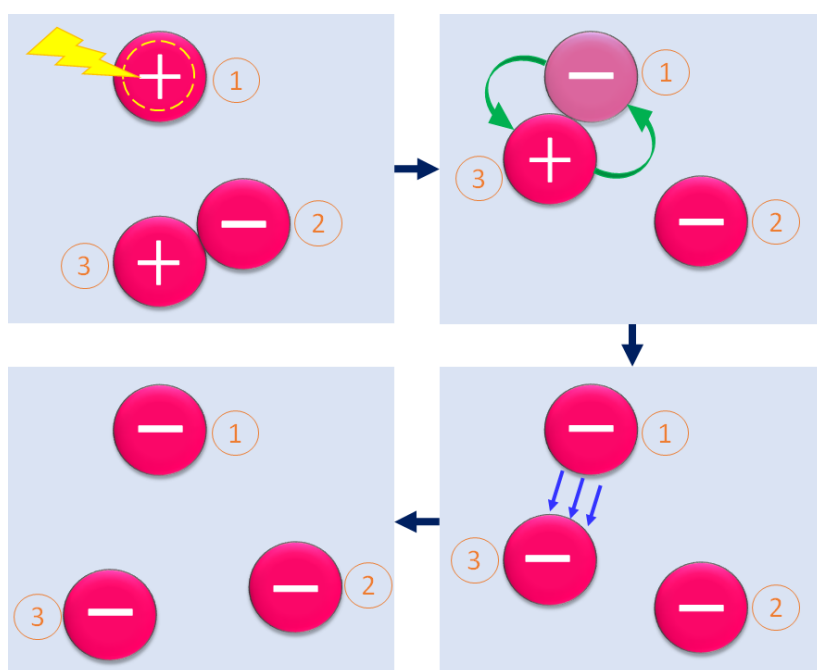


Figure A.2 Schematic illustration of the effect of FRAP on the modification and redistribution of the surface charge of Rhodamine B-doped ZnO microspheres.

Appendix B

Anisotropic distribution of charged dyes in ZnO microspheres

We have shown previously that the use of charged dyes, such as rhodamine B, can change the electrical polarization of zinc oxide microdroplets. To better understand how charged molecules interact with condensed ZnO microspheres, and which role they play in the final organization of ZnO microcapsules, we examined the effect of two widely used electrically charged fluorescent probes, which are rhodamine B and fluorescein.

In the first experiment, we prepared separately two samples of ZnO nanoparticles, to which we added separately the two dyes, at a concentration of 0.1 mM. Then the two mixtures were injected in the microfluidic device in order to produce two types of ZnO microdroplets, one which doped with rhodamine B and the second with fluorescein.

In the second experiment, we add successively rhodamine B and fluorescein dyes, at a concentration of 0.1 mM, to ZnO nanoparticles dispersion, then the

mixture is injected in the microfluidic device in order to produce monodisperse rhodamine B/Fluorescein doped ZnO droplets. In the second experiment, droplets were produced by mixing and stirring, immediately for approximately 1 minute, ZnO nanoparticles dispersion with rhodamine B and fluorescein at the same concentration (0.1 mM). All the droplets, in both experiments, were stabilized using dSURF at 0.5 %.

Figure B.I(a) shows a confocal image of ZnO microspheres doped with rhodamine B (red spheres) and fluorescein (green spheres) obtained using the first approach, which was carried out through a separate synthesis of two types of particles. The latter have been mixed together and visualized using confocal microscopy. In both cases, uniform distribution of the dyes within the microspheres can be seen in Figure B.I(a). Moreover, rhodamine B and fluorescein have been well-confined. Surprisingly, the analysis of the results obtained when ZnO colloidal nanoparticles have been doped simultaneously with both dyes, reveals that the latter were distributed in an astounding way, where we can see the fluorescein well trapped in the center of the microspheres while rhodamine B occupied the outer contour of the microspheres, as depicted in Figure B.I(b).

In these experiments, rhodamine B molecule plays an important role since it has the property of carrying both positive and negative charges. Its counter-ion has also been used to further stabilize the suspension of ZnO nanoparticles in the presence of fluorescein in its zwitterionic form. In addition, the ZnO nanoparticles surface has a positive charge characteristic. The negative molecular chains of fluorescein molecule can therefore easily adsorb by electrostatic forces to the surface of colloidal ZnO nanoparticles, and rhodamine B molecule can then come

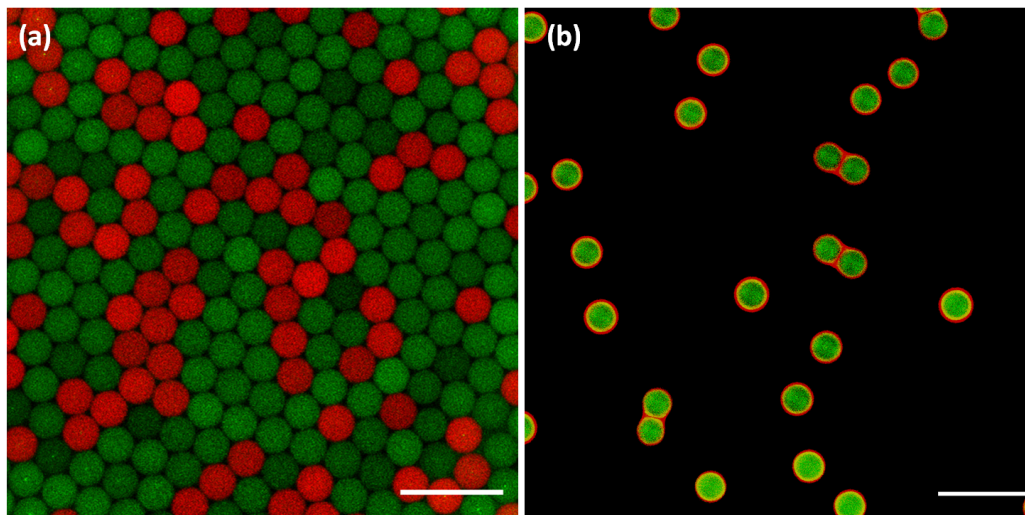


Figure B.1 Confocal images of (a) uniform rhodamine B-doped ZnO microspheres (red particles) and fluorescein-doped ZnO microspheres (green particles) synthesized using the first approach, (b) rhodamine B/fluorescein-doped ZnO microspheres prepared using the second approach. Scale bars 50, μm .

into contact; using its positive counter-ion, with fluorescein. Finally, rhodamine B molecule is deposited on the surface of the microspheres. And so these charged molecular chains that were present in each single droplet may have connected together, resulting on a selective uptake of rhodamine B and fluorescein all around the microsphere.

In this context, Liu et al. studied the effect of the charges on the dipolar field of apple-shaped ZnO microspheres²⁰. These authors used two different dyes: a positively charged dye (Safranin T) and a negatively charged dye (Congo Red). They observed a significant selective absorption of the positively charged dye (Safranin T) on the polar surfaces of the specific negatively charged ZnO microspheres.

Appendix C

Diffusion driven merging of ZnO microcapsules

Remarkably, when we visualized rhodamine B- and fluorescein-labeled microspheres in confocal microscopy without giving the particles the time to reach their final size, a fusion between two different types of particles took place (Figure C.1). In fact, the results shown in Figure C.1 clearly reveal a quick diffusion of fluorescein in ZnO microsphere to rhodamine B-labelled particle. Indeed, fluorescein diffusion takes place in ZnO microsphere faster than rhodamine B which is in good agreement with the experimental results stated above.

Here, we would like to point out that interesting considerations can be drawn from the comparison of the diffusion coefficients estimated utilizing FRAP experiments (see Figure C.2). Based on the above observations, a plausible explanation concerning the impact of charged fluorescent probes can be developed. We speculate that under our synthesis conditions, the fluorescein dye exhibits a negatively charge on its phenol and/or carboxylic acid functional groups ($pK_a = 6.4$) (see

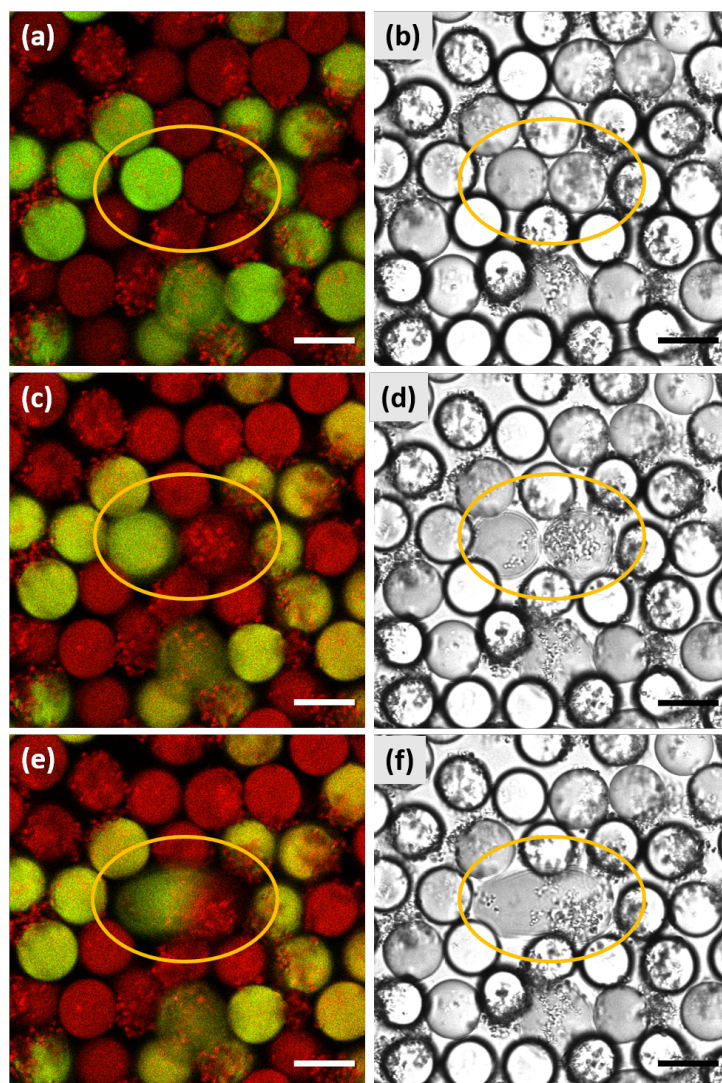


Figure C.1 Confocal microscopy micrographs (a, c, e) and bright field (BF) (b, d, f) optical images of a combination of differently stained fluorescent ZnO microspheres mainly rhodamine B-doped (red particles) and fluorescein-doped (green particles). Scale bars, 20 μm .

Figure 4.1). Such a charged dye can land slightly onto positively polar planes of ZnO microspheres. Negatively charged fluorescein (anionic/dianionic) may allow probing contribution of attraction at the positively charged surfaces of ZnO building blocks.

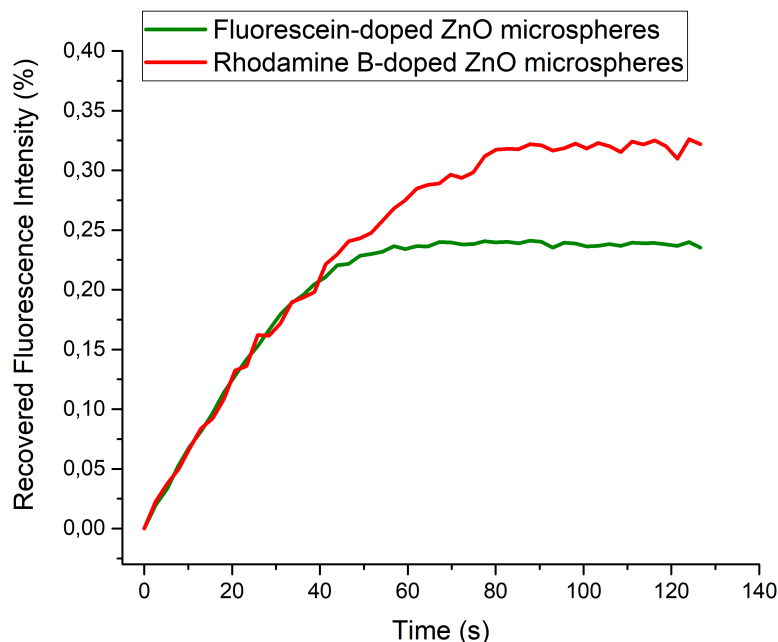


Figure C.2 Fluorescence recovery after photobleaching (FRAP) was measured for two charged fluophores (rhodamine B in red, and fluorescein in green). The graph shows recovered fluorescence intensity curves over time calculated for two types of ZnO samples.

The results also give a clear indication of an electrostatic interaction repelling zwitterionic molecules from the negative ZnO surface, yet additionally long-range electrostatic forces between the negatively Fluo-doped ZnO particles and the zwitterionic rhodamine B-doped ZnO particles. As a consequence of this fact, the repellent interactions promote destabilization of the equilibrium distributions of the dyes with polar ZnO planes, and forces fluorescein molecules to swell basically in the outer of the microsphere, resulting in a diffusion towards rhodamine B-doped ZnO.

Appendix D

Self-organization of nanoparticles and birefringence of microcapsules

We suggested that the microcapsules are made of the stacking of ZnO nanoparticles in the shell. Since such building units (with a thickness of about $0.1 \mu m$) are assumed to have a non spherical shape and an intrinsic dipole moment polar, one may wonder how such anisotropic building units self-organize inside the shell.

To better understand such organization, we used polarizing optical microscopy to investigate birefringence properties of ZnO microcapsules, as shown in Fig. [D.1](#), where we see clearly the coexistence of birefringent and non birefringent microcapsules. Likewise what has been observed in droplets and shells of liquid crystals, different orientations of the building units inside and on the surface of the microcapsule shell should be considered: i) a random (isotropic) orientation of ZnO nanoparticles (or their aggregates) inside the shell, ii) a radial orientation and iii) the so-called bipolar orientation of the nanoparticles by respects to the surface of the microcapsule as sketched in Figure [D.2](#). For symmetry reasons,

random and radial radial orientations should lead to isotropic microsphere (without birefringence), whereas the second orientation should lead to a birefringent microsphere.

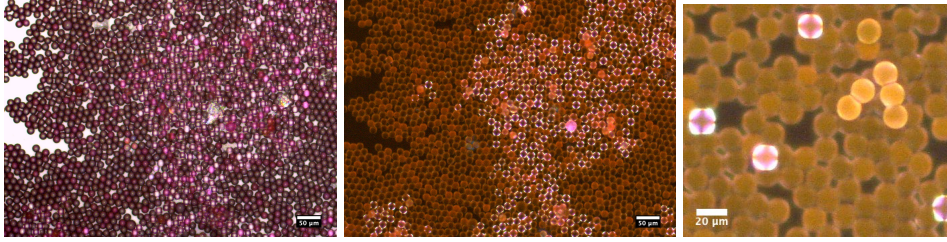


Figure D.1 Polarizing optical microscopy images of RhB doped ZnO microcapsules, under no polarizers (left) and crossed polarizers (middle and right).

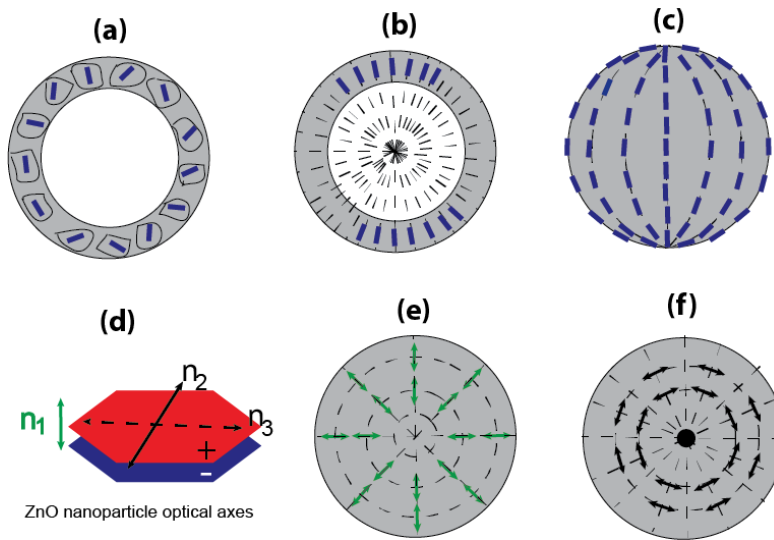


Figure D.2 Schematic representation of different optical axes of ZnO nanoparticles; schematic representation of different optical axes of ZnO nanoparticles; (a) random orientation of domains of ZnO nanoparticles (isotropic), (b) homeotropic orientation of ZnO nanoparticles where the nanoparticles orient their principal optical axes (n_2 or n_3) perpendicular to the surface of the microcapsule, (c) planar orientation of ZnO nanoparticles where n_2 or n_3 optical axes are parallel to the surface of the microcapsule, (d) schematic representation of different optical axes of ZnO nanoparticles, (e) top view of the microcapsule with a homeotropic orientation showing the orientation of n_1 optical axes by respects to the equatorial planes of the microcapsule, (f) top view of the microcapsule with a planar orientation showing the orientation of n_2 (or n_3) optical axes by respects to the equatorial planes of the microcapsule.

General Conclusion

In this thesis work, we have developed an original droplet-based microfluidic approach to generate zinc oxide microcapsules with well-controlled size and morphology in a micro-metric range from 10 μ to 30 μ . We have shown that microcapsules have an envelope whose thickness depends on the initial size of the microdroplets.

We have studied the effect of different parameters on droplet formation, and on the size and stability of the resulting microspheres, such as the effect of surfactants, dispersed and continuous phase flow rates, addition of electrical charges (ions, dyes, ...), surfactant type (TB-Krytox and dSUR) and the kinetics (solvent evaporation rate), as well as on the study of their optical and electrical properties. We have characterized the as-synthesized zinc oxide microspheres using optical microscope, scanning electron microscopy (SEM), energy dispersive X-Ray spectroscopy (EDS), transmission electron (TEM) microscopy, photoluminescence (PL), and confocal microscopy (CM).

We have discussed the kinetics and real-time monitoring of uniform-sized and stable zinc oxide microdroplets and showed that the decrease of droplets size versus time follows two steps. In this regard, we have determined the relationship between droplets size and particles size and provided a detailed description of the

formation mechanism of zinc oxide microcapsules.

We have also shown the polarity feature of zinc oxide nanoparticles and their self-organization at the microdroplet interface. We have shown that the electric charges carried by the primary units of zinc oxide nanoparticles play a vital role in the droplet interfaces stability, thus in the presence of charged molecules, and the size of the synthesized zinc oxide microcapsules.

Moreover, we explained the colloidal growth processes and the self-organization of zinc oxide nanoparticles using a microfluidic approach. In addition, we suggested that both orientation and self-organization of zinc oxide nanoparticles in microdroplets and microparticles should be governed by a balance between interfacial forces and electrostatic forces.

We have presented experimental evidences of the preferential adsorption of charged fluorescent dyes at the zinc oxide droplets interface and establish different models to support our findings. The optical and electrical properties of these microspheres have been studied both experimentally and theoretically.

Furthermore, we have developed a new approach to measure the temperature inside optofluidic microsystems at the millisecond (ms) time scale in a reliable way using dye-doped Zinc oxide microcapsules. In addition, we used rhodamine B and rhodamine 6G as molecular temperature probes for high throughput fluorescence-based thermometry. We have demonstrated that rhodamine B and rhodamine 6G can be used efficiently as molecular probes for measuring temperature in accurate and quick manner at the microscale of flowing zinc oxide microparticles inside microfluidic devices. In addition, we have also demonstrated that the as-synthesized doped zinc oxide microspheres can be used to study the

dynamics of fluorescent probes mobility, and to measure the diffusion coefficients of rhodamine B and fluorescein dyes inside zinc oxide microcapsules.

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Titre : Synthèse, structure, organisation et premières applications des microcapsules colloïdales de ZnO élaborées par voie microfluidique.

Mots clés : Microfluidique à base de gouttelettes, Oxyde de Zinc, ZnO, Microcapsules, Microsphères.

Résumé : Ce travail se concentre sur le développement d'une approche microfluidique originale à base de gouttelettes pour générer des sphères de ZnO de taille micrométrique très monodispersées avec une taille et une morphologie bien contrôlées. Cette approche est simple et prometteuse non seulement pour la fabrication de microcapsules de ZnO de taille uniforme, avec une taille ajustable et un contrôle précis à l'échelle micrométrique, mais aussi pour acquérir de nouvelles connaissances sur la compréhension des processus de croissance colloïdale et l'auto-organisation des nanoparticules de ZnO par la voie microfluidique. En outre, ces microparticules peuvent trouver des applications intéressantes dans de nombreux domaines tels que la photonique, le photovoltaïque ou la biomédecine.

Ce travail porte sur l'effet des paramètres de fabrication sur la formation de gouttelettes, la taille et la stabilité des microsphères résultantes, ainsi que sur l'étude de leurs propriétés optiques et électriques, en couplant les travaux expérimentaux et théoriques. Nous avons montré la synthèse, dans une gamme micrométrique allant de 10 μm à 30 μm , de microcapsules de ZnO mésoporeuses à enveloppe fine et flexible.

Nous étudions la caractéristique polaire des nanoparticules de ZnO et leur auto-organisation interfaciale. En outre, nous révélons que les charges

électriques portées par les unités primaires de ZnO jouent un rôle crucial dans la stabilité des gouttelettes en présence et en absence de molécules chargées. Elle joue également un rôle clé tout au long du processus d'assemblage, de la création des nanoparticules colloïdales de ZnO aux microgouttelettes, et enfin aux microsphères.

Nous rapportons, pour la première fois, l'auto-organisation de micro-gouttelettes de ZnO liquide dopé en réseaux carrés. Nous démontrons qu'un tel résultat révèle l'aspect polaire des microgouttelettes de ZnO et corrobore un changement d'équilibre entre les forces motrices contrôlant l'organisation des nanoparticules de ZnO à l'échelle nanométrique. Nous avons développé différents modèles, en très bon accord avec le champ dipolaire et les mécanismes de forces interfaciales, pour étayer les résultats expérimentaux mis en avant, et pour expliquer l'organisation interfaciale des nanoparticules de ZnO/RhB sur la base des propriétés d'organisation des gouttelettes de ZnO.

À partir de nos résultats et de la dépendance constatée de la taille des microcapsules, de l'épaisseur de la coquille et de la densité de surface des nanoparticules par rapport à la taille des gouttelettes, nous fournissons un modèle original de la contribution des facteurs impliqués dans le mécanisme de formation de la coquille.

Title : Microfluidic-based colloidal ZnO microcapsules: synthesis, structure, organization and first applications.

Keywords : Droplet-based microfluidics, ZnO, Microcapsules, Microspheres.

Abstract : This work focuses on the development of an original droplet-based microfluidics approach to generate highly monodisperse micrometer-sized ZnO spheres with well-controlled size and morphology. This approach is straightforward, and promising not only for the fabrication of uniform-sized ZnO microcapsules, with adjustable size and precise control at the microscale, but also for gaining new insights into the understanding of colloidal growth processes and self-organization of ZnO nanoparticles by the microfluidic route. In addition, such microparticles may find interesting applications in many areas such as photonics, photovoltaics, or biomedicine.

This work deals with the effect of handling parameters on droplet formation, size, and stability of the resulting microspheres, as well as the study of their optical and electrical properties coupling experimental and theoretical works. We have shown the synthesis, in a micrometric range from 10 μm to 30 μm , of mesoporous ZnO microcapsules with a thin and flexible shell.

We investigate the polar feature of ZnO nanoparticles and their interfacial self-organization. Besides, we reveal that the electric charges carried by

ZnO primary units play a crucial role in the stability of the droplets in the presence and in the absence of charged molecules. It also plays a key role throughout the assembly process from the creation of the colloidal ZnO nanoparticles to the microdroplets, and finally the microspheres.

We report, for the first time, the self-organization of doped-ZnO liquid microdroplets in square arrays. We demonstrate that such a result discloses the polar aspect of ZnO microdroplets and corroborate a shift in the balance between the driving forces controlling the ZnO nanoparticles organization at the nanoscale. We have developed different models, in very good agreement with the dipole-field and interfacial forces mechanisms, to support the experimental results put forward, and to explain the ZnO/RhB nanoparticles interfacial organization based on ZnO droplets organization properties.

Based on our findings, and on the stated dependence of the microcapsules size, shell thickness, and nanoparticles surface density versus the droplets size, we provide an original model for the contribution of the involved factors in the shell formation mechanism.