A microfluidic chip for generation a lipid phase in an aqueous dispersion

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Abstract. The subject of study involves the handling of liquids and gases in the order of micro-litres and in micro scale resolution. Microfluidic structures are very fine in the context of the macro world, and standard dimensions are around 100 µm, but even finer, depending on the manufacturing process. Visualization are used to study events and processes inside microstructures. Events taking place in fine structures are influenced by parameters such as liquid resistance, interfacial forces, diffusion transfer of molecules and mutual interactions both at the chemical and thermodynamic levels. The analysis of the behaviour of liquids in microfluidic systems involves a change of approach in deriving the influence of input conditions on the resulting behaviour and the course of interactions both between the wall of the structure and the liquid, but also in the liquid itself. The methodology and production of a microfluidic device usually uses a number of technologies. The process often combines a lithographic technique with a controlled etching or laser engraving method. This article introduces the initial experiments with droplet microfluidic generators, which took place at TUL - CXI and subsequently led to the construction of your own implementation in the form of a capillary chip that can be used in pharmacy.

1 Introduction and Motivation

Microfluidic structures can be as thin as the human hair, i.e. around 100 µm, and unlike the macro scale world, where inertial (bulk) forces and momentum dominate, fluid behaviour is influenced by fluid resistance, surface forces, interfacial interactions and other effects at the molecular level. Microfluidics in itself is a complex discipline requiring not only physical, but also chemical and engineering knowledge. It is one thing to understand the phenomena of fluid flow on such a small scale, but it is another thing to be able to design tiny structures and produce them in a repeatable and defined manner. The design of microfluidic circuits includes not only the creation of structures, but also their development, implementation and connection to hydraulic systems.

If such a solution is unified on one chip, then we call the integrated system Lab-On-Chip (LOC). The reason and motivation for the miniaturization of individual elements is their inclusion in a fully automated and portable system. The fundamental reason for miniaturization, if we consider working with liquids, is very small amounts of expensive and rare chemical reagents. If we limit the term microfluidics only to the area that works with liquids and exclude a number of biological applications where cells or bacteria are manipulated, then the leading role is currently played by droplet generators and reactors. The formation of defined dispersions, combined drops or mixing of liquids or mutual reactions and kinetics of liquids finds application in the development of biological drugs.

LOC microfluidic platforms for generating dispersions are based on liquid transport through a channel or capillary. These systems can be stored in 2D or 3D structures. The basic geometric arrangement for liquid interaction study is T-junction. Liquids are supplied to the individual branches in a controlled continuous flow using linear pumps. It is the dosing of liquids that is currently the biggest problem, as it is the source of a large dead volume of liquids.

The behaviour of liquids in microstructures seems to be mysterious if we consider only quantities that we know for sure, i.e. gravity, inertia forces. Simply changing the surface tension of a liquid, e.g. by adding a surfactant, can completely change the behaviour of the liquid in the system – its affinity to the surface, which can be stronger than the forces caused by the movement of the liquid, different viscosity or density.

Dominant interaction in microfluidics is interfacial interaction. Surface tension is caused by the cohesion of fluid molecules at their interface. While cohesive forces act on the surface of the fluid, adhesive forces act on the solid walls. These are manifested by capillary phenomena. The surface tension of a liquid causes the surface of the liquid to curve to compensate for the normal pressure difference. The relationship between the surface tension and the normal pressure difference at

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the interface of a thin liquid layer is given by the Young-Laplace equation.

Another parameter that changes the behavior of the liquid is that the channel walls are very close to each other due to miniaturization. Since the walls are immobile, they impede the flow of the fluid layers near the wall and thereby reduce the overall flow rate in the microfluidic channel. The resistance of the channel depends on the viscosity of the liquid and the dimensions of the channel.

\[ p = \frac{\lambda}{D_H \mu} \frac{L}{v^2} \], where \( \lambda \) friction coefficient, \( L \) channel length, \( D_H \) hydraulic dimension, \( \rho \) liquid density, \( v \) is mean flow velocity.

As the Reynolds number can be calculated as: \( Re = \frac{\rho \rho D_H v}{\mu} \), where \( \rho \) is density of liquid, \( v \) is mean flow velocity, \( \mu \) is dynamic viscosity.

For example, the dimensionless Reynolds number (Re), which is used to identify the importance of inertial forces in a fluid compared to viscous forces, is typically in the order of 100, very often close to 1 or even less. In the case of the microfluidic channel (T-junction with square cross section: \( b = 1000 \mu m, a = 100 \mu m, L = 16 \) mm), the Re for a flow of 1 ml/h water is 0.51 at mean velocity of 2.78 mm/s, the channel resistance should be 0.53 mBar. For a maximum flow rate of 100 ml/h, \( Re = 50.51, v = 277.78 \) mm/s and channel resistance \( p = 53.33 \) mBar.

From the experience of experimental practice, however, the internal resistance of the channel is greater than this value, which, however, has not yet been determined exactly. However, the influence of flow stratification is assumed. At high Reynolds numbers in the macro world, inertial forces are dominant, leading to the emergence of turbulent structures. In the case of microfluidic flows, viscous forces prevail over inertial forces and liquid layers flow side by side in the axis of the channel and do not mix convectively. Mutual mixing occurs only due to the influence of liquid molecules at the interface of two mutually miscible liquids. This principle is diffusion. This is the process of movement of molecules from an area with a higher concentration of a substance to a lower one due to Brownian motion.

At the TUL - CXI workplace, the task of describing and subsequent optimization of microfluidic chips for the creation of lipid particles is solved as part of the project. The motivation of use of microfluidic chips is the preparation of lipid particles in a defined range of sizes created in the required amount. To this end, different geometries of microfluidic chips, layouts and types of dispensers have been tested.

The formation of dispersions can be achieved by various emulsification methods. Conventional techniques, working with large volumes of liquid (min. 200 ml) provide little control over the resulting dimension. This is accompanied by a wide distribution of droplet sizes from a few micrometres to millimetres.

The requirement for monodispersed droplets can be solved by their production using microfluidic structures. The basic arrangement for the formation of droplets is the T-junction. These are two orthogonal inputs for mutually immiscible phases. The continuous phase is the carrier liquid, usually passing through a wider, continuous branch. The second phase is dispersed. The mechanism of dispersion is compression, dripping or jetting of the immiscible phase. In all cases, the effect is influenced by the forces at the interfacial interface, which also determine the limits of the method.

Another type of microfluidic setup is based on capillary impact, when the capillary of orifice in micrometres is placed into the channel and the carrying liquid acts in the sense of Kelvin-Helmholtz and Rayleigh–Taylor instabilities.

The resulting droplet size can be influenced by the following parameters: 1. the ratio of the width and height of the sides of the channels, 2. the material of the channels, i.e. wettability and surface affinity, 3. the ratio of continuous and dispersed phase flow rates, 4. the shape of the capillary, channels and Reynolds number, 5. by the ratio of viscosity and density of both phases.

### 2 Design of Microfluidic Experiments

In the first phase, a commercial microfluidic chip from Microfluidic ChipShop was used, specifically the Fluidic 285 type. These types of chips were chosen mainly because they allow testing several variants of mixing two liquids in different arrangements of angles and diameters of individual branches. For a more detailed investigation, the geometry in detail E (see Figure 1) has proven to be the best in terms of particle production.

![Sketch of Microfluidic ChipShop's Fluidic 285](Image)

In order to accurately describe the ongoing process of the formation of lipid particles in the microfluidic channel, it was necessary to design and compile a visualization methodology. The methodology is based on an inverted microscope with optional objectives for the variability of observed details and with a lower and additional upper LED illuminator. Furthermore, the microscope is equipped with a Basler digital camera for...
In the selected chips, drops of oil were added to the main stream of water, and vice versa – drops of water were added to the stream of oil. A precise description of liquid mixing interactions in both mentioned arrangements provides relevant information for the design and creation of custom chips and input data and boundary conditions for numerical simulations. During the initial simulations, several parameters were traced that could significantly influence the dispersion process. During the experiments, some were excluded as irrelevant.

### Water flow in main branch \( b \)

- **3 ml/hour**
- **33 ml/hour**

**Fig. 2.** Development of mixing of liquids behind T-junction of water – oil phases, constant oil flow, i.e. 0.3 ml/hour.

**Fig. 3.** a) Formation of individual separation of water droplets in oil, b) transport of individual drops by the main stream of liquid – oil.

Branch E (see Figure 1) has three consecutive branches assigned to the main channel. The ratio of the diameters of the main and secondary branches is 10:1. In the first step, the behavior during the activation of one branch was monitored. Both variants of the mixing of liquids flowing through individual branches in a simple T-branch arrangement were monitored (square cross-section with sides and channel length: \( b = 1000 \mu m, a = 100 \mu m, L = 16 \text{ mm} \)). The experiment was aimed at determining the effect of liquid flow on the size of the resulting drops and the frequency of their formation. It has been shown that if the carrier liquid is water, dispersion does not occur. File of visualizations see Figure 2 shows individual liquid states at selected water flow values in the main branch of 3 ml/hour and 33 ml/hour. The oil flow in the secondary branch was maintained at 0.3 ml/hour. In this case, the water is coloured blue, a branch of the secondary branch is connected to the upper wall of the main branch.

In the next step, an inverse experiment was carried out, in which oil flowed through the main branch and water, also dyed with blue dye, was dosed from the secondary branch \( b \). After the critical volume of the water drop has been reached, due to inter-phase and inter-surface forces, it is detached, the drop is formed into a spherical shape and is subsequently transported by the flow of the main liquid. In this way, it is possible to produce significantly monodisperse drops. Using this inverse example, it was proven that by changing the flow rates in the main and secondary branches, the size of the drops and the frequency of their formation can be regulated.

### 2.1 Numerical Support

The experimental results were also supported by numerical simulation. The geometry for the numerical simulation tests includes a short section (approx. 0.3 mm) of a wide horizontal channel, an inlet of a thin vertical channel and a 1.2 mm outlet horizontal channel where the droplet is formed. Several computational meshes with different number and size of elements were generated in the ANSYS Fluent software. The first numerical tests revealed that the Volume of Fluid solver is potentially unstable when calculating the interphase interface in areas where elements of very different sizes are adjacent, so in the end the mesh was not refined in the boundary layer area and is isotropic throughout the volume. However, a fully developed laminar profile can be expected in a microfluidic chip, and inserting structured layers in the area near the walls is not necessary anyway. The mesh used for the calculations presented here has 63 thousand elements, the characteristic size of the element is 5 μm.

The physical constants for the used liquids were specified according to tabular values and technical sheets, further determined based on the values set during the experiment. The aqueous phase (density 993 kg/m³, kinematic viscosity 0.696·10⁻⁶ m²/s) constants correspond to pure water without surfactants at 37°C, the data for the oil phase (density 970 kg/m³, kinematic viscosity 1.000·10⁻⁶ m²/s) were taken from the datasheet of Lukosiol M100 oil. The problem is the value of the interfacial tension between oil and water and the contact angle at the oil-liquid-Plexiglass interface, which are not available and were chosen by estimation in the first numerical test. The boundary conditions for the flow at the horizontal and vertical inlet were calculated based on the values set during the experiment: - aqueous phase flow rate in the thin vertical channel 0.5 ml/hr: \( u_{\text{water}} = 0.00992 \text{ m/s} \); - oil phase flow rate in the wide horizontal channel 2.6 ml/hr: \( u_{\text{oil}} = 0.00515 \text{ m/s} \). A constant zero pressure (pressure outlet) was prescribed at the outlet.

An unsteady simulation of the flow of two incompressible immiscible fluids in the solver is fully implicit, the time step is automatically adjusted so that the Courant number \( \text{Co} > 1 \). In the initial state, the computational area is completely filled with the oil phase, i.e. \( \alpha = 1 \).
A 3D non-stationary simulation of the flow of two incompressible immiscible fluids was set up and debugged in the OpenFOAM computational library using the Volume of Fluid method. This method works with a stationary invariant network and captures the shape of the interface between two (or more phases) using the quantity volume fraction \( \alpha \). This scalar field has a value of 0 in the elements where the first phase is present (in our case the aqueous phase), 1 in the elements where the second phase is present (in our case the oil phase) and which reaches values between 0-1 in the elements where the interfacial interface is present at any given moment. The VOF method is capable of capturing interfacial interactions - surface tension at the liquid-gas interface, interfacial tension at the liquid-liquid interface and interactions at the interface between two liquids and a solid wall (contact angle). The solver is fully implicit, the time step is automatically adapted. It is known that for the numerical stability of the VOF solver, it is necessary to keep the maximum time step size,

\[
dt \leq \max(10\tau_\mu, 0.1\tau_\rho),
\]

where

\[
\tau_\mu = \sqrt{\frac{\nu \rho dx}{\sigma}}, \quad \tau_\rho = \sqrt{\frac{\rho d x^2}{\sigma}}
\]

and where \( dx \) is the characteristic grid dimension. For the finer grid mgm13 and the physical constants used in the sgm305 simulation yields a stable time step method \( dt = 1.4 \cdot 10^{-6} \), which at the simulated time \( T = 0.4s \) represents a rather severe limitation in terms of simulation length and computational complexity.

The results of the numerical simulation are demonstrated in Figure 4, which shows the evolution of the interphase interface and volume fraction \( \alpha \) over time. When compared with the image from the experiment in Fig. 5, it can be seen that a drop of approximately the same shape is first formed at the mouth of the vertical capillary. A drop of water is carried by the flow of oil in the main channel. However, in the numerical simulation, this drop continues to lengthen, does not break off, and eventually reaches the exit of the area.

Fig. 5. Evolution of the interfacial interface with time. Red – oil phase, blue – water phase.

There are probably two reasons why the droplet development dynamics in the numerical simulation does not match the experiment in the later stages. First, no reliable data are available regarding the magnitude of the interphase voltage and the contact angle. The second and perhaps even more important reason is that the simulations were only performed in 2D. Although the geometry of the experiment is pseudo-two-dimensional, surface phenomena at the water-oil-solid wall interface play a significant role in the movement and detachment of drops. The shape of the droplet is largely determined by the contact angle. In a real 3D experiment, interaction occurs not only on the side walls, but also on the front and back walls of the microfluidic chip. Apparently, the surface phenomena on these walls cannot be neglected, and further simulations will be carried out in 3D.

2.2 Capillary LOC

Based on knowledge of surface affinities and interactions of individual phases, we decided to create a system that would create lipid particles in water. From the results of the research, it was evident that achieving a stable dispersion is not possible with continuous flow and it is necessary to induce pulsatile flow of at least one phase. Another condition that was difficult to meet is the separation of the lipid phase from the channel walls, which have a strong mutual affinity. This could only be realized in the form of a so-called capillary chip.

The capillary microfluidic chip is made of three layers of extruded or cast PMMA, which are firmly connected to each other by bonding technology to form strong covalent bonds. It is a sandwich system consisting of a base layer, a middle layer with a defined structure and a cover layer with openings for the entry of liquid and preparation for fitting ports, see Figure 6.

In the realization of the chip, PMMA material was used, which can be easily processed using laser cutting, and the individual layers can be connected using SolventVapour Annealing (SVA).
Laser cutting is a very efficient and time-saving method of processing plastic materials. Complex structures can be created in one step using the energy of a focused laser beam. The beam generated by the CO₂ laser is stable with a cut width of several tens of micrometres. When processing PMMA plates up to a thickness of 3 mm, a laser power of 20 W is used with a laser head speed of 5 mm/min. All plates are machined with this technology. After machining, the material must be annealed in order to reduce the residual material stress on the cutting edges. It is recommended to place the workpieces in an oven heated to a temperature of 70 °C and anneal for 1 h with every 1 mm of material thickness. After the specified time, it is advisable to let the workpieces naturally temper to room temperature.

Fig. 5. The complete assembly of the capillary microfluidic chip and its real form fitted with ports and connected to the hydraulic circuit during the experiment.

Before joining the layers, it is necessary to remove the protective film and degrease the surface with isopropyl alcohol and remove dust particles preferably with clean compressed air. Surface treatment to create a strong bond between the layers using the SVA method. This is a method in which the surface is gently etched in a controlled manner, the binding polymer structures are necessarily broken, and by the subsequent application of layers treated in this way, a very strong bond is formed at the molecular level. Such a joint is optically clean, which is necessary for the device, which is to be further used for a detailed optical analysis of the processes, taking place inside, under a microscope. We used chloroform to etch PMMA.

2.3 Fluidic System

Linear pump connection
During continuous dosing of the lipid phase, the dispersion is defined by the inner diameter of the capillary, the separation of individual drops occurs due to the interphase interface forces of the capillary – lipid phase – aqueous phase.

The size of the drops of the lipid phase is further defined by the flow rate of the lipid phase and the flow rate of the aqueous phase when passing around the mouth of the capillary.

Standard parameters for setting the flow in linear mode are: flow rate of 0.5 ml/h lipid phase and 50 ml/h aqueous phase. At this setting, the droplet size of the lipid phase corresponds to the inner diameter of the capillary, i.e. 0.159 mm, and the break-off production frequency is 1 Hz. A higher flow rate of the aqueous phase will increase the frequency of droplet detachment and at the same time slightly reduce their size. By maintaining the flow of the lipid phase through the capillary and increasing the flow of the aqueous phase to 150 ml/h, the frequency of droplet production will increase to 2 Hz and their size will decrease by about a third, i.e. 80 μm.

Peristaltic pump connection
During pulsed dosing of the lipid phase by means of a peristaltic pump, backflows occur at the capillary mouth and, due to local eddies, the lipid phase disintegrates into finer droplets, which are created by the significant acceleration of the liquid during the pump discharge and are further carried away by the steady flow of the aqueous phase during the rest part (suction) operating mode of the peristaltic pump. This corresponds to the occurrence of Kelvin-Helmholtz instability at the interface of two different fluids with different flow velocities. This instability certainly arises in cooperation with the Plateau-Rayleigh instability arising at the interface of two phases with different surface tension, and the resulting jet disturbances are supported by a pulsating regime. In the pulse dosing mode, the lipid phase is released into the aqueous phase in clusters of droplets with a frequency that corresponds to the operating frequency of the peristaltic pump.

The volume of the liquid of the lipidic phase is constant and is determined by the parameters of the peristaltic pump, i.e. the volume of the dosed liquid between the blades defined by the diameter of the working tube and the distance between the blades. The working setting of the PCD 61 peristaltic pump generating the droplet size of the lipid phase with a diameter of about 10 μm is 7.1 ml/min with a clustering frequency of 1.5 Hz. A cluster is formed during one pulse and consists of 140 drops.

Thanks to the pulsation mode, the corresponding amount of liquid can be processed into drops of the required size in a very short time. Immediately after formation, the drops are removed by a continuous flow of the aqueous phase, which is generated by a linear pump and corresponds to 50 ml/h.
2.4 Working Liquids

The microfluidic chip is designed and implemented for the need to disperse the lipidic phase into an aqueous environment, at the same time ensuring and stabilizing this dispersion is required. The resulting dispersion is discharged through the outlet port into the collection container. This sampling is free and open, which allows continuous analysis of production, especially if the system is being debugged and the individual flow rates of working fluids are changed.

**Lipid phase**

The basic requirement for the lipid phase is the composition, where the main component was determined to be 1-tetradecanol, as the carrier of the medicinal component. A condition for successful dispersion is maintaining both phases of the system in liquid form. The table melting point of 1 – tetradecanol is at least 38°C. Maintaining this temperature in the entire fluidic system turns out to be unrealistic. For that reason, a mixture was used consisting of 60% by weight of 1-tetradecanol, which is melted at a higher temperature and 10% by weight of ethanol is added to it, the entire solution is supplemented with 30% by weight of oleic acid. The resulting mixture is liquid at elevated room temperature, i.e. 25°C.

**Aqueous phase**

A condition for maintaining the stability of the dispersion is the presence of surfactants. A suitable addition to the aqueous phase is the anion-active ionogenic surfactant SLS (Sodium Lauryl Sulfate), which is commonly used in cosmetics and the food industry. To ensure a stable dispersion, 0.5% by weight of surfactant in an aqueous solution is sufficient.

The stability of the dispersion is tested on samples taken 30 min, 1 h and 24 h after sampling from the microfluidic chip. The samples are evaluated under a microscope. Particle size distribution characteristics were evaluated with the help of image analysis.

The optimization of the production of lipid particles using fluidic microchips is currently at such a level that it can already efficiently produce lipid particles with a size of 5 µm - 30 µm. There is still a non-negligible percentage of larger particles. The production process must therefore be further optimized with clarification of the requirements for the production range of sizes.

3 Conclusion and Summary

The material composition and the composition of the individual mixing phases are part of the optimization and validation of the fluidic microchips together with their geometry. Thus, the materials for the base of the microchip and the technology for producing channels in this material became part of the solution described in the submitted utility model [4,5]. In the course of optimization and validation of the best solution, both polycarbonate and glass solutions were investigated, and laser engraving (laser ablation) and etching using different types of acids proved to be suitable as variants for the preparation of channels in the base. The admixtures of the final phase formulations were selected based on two requirements. The first consideration followed the purpose of using the admixtures, namely to ensure the lipid phase dripped into the aqueous base and to keep the solidification droplets separated without subsequent clumping. The second consideration is the healthfulness of the admixtures used at the required concentrations. To this end, the aqueous phase sorbents used as well as other lipid phase admixtures are described in the solution.

The results of the analyses of the particles produced by the fluidic microchip are described in the submitted utility model as part of the validation of the technical solution, including the determination of the production ranges (volumetric dosage definitions) and the sizes of the lipid particles. The optimization and validation of the fluidic microchips, together with their geometry, also includes the material composition and the composition of the individual mixed phases. Thus, the materials for the base of the microchip and the technology for producing channels in this material became part of the solution described in the submitted utility model. In the course of optimization and validation of the best solution, both polycarbonate and glass solutions were investigated, and laser engraving (laser ablation) and etching using different types of acids proved to be suitable as variants for the preparation of channels in the base. The admixtures of the final phase formulations were selected based on two requirements.

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It has been shown that 2D simulations cannot capture the dynamics of interfacial interfaces in a microfluidic channel of a given thickness and that numerical simulations must be performed in 3D. These need to be run in parallel on a computational cluster, and from a licensing point of view the use of the open-source library OpenFOAM seems to be the most appropriate here. A sensitivity study on the computational mesh has been performed, and a mesh with a characteristic element size of 5 μm, i.e. 1.8mil elements on a given geometry, appears to be optimal.

A fundamental problem with existing numerical simulations is that we do not know the exact values of the key physical quantities for multiphase flow - the magnitude of the interfacial tension at the interface between the aqueous and oil phases and the magnitude of the contact angle at the interface between the aqueous phase, oil phase and the plexiglass channel wall. Analytical considerations and numerical simulations show that these quantities have a significant influence on droplet formation.

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