Droplet formation and shrinking in aqueous two-phase systems using a membrane emulsification method

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Using a membrane emulsification method based on porous hollow-fiber membranes in combination with an aqueous two-phase system (ATPS), we are able to produce “water-in-water” droplets with narrow-dispersed size distributions. The equilibrium phases of the aqueous two-phase system polyethylene glycol-dipotassium hydrogen phosphate are used for this purpose. The droplet diameter of a given fluid system is determined by the flow rates of the continuous and disperse phase as well as the hollow fiber dimensions. When diluting the disperse phase and thus moving the ATPS system out of equilibrium, the droplet size can be further reduced in comparison to the equilibrium case. Generally, droplets formed with this method have diameters 20%–60% larger than the inner hollow fiber diameter. The new strategy of diluting the disperse phase allows the production of droplet diameter below the inner diameter of the membrane. © 2015 AIP Publishing LLC.

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I. INTRODUCTION

Aqueous two-phases systems (ATPS) or aqueous biphasic systems are well-established multi-phase systems studied and applied in biotechnology since the 1950s for the purification and separation of proteins, enzymes, and other biomolecules. In contrast to the conventional organic-water extraction, these systems have the advantage of forming of two immiscible water-based phases thus avoiding potential denaturing effects of the organic phase on biomolecules. The phase separation of water can be achieved by mixing two aqueous polymer solutions with particular properties or by mixing one aqueous polymer solution with certain salts (e.g., phosphates and sulfates) or surfactants.^{1,2}

ATPS have been utilized in the last few years in combination with microfluidic processes for the continuous extraction purposes. This unit operation can be accomplished as two parallel laminar streams or as segmented flow in the form of droplets.³ For instance, Munchow *et al.* utilized electrophoresis in a microchannel to extract proteins in a parallel flow of a poly(ethylene glycol)-dextrane ATPS.⁴

By segmenting the flow into droplets, both the mass transfer and specific interfacial area can be increased to improve the extraction process efficiency.⁵ Unfortunately, stable droplet generation is significantly more difficult in ATPS than in oil-water systems due to the low interfacial tension between the two aqueous phases.^{6,7} Typical interfacial tensions of ATPS are in the range of 10^{-6} – 10^{-3} N/m depending on phase composition, which is usually several orders of magnitude smaller than organic-water interfaces.^{8,9} The interface of an annular or parallel flow of ATPS systems is quite stable and remains undisturbed by Rayleigh-Plateau instabilities. The latter usually lead to droplet formation in regular oil-water systems, but are not as effective in ATPS.

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A few methods relying on external forces to produce droplets out of aqueous two-phase systems have been published over the last few years. These publications have shown that downstream processing of ATPS droplets is feasible, e.g., for microgel synthesis or selective extraction. Choi *et al.* proposed the use of an electric field on a T-junction to create droplets of an ammonium sulfate rich phase which were then used to extract ruthenium red from a tetrabutylammonium bromide rich phase.¹⁰ Ziemecka *et al.* implemented a piezoelectric actuator in a microfluidic flow focusing device to impose an oscillation on the interface which forces droplet formation out of an otherwise stable liquid jet. Droplets of a modified dextrane solution were then generated in a PEG outer phase and cross-linked to form microgels.^{11,12} The production of hydrogel particles¹³ is also a promising application of ATPS, for instance, for drug delivery applications¹⁴ or cell immobilization^{15,16} without the need of organic solvents that are commonly used as the outer phase. A similar approach was published by Shum *et al.* who induced the required fluctuations by mechanically shaking the tubing of a disperse phase with the help of an orbital shaker. A glass-capillary device was employed for the jet formation in coflowing streams. In addition, resulting droplets of a poly(ethylene glycol)-diacrylate solution were photo-crosslinked to form hydrogel particles.¹⁷ A pulsation of the system inlets was also employed by Moon *et al.* for the ATPS droplet generation in a flow-focusing device. In this case, the pressure fluctuations on the disperse phase were generated with a solenoid valve connected to a pressure regulator as used also for stop-flow lithography.¹⁸

Filtration membranes have been extensively used to produce emulsions.¹⁹ In a particularly precise membrane-based process, a hollow fiber membrane is used to create an accelerated coflowing stream that, in turn, leads to thread break-up and the production of droplets with very narrow size distributions even below 1% standard deviation.^{20,21} This method can also be used in a microfluidic channel configuration and allows potential downsizing into the micrometer scale.²² As opposed to today's microfluidic processes, this ATPS droplet formation process does not rely on an external trigger as will be presented below.

Motivated by the advantages of "water-in-water" droplets and their significance for future green chemistry processes, we report in this paper the use of the hollow-fiber emulsification method for the droplet generation of a potassium phosphate/poly(ethylene glycol) aqueous two-phase system.

One of the potential limitations of the hollow-fiber emulsification process is the fact that the emerging droplets have dimensions dominated by the channel size resulting in diameters up to twice the diameter of the channel. So far, we have not succeeded in reaching droplet dimensions below this limit. In the context of ATPS, shifting the system equilibrium by varying the concentration of one or both phases may lead to a phase volume change. This has been applied by the Takayama group for the visualization of enzymatic activity of dextranase in droplets of a dextrane solution formed in a PEG solution.²³ Here, the dextranase hydrolyzes the dextrane chains and as a consequence the two-phase system shifts beyond the binodal converting it to a single phase system. Droplets were produced both by micropipetting and with an actuated flow focusing device. Also on this topic, this group has presented works on the rehydration of dextran spots dried on the floor of a microwell with a poly(ethylene glycol) solution²⁴ and on the dehydration of a single droplet via an oil phase for the optical determination of phase diagrams.²⁵ These studies are practical proof that out-of-equilibrium ATPS allow size variation of phases.

Here, we demonstrate that the use of two ATPS phases that are out of equilibrium by diluting the disperse phase allows a significant droplet size reduction in hollow-fiber emulsification. Once the out-of-equilibrium phases are in contact, both phases move back to the binodal on a new tie-line and as a consequence the droplets shrink, thus achieving smaller droplet sizes without any modification of the device geometry.

II. MATERIALS AND METHODS

A. Preparation of aqueous two-phase system PEG-K₂HPO₄

The aqueous two-phase system used for the droplet formation and the subsequent droplet shrinking experiments is prepared from anhydrous dipotassium hydrogen phosphate K₂HPO₄

(Applichem GmbH) and polyethylene glycol (PEG) with a molecular weight of 4000 g/mol (Merck KGaA). Both components are dissolved in deionized water and let equilibrate over night. The two phases are then centrifuged at 5000 rpm for 5 min to ensure total separation.

In order to define the composition of both phases, the phosphate concentration in each phase is determined by conductivity measurements of a ten-fold diluted sample using a conductometer. The PEG concentration is then obtained from balancing the total dry weight of a freeze-dried sample with the previously calculated amount of phosphate.²⁶ The region around the critical point is estimated by cloud point approximation and interpolation from the top and bottom phases.²⁷

For the droplet size reduction experiments, the bottom phase (phosphate rich) is diluted with deionized water. The obtained solutions are presented in weight percentage of the original bottom phase to the final diluted phase. In other words, a 76% solution is composed of 76% original bottom phase and 24% added water.

B. Experimental setup for droplet formation

The principle of the droplet formation in the hollow fiber membrane is presented in Figure 1 for two ATPS phases in equilibrium (A) and for the non-equilibrium case where the disperse phase is diluted (B).

An important requirement for a stable droplet formation in the membrane channel is a poor wettability of the to-be-dispersed phase towards the membrane material. Since both phases are largely made of water, their wetting properties are similar, thus the selection of the material is not trivial as in oil-water systems.^{20,21} A membrane with a high wetting affinity towards a polar (aqueous) phase such as polyethersulfone (PES)/polyvinylpyrrolidone (PVP) membranes would render the droplet formation impossible since it is susceptible to wetting by both phases. In contrast, a membrane material with poor water wettability can minimize the risk of wetting by the droplet phase. A polypropylene microfiltration hollow fiber membrane (Accurel® PP Q3/2 from Membrana GmbH, nominal pore size $0.2\ \mu\text{m}$) with a nominal inner diameter of $d_i = 600\ \mu\text{m}$ satisfies these wetting properties and for this reason is used in all the experiment described here. In addition, the risk of wetting is minimized by the pressure gradient applied through the membrane wall from the outside. The permeating continuous phase forms an annular flow of the outer phase around the disperse-phase thread.

FIG. 1. Droplet formation inside a hollow-fiber membrane by applying the top phase (PEG-rich) through the membrane wall and the bottom phase (phosphate-rich) through one end of the lumen side for both phases in equilibrium (A) and out of equilibrium by diluting the bottom phase (B).

The hollow fiber membrane is potted at both ends to two metal tubes and placed in a transparent acrylic housing by fixing both metal tubes with bored-through fittings. This leaves an effective membrane length of 10.9 cm in wetted state (10.5 cm in dry state). One end is connected to a syringe pump (Harvard Apparatus, PHD Ultra) with the disperse phase, while the other end is cut in an approximate 60° angle to improve droplet release.²¹ This tip is then inserted in a glass tube with an inner diameter of 1.6 mm where the droplet train can be observed and captured with a CCD camera (Allied Vision Technologies, Stingray F046C IRF) at an effective resolution of $3.3 \mu\text{m}/\text{px}$. The end of the glass tube is submerged in a beaker with the bottom phase filling it just above the opening. The shell side is also connected to another syringe pump responsible of feeding the continuous phase. A retention of the PEG 4000 or K_2HPO_4 by the porous membrane is not expected due to its low molecular weights. Further details on the experimental setup can be found in a previous publication.²⁰

C. Experimental procedure

Following the considerations described above, the more hydrophobic²⁸ and lighter PEG-rich top phase is used as continuous phase (cf. Figure 1) The module is placed with the outlet pointing downwards due to the higher density of the phosphate-rich bottom phase ($\rho_B = 1151.4 \text{ g/l}$ vs. $\rho_T = 1078.9 \text{ g/l}$, both measured in a 5 ml pycnometer).

Since the dry membrane can hardly be wetted with an aqueous phase, the membrane needs to be first flushed with a water-miscible organic phase, which then can be replaced by the aqueous phase. The module is flushed with ethanol (Merck KGaA) until all air bubbles have been flushed out from both the lumen (inside) and the shell (outside) sides of the hollow fiber membrane. Before filling the module with the PEG-rich phase, both lumen and shell are flushed over 30 min with deionized water to eliminate any traces of ethanol.

At running PEG-rich continuous phase, the phosphate-rich phase is fed as disperse phase at a given flow rate. Three volume flow rates of the continuous phase $\dot{V}_c$ are tested for the experiments in equilibrium (8 ml/h, 16 ml/h, and 24 ml/h). For each one, the disperse phase flow rate $\dot{V}_d$ is varied randomly from 0.2 ml/h up to 2.4 ml/h with a step size of 0.2 ml/h. The shrinking experiments are carried out for three diluted phases (76%, 84%, and 92%) and varying continuous phase flow rates (8 ml/h and 16 ml/h). The disperse phase flow rate is also varied within the interval mentioned above.

Since the membrane is not transparent, the droplets can only be measured once they leave the membrane tip. The droplet train is recorded directly under the membrane tip, whereas 40 to 160 droplets are captured for each set of parameters. The resulting droplet images are evaluated using the Image Processing Toolbox of MATLAB[®] which calculates the mean diameters of an ideal spherical droplet and their respective standard deviations.

III. RESULTS AND DISCUSSION

A. Phase diagram

The resulting phase diagram for the PEG4000- K_2HPO_4 system is presented below in Figure 2. The phosphate-rich phases show very low concentrations of PEG, which complies with previous published data of potassium phosphate-PEG aqueous two-phase systems.²⁷ As described above, the top phase marked as T_0 is used as the continuous phase permeating through the membrane wall and the bottom phase B_0 (or the diluted version of it B'_0 , cf. Figure 4) as droplet phase.

B. Influence of flow rates on droplet formation

Figure 3 shows the resulting mean droplet diameter for different volume flow rates of the disperse phase and continuous phase (experimental image can be found in the supplementary material).³² Missing data points at low flow rates of the disperse phase in the 16 ml/h and 24 ml/h data series respond to unstable and unreproducible droplet formation. The upper limit at high flow rates was either defined by unreproducible droplet formation or instant coalescence

FIG. 2. Phase diagram for PEG4000 and K₂HPO₄. Square ($\square$) points are determined via dry weight and conductivity measurements of the equilibrium phases. The triangle points ($\triangle$) represent measurements by cloud point. Point A depicts the initial global concentration before separation. Points T₀ and B₀ depict the PEG-rich top phase and phosphate-rich bottom phase, respectively, at both ends of the tie-line. These phases are used for the droplet formation experiments.

at the tip of the membrane due to the significant deceleration of the droplet train when leaving the membrane. This renders image capturing of isolated droplets impossible. In this case, it cannot be resorted to surfactants to avoid coalescence since these barely adsorb at the interface.

In the parameter set investigated here, droplets are always bigger than the inner diameter of the membrane. This fact and the trends of the presented curves are in well agreement with previous studies on oil-water systems where a droplet size reduction has been observed by increasing the flow rate of the continuous phase.²¹ Moreover, the effect of flow rate of the disperse phase is also consistent with previous observations, in particular, the presence of a droplet size maximum as can be seen from the 8 ml/h and 16 ml/h series. Even though the trend of a decreasing droplet diameter with increasing continuous phase flow rate appears to contradict the theory on droplet formation in coflowing streams,^{29,30} it can be well explained with the observations of these studies. It is important to bear in mind that the continuous phase inside the hollow fiber is being constantly accelerated over the entire membrane length due to permeation. By increasing the disperse phase flow rate and thus the inertial forces of the disperse phase, the break up point moves further downstream. This means that the droplet generation will occur at a point in the hollow fiber where the local flow velocity of the surrounding phase is higher and by that it will generate smaller droplets.²⁰

The range shown here where stable droplet formation occurs is located at lower flow rates of both phases when compared to oil-water systems. This responds to different fluid properties such as viscosity, but in particular, to the significantly lower interfacial tension of ATPS systems. If we account for spontaneous droplet formation at certain capillary numbers ($Ca = \frac{v\eta}{\gamma}$), a lower interfacial tension γ will require lower velocities v at constant viscosity η for droplet formation as has been previously discussed in literature.^{12,31} A dimensional analysis is here only of limited benefit since the local hydrodynamic conditions at the break-up point are unknown. A further effect that is also present in this case is a sudden droplet size increase at high flow rates of the disperse phase (e.g., $\dot{V}_c = 24 \text{ ml/h} / \dot{V}_d = 2.4 \text{ ml/h}$). While this range of increasing droplet size showed relatively high size distribution in the oil-water case (cf. Breisig *et al.*²⁰); in this case, the size distribution remains low with a coefficient of variation (C.V.) of 1.95%.

FIG. 3. Influence of different flow rates of the disperse and continuous phases on the mean droplet size. Data point in gray (*) represents the mean droplet size of a bimodal distribution. Error bars depict the standard deviation of the droplet size distribution.

The droplet size distribution of all operating points is calculated from a population of successive droplets as a means to follow any possible patterns in the droplet consecution. Most points present rather random distributions if the end of the observation glass tube is submerged in a beaker with the continuous phase. If the tip is not submerged (free-standing), a distinctive periodic behavior is appreciable. This periodicity responds to the accumulation of both phases at the capillary opening that form a large drop that after a certain time detaches and falls (see supplementary material).³² Such weak pressure fluctuations show a significant impact on the droplet formation taking place several centimeters above this point. In this sense, any pressure fluctuations should be avoided to ensure a narrow size distribution when applying this method. Another interesting pattern arises at the highest flow rate of the $\dot{V}_c = 16$ ml/h series (in Figure 3, data point in grey). Here a bimodal distribution is observed, shown by a zigzag pattern in the droplet consecution (see supplementary material).³² Considering that both droplet sizes are comparable in terms of magnitude with other data points, it is clear that these droplet pairs are not produced from a single larger droplet, but from periodic variations in the droplet formation conditions. A periodic pressure variation may modify the velocity profile of the continuous phase and thus the local velocity at the break-up point.

In terms of the general size distribution of the parameter range presented in Figure 3, this method allows extremely narrow-dispersed droplet sizes (marked with the error bars). Most data points present coefficients of variation in the order of 1%–3%, reaching down to 0.75% in some cases.

C. Influence of dilution of the disperse phase on the droplet size

Figure 4 presents a schematic phase diagram describing the process occurring when a diluted bottom-phase droplet is contacted with the equilibrium top phase. The phase diagram in Figure 2 is not used here for practical reasons.

The initial top phase T_0 at the upper end of the tie-line (in equilibrium) is contacted with a diluted version B'_0 of the equilibrium bottom phase B_0 . The ratio between these two phases is set by the applied mass flow rates, which add up to a hypothetical global concentration M . The

FIG. 5. Mean droplet size for different dilutions and flow rates of the disperse phase at a flow rate of the continuous phase of $8 \frac{\text{mL}}{\text{h}}$.

direction of change of the interfacial tension is not trivial, we have measured it for the equilibrium system and the most diluted bottom phase (76%) using the pendant drop method (DSA100, Krüss GmbH). Resulting interfacial tensions $0.51 \times 10^{-3} \text{ N/m}$ (undiluted) and $0.22 \times 10^{-3} \text{ N/m}$ (diluted) are in good agreement with previous studies⁸ and demonstrate that in this particular case, a decrease is expected. While a reduction of the viscosity should yield larger droplets, smaller interfacial tensions decrease droplet size.^{20,21} This could also be a

FIG. 6. Mean droplet size for different dilutions and flow rates of the disperse phase at a flow rate of the continuous phase of $16 \frac{\text{mL}}{\text{h}}$.

possible source of size reduction. In this sense, size reduction cannot be exclusively traced back to water transport between phases since a change in fluid properties might also influence the outcome.

IV. CONCLUSION

In this paper, we present an alternative method for producing aqueous two-phase systems (ATPS) droplets with very narrow disperse size distributions in a range below 1% deviation. Top and bottom equilibrium phases of a phosphate/PEG ATPS system have been used to produce droplets with the method of hollow fiber emulsification. The influence of flow rates of the disperse and continuous phase on droplet diameter was studied for this system and proved to behave similar to conventional oil-water systems. In addition, a strategy for obtaining smaller droplets even below the channel diameter has been presented as a proof of principle.

This method for ATPS droplet production can serve as a useful tool for microgel production, encapsulation of cells or bioextraction. Thanks to the simple device design and availability of hollow-fiber membranes, this method can be easily scaled up by parallelization of several membranes in one single device. By taking advantage of out-of-equilibrium ATPS, the droplet diameter can be decoupled from the diameter of the hollow-fiber membrane and sizes below membrane diameter are attainable. This droplet reduction approach can be extrapolated also to oil-water systems where the selective extraction of a component from one phase to the other can result in a significant volume shift and thus, in smaller droplets sizes.

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³²See supplementary material at <http://dx.doi.org/10.1063/1.4929519> for an image of the produced droplet leaving the membrane and plots of the measured droplet diameter in a droplet consecution.