Stabilisers for water-in-fluorinated-oil dispersions: key properties for microfluidic applications

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1 Introduction

Simple liquids do not necessarily mix. It reflects the fact that various compounds interact differently at the molecular level. However, immiscible liquids transiently exist in mixtures in the form of dispersions. An emulsion, is a dispersion of small droplets into a continuous phase, stabilised by a third compound, typically surfactant molecules [1]. The properties of the resulting mixture – mechanical, rheological, chemical... – are essentially different from those of both individual liquids, creating complex fluids of practical interest for applications. Many products of our daily life are based on these disperse systems, from food colloids to pharmaceutical and cosmetic formulations, drug delivery systems, to just cite a few applications [2]. The kinetic stabilisation of dispersions is essential to maintain the properties of the mixture over time. Recently, the enormous potential of emulsion droplets as miniaturized reaction vessels has been exploited to provide novel assay systems [3, 4]. Interestingly, the idea of using droplets as microreactors has already been brought up in the middle of the 20^{th} century [5]. The real breakthrough came with the recent advances in the droplet-based microfluidic technology [6, 7, 8, 9, 10, 11].

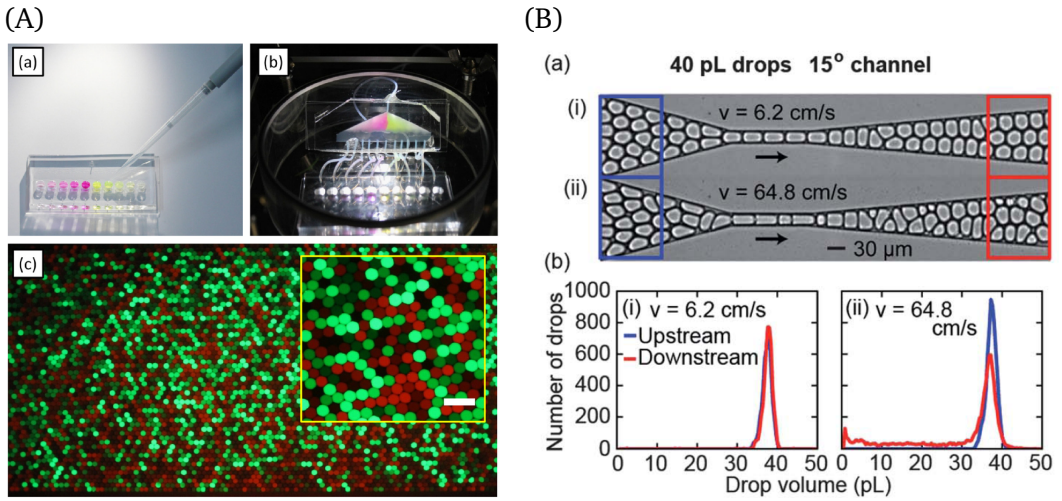

Fig. 1. Microfluidic manipulation of emulsions. (A) Complex emulsions are produced using microfluidics. Each droplet in the emulsion can have its own individual composition as shown here with fluoresent dyes (Reprinted with permission from Lim *et al.* [20] Copyright 2015, AIP Publishing LLC). (B) The manipulation of emulsions in microchannels leads to new types of ageing processes that need to be understood and controlled (Reproduced in part from Rosenfeld *et al.* [21] with permission of The Royal Society of Chemistry).

Droplet-based microfluidics emerged at the very beginning of the 21^{st} century as a subdomain of microfluidics [6]. It employs immiscible phases that are flowed through microchannels such that homogeneous shearing of the liquids results in the formation of emulsions with discrete monodisperse droplets. The technique allows for the production and precise manipulation of calibrated emulsion droplets at high rates (up to several kHz), unleashing an enormous potential for high-throughput screening applications, single cell analysis, DNA-based diagnostics or drug screening [12, 13, 14, 15, 16, 17, 18, 19].

The emulsions produced in microfluidics are unconventional from a material point of view: each droplet has typically an individual composition at every time step, depending on the initial loading of compounds and on the biochemical processes taking place in the droplet (Figure 1(A)).

As a result, new types of ageing mechanisms are to be expected in these emulsions. First, the flow of droplets in microchannels

affects the stability of the droplets (Figure 1(B)), and induce ageing of the emulsion by manipulation of individual droplets [22, 23, 21]. Understanding and controlling these ageing processes is a prerequisite for an efficient use of the technology [11]. It is therefore important to understand the dynamics of surfactant-laden interfaces on the flow of droplets in confinement, at the time-scale of droplet manipulation (typically ~ 1 ms) and at the lengthscale of the microchannels (typically 1 - 100 μm). As an emulsion, the droplet assembly ages according to the classical ageing processes, such as flocculation, coalescence, gravitational separation, and Ostwald ripening [1]. In addition, molecular transport of solutes between the droplets – driven by differences in chemical potential of encapsulated molecules – is driving the system towards its equilibrium. This process is not really crucial for emulsions used in material science as all droplets are virtually identical in composition. Here such a transport process leads to cross-talk between droplet microreactors [24, 25, 26, 27]: the concept of individual, independent microreactor ultimately breaks down at sufficiently large time-scale, compromizing the feasibility of assays based on the compartmentalization approach.

The understanding of mass transfer – and ageing processes in general – in these emulsions is essential for the establishment of platforms usable for biotechnological high-throughput applications. Reversing the viewpoint, the control of these transport processes between droplets can also open new ways to temporally program the composition of droplet microreactors and design novel materials and microsystems.

2 Manipulation of emulsions in microfluidics

2.1 Droplets in microchannels

The most widely used channel geometries for microfluidic droplet production are the T-junction and the flow-focussing geometries

– where the breakup of a stream of a first fluid is induced through shearing by a second fluid[6, 28, 10] – or step emulsification – where capillary forces at a step change in the height of a microchannel drives droplet formation [29]. In all cases, highly monodisperse droplets are formed due to the homogeneous shearing and the controlled emulsification conditions. Droplet production frequencies are ranging from a few to more than 10 kHz[30, 31, 20] with volumes down to the femtolitre range [31, 32]. Several techniques have been developed to further manipulate, sort, split, trap or fuse droplets in microfluidic devices [10]. Besides their interest for applications [15], the tools for immobilizing, arranging and spacing droplets in a predefined way, allows to significantly reduce the degree of freedom of an emulsion system and quantitatively address the dynamics of interfaces at small scales. Such tools appear especially interesting as a means to study physico-chemical processes in emulsions at the length-scale and time-scale of relevance. From a technology view-point, controlling the physico-chemical properties of the formulations used in microfluidics is essential to guarantee that droplet manipulation in channels is effective and reliable.

The manipulation processes in microfluidics are controlled by several dimensionless numbers. The viscosity ratio between both phases, the ratio of the droplet size to the channel dimension, and all the hydrodynamic dimensionless numbers control the droplet behaviour, in addition to all the dimensionless numbers defined to account for channels geometry. Among others, the capillary number $Ca = \eta U/\gamma$, where η is the viscosity (usually taken for the continuous phase), U the droplet velocity and γ the interfacial tension, has a crucial role. As an example, the capillary number controls the splitting of droplets at a constriction during flow (Figure 1(B)) [21]. For a fixed processing speed (or throughput), reducing the capillary number is favorable to guarantee that interfacial effects dominate the physics of the system. Therefore η should be 'small' and γ 'large'. This condition determines what an efficient surfactant formulation should be for a reliable ma-

nipulation of the droplets: the continuous phase should have a viscosity as low as possible while the interfacial tension should be as high as possible. We will see below how formulations based on fluorinated oils match these requirements.

2.2 Understanding the dynamics of surfactants at interfaces

The surfactant play a key role in the stabilisation of the interfaces. The dynamics of the droplet deformations will be determined by the properties of the surfactant. Classically, surfactant adsorption is measured using tensiometry on large volumes. It was, however, shown that the dynamics of adsorption of surfactant is essentially different at 'large' scales compared to 'small' scales [33]. Here, large and small are defined by a discussion on the two limiting cases for adsorption: the adsorption is either limited by the bulk diffusion of the surfactant to the interface (diffusion-limited adsorption) or by the reaction rate of adsorption of molecules to interface (kinetic-limited adsorption). The crossover between both regimes occurs for a droplet size $R^* = D/k_{ads}\Gamma_\infty$ where D is the diffusion constant, k_{ads} the forward rate of adsorption and Γ_∞ the maximum interfacial concentration of the surfactant. Typically, R^* is of order 10-100 μm [33]: At small scales, adsorption / desorption controls the dynamics of surfactant. Tensiometry on large volumes (even using pendant droplets with volumes of $\sim$ 1 μL) is diffusion limited and does not provide the relevant information to understand the surfactant dynamics at the scale of emulsion droplets. Over the past years, microfluidic systems have been designed to address the questions dealing with interfacial tensiometry at the relevant scales [34, 35] with a recent focus on the dynamics of surfactant-laden interfaces [36, 37, 38]. In this context, a full understanding of droplet flow in the presence of surfactant is far from being reached. Open-questions involve the dynamics of interfaces in confinement and the role of Marangoni stresses and interfacial rheology on the behaviour of droplets in confinement.

3 Mass transport in emulsions

The second class of problems related to ageing deals with the transport of compounds between the droplets. Mass transfer between emulsion droplets occurs as a result of phase partitioning due to a finite solubility of the dispersed phase (or its solutes) in the continuous phase [39, 40] or alternatively in the bilayers possibly forming between droplets [41].

3.1 Ostwald ripening

In brief, the chemical potential $\mu(r)$ of molecules of the dispersed phase is a function of the droplets radius r [42]:

$$\mu(r) = \mu_\infty + \frac{2\gamma V_m}{r} \tag{1}$$

μ_∞ being the chemical potential in bulk, V_m as their molar volume and γ as the interfacial tension. The dependence of the solubility S of an emulsion droplet on its size is then described by the Kelvin equation:

$$S(r) = S_\infty \exp \frac{2\gamma V_m}{rRT} \tag{2}$$

with S_∞ as the bulk solubility of the dispersed phase in the continuous phase, R as the ideal gas constant and T as the absolute temperature. In polydisperse emulsions, heterogeneities in chemical potential and solubilities must equilibrate. The diffusion of the solutes of the dispersed phase in the continuous phase results in a net mass transport from smaller to larger droplets. Hence, small droplets shrink on the expense of larger droplets that grow in size, ultimately resulting in an temporal increase of the average droplet size and a reduction of the interfacial area of the emulsion (Ostwald ripening). In the presence of a third species, insoluble in the continuous phase, its osmotic pressure

$\Pi \approx RT\Delta c$ will oppose the Laplace pressure. Both contributions should be considered to determine the evolution of droplet sizes and the conditions where monodisperse emulsions can be stabilized [42]. Interestingly, such transport processes are used to measure metabolism of micro-organisms in a quantitative maner making use of the variation of composition in individual droplets as a biochemical process is taking place in the droplet (in this case, sugar consumption by yeast) [43].

3.2 Solute transport

According to similar considerations, the net transport of solutes between emulsion droplets is a consequence of heterogeneities in the chemical potential of solutes among the droplets. A finite solubility of solutes in the continuous phase generally results in the leakage of compounds from the emulsion droplets. The release rate was described to be dependent on the partition coefficient of the solutes between the dispersed and the continuous phase [39, 40] while interfacial properties were shown to influence the rate of release [44, 45, 46, 47, 48, 49]. A decrease in the rate of release was also observed when replacing hydrogenated with fluorinated components as the continuous phase [50, 45, 48]. This effect was attributed to changes in the interfacial tension, the size of the surfactant molecules and a higher cohesive energies between the fluorinated surfactant molecules [47]. However, the partitioning coefficient of most organic molecules in the investigated water-in-oil emulsions is significantly altered when replacing hydrogenated with fluorinated components [51, 52]. The hypothesis that interface acts as an effective barrier to the diffusion of molecules was revised by some authors recently [53], suggesting that no significant energy barrier for molecules crossing an interface exists [54]. In this limit, the transport of molecules between emulsion droplets is controlled by the diffusive flux in the continuous phase. The concentration of solutes close to the interface (but in the continuous phase) is then given by the con-

centration in the dispersed phase and the partition coefficient $K = c_{eq,cont}/c_{eq,disp}$ between both phases. The permeability P of the oil is then defined as:

$$P = \frac{KD}{d} \tag{3}$$

where d is the thickness of the permeable layer. This equation is known as Overton's rule, frequently used to described the rate of transport through biological membranes [55, 56]. In fluorinated oils, the transport of small molecules in emulsions was shown to depend on surfactant concentration [57], an indication that there is no significant energy barrier to partitioning in emulsions – or at least in fluorinated oils. This point is consistent with the scaling observed for the decrease of transport rate upon the addition of Bovine Serum Albumine to the dispersed phase [57].

3.3 Transport through bilayers of surfactant

The transport processes based on phase partitioning, might also arise from transport through bilayers of surfactant possibly forming between emulsion droplets[41]. Such bilayers form upon the interaction of surfactant monolayers adsorbed at the interface of emulsion droplets [58]. After formation of a bilayer, the droplets become strongly adhesive without coalescing, as a result of the molecular interactions between the surfactant molecules [58]. Whether or not bilayers of surfactants form, in a given emulsion system, is dependent on several parameters. For example, the solubility of the surfactant molecules in the continuous phase plays an important role. It was shown that changing the composition of the continuous phase significantly alters the adhesion energy between the droplets. The energy of adhesion is essentially zero in good solvents [58] resulting in the absence of bilayers. Hence, the adhesion energy between monolayers of surfactant molecules is significantly increased by decreasing the solubility of the surfactants in the continuous phase[41]. For mass transport across bilayers, two

distinct mechanisms have been suggested. One is based on the partitioning into and diffusion through the bilayer [59], the other one is based on transient pores in the bilayer occurring due to thermal fluctuations[60]. It has been suggested that the transient pore mechanism is dominant for inorganic ions while the partitioning and diffusion mechanism is more relevant for neutral molecules[61]. It was recently shown that an increase in adhesion energy results in a lower membrane fluidity and ultimately in a lower permeability [41].

4 Emulsions with a fluorous phase

The most promising formulations are based on fluoro-surfactant and fluorinated oils. Controlling these formulations is crucial for applications. We will discuss in more details the specificities and the relevance of fluorinated emulsions for their use in droplet-based microfluidics. We will not focus on the biocompatibility aspects but rather on the physical-chemistry of the system [11].

4.1 Organofluorine chemistry

Starting from terminology, fluorocarbons exclusively contain carbon and fluorine while perfluorinated compounds are characterized by the replacement of all carbon-hydrogen bonds with carbon-fluorine bonds (it should be noted that this terminology is not necessarily strictly followed [62]). We focus here on perfluorocarbon systems as defined above. The C-F-bond is highly polarized due to the high electronegativity of the fluorine. Fluorine is not a very good hydrogen bond donor and does not significantly interact with hydrogen-bonding acceptors [63]. The low polarizability of the C-F-bond, results in relatively weak London dispersion forces between the molecules, which scale with the square of polarizability [62]. Perfluorocarbon compounds were reported as 'extremely nonpolar' [64] and even the least polar existing flu-

ids [65]: teflon, for example, has a relative permittivity of only 2.1 [66].

As a consequence of the generaly very weak interactions of fluorinated compounds, they have a low cohesive energy (energy of vaporization). This results in a low value of the solubility parameter in the thermodynamic description of liquid-liquid mixtures [51]. Fluorocarbon are therefore miscible with aliphatic hydrocarbons [51] – at least above a critical temperature as expected for binary mixtures [67, 68]. The critical temperature increases with the length of the hydrocarbon chain and the length of the fluorocarbon chain: the solubility of aliphatic hydrocarbons in fluorocarbons therefore decreases for larger molecules [68]. The solubility of small organic molecules is usually considered to be small: as an example napthalen has a solubility in fluorocarbon derivatives ($(C_4F_9)_2)O$ and $(C_3F_7)_3)N$) of the order of 0.003 (mole fraction) at 25^oC [52]. The weak intermolecular forces result in a relatively high compressibility of the fluids, which reflects the availability of interstitial space[62]. For that reason, respiratory gases such as oxygen and carbon dioxide are generally highly soluble in fluorous fluids. The solubility of oxygen in fluorocarbons is about three to ten times higher than in the parent hydrocarbons [69]. This characteristics makes them highly valuable for use as blood substitutes or breathing liquids [70, 71, 72]. Furthermore, using perfluorinated compounds as a continuous phase, cells can be cultured in aqueous emulsion droplets[73, 74]. For droplet-based screening applications, these properties are highly valuable. The low solubility of organic molecules in fluorous fluids results in restricted cross-talk between emulsion droplets [25] by a reduction of the partitioning coefficient while the high solubility of respiratory gases is a key for cell survival in droplets[75, 76, 77, 78]. Furthermore, while conventional hydrocarbon compounds may swell the microfluidic core material PDMS (PolyDiMethylSiloxane), leading to device delamination or channel deformation, fluorinated compounds are highly compatible with PDMS [79]. Water-in-fluorinated-oil

emulsions are therefore considered to be the most promising systems for the miniaturization of biochemical assays in emulsion droplets[11].

4.2 Phase partitioning into fluorous fluids

Due to the very low polarizability, fluorinated compounds are generally of extremely nonpolar character. Non-fluorous solutes, with the exception of small gases, are in general virtually insoluble in fluorous solvents[80]. These liquids can be valuable for the selective extraction of molecules covalently modified with fluorous tags[81, 82] Specific non-covalent interactions result in an increased solubility of organic molecules in fluorous liquids[65]. The increased solubility is the result of noncovalent associations of perfluororinated molecules and organic molecules based on hydrogen bonding or ion pairing. Attention has for example been drawn to fluorous carboxylic acids. They were shown to act as molecular receptor for organic molecules significantly increasing their solubility in fluorous liquids. One of the first reports came from Palomo *et al.* [83]. The authors found a dramatic solubility increase in fluorous solvents for fluorinated urea in the presence of fluorinated carboxylic acids. In the absence of any other functional groups, carboxylic acids were shown to exist as hydrogen bonded dimers in fluorous fluids [84]. However, it has been demonstrated that hydrogen bonds with the lone pair of nitrogen are more stable than the hydrogen bonds present in cyclic carboxylic acid dimers [85, 86]. As a consequence, most nitrogen H-bond acceptors are more successful at competing for the carboxylic acid H-bonds than the carboxylic acids themselves [87]. This in turn results in the effective extraction of organic molecules comprising Lewis base characteristics as shown for pyridines [87, 80]. The presence of equimolar amounts of fluorinated carboxylic acids in the fluorous phase leads to an almost complete extraction (up to 99%) of pyridine derivatives from chlorofrom into the fluorous phase [87]. Furthermore, it was

demonstrated that the extraction of aniline in similar conditions is much less efficient (5%). This was explained by the fact that pyridyl nitrogen acts as a better hydrogen bond donor than primary amines. Moreover, it was argued that the highly selective and effective extraction is based on the fact that the substrate-receptor interactions are reinforced in fluorous liquids, as they are considered to be ultimate noncompetitive solvents [80]. Similar effects are observed with dyes (Figure 2). Surfactant formulations should therefore be optimized to control the emulsion stability while avoiding transport enhancement.

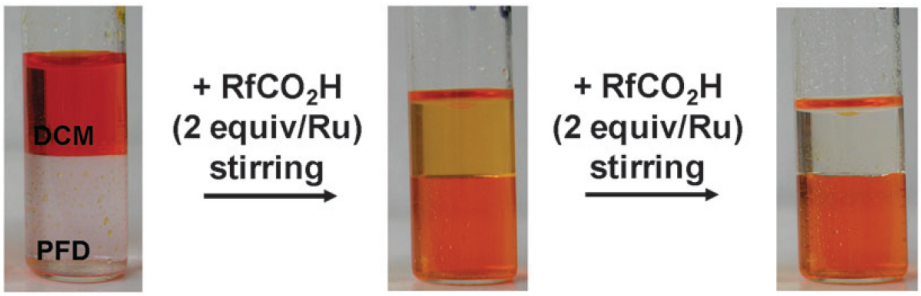

Fig. 2. Switch of partitioning induced by surfactant between a hydrocarbon (DCM = Dichloromethane) and a fluorous phase (PFD = Perfluorodecalin). A complex is formed between the red dye [Rubipy$_3$]2+ (Ru = Ruthenium, bipy = bipyridine) and the perfluoropolyether carboxilic acid RfCOOH leading to the extraction of the Ruthenium through complexation in the fluorous phase. (Reprinted from Correa *et al.* [88], with permission of The Royal Society of Chemistry).

In summary, noncovalent interactions significantly improve the extraction of organic molecules into a fluorous phase. The efficiency is strongly dependent on the compatibility of substrate and receptor. Particularly fluorous carboxylic acids have been studied that were shown to form strong hydrogen bonds with nitrogen containing Lewis Bases. Understanding these interactions is important for applications and microfluidics provides the tools to address these questions quantitatively.

4.3 Mass transport studied in microfluidics

One of the first microfluidic studies about mass transfer in two-phase systems was presented by Burns *et al.* [89]. The authors

have shown that in droplet-based microfluidic systems the mass transfer rates between the continuous and the dispersed phase can be up to several orders of magnitude faster than in non-miniaturized systems. The enhancement in the interfacial mass transfer was explained by internal convective circulation resulting from shear forces [89, 90, 91]. Courtois *et al.* [24] were among the first to study the retention of organic molecules in emulsion droplets in microfluidic environments, using an on-chip storage system to quantify the exchange rates. Qualitatively it was shown that the mass transfer of fluorophores between aqueous droplets dispersed in mineral oil was dependent on the nature of the compounds, the surfactant concentration and the number and composition of neighbouring droplets. Furthermore, it was stated that the release of fluorophores to the continuous phase "is a consequence of diffusion into the oil phase as well as formation of reverse micelles" but the mechanism of mass transfer remains mostly unclear. Strikingly, a method based on the addition of the protein bovine serum albumin was presented to decrease the leakage of compounds from aqueous emulsion droplets. The effect was considered to be based on the formation of a protein layer at the droplet interface acting as a kinetic barrier.

Bai *et al.* [25] have developed a double droplet trap system to study mass transport between emulsion droplets. The authors suggested that the transport of small molecules is occurring "across the resultant surfactant bilayers formed between droplet pairs". This was justified with the observation that "the droplets were clearly deformed, strongly suggesting the formation of a surfactant bilayer". However, as their trapping strategy is relying on constant fluid flow through the experimental zone to keep the droplets in contact, such deformations might be the result of the hydrodynamic drag force acting on the droplets. In contrast, it was shown that the transport of the fluorophore fluorescein between neighbouring droplets is significantly faster with a hydrocarbon continuous phase (mineral oil, 1% Span80 (sorbitan monooleate)) compared to a perfluorinated continuous phase

(FC-77 (a mixture of perfluoro-octane and perfluoroctane-ether), 1% 'EA' surfactant (Polyethylenoxide-perfluoropolyether block-copolymer)). It was reasoned that "the nature of the bilayer determines the transfer rate of molecules".

However, the solubility of fluorescein in hydrocarbon and fluorocarbon liquids is expected to differ dramatically. Therefore it can not be excluded that these observations are a result of a transport mechanism based on phase partitioning rather than transport through surfactant bilayers. Woronoff *et al.* [27] have shown in their experiments that the exchange rate of small molecules between droplets is dependent on their hydrophobicity. Their study was based on the measurement of the retention of several coumarin derivatives in water-in-fluorinated-oil emulsion droplets. A direct link between half-life of retention of the fluorophores in the emulsion droplets and the predicted partition coefficient of the dye was found. Recently the modulation of exchange rates with various buffers and additives was also demonstrated [49, 92].

Two limiting situations emerge to explain the exchange kinetics. Either the exchange is limited by the diffusion of the molecules between the droplets (diffusion limited transport) or by the kinetics of partitioning accross the interface (the kinetic limited transport, corresponding to an 'energy barrier' to partitioning). Chen *et al.* [93] used numerical methods to model the transport of fluorophores between water-in-hydrocarbon oil or alternatively in water-in-fluorinated oil emulsion droplets arranged in a two-dimensional hexagonal packing. The authors used a model assuming an effective permeability of fluorophores across the droplet interface of 10^{-8} m s^{-1}, which is based on the permeability of rhodamine B across the cornea, measured in another study[94]. The authors found, for the examined case, that "the leakage process was rate-limited by the transport of the probe across the droplet boundary, rather than by diffusion through the continuous phase [...]". In contrast, Dunstan *et al.* investigated the transport of reagents between water-in-hydrocarbon oil emulsion droplets in two-dimensional hexagonal packing and report diffusion through

the continuous phase as the limiting process[54] assuming that no significant energy barrier for molecules crossing the droplet interface exists. Accordingly, they find that the rate limiting step of transport is the diffusion across the continuous phase.

It is likely that both limits exist in different systems. However, the quantitative scaling relationship for the exchange rate as a function of surfactant concentration strongly suggests that transport in emulsions is diffusion limited [57]. The transport rate decrease observed with BSA is also fully compatible with a modification of the partitioning coefficient and it can therefore be concluded that there are – at the moment – no experimental data showing that kinetic barriers at interfaces are affecting the transport rates in emulsions. Surfactant formulations should therefore be optimized to control the emulsion stability while avoiding transport enhancement. The situation is, however, different for interfaces that are more complex than single molecular layers, as is the case for capsules or particle-laden interfaces.

4.4 Materials beyond surfactants

Surfactants are the basic systems usable to control the stabilisation of interfaces. To date, the family of molecules that are usable for droplet-based microfluidics is still limited (Table 1). Alternative strategies have emerged to stabilise droplets and circumvent the problems related to molecular interactions between the surfactant and the solutes (Figure 3). Interfaces can be stabilised by particles as in armored droplets or pickering emulsions.

A series of stabilising agents have been designed to stabilise aqueous dispersions in pefluorinated oils. The successful system involve gold nanoparticles [96] or silica nanoparticles [95]. In the first case, a full control of the mechanical properties was demonstrated with the possibility to attach cells at the droplet interface. In the latter case, the stabilisation of the emulsions is combined to a decrease of the leakage rate of fluorophores. Indeed the ab-

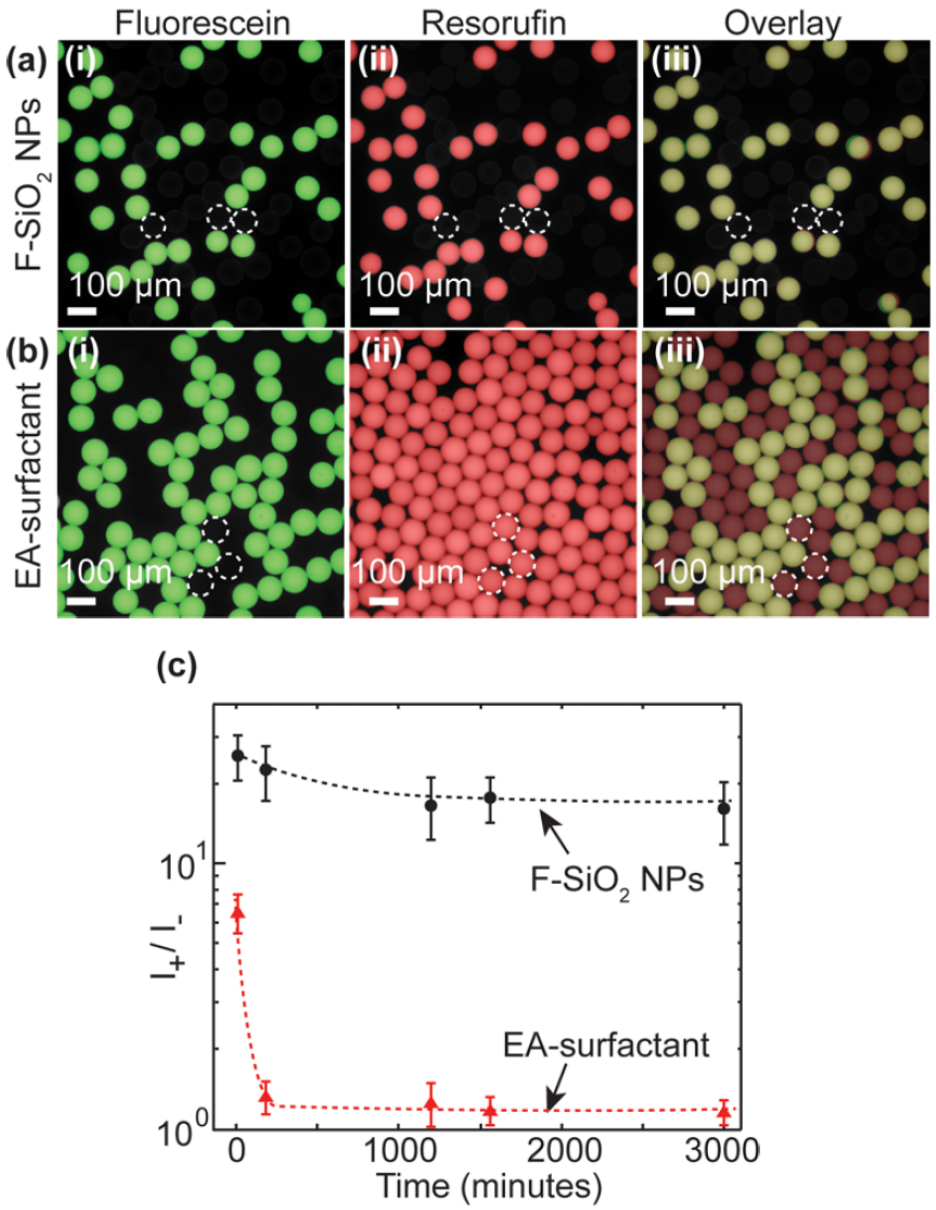

Fig. 3. Retention comparison between surfactant-stabilised emulsions and Pickering emulsions (Reprinted (adapted) with permission from Pan *et al.* [95] Copyright (2014) American Chemical Society). Two dyes (Fluorescein, green and resorufin, red) are encapsulated in nanoparticles stabilised droplets (top) and surfactant stabilised droplets (bottom). With the surfactant, resorufin is exchanged (yellow droplets) over time while with nanoparticles, the compartmentalization of the dye is more effective. Bottom graph: quantitative measurement of the exchange process showing how retention is improved using nanoparticles at the interface.

sence of surfactant in the oil phase reduces drastically the partitioning [57] making this strategy efficient to reduce the cross-talk. An additional interesting point is that nanoparticles-laden interfaces are expected to have the same interfacial tension as

Stabilisers	Use and typical application	References
PFPE-based Surfactant Synthesis	Stabilisation of droplets in microfluidics	[97] [98] [99] [100]
PFPE-based Surfactant	Self-assembly of surfactant at droplet interfaces	[101]
PFPE-based Surfactant	Microemulsification of Water in Supercritical CO_2	[102]
PFPE-based Surfactant	Catalysis of chemical reactions	[103] [104]
Gold-based Nanoparticles	Controlling the mechanics of the interfacial layer	[96]
Silica-based Nanoparticles	Pickering emulsions with functionalised silica nanoparticles (fluorosilane coating)	[95]

Table 1
Stabilisers for water-in-perfluorinated-oils dispersions used in droplet-based microfluidics. (PFPE=Perfluoropolyether). Additional information for the case of surfactant is available in ref [11]

the bare interface. Therefore if we compare these interfaces with surfactant-laden interfaces, at fixed capillary number, the velocity of droplets can be increased and therefore higher throughputs are to be expected for the manipulation of these objects.

5 New doors opening

Transport processes in emulsions are a potential problem for biotechnology applications. In contrast, they can also be seen as an interesting concept for new applications. Three of these applications will be discussed here as promising new avenues for fluorinated emulsions, in combination with microfluidics.

5.1 Switchable systems

The orthogonality in the properties of organic, aqueous and perfluorinated compounds also offers new means to control interfaces. A very striking result was obtained with three-phases sys-

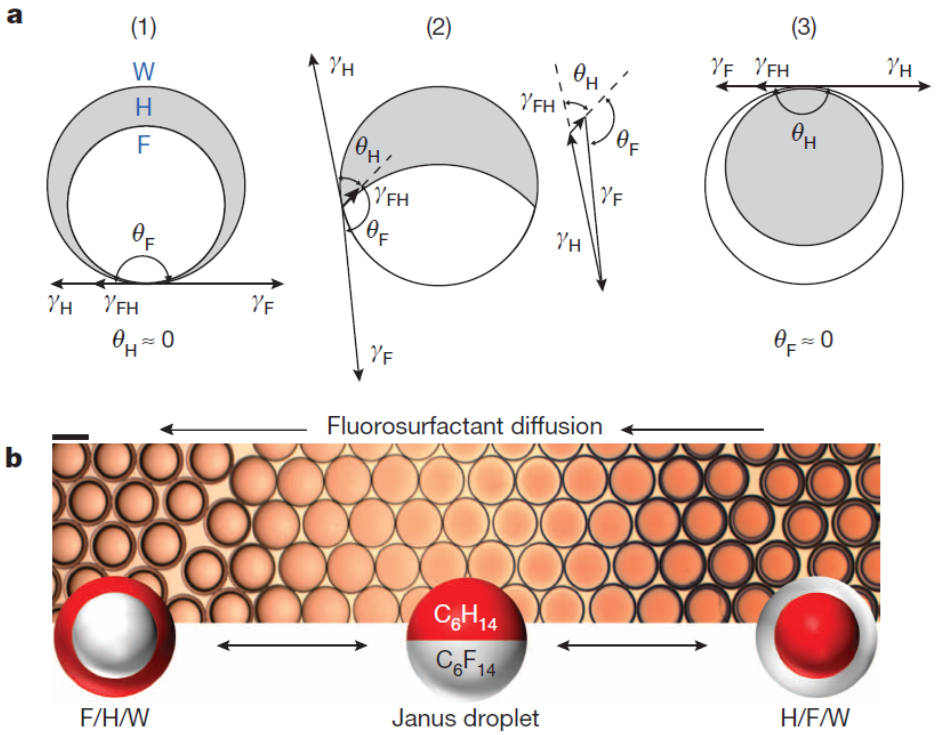

Fig. 4. Switchable emulsions. In a three phase system, surfactants modulate the spreading parameter for the three interfaces and a reversible switching between the configurations is achieved (Reprinted by permission from Macmillan Publishers Ltd: Nature, Zarzar *et al.* [105], copyright 2015).

tems involving fluorinated compounds (Figure 4). Switchable systems were designed in which morphological transitions in the emulsion are reliably controlled [105]. The order of the encapsulating phases is reversibly controlled by external parameters (for example temperature or surfactant concentrations) which offers promising switchable systems usable as new types of materials. The crucial role of microfluidics to control this system should be mentioned.

5.2 Relevance as biomimetic models

Compartmentalization is an essential step in the apparition of life and in biological processes [106]. The ability to control compartmentalization will provide platforms usable to experimentally test hypotheses on chemistry in simple minimal systems. Recently the

coupling between transport processes in emulsions and oscillating reactions in emulsion droplets led to a conversion of chemical patterns into morphological patterns [107] (Figure 5). This result is a step towards a better understanding on how transport processes can be coupled to mechanical systems to explain features related to morphogenesis. The use of this concept can become extremely

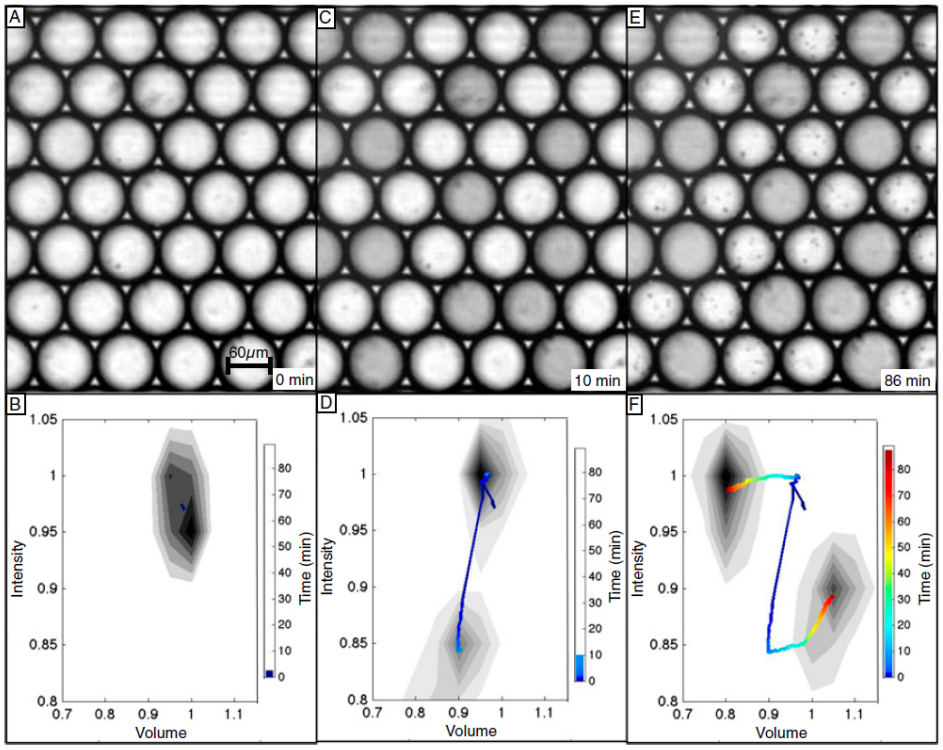

Fig. 5. Coupling of an oscillating chemical reactions to size modulation by transport processes. Starting from individual droplets of water in fluorinated oil (top left), a chemical pattern emerges in the emulsion (revealed by the different gray levels between the droplets in the central top figure). This chemical pattern finally lead to a morphological pattern with droplets of different sizes through coarsening and transport of water between the droplets (top right). The bottom graphs show how the gray level and droplet size evolve in time with first the establishement of the chemical heterogeneity (two populations with the initial droplet volume) and then the coarsening with the growth of the clear droplets and the shrinkage of the dark droplets. The conversion of chemical patterns into mechanical changes is a promising model for morphogenesis studies or mechanical actuations through chemical reactions (Reprinted with permission from Tompkins *et al.* [107]).

powerful to design macroscopic active systems and control their behaviour through chemical programing and self-organized processes. Here again the role of microfluidics is to provide the tools

to prepare monodisperse emulsions leading to organized patterns.

5.3 Catalysis and enhancement of reactions in compartments

Besides the interest of interfaces as catalyst, especially in fluorinated phases[104], chemical reactions are modulated by the presence of an interface. It was recently demonstrated that an interfacial reaction can contribute to the enhancement of a chemical reactions and significantly improve the efficiency of the given reaction [108]. The basic concept to understand the process is to compare the time it takes for a molecule to diffuse from the interface where it is produced from two reagents to the center of the droplet with the time scale of the backward reaction in bulk (Fig. 6). From this comparison, a lengthscale emerges below which the bulk concentration is dominated by the interfacial reaction. The coupling between reaction, diffusion and desorption therefore enhance a chemical reaction. This generic result

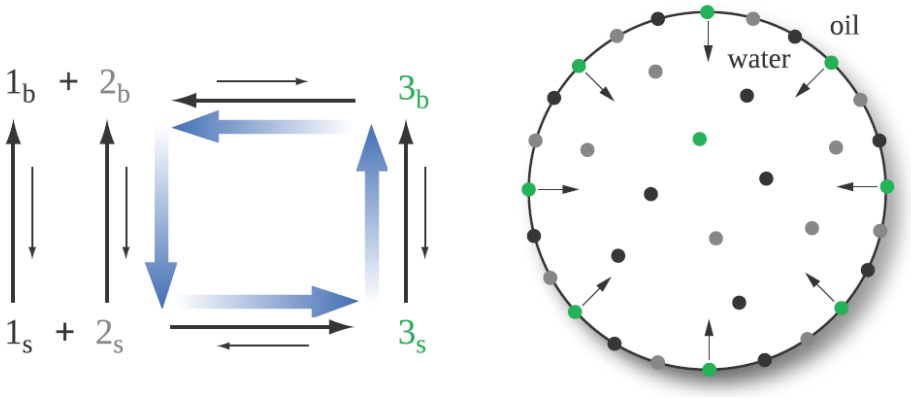

Fig. 6. A simple bimolecular reaction is enhanced when performed in microcompartments. The coupling of adsorption at interfaces, reaction, and diffusion leads to an increase in product formation in microdroplets (Reprinted with permission from Fallah-Araghi *et al.* [108], Copyright 2014, American Physical Society)

might help to understand the crucial role of compartments in the synthesis of large molecules, a problem of interest for prebiotic chemistry. Here, the role of microfluidics is to provide the tools to prepare calibrated emulsions for a quantitative measurement of the reaction kinetics in monodisperse droplets.

6 Conclusion

In summary, droplet-based microfluidics is the key technology to manipulate emulsions with new applications emerging in biotechnology, material science and chemistry. The success of microfluidics over the past ten years is largely correlated with the development of surfactant formulations in fluorinated oils. Those are especially adapted to microfluidics for the low solubility of organic molecules in fluorocarbon (significantly lower than in organic or mineral oils) which favors compartmentalization of organic molecules. In addition, new experimental strategies provide a whole range of solutions to further improve the systems. A whole new range of experimental systems can now be envisioned for the study of fundamental and applied questions in a wide variety of fields by the coupling of microfluidics and soft-matter systems based on microcompartments.

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Of special Interest*:
[22]: In microfluidics, droplet flow is shown to be a source of destabilisation of emulsions: counterintuitively, it is not when droplet approach that they coalesce but rather when they start to separate.
[57]: Surfactant is shown to control the transport of small organic molecules in water in fluorocarbon emulsions. Proteins in the dispersed phase are slowing down the transport because they act on the partitionning coefficient between both phases, not because they form a layer at the droplet interface.
[95]: The stabilisation of interfaces by nanoparticles solves two problems at once: transport in the continuous phase is significantly reduced and cells have a solid substrate where they can anchor.
[88]: Inter molecular association between solutes in one phase and surfactant in the other phase is a mechanism strongly affecting the partitioning between phases.

Of outstanding Interest**:
[107]: This paper shows how chemical patterns can lead to morphological patterns in emulsions through transport and ageing processes.
[105]: This paper shows how the control of a three phase system leads to switchable systems in a soft matter system, while maintaining the overall structure of the system.