SUPERCRITICAL PARTICLE TECHNOLOGY IN MICROFLUIDIC DEVICES

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This article reports the fast potential of the transfer of supercritical fluid processes for particle generation from steel vessels to transparent microfluidic devices for the purpose of analyzing and understanding the functioning chain of the involved mechanisms. Preliminary investigations with respect to supercritical fluid extraction from emulsions SFEE were carried out in glass capillaries as well as in microfluidic glass chips.

INTRODUCTION

The use of conventional particle technology e.g. milling, sol-gel-, deposition- or evaporation-techniques suffer from many disadvantages concerning high mechanical stress, high temperatures and a great amount of organic solvents especially when it comes to extraction techniques. In the production of pharmaceuticals very strong restrictions exist in terms of solvent residuals. Additionally the temperature instability of many pharmaceuticals as well as the control of the specific size and morphology for optimal admittance and bioavailability has to be considered [1]. Most of the techniques are not applicable when it comes to composites or encapsulates where an active material has to be incorporated in a polymer matrix.

Supercritical fluids enable a field of generating micro- and nanoparticles with low stresses and at moderate temperatures. The most beneficial feature of supercritical fluids is their tunable properties with only small changes in pressure and/or temperature and with this a direct influence on their solvent/antisolvent power and phase behavior. These tunable properties with process parameters allow a direct influence on the overall resulting particle size, morphology and particle size distribution (PSD) and gives rise to many different processes and applications in the supercritical particle technology in the past two decades [2-4].

The production of composites and encapsulates of an active component in a polymer matrix was realized and investigated using many different applications where supercritical CO_2 was used as solvent, antisolvent or solute [5]. A very promising approach in this field of research is the extraction of an organic solvent from an o/w-emulsion with supercritical CO_2. The combination of an emulsion technique with supercritical CO_2 was firstly presented by Perrut et al. [6] and extended to the supercritical fluid extraction of emulsions (SFEE) process, patented by Chattopadhyay et al [7]. During the SFEE process an o/w-emulsion is formed, where an organic solvent solution with dissolved solute and polymer represents the dispersed phase and water the continuous phase. This emulsion is then brought in contact with supercritical CO_2 which is very good miscible with most organic solvents. During the process the CO_2 acts on the one hand as an antisolvent thus saturating the organic phase and as an extraction agent, extracting the organic solvent out of the emulsion into the CO_2 phase. Both

effects cause the generation of micro- and nanoparticles that are finally dispersed in the water phase. In the past years composite particles [8-14] and nanoparticles [15, 16] were successfully produced. A very recent publication shows the behavior of single solvent droplets in water in contact with supercritical CO_2 [17]. Besides the usual characterization parameters namely particle size, PSD and morphology for co-formulations also the drug-load, the drug-release, the drug incorporation quality and the properties of the polymer itself have to be considered. According to these authors in addition to the pressure, the temperature and the composition of the components other important influence parameters on the properties of the resulting particles are the droplet size and the distribution of the emulsion droplet (DSD). Even though the process was applied successfully to different systems, a lot of open questions remain concerning the behavior of emulsion droplets during extraction and during precipitation to better understand the mechanism of single process steps.

The present work shows an approach to apply the supercritical extraction technique to microfluidic-glass-devices, to visualize single droplets during the whole SFEE process under a microscope. This includes the generation of the emulsion, the contact of the o/w-emulsion with supercritical CO_2, the extraction of the solvent and in future studies also the particle formation process. This information can be gathered at single droplets during the process, where the local position in the microfluidic-system represents a specific residence time in the conventional process. Additionally it has to be pointed out that the use of different kinds of microfluidic nozzles and nozzle geometries enable the production of single- [18-20] or double [21-23] emulsions with almost uniform droplet sizes. The adjustment of different emulsion droplet sizes is done by changing the flow rates of the dispersed and the continuous phase, so that the whole SFEE process can be carried out on one pressure level in a continuous way. Only few applications concerning supercritical fluids in microfluidic devices can be found in literature [24, 25] a review article is given by Verboom [26].

This work focuses on the construction and description of the setup and the investigation of the phase behavior of a common system in the SFEE process containing ethyl acetate, water and CO_2. Ethyl acetate dissolves most biopolymers like PLGA and many data can be found in the literature to allow comparisons with gathered results.

MATERIALS AND METHODS

Materials:

In this work, ethyl acetate (CHROMASOLV® Plus, for HPLC 99.9%, purchased from Sigma Aldrich) was used as the organic solvent to produce the dispersed phase, deionized water was used to form the continuous phase, poly (lactic-co-glycolic acid) (PLGA lactide : glycolide (75:25), molar wt. 66.000 – 107.000, purchased from Sigma Aldrich) was used as solute and CO_2 (99.5%, purchased from Linde) as antisolvent.

Methods:

Figure 1 shows a schematic representation of the experimental setup that was used for the first experiments on the extraction of organic solvent from emulsions by supercritical CO_2. The microfluidic systems, being the central part of the setup is not shown in this figure but will be discussed in detail afterwards. Three important influence parameters on the particle properties were stated previously, namely the pressure, the temperature and the composition in the

system during the process. In addition the flow rates of the organic solvent and water define the emulsion droplet size. So it is of great importance to maintain these parameters constant. The system pressure is adjusted with three independent working high-pressure syringe pumps with nearly zero pressure pulsations. To keep the system pressure on a constant level, an automated back pressure regulator is used.
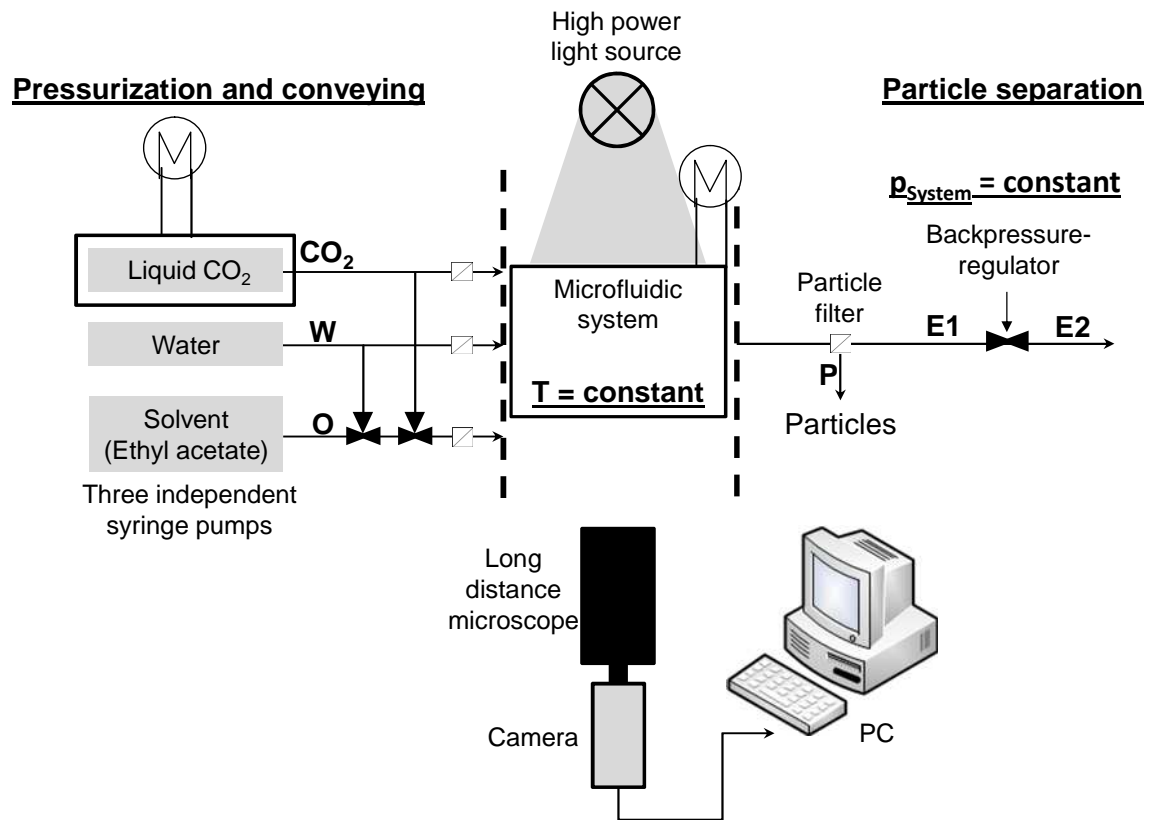

Figure 1: Experimental and optical setup for the detection of the flow behavior in the microfluidic systems

The microfluidic devices are placed in a small housing with glass windows to allow optical access. This housing is constantly purged with temperature controlled air to keep the temperature of the whole microfluidic system constant. By controlling the volumetric flow rate of the pumps the overall composition is set up for the three component system. For water and solvent the density does not change essentially with the pressure as they are liquid. To get reproducible compositions the CO_2 has to be cooled keeping it in a liquid state during pumping. This is achieved by a thermostat running constant at 0°C. Three inline sinter filters with a pore diameter of 0.5 µm are placed between each pump and the microfluidic system to avoid clogging of the small channels or capillaries. The two plug valves are used to purge the solvent line either with CO_2 or with pure water. On the downstream side of the microfluidic device a particle filter is installed to separate particles from the solvent/antisolvent/water mixture (not used in these experiments).

The optical investigation of the process is done qualitatively by imaging the phase boundaries between the organic-, the water- and the CO_2-phase and their change during the process. For visualization, a very intense white-light source is used to illuminate the microfluidic system. On the back side of the system the shadows of all appearing phase boundaries are imaged with a camera through a long distance microscope. The acquisition of the single images at fixed positions of the system were done with an interline transfer CCD camera that facilitates

an illumination time of the camera-chip down to 500 ns and allows a freezing of the very fast moving droplets on a picture. The spatial resolution of the long distance microscope images approximately 500 nm x 500 nm on one pixel of the camera what will be suitable to visualize at least the beginning of the precipitation process.

A sketch of the single steps that take place during the supercritical extraction of emulsion in a microfluidic system is presented in Figure 2. First of all it is to mention that microfluidics are used and gained great popularity because of their small channel geometry and due to that, very defined flow conditions. Single droplets can be seen as small individual reactors where the progress of a process in time is transferred to a certain location in space downstream in the capillary. At camera position 1 the organic solvent mixes with water provided from the opposing direction. This so called focusing flow configuration forms o/w-emulsions in the nozzle and further downstream. The emulsion stabilizes until position 2. This is labeled with emulsion formation-section in Figure 2. The stabile o/w-emulsion gets in contact with CO_2 at position 3. The second nozzle accelerates the emulsion causing a separation in single water droplets containing organic droplets in the CO_2 as the new continuous phase. Between position 4 and 5 the extraction and saturation process takes place and is visualized at different positions to gather information about the state of the extraction- and the particle precipitation process. The diameter of the extraction line defines the flow speed of the mixture. The length of the extraction line is than a function of the amount of CO_2 and the contact time needed to extract the solvent from the droplets into the CO_2 phase.
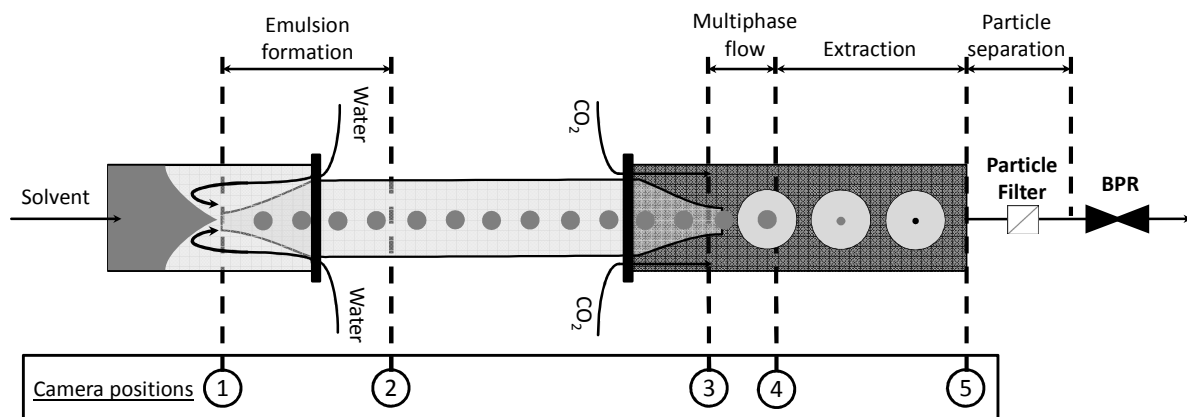

Figure 2: Representation of the different process steps in the SFEE process in a microfluidic device

Two different types of microfluidic devices were used in this study having some pros and cons concerning high pressure applications. On the one hand microfluidic glass chips were used with rectangular shaped channels. In this system a great benefit is the robustness of the system where all needed crossings are located on one single microfluidic device. A challenge is the sealing of the steel capillaries connecting the pump and the microfluidic-chip by a rubber PEEK connector. This connection can cause leakages at elevated pressures and there is a high risk in damaging the glass ports by tightening the sealing for high pressures. On the other hand glass capillaries can be used and connected with T-crossings to concentric tube systems. A possible system is shown in Figure 2. The both outer tubes have a bigger inner diameter than the connection tube in between. The small capillary has a squared outer diameter to allow a central positioning in the two outer capillaries. The nozzles are formed by capillary pulling where the glass is heated up until melting temperature and pulled at the same time. Different capillary nozzles are shown in Figure 3.
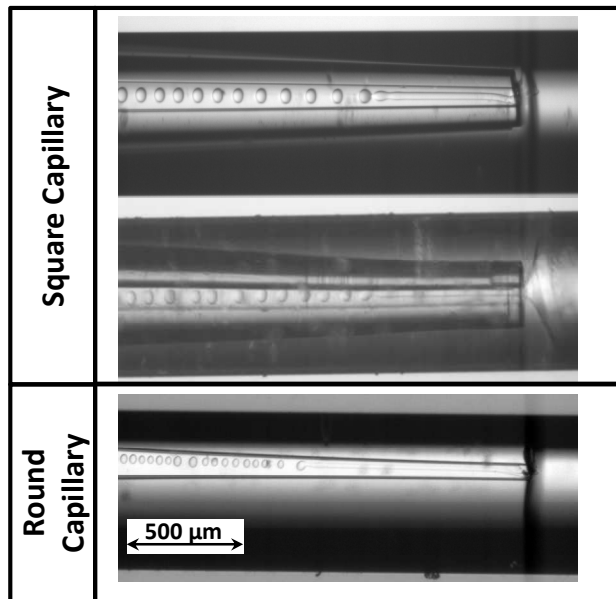
Figure 3: Capillaries with different nozzle diameters, manufactured by capillary pulling

The great advantage of the capillary systems is the complete freedom in diameters, lengths and nozzle geometries. Also the application of different hydrophobic or CO_2-philic coatings at the inner capillary walls is easier to apply. Problems occur with the fragility of complex systems and the connection of the glass tubes to normal 1/16" steel ports that are carried out with tubing sleeves sealed with epoxy. Two pictures of the different experimental setups are shown in Figure 4.
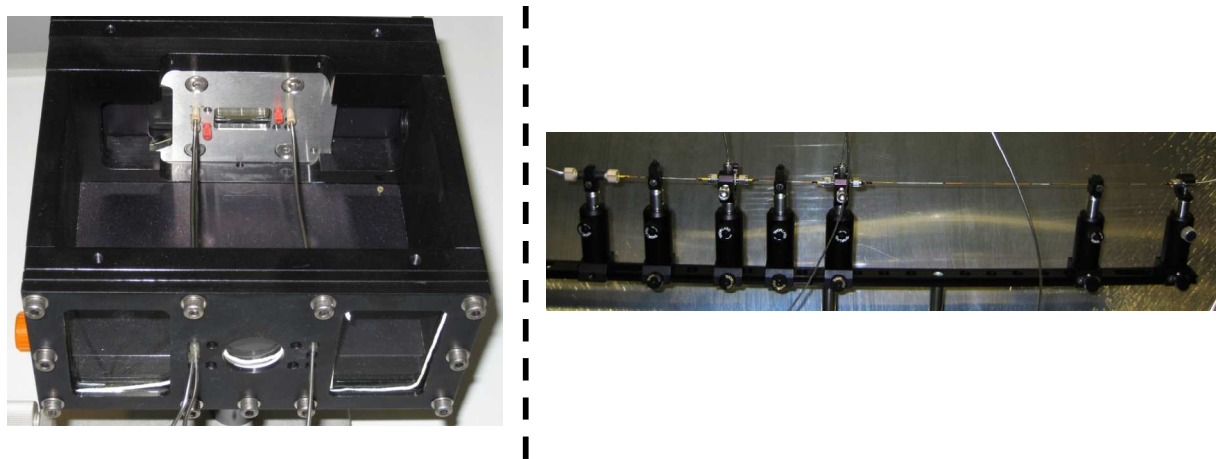

Figure 4: Pictures of the two different microfluidic experimental setups, on the left side the microfluidic-chip setup and on the right side the glass capillary system

The experimental procedure always follows the same steps. After filling the pumps, the water and the ethyl acetate pumps are started and the BPR is closed to elevate the system pressure to the desired value and maintains it when it's reached. When the system pressure is reached the CO_2-pump is started. Steady state conditions are reached after approximately two hours. Changing the flow conditions of a running system at constant pressure only needs 5-10 minutes time for steady conditions again. In this work only some first results are shown that describe the extraction process without the use of PLGA as solute. The results are depicted below.

RESULTS

As a first step the two systems namely the formation of o/w-emulsions and the behavior of water in supercritical CO_2 were investigated separately. For these experiments microfluidic glass chips were used with a single focusing flow configuration on one chip. The channels had a rectangular shape with a channel width of 100 µm and a channel height of 20 µm. All the experiments were run at a system pressure of 8 MPa and a temperature of 40°C. On the left side in Figure 5 two main factors influencing the size and the number of droplets formed per time can be seen, namely the total flow rate and the ratio between water and the organic phase. This is in good agreement with the literature [27]. No surfactant was used to stabilize the droplet. Far downstream no coalescence of the droplets was observed only if the distance between the droplets was more than several droplet diameters, like shown on the first image on the left side. The number of droplets ranged from 500 – 4000 droplets per second at total flow rates of 30 – 70 µl min^{-1}, resulting in flow velocities of 0.25 – 0.6 m s^{-1}.
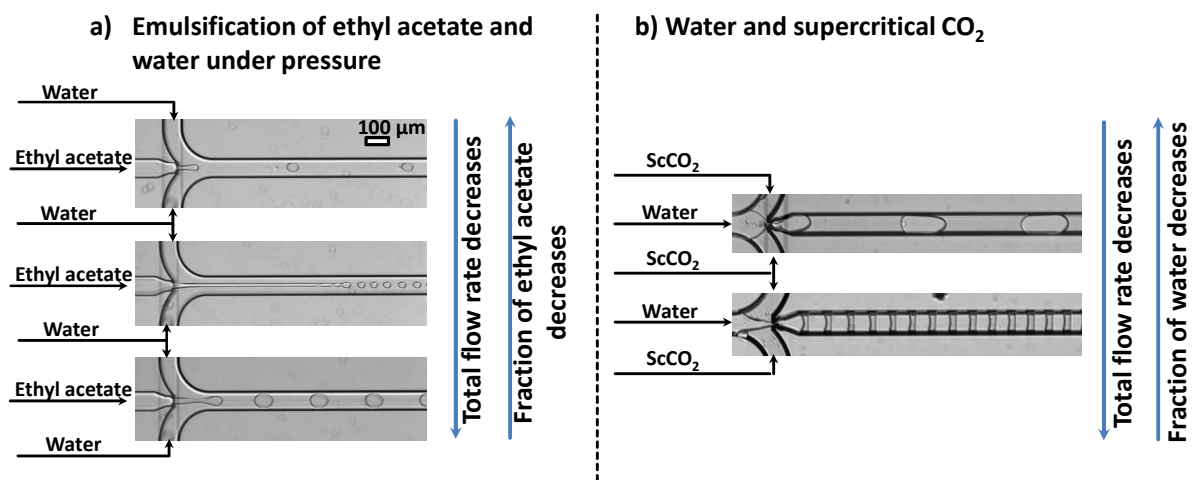

Figure 5: Flow pattern in microfluidic glass chips, a) ethyl acetate in water emulsions produced at different flow rates, b) water in supercritical CO_2 at different flow rates in hydrophobic coated channels, all experiments run at 8 MPa and 40°C.

Many experiments were carried out using supercritical CO_2 as the disperse phase and water as the continuous phase with uncoated channel walls. The results are not shown here but were in very good agreement with the investigations of Marre et al. [25]. On the right side of Figure 5 the behavior of water in supercritical CO_2 is shown in perfluoro surface coated channels (according to the distributor). The formation of water droplets could be seen on the first image but the stabilization of water droplets in supercritical CO_2 could not be achieved until now and is a field of current investigations. Some trends are given in Figure 5 describing how the water droplets behave with different flow rates directly after the nozzle but some millimeter downstream the water always touches the wall and forms water compartments as shown on the second picture on the right side.

The next step was to combine the two systems and to visualize the whole process from the emulsification of ethyl acetate in water, the contact of the emulsion with CO_2 and the extraction of organic solvent from the water phase. A series of images is shown in Figure 6 that shows all important steps of the process at an exemplarily chosen parameter set.
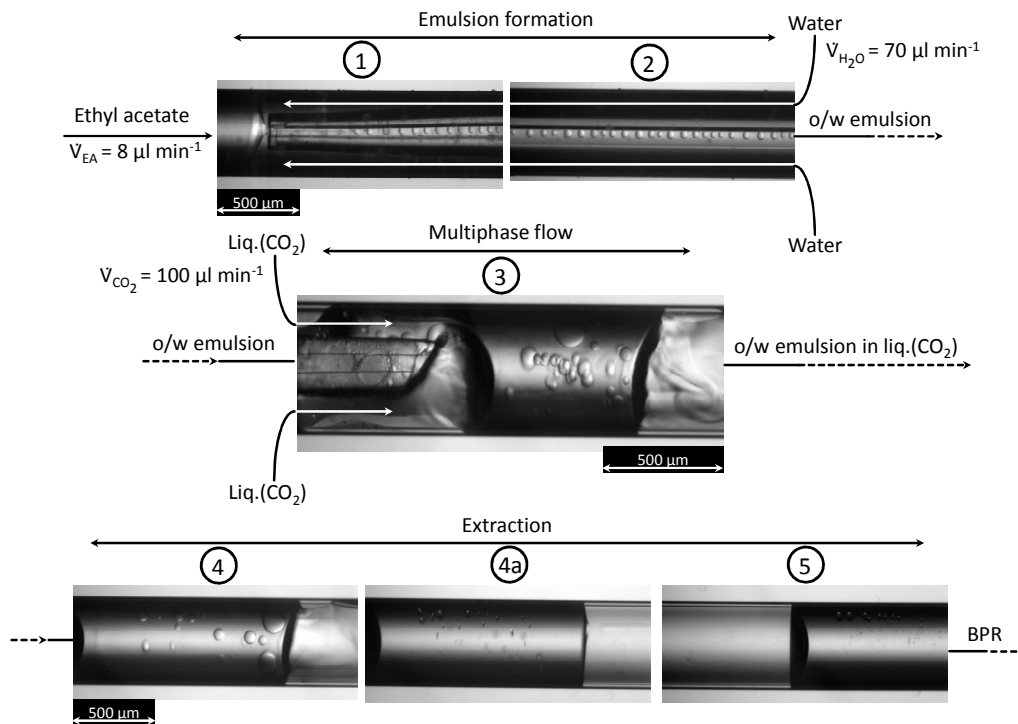
Figure 6: Series of images of an experiment at T = 25°C, p = 7 MPa, flow rate (ethyl acetate) = 8 µl min^{-1}, flow rate (water) = 70 µl min^{-1}, flow rate (CO$_2$) = 100 µl min^{-1} at the previously explained camera positions

For the acquisition of these images a concentric capillary system was used as it was described above. The inner diameter of the two bigger capillaries was 450 µm, the connecting capillary had a squared cross section with an outer "diameter" of 370 µm and an inner "diameter" of 100 µm. The nozzle tips on both sides had an inner diameter of approximately 45-50 µm. All capillaries were used with untreated glass surfaces. Ethyl acetate was delivered with a flow rate of 8 µl min^{-1}. Water was focused on this flow with a flow rate of 70 µl min^{-1} forming o/w-emulsion droplets of 30 µm in diameter. In the emulsion formation area, position 1 was located directly at the nozzle and position 2 was located 2 cm downstream of the nozzle. At position 3 the acceleration of the o/w-emulsion in the nozzle while getting in contact with liquid CO$_2$ splits the continuous water phase in small dispersed segments separated by CO$_2$. It can be seen that the water instantaneously gets in contact with the wall resulting in small plugs instead of droplets. The image at position 3 has a different magnification than all other pictures to better see details of the mixing process. In the extraction capillary it can clearly be seen that the size of the organic droplets in the water phase decreases. At position 5 the droplets are almost completely vanished. This has to be caused by the extraction effect of the CO$_2$. The distance between the second nozzle and position 5 is 25 cm. At given flow rates the velocity is 1.25 cm s^{-1} resulting in a contact time of water and CO$_2$ of 20 seconds.

CONCLUSION

Nearly all publications on supercritical fluid based particle technology are carried out in steel high pressure vessels. The aim of this contribution was to show the possibility to combine the microfluidic with a supercritical extraction process as a very versatile tool to investigate process steps during the high pressure process in optical accessible systems. Additionally microfluidic devices allow the formation of uniform emulsion droplet sizes adding a new

feature to the process. First experiments could be presented showing the emulsification and the extraction process in one microfluidic device. A detailed investigation of all parameters concerning the process in microfluidic systems also with the presence of solute is in development and will be presented soon.

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