Open Droplets: Programming chemical flow in microfluidics

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ABSTRACT

Droplet-based microfluidics is the key technology for the miniaturization and automation of assays in controlled microcompartments. It relies on the use of emulsion droplets as microreactors each encapsulating a biological system of interest (genes, cells, drugs,...) which can be analysed at a ultra-high throughput. However, emulsions are metastable dispersions in which molecular transport is a major mechanism driving the system towards its state of minimal energy. In practice, such a molecular transport leads to the breakdown of the compartmentalization principle. Determining the underlying mechanisms of molecular transport between droplets is therefore essential. It is however in general challenging due to the complexity of a typical emulsion system. Here we introduce the concept of 'minimal emulsions', which are controlled emulsions produced using microfluidic tools, simplifying an emulsion down to its minimal set of relevant parameters. We use these minimal emulsions to unravel the fundamentals of transport of small organic molecules in water-in-fluorinated-oil emulsions, a system of great interest for biotechnological applications. Our results are of practical relevance to guarantee a sustainable compartmentalization of compounds in droplet microreactors and to design new strategies for the dynamic control of droplet compositions. They also lead to new means to actively control the transport of molecules from the interior of the droplets to their exterior, which is a key elementary for the build up of a new generation of cell-like microcompartments, opening the door to chemical programming in soft matter systems [1].

Keywords: droplets, emulsions, chemical control

1 Introduction

An emulsion is the dispersion of one fluid into another, stabilised by surfactant molecules [2, 3]. Emulsions have a wide range of technological applications, including the use in food products, paints, cosmetics, chemical synthesis, and drug delivery [4]. In recent years, droplet-based microfluidics has been proposed as a means for the miniaturization and automatization of biochemical assays. The billions of microcompartments contained in an emulsion provide an environment ideal for the parallelisation of assays [5, 6, 7, 8, 9, 10]. This concept was shown to be very powerful for applications relying on high-throughput parallelized measurements such as drug screening [11, 12], biomarker analysis [13, 14, 15], cell screening [16, 17, 18] or protein engineering [19, 20].

We consider the kinetics of equilibration of concentration differences between droplets containing solutes which are poorly soluble in the continuous phase. We use fluorinated oils as the continous phase and aqueous droplets as the dispersed phase. This system is of particular interest for biochemical applications [21, 22]. Although the solubility of organic molecules in fluorinated oils is normally very low [23, 24], surfactant molecules mediate their solubility, as they do for organic systems [25], through their amphiphilic character [21]. Thus, there is a finite solubility of encapsulated compounds in the continuous phase, which can lead to cross-talk between droplets.

2 Results

We introduce the concept of minimal emulsions made of an assembly of fixed monodisperse droplets, with controlled center to center distances. The microenvironment of each droplet is precisely controlled, to a level unreachable in bulk emulsification. We use this system to study the chemical equilibration of organic molecules in emulsions. We use fluorophores as model molecules. We focus on three molecules, Fluorescein, Resorufin and Rhodamine 6G which are all exchanged but exhibit timescales of exchange well separated, from minutes for Rhodamine 6G to to days for Fluorescein. Resorufin is known to display exchange with a time-scale of hours and will be used as a model dye for convenience [32]. Two droplet populations of identical size are produced, where fluorophores are only present in one population (resorufin sodium salt, 100 μM). The surfactant is a perfluoropolyether - polyethylenoxide block copolymer (PFPE-PEG-PFPE) stabilising our emulsions against coalescence. The intensity of the emitted fluorescent light is proportional to the concentration of the fluorophore in the relevant concentration range between

0.1 to 100 μM. Hence, the concentration of resorufin sodium salt in individual droplets is determined by the fluorescence intensity. Droplets are stored in our arrays and fluorescence images are recorded every five minutes until the fluorophore concentrations between the two populations are equilibrated. The dynamics of transport of fluorophores from the initially 'filled' towards the initially 'empty' droplets are measured by analysing time sequences of fluorescence images (Fig. 1).

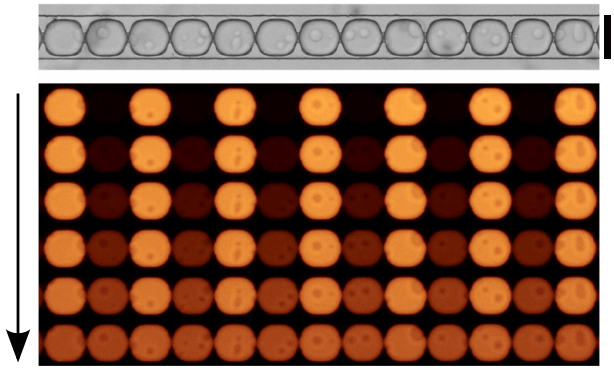

Figure 1: Equilibration of concentration of resorufin between ajacent droplets. The process is controlled by the diffusion through the oil phase. (scale bar 100 μm)

We repeated the experiments for various surfactant concentration and droplet spacing. Our experiments were consistent with a diffusion-limited model, showing that the kinetics of partitionning between the oil and the water is locally infinitely fast compared to the diffusive flux between the droplets [1]. From this result, we derived two methods to control chemicals in emulsion:

Emulsion-based targetted delivery – In this first method, the local control of partitionning between adjacent droplet is achieved with additivies in the aqueous phase (here salt) which provides means to fully displace our chemical from one droplet to the next (Fig. 2).

Partitioning control for extraction – In this second method, the extraction of dyes is achieved by using additives solubilized in the oil (Fig. 3). By adding carboxylic acids soluble in the oil, we control the extraction of the dye.

Flow induced delivery – In this third example, the same principle of the control of partitionning by additives can then be inverted to introduce a dye into droplet using a dye saturated surfactant-oil mixture (Fig. 4).

3 Discussions

Our experiments show that microfluidics provides tools to efficiently manipulate droplets to prepare, order and store them in a controlled manner; emulsions with a precisely defined microstructure are obtained for quantitative studies of physicochemical processes at the microscopic level. We show that the fluorophore transport in fluorinated emulsions, used as a model for organic molecule transport, is, in all our experiments, limited by the diffusive transport through the continuous phase. The dependence of the transport process on the droplet spacing is fully consistent with an analytical model based on the proper description of the permeability of the oil membrane separating the droplets. Increasing the spacing between droplets is an efficient strategy in reducing the exchange of material between

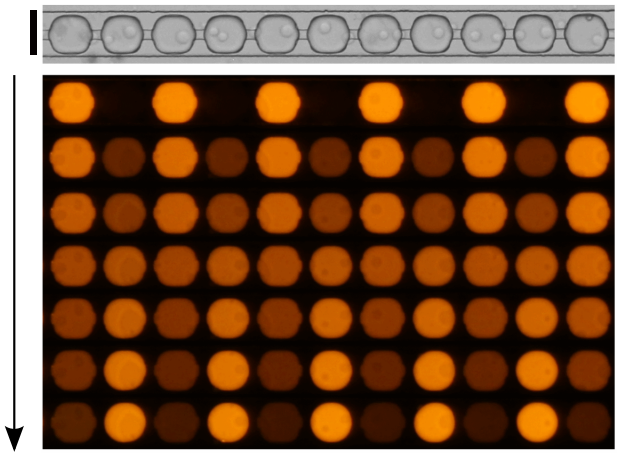

Figure 2: Targetted delivery from filled droplets towards empty droplets. Using additives (here salt) we act on the partitionning coefficient between droplets at the scale of the droplet. We induce a full transfert of the dye towards the neighbouring droplets. (scale bar 100 μm)

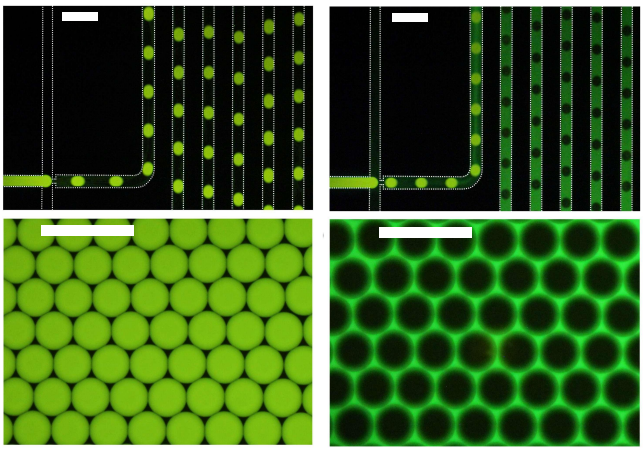

Figure 3: Extraction of organic molecules by fluorocarbon additives. We can tune the extraction process by adding carboxylic acid to the oil-surfactant mixture. Left: low carboxylic acid concentration, right: high concentration (scale bar 300 μm)

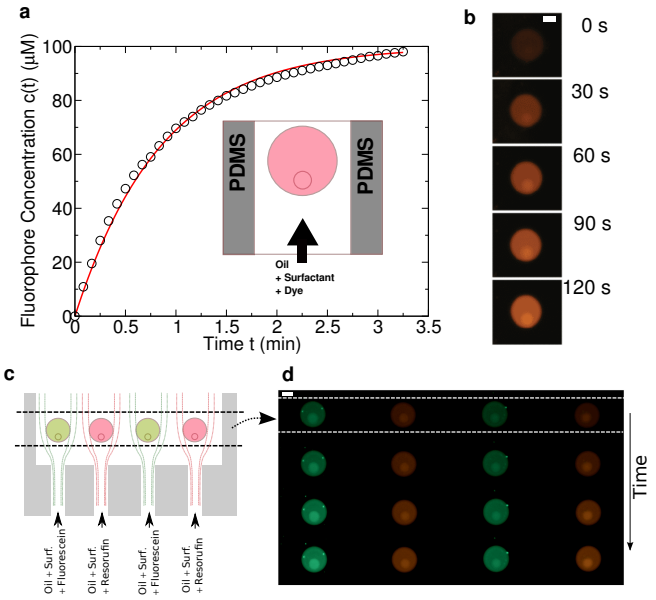

Figure 4: Targetted delivery of organic molecules into droplets. (a) Principle of the transfert and measurement of the concentration in the droplet over time. (b) micrographs of the droplet in fluorescence mode. (c) Principle of the parallelization and (d) micrograph of a parallelized uptake experiment (scale bar 40 μm)

droplets. In combination with a decrease of concentration of surfactant, we have shown a decrease in the rate of transport by a factor of about 30. In a bulk emulsion, the equivalent strategy would be to increase the continuous phase volume fraction which is technically challenging. The values of the diffusion coefficient of the fluorophores in the continuous phase obtained experimentaly show that the transport of fluorophores is mediated by large assemblies of surfactant molecules. Simple additives, such as sodium chloride, BSA [32] or sugars [30], not only affect the rate of transport but also the distribution of organic molecules among the droplets. We demonstrate how to use this concept for the targeted delivery of compounds, a potential new mechanism for actively feeding droplets from external sources. In practice, special care should be taken when changing buffer conditions in biochemical applications or using additives such as encoding fluorophores which might affect interactions and partitioning. In contrast, understanding and controlling this process is essential to deliver molecules from one droplet to the next and might provide new tools for the chemical control of the content of emulsion droplets. Besides straightforward applications in droplet-based microfluidic systems, we believe that our approach will be applicable to emulsion-based synthesis where transport of reagents between compartments is crucial. Our system might also provide additional insights to understand how organic molecules can be concentrated in a population of microcompartments, a question relevant for compartmentalisation through phase separation in cells [33], for prebiotic chemical systems [34, 35] and for the design of minimal functional micro-compartments [34, 36, 37].

4 Conclusions

We produce, order, and immobilize droplets in a controlled fashion [8, 31]. As a result, we access fundamental information on the rate-determining step of transport. We demonstrate that the transport processes follow a universal law based on Fickian diffusion, described using simple thermodynamic arguments. We further use our understanding of the process to effectively control chemical transport between microreactors. We demonstrate the simple control and programming of chemicals in emulsions for targeted delivery into droplets at rates compatible with the typical timescales of biochemical assays and could be used as a building block for Synthetic Biology applications.

REFERENCES

[1] P. Gruner, *et al.* Controlling molecular transport in minimal emulsions. *Nat Commun*, 7:10392, 2016.

[2] J Bibette, F Leal-Calderon, and P Poulin. Emulsions: basic principles. *Rep. Prog. Phys.*, 62:696–1033, 1999.

[3] D.J. McClements. Nanoemulsions versus microemulsions: terminology, differences, and similarities. *Soft Matter*, 8:1719–1729, 2012.

[4] M. J. Rosen. *Surfactants and Interfacial phenomena*, pages i–xiii. John Wiley & Sons, Inc., 2004.

[5] D. S. Tawfik and A. D. Griffiths. Man-made cell-like compartments for molecular evolution. *Nat. Biotechnol.*, 16(7):652–656, Jul 1998.

[6] V. Taly, B. T Kelly, and A. D Griffiths. Droplets as microreactors for high-throughput biology. *ChemBioChem*, 8(3):263–272, Jan 2007.

[7] SY Teh, R Lin, L.-H. Hung, and A. P Lee. Droplet microfluidics. *Lab Chip*, 8(2):198–220, Feb 2008.

[8] R. Seemann, M. Brinkmann, T. Pfohl, and S. Herminghaus. Droplet-based microfluidics. *Reports on Progress in Physics*, 75:016601, 2012.

[9] M T. Guo, A. Rotem, J. A. Heyman, and D. A. Weitz. Droplet microfluidics for high-throughput biological assays. *Lab Chip*, 12:2146–2155, 2012.

[10] A.B. Theberge, *et al.* Microdroplets in microfluidics: An evolving platform for discoveries in chemistry and biology. *Angew. Chem., Int. Ed.*, 49:5846–5868, Jun 2010.

[11] J.-C. Baret, *et al.* Quantitative cell-based reporter gene assays using droplet-based microfluidics. *Chem. Biol.*, 17(5):528–536, May 2010.

[12] O. J Miller, *et al.* High-resolution dose-response

screening using droplet-based microfluidics. *Proc Natl Acad Sci U S A*, 109(2):378–383, Jan 2012.

[13] D. Pekin, *et al.* Quantitative and sensitive detection of rare mutations using droplet-based microfluidics. *Lab Chip*, 11:2156 – 2166, May 2011.

[14] V. Taly, *et al.* Multiplex picodroplet digital pcr to detect kras mutations in circulating dna from the plasma of colorectal cancer patients. *Clin Chem*, 59(12):1722–1731, Dec 2013.

[15] A. Didelot, *et al.* Multiplex picoliter-droplet digital pcr for quantitative assessment of dna integrity in clinical samples. *Clin Chem*, 59(5):815–823, May 2013.

[16] J.-C. Baret,*et al.* Fluorescence-activated droplet sorting (fads): efficient microfluidic cell sorting based on enzymatic activity. *Lab Chip*, 9(13):1850–1858, Jul 2009.

[17] B. El Debs, *et al.* Functional single-cell hybridoma screening using droplet-based microfluidics. *Proc Natl Acad Sci U S A*, 109(29):11570–11575, Jul 2012.

[18] S. L Sjostrom, *et al.* High-throughput screening for industrial enzyme production hosts by droplet microfluidics. *Lab Chip*, 14(4):806–813, Feb 2014.

[19] J. J Agresti, *et al.* Ultrahigh-throughput screening in drop-based microfluidics for directed evolution. *Proc. Natl. Acad. Sci. U. S. A.*, 107(9):4004–4009, Mar 2010.

[20] B. L Wang, *et al.* Microfluidic high-throughput culturing of single cells for selection based on extracellular metabolite production or consumption. *Nat Biotechnol*, 32(5):473–478, May 2014.

[21] M. P. Krafft. Fluorocarbons and fluorinated amphiphiles in drug delivery and biomedical research. *Adv Drug Deliv Rev*, 47(2-3):209–228, Apr 2001.

[22] J.-C. Baret. Surfactants in droplet-based microfluidics. *Lab Chip*, 12(3):422–433, Feb 2012.

[23] R.L. Scott. The solubility of fluorocarbons. *J Am Chem Soc*, 70(12):4090–4093, Dec 1948.

[24] Simons JH and Linevsky MJ. The solubility of organic solids in fluorocarbon derivatives. *J. Am. Chem. Soc.*, 74:4750–4751, 1952.

[25] AS Kabalnov. Can micelles mediate a mass transfer between oil droplets ? *Langmuir*, 10:680–684, 1994.

[26] L. Mazutis, *et al.* Multi-step microfluidic droplet processing: kinetic analysis of an in vitro translated enzyme. *Lab Chip*, 9(20):2902–2908, Oct 2009.

[27] F. Courtois, *et al.* Controlling the retention of small molecules in emulsion microdroplets for use in cell-based assays. *Anal. Chem.*, 81(8):3008–3016, Apr 2009.

[28] Y. Bai, *et al.* A double droplet trap system for studying mass transport across a droplet-droplet interface. *Lab Chip*, 10(10):1281–1285, May 2010.

[29] N. Wu, *et al.* Management of the diffusion of 4-methylumbelliferone across phases in microdroplet-based systems for in vitro protein evolution. *Electrophoresis*, 31(18):3121–3128, Sep 2010.

[30] P. A Sandoz, A. J Chung, W. M Weaver, and D. Di Carlo. Sugar additives improve signal fidelity for implementing two-phase resorufin-based enzyme immunoassays. *Langmuir*, 30(23):6637–6643, Jun 2014.

[31] E. Fradet, *et al.* Combining rails and anchors with laser forcing for selective manipulation within 2d droplet arrays. *Lab Chip*, 11(24):4228–4234, Dec 2011.

[32] Y. Skhiri,*et al.* Dynamics of molecular transport by surfactants in emulsions. *Soft Matter*, 8:10618–10627, 2012.

[33] A. A Hyman and K. Simons. Cell biology. beyond oil and water–phase transitions in cells. *Science*, 337(6098):1047–1049, Aug 2012.

[34] A. I. Oparin. The origin of life and the origin of enzymes. *Adv Enzymol Relat Areas Mol Biol*, 27:347–380, 1965.

[35] A. Fallah-Araghi,*et al.* Enhanced chemical synthesis at soft interfaces: A universal reaction-adsorption mechanism in microcompartments. *Physical Review Letters*, 112(2):028301, JAN 13 2014.

[36] E. Sokolova,*et al.* Enhanced transcription rates in membrane-free protocells formed by coacervation of cell lysate. *Proc Natl Acad Sci U S A*, 110(29):11692–11697, Jul 2013.

[37] E. Spruijt, E. Sokolova, and W. T S Huck. Complexity of molecular crowding in cell-free enzymatic reaction networks. *Nat Nanotechnol*, 9(6):406–407, Jun 2014.

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