Emulsification on a Microscale: Faster, Better, and More Effective

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Abstract

Emulsions are traditionally prepared with the application of high shear forces generated by the use of static mixers, homogenisers, or ultrasound. The resulting emulsions are sensitive to change of process conditions. The application of high forces and temperatures can significantly affect the constituents of the emulsions and their final stability. Microfluidic technology seems to be a very efficient alternative to classic emulsification methods. The dimensions of microdevices in combination with continuous processes offer a great advantage over classic batch emulsification processes carried out on a larger scale. The small dimensions of the microdevices allow easy transport of equipment, better control and safety of the process, and intensified mass and energy transfer. The mixing time in microdevices is reduced to a few milliseconds because the molecules in the microchannels have a short diffusion path. In this paper, an overview of emulsification processes, the advantages of use of microfluidics in emulsification, and future perspectives of microemulsification are presented.

Keywords
Emulsion types, emulsification mechanism, emulsion instability, microfluidic systems, continuous emulsification on a microscale

1 Introduction

Emulsions are used in various industrial fields, from the production of fuels, detergents, food and cosmetic products to preparation of pharmaceuticals and process implementation in biotechnology and biomedicine.1 They are usually developed with the aim of encapsulating lipophilic components dispersed in an aqueous medium.2 Emulsions are dispersed systems consisting of two immiscible liquids. One liquid is present in the form of droplets (dispersed phase) dispersed in another liquid (continuous phase).3 The ability of a system to maintain dispersion of one phase in another depends on emulsion stability. Stability can be enhanced by adding surfactants/emulsifiers (amphiphilic compounds) that block droplet coalescence at the liquid-liquid interface or by adding stabilisers that increase viscosity of continuous phase and delay coalescence.

The most common emulsion types include oil-in-water (such as milk, sauces), and water-in-oil (like mayonnaise and Hollandaise sauce, containing egg yolk lecithin as the emulsifier). Emulsions are usually classified based on their structure: oil-based and water-based emulsions (Fig. 1).

As already mentioned, both types of emulsions have three distinguished areas: dispersed phase, continuous phase, and interfacial layer. Besides the types of emulsions shown in Fig. 1, emulsions can also be classified based on their size and structure:

- Oil-in-water (O/W) and water-in-oil (W/O) macroemulsions are characterised by a size range of 0.1–5.0 μm, and are usually non-transparent due to the larger particles.
- Nanoemulsions have a size range of 20–200 nm. Depending on the particle size, they can be transparent and opaque. They are kinetically stable.

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• Multiphase emulsions are “emulsions within emulsions”, such as water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) systems. They are produced in multi-stage processes. For example, the W/O/W system begins with the preparation of a W/O emulsion, which is then emulsified in water.

• Mixed emulsions are systems containing two different dispersible phases that are not mixed in a continuous phase.

• Micellar emulsions or microemulsions have a particle size range of 5–50 nm and are thermodynamically stable.\(^5\)

Furthermore, another classification of emulsions can be made according to the nature of the emulsifier.\(^6\)

• simple molecules and ions
• nonionic surfactants
• surfactant mixtures
• ionic surfactants
• nonionic polymers
• polyelectrolytes
• mixed polymers and surfactants
• liquid crystalline phases
• solid particles.

1.1 Emulsification mechanism

Emulsions are commonly formed by homogenisation of two immiscible phases in the presence of one or more surfactants.\(^7,8\) During homogenisation one phase is being dispersed as small droplets into the other phase.\(^8\) To form an emulsion, the interfacial surface area between two immiscible liquids must be increased. Depending on the force applied to increase the interfacial surface area, high or low energy methods are used to produce emulsions.\(^8\) High-energy methods are typical for devices such as high shear homogenisers, colloid mills, high-pressure homogenisers, and ultrasonic homogenisers.\(^9-11\) The problem with such approaches is that the application of high pressures and temperatures can lead to depolymerisation and denaturation of polysaccharides and proteins.\(^12\) Low-energy approaches rely on the spontaneous formation of emulsions under controlled conditions based on specific physicochemical properties of the emulsion components. Such methods are phase inversion temperature (PIT), and spontaneous emulsification (SE). With of the increase in temperature, the affinity of the surfactant for water and oil gradually changes, resulting in phase inversion.\(^13\) PIT emulsification can only be used in systems containing temperature-sensitive surfactants such as polyoxyethylene-type nonionic surfactants.\(^14,15\) Temperature-sensitive surfactants are water soluble at low temperatures, and the surfactant layer at the droplet interface has a positive curvature. At high temperatures, surfactants become oil-soluble, and the curvature of the surfactant layer at the droplet interface becomes negative. At an intermediate temperature, known as the PIT, surfactants have equal affinity for the aqueous and oil phases. At PIT, the spontaneous curvature of the surface layer at the droplet interface is zero, resulting in complete oil solubility in a bicontinuous or lamellar liquid crystalline phase.\(^16,17\) Spontaneous emulsification increases the entropy and decreases the Gibbs free energy of the system. Spontaneous emulsification is a process-based diffusion of the solute into the more soluble phase after mixing the two phases. To increase the stability of emulsions, emulsifiers are used to create an energy barrier between dispersed and continuous phases, thus achieving a reduction in surface tension. Their main properties are adsorption on the interface and self-organisation in various supramolecular structures (associates).\(^1\) According to the structure of the molecule, one molecule end is lipophilic (hydrophobic) and the other is hydrophilic. Emulsifiers can be divided according to their charge in the aqueous system, solubility, hydrophilic/lipophilic balance and the chemical structure of functional groups. The choice of emulsifier depends on the product formation, process conditions, and the desired final properties. To make the product more appealing to the customer, different ingredients can be added to the emulsion to modify its texture, flavour, colour, stability, etc.\(^18\)

Once the emulsion is formed, typical droplet size is in the range 0.1–100 \(\mu\)m.\(^19\) In order to characterise emulsions based on droplet size, the distribution coefficient of variation (CV) is considered. For research and industrial application, monodisperse emulsions with CV value less than 25 % are the most interesting. Small droplet size and narrow size distribution is crucial for good process management, especially in the pharmaceutical industry and drug delivery, or in the food industry where better control of the release of bioactive compounds can be achieved by emulsification.\(^20\)

1.2 Emulsion instability

One of the greatest challenges for emulsion preparation is maintaining the physical stability of the emulsified sys-

![Fig. 2 – Emulsion instability (according to Espinosa-Álvarez et al.\(^21\))](image-url)

\(\text{Slika 2 – Nestabilnost emulzija (prema Espinosa-Álvarez i sur.)\(^23\)}\)
The mechanisms of emulsion separation (Figs. 2 and 3) can be divided into: gravitational separation (creaming and sedimentation), droplet aggregation (or flocculation), and Ostwald ripening and droplet coalescence.\(^2\)

Phase separation in emulsions is determined by the frequency of collision between droplets, which depends on phenomena such as Brownian motion, gravity, and shear force.\(^2\) The combination of these phenomena can result in a concentration gradient that causes larger droplets to migrate more rapidly; upward when their density is less than that of the medium (stratification – incineration); or downward when their density is higher than that of the medium (sedimentation). Flocculation is defined as the association of small emulsion particles into a large aggregate that disperses when shaken. It is a reversible process in which the droplets remain intact.\(^2\) Flocculation is caused by van der Waals attraction; van der Waals attraction increases as the distance between droplets decreases, leading to droplet aggregation when the distance is small.\(^2\)

The pressure difference between large and small droplets causes Ostwald ripening, which results in mass diffusion from the smaller to the larger droplets.\(^2\) All of these instability processes can subsequently lead to coalescence of the droplets. This is an irreversible process in which two droplets coalesce due to the loss of the stabilising layer, resulting in the development of distinct oil and water phases.\(^2\)

### 2 Microfluidic systems

Microfluidic systems refer to technologies that enable automation and linkage of processes on a small scale.\(^2\) Such systems consist of a series of elements. The base is a microdevice that contains a network of interconnected microchannels with diameters of less than 1 mm. Usually, the range refers to sizes from 10 to 500 μm.\(^2\) A microsystem may consist of one or more subunits. The basic microdevice consists of a microchip, a holder, and capillaries connecting the elements (Fig. 4). Apart from the aforementioned elements, the microsystem may contain additional devices such as pumps, tanks for reagents, micromixers, micro heat exchangers, and microseparators.\(^2\) Small dimensions of microsystems allow for their several advantages over conventional systems,\(^2\) which include:

- effective mass and heat transfer
- large surface-to-volume ratio
- lower costs per analysis
- lower number of steps in the process
- higher specificity, sensitivity, reliability of the process.\(^2\)

The advantages of microsystems allow easy process optimisation, transfer of the process to a larger scale (scale-up), combining the elements of the system in sequence and continuous processes.\(^2\) Connecting the elements of a microsystem can be realised in parallel or in series, and with internal and external increase in the number of subunits.\(^2\)

Many different materials (i.e., silicon, glass, ceramics, polymers, hydrogels, paper, biodegradable materials, silk) can be used for fabrication of microdevices. Polymers and glass are most widely used. During emulsion preparation, optical transparency is an important property of the material from which the microsystem is constructed, because transparent materials allow observation of emulsion formation. Therefore, glass and suitable polymers are used most often in the construction of microdevices for preparation of emulsion. The choice of material on such a small scale can also have a significant effect on the functionality of the microfluidic system. For example, if the channel surface is hydrophobic (such as polymer surface), it is more suitable...
3 Emulsification in microfluidic systems

With the application of microfluidic systems in emulsion formation, completely new and versatile approaches emerge. One of the greatest advantages of microfluidic application is the precise control of droplet formation and the possibility to create highly monodispersed emulsions. Emulsification is performed using a variety of systems, from direct phase mixing (shear-based systems) to application of membranes, different geometries and high pressures (geometry-induced capillary breakup systems). Microdevices for direct mixing have different geometries, referred to in the literature as T-, Y-, and X-shaped microdevices, according to the construction of the inlet streams (Fig. 5a–c). T-shaped microdevices are the simplest systems for droplet formation. The continuous phase is introduced through a horizontal channel, while the inlet of the dispersed phase is perpendicular to the flow of the continuous phase. The flow of the continuous phase generates shear forces and a pressure gradient allows the gradual entry of the dispersed phase and droplet formation. For higher efficiency, it is possible to combine multiple systems and add barriers for better mixing. In the Y-shaped microdevices, continuous two-phase flow of two liquids and rapid mixing of the liquids is possible. The X-shaped microdevices have three inlets, a continuous phase enters from the side and is adjacent to the dispersed phase, which enters the system in the form of droplets. Another attractive advantage of microfluidics is the possibility to fabricate such devices that enable construction of multiple-layer emulsion in highly controlled environment (Fig. 5d).

Fluid flow in microchannels is usually laminar (low values of Reynolds number) and mixing takes place by diffusion. To overcome the dominant effect of viscous forces over inertia forces, and to increase systems throughput, different methods of mixing at the microscale have been proposed. Intensification of mixing is supported by the introduction of various elements in microsystems. Most elements that promote mixing aim to shorten the diffusion path of molecules. Diffusion of a small organic molecule in an aqueous system occurs in 5 s for a path of 100 μm. One of the best-known mixing systems was developed at the Mainz Institute of Microtechnology, a multilaminar type micromixer. Mixing is based on splitting the main stream into even smaller laminar streams. Smaller streams have shorter diffusion times and faster phase mixing. Some modifications include construction of microchannels and merging of laminar streams at the outlet. Certain types of systems are constructed by dividing the main flow into smaller flows, which are then mixed and divided again, repeating the process. By repeating the separation and mixing sequence, phase streams are obtained in 32 layers. A more efficient emulsification process is achieved with microchannels etched in series parallel or perpendicular to the microdevice plate. Microdevices contain multiple layers that allow separation of droplets at the exit of the microchannel.

3.1 Application of emulsification processes in microsystems

The use of emulsions is widespread, from consumer products to industrial products. In the conventional methods, homogenisers, colloidal mills and mixers are usually used for the preparation of emulsions. The classic methods for the production of emulsions have shortcomings which are a result of the inadequate ability to control the process. Equipment that applies high forces is used to produce emulsions. Moreover, only 1–5 % of the energy used in the process is used to disperse one phase into another, while the rest of the total energy used is lost in the form of heat. Additionally, the classic process results in emulsions polydispersed in size, with a partition coefficient of 40 %, which are therefore very unstable. Poor regulation of parameters such as temperature, applied forces, and droplet size distribution in the emulsion has an impact on the components of the emulsion (e.g., starch molecules, proteins) and the long-term stability of the emulsion. Transferring the process to a smaller scale has the potential to eliminate the existing problems. Compared to classic technologies, microsystems apply less force, less pressure, and less energy. The flow is laminar and there is no turbulence or cavitation, making the process easier to control. Theoretically, by connecting multiple microunits to the system, it is possible to achieve productivity similar to those of standard emulsification methods.
Published research shows that microsystem emulsification can be used successfully for product recovery and process improvement. Yeh et al. achieved more than 98% conversion of oil to biodiesel in the microsystem. Microemulsions were also used in biodiesel production in the work of Šalić et al.. The authors studied oil/water and methanol/oil emulsions, and tested the effect of different emulsifiers (SDS, PEG 1550, PEG 6000, Tween 80, Triton X-100, gum arabic, mustard, and egg white) on emulsion stability. Cuk et al. compared an emulsion composed of enzyme lipase and oil to maintain high enzyme activity when methanol was added in high excess during biodiesel production in a microreactor. Gojun et al. also compared different strategies of biodiesel synthesis in microreactors. They compared the use of waste and edible cooking oils, commercial and produced enzyme, systems with and without emulsions. The highest biodiesel yield of 32% at a residence time \( \tau = 30 \) min was obtained in the microreactor system with an emulsion of waste oil and commercial enzyme suspended in a water buffer. Saito et al. compared the stability of an O/W emulsion stabilised with BSA serum obtained in the microsystem and in the homogeniser. The emulsions prepared in the microsystem showed higher stability compared to the emulsions prepared in the homogeniser. The application of microsystems for preparation of emulsions and multiphase emulsions in the pharmaceutical industry has also been studied. Multiphase emulsions are produced in multistage emulsification processes where turbulent mixing occurs, making control of the droplet size in the emulsion difficult. In the preparation of multiphase emulsions, for example W/O/W, a size of 40 to 200 \( \mu m \) of the droplets has been achieved, while nanometer droplet diameters have been achieved for standard emulsions.

For the microsystem proposed by Okushima et al., the control of the emulsification was facilitated with laminar phase flow in the system. The result was a W/O/W emulsion with the coefficient of variation of 3%, and droplet diameter of 52 \( \mu m \) for the aqueous phase, and 83 \( \mu m \) for the organic phase.

The investigations published so far on the applicability of emulsions in medicine and pharmacy mainly deal with microemulsions used in the treatment of tumors, cardiovascular diseases, neurological disorders, and inflammatory processes. Moreover, the resulting emulsions are converted into microcapsules containing drugs. Microemulsions are also studied in the food and chemical industry for microencapsulation of bioactive compounds, flavours, dyes, preservatives, enzymes, and agrochemicals. Emulsification in microsystems allows less use of emulsifiers and preservatives. This is the case, for example, in the production of creams in cosmetics, resulting in a dispersed phase size of 0.8–2.5 \( \mu m \), with a lower emulsifier concentration compared to standard production technology. In addition, attempts to combine multiple microchannels to increase productivity have shown success in producing monodisperse emulsions. An example is the 126-input system that allows the production of 96.4 \( \mu m \) emulsions with a dispersion coefficient of 1.3% at a productivity of 320 ml h\(^{-1}\). Tetradis-Meris et al. presented a system with 180 connected units, achieving a droplet size of 21.14 \( \mu m \) and a dispersion coefficient of 4.74%.

4 Conclusion

The great potential of microfluidic application in the emulsion production is described in many studies. Advanced microfluidics have the potential to produce, analyse, and characterise emulsions, and all these steps could be performed in a single microdevice. By choosing appropriate material for production of microdevices, emulsion preparation can be enhanced and highly controlled in order to produce uniform droplets of desirable dimensions ranging from a few microns to a few hundreds of microns.

Despite all the advantages, there is still a number of factors the influence of which needs to be investigated for the implementation of a microfluidic emulsification process in commercial application (from the choice of material and system design to the parameters of the emulsification process).

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Tradicionalno se emulzije pripremaju primjenom jakih smičnih sila koje nastaju upotrebom stačitih miješala, homogenizatora ili primjenom ultrazvuka. Tako proizvedene emulzije osjetljive su na promjenu procesnih uvjeta. Primjena jakih sila i povišenih temperatura može značajno utjecati na komponente emulzija i njihovu konačnu stabilnost. Primjena protočnih mikrosustava pokazala se učinkovitom alternativnom tehnologijom klasičnim metodama emulgiranja. Male dimenzije mikrouređaja u kombinaciji s kontinuiranom provedbom procesa omogućuju brojne prednosti u odnosu na klasične šaržne procese emulsifikacije koji se provode u većem mjerilu. Male dimenzije mikrouređaja omogućuju lakši transport opreme, bolju kontrolu i sigurnost procesa te intenzivniji prijenos tvari i topline. Vrijeme miješanja u mikrouređajima smanjeno je na nekoliko milisekundi zbog kratkog difuzijskog puta molekula u mikrokanalima. U ovom radu dan je pregled procesa emulgiranja, prednosti primjene protočnih mikrosustava u provedbi procesa emulgiranja te potencijalnih novih područja primjene te tehnologije.

Ključne riječi
Vrste emulzija, mehanizmi emulgiranja, nestabilnost emulzija, protočni mikrosustavi, kontinuirano emulgiranje na mikrorazini

SAŽETAK
Emulgiranje na mikrorazini: brže, bolje i učinkovitije

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Tradicionalno se emulzije pripremaju primjenom jakih smičnih sila koje nastaju upotrebom stačitih miješala, homogenizatora ili primjenom ultrazvuka. Tako proizvedene emulzije osjetljive su na promjenu procesnih uvjeta. Primjena jakih sila i povišenih temperatura može značajno utjecati na komponente emulzija i njihovu konačnu stabilnost. Primjena protočnih mikrosustava pokazala se učinkovitom alternativnom tehnologijom klasičnim metodama emulgiranja. Male dimenzije mikrouređaja u kombinaciji s kontinuiranom provedbom procesa omogućuje brojne prednosti u odnosu na klasične šaržne procese emulsifikacije koji se provode u većem mjerilu. Male dimenzije mikrouređaja omogućuju lakši transport opreme, bolju kontrolu i sigurnost procesa te intenzivniji prijenos tvari i topline. Vrijeme miješanja u mikrouređajima smanjeno je na nekoliko milisekundi zbog kratkog difuzijskog puta molekula u mikrokanalima. U ovom radu dan je pregled procesa emulgiranja, prednosti primjene protočnih mikrosustava u provedbi procesa emulgiranja te potencijalnih novih područja primjene te tehnologije.

Ključne riječi
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