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Nanoemulsions: the properties, methods of preparation and promising applications

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Abstract. The properties of nanoemulsions and various methods for their preparation including the high-energy and low-energy emulsification methods and the combined methods are reviewed. Among the high-energy methods, the emphasis is placed on high-energy stirring, ultrasonic emulsification, high-pressure homogenization including microfluidics and membrane emulsification. Among the low-energy emulsification methods, the attention is focused on the phase inversion temperature method, the emulsion inversion point method and the spontaneous emulsification. Using a combined method, which includes the high-energy and low-energy emulsification, it is possible to prepare reverse nanoemulsions in highly viscous systems. Main advantages and limitations of different methods of nanoemulsion preparation are discussed and the potential fields of nanoemulsion applications are considered. The bibliography includes 255 references.

I. Introduction

In the last few decades, considerable interest has grown in nanoemulsions (NEs) and methods for their preparation. First of all, this is related to the prospects of applications of such disperse systems in medicine, in pharmaceutical and cosmetic industries, *etc*. A number of reviews on various methods for preparation of NEs with emphasis placed on the low-energy methods are available.[1 – 3]

The term 'nanoemulsions' is often used to designate emulsions with the internal phase droplets smaller than 1000 nm.[4, 5] However, it is more correct to apply the term 'NEs' only to the disperse systems with less than 100-nm droplets. Nanoemulsions are sometimes called as ultrafine emulsions or miniemulsions. A characteristic feature of NEs is their kinetic stability. Preparation of NEs requires that energy be supplied to the system.

There is yet another type of disperse systems representing one liquid phase dispersed in the other; the droplet size in them is at most 100 nm. These systems form spontaneously and are thermodynamically stable. Usually, they are called microemulsions despite the fact that the droplet size lies in the nanometre range.[6] The major difference between microemulsions and nanoemulsions is that the former are thermodynamically stable, while the latter are not. Qualitative characteristics of the stability of NEs and conventional macro- and nanoemulsions are presented in Table. 1.

In spite of some semantic inconsistency of the terms 'microemulsion' and 'nanoemulsion', this terminology is commonly accepted in the literature, especially abroad. Therefore, in this review the term 'NEs' refers to the kinetically stable emulsions only. Emulsions with droplet sizes slightly exceeding a value of 100 nm will also be considered here. However, we shall emphasize again that it is more correct to apply the term 'NEs' only to the emulsions with droplet sizes less than 100 nm.

II. Properties of nanoemulsions

Nanoemulsions are thermodynamically unstable systems because the change in the Gibbs free energy (ΔG) upon their formation is greater than zero. As a rule, the interfacial tension at the phase boundaries in NEs is $1 - 10$ mN m^{-1}. Therefore, the magnitude of the term $\sigma\Delta F$ [see Eqn (1)] describing an increase in the surface energy

Table 1. The characteristics of nano-, macro- and microemulsions.

System	Droplet size /nm	Stability type
Nanoemulsion	< 100 nm	kinetic
Macroemulsion	> 100 nm	"
Microemulsion	< 100 nm	thermodynamic

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upon the formation of the new surface of the dispersed droplets is rather large and cannot be compensated by an increase in entropy.

$$\Delta G = \sigma \Delta F - T\Delta S, \quad (1)$$

where ΔF is the change in the surface area of the interface, σ is the interfacial tension at the boundary between the droplet of the internal phase and the dispersion medium, T is temperature, and ΔS is the change in the entropy.

In contrast to NEs, the interfacial tension in microemulsions is only $10^{-1}-10^{-3}$ mN m^{-1} (Refs 6–8). Under certain conditions, the term related to the change in the surface area of the interface in Eqn (1) becomes smaller than the entropy change term, *viz.*, $\sigma\Delta F < T\Delta S$. In this case, microemulsions (thermodynamically stable disperse systems) are formed.

In the formation of NEs, the Gibbs free energy change is positive and the disperse system tends to reduce the surface energy. Therefore, the processes occurring in NEs are analogous to those in the separation of conventional macroemulsions.

Sedimentation and creaming occur under the action of gravity and centrifugal force, respectively. If the density of the droplets of the internal phase is higher than that of the dispersion medium, sedimentation takes place. *Vice versa*, if the density of the dispersed droplets is lower than that of the dispersion medium, creaming occurs and the droplets of the internal phase collect in the upper part of the vessel. In the absence of flocculation of the droplets of the internal phase, these processes are atypical of NEs because the droplets are involved into Brownian motion of molecules of the dispersion medium. Owing to the tendency to an increase in the entropy of the system, the gradient of the droplet concentration arising due to their sedimentation or creaming results in their diffusion into the opposite direction, and no sedimentation occurs in NEs.

Coagulation occurs if the attractive forces between droplets of the internal phase exceed the repulsion forces. It results in association of a few or many droplets into aggregates. The stability of NEs against flocculation can be estimated on the basis of analysis of the surface forces acting in the dispersion medium layers between droplets of the internal phase.

Depending on the emulsifier type, electrostatic and steric stabilization of NEs are distinguished.[9] Electrostatic repulsion occurs upon absorption of ionic surfactants on the surface of droplets of the internal phase. Stabilization of droplets in NEs by electrolytes is theoretically possible, but only if the fraction of the internal phase (φ) is low. However, there is no experimental confirmation of this possibility as yet.

Steric stabilization of NEs is due to adsorption of nonionic surfactants or polymers on the droplet surfaces. In these systems, when droplets draw together and the thickness of the layer between them decreases, the space necessary for movement of the polymer chains becomes restricted, *i.e.*, the entropy term in the expression of free energy decreases and droplets are repulsed.

According to the Flory–Huggins theory, the free energy of mixing of polymer chains with the substance of the dispersion medium obeys the following equation:[10]

$$G_{\text{mix}} = kT\left(\frac{4\pi}{V_{\text{ex}}}\right)\phi^2 N_{\text{A}}\left(\frac{1}{2}-\chi\right)\left(3r+2\delta+\frac{H}{2}\right)\left(\delta-\frac{H}{2}\right)^2, \quad (2)$$

where k is the Boltzmann constant, V_{ex} is the molar volume of the substance forming the dispersion medium, ϕ is the volume fraction of polymer chains with the thickness δ, N_{A} is the Avogadro constant, r is the droplet radius, χ is the Flory–Huggins interaction parameter, and H is the distance between the droplets.

At $\chi > 0.5$, the value G_{mix} becomes negative, and droplets are attracted. At $\chi < 0.5$, the value G_{mix} is positive and droplets of the internal phase are repelled.[9]

If the free energy of mixing is positive, approach of droplets to one another (due to, *e.g.*, Brownian motion) results in the appearance of depletion forces because the osmotic pressure in the layer increases. If the sizes of the dispersed particles and the correlation length of polymer molecules in solution are similar, the depletion forces can be much stronger than the interaction forces acting between macroscopic particles. In addition, consideration of the stability of nanosystems requires the introduction of corrections for the curvature of the particle surfaces into calculations of van der Waals and ion-electrostatic interactions, as well as corrections for specific features of the charge distribution over the particle surfaces and in the interparticle layers.[11]

Direct NEs [oil-in-water (O/W) type] are often stabilized using ionic and nonionic ethoxylated surfactants. As a rule, such disperse systems are stable against flocculation due to electrostatic and steric stabilization.[12]

Reverse NEs [water-in-oil (W/O) type] are much less stable to flocculation. The dispersion medium in such NEs is usually nonpolar. Because there is no strong electrostatic repulsion, collisions of droplets of the internal phase result in their flocculation and subsequent sedimentation of floccules formed by the internal phase droplets. Effective stabilization of reverse NEs is only possible due to steric factors.

Coagulation and sedimentation are characterized by only slight changes in the surface free energy of NEs because droplets preserve their initial size. However, these processes lead to a local increase in the droplet concentration, while the thickness of the layers of the dispersion medium between droplets decreases. In this case, the role of coalescence (*i.e.*, association of two or more droplets resulting in a larger droplet) becomes more important. The influence of various physicochemical factors on the stability of emulsion films to breaking has been discussed in reviews,[13,14] where the thermofluctuation mechanism of breaking of thin films between the internal phase droplets and asymmetric stability of the emulsion films has been considered. Taking into account the curvature of the surfactant monolayer in lyotropic mesophases, the authors of Refs 13 and 14 analyzed the conditions for phase inversion [phase inversion temperature (PIT) method] resulting in the formation of highly concentrated direct and reverse emulsions. As shown below, the PIT method is also used for the preparation of NEs.

For most O/W NEs, the main mechanism of reduction of the free energy of the system consists in Ostwald ripening (or isothermal distillation), *i.e.*, growth of droplets of the internal phase in such a manner that small droplets dissolve, whereas large droplets grow.[15]

In the formation of small droplets, the chemical potentials of their components increase, depending on the radius of curvature of the droplet, by

$$\Delta\mu = \frac{2\sigma V_m}{r}, \quad (3)$$

where V_m is the molar volume of the dispersed phase.

Therefore, as the droplet size of the internal phase decreases, their solubility in the dispersion medium increases:

$$c(r) = c_0 \exp\left(\frac{2\sigma V_m}{rRT}\right), \quad (4)$$

where c_0 is the solubility of the compound in the phase with the flat boundary and R is the universal gas constant.

Even if the degree of polydispersity of NE is rather low, there is some size distribution of droplets of the internal phase and, hence, the chemical potentials inside the droplets are different. This results in Ostwald ripening of NE, which inevitably leads to subsequent emulsion breakdown.

There are methods to decrease the rate of the Ostwald ripening in macroemulsions and NEs. For instance, this can be attained using a compound less soluble in the dispersion medium as the internal phase. Taking O/W emulsions with hydrocarbons of various molecular masses as examples, it was shown that the stability of the emulsions against the Ostwald ripening significantly increases with decreasing the hydrocarbon solubility in water as dispersion medium.[16]

A more efficient method was also proposed.[17] It was demonstrated that the use of a binary internal phase (solubility of the second component is much lower than that of the first component) decreases the rate of the Ostwald ripening. The more soluble compound is faster transferred from smaller to larger droplets. As a result, the composition of the particles is changed, *viz*., the concentration of the poorly soluble component increases in small droplets and decreases in large ones. Now diffusion of the less soluble component becomes the rate-limiting step, and the emulsion becomes kinetically stabilized against the Ostwald ripening.[18]

The Ostwald ripening in reverse NEs can be stopped by adding an electrolyte to the internal phase. Water diffusion from smaller to larger droplets of the internal phase leads to an increase in the electrolyte concentration in the smaller droplets and, therefore, an increase in the osmotic pressure. This prevents further water transfer, and the Ostwald ripening ceases. The amount of electrolyte sufficient to stop the Ostwald ripening in reverse emulsions can be found from the pressure balance in a polydisperse reverse NE.[19]

Despite the fact that both direct and reverse NEs are kinetically stable disperse systems, they do not undergo breakdown for several months or even years.

Comparing nano- and microemulsions, one can conclude that the thermodynamic stability of the latter is their undoubted advantage. However, microemulsions are formed at much higher surfactant concentrations which is undesirable, especially, in medicine.

Nanoemulsions differ from macroemulsions in appearance, namely, NEs with a droplet size of about 100 nm are semi-transparent and exhibit slight opalescence (Fig. 1); as the droplet diameters further decrease, NEs become transparent.[20] This fact predetermines an additional interest in NEs in their utilization in pharmaceutical and cosmetic industries.

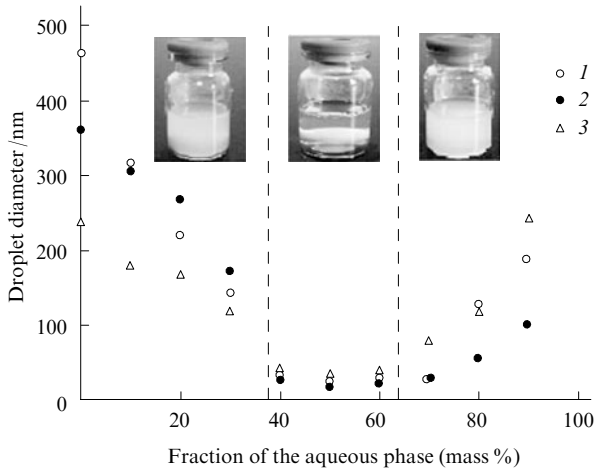


Figure 1. The droplet sizes and appearance of O/W nanoemulsions as functions on the fraction of the aqueous phase at oil : surfactant ratio equal to 0.9 (*1*), 1.0 (*2*) and 1.1 (*3*).[20]

An increase in the fraction of the internal phase makes the system more viscous. Photographs of O/W NEs with different fractions of the organic phase are shown in Fig. 2.[21]

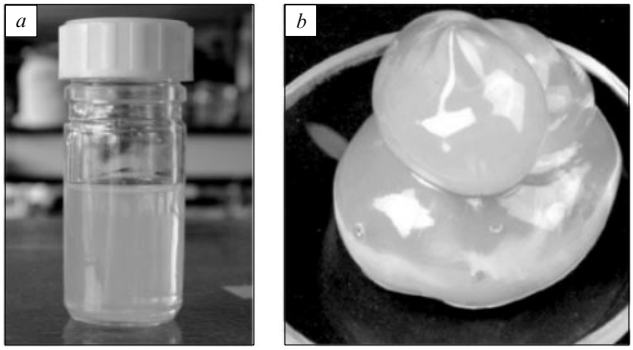


Figure 2. The appearance of direct nanoemulsions. The fraction of the internal phase is 15 (*a*) and 25 vol.% (*b*).[21]

III. Methods of nanoemulsion preparation

Nanoemulsions are prepared using low-energy, high-energy and combined methods. The high-energy methods include high-shear stirring, ultrasonic emulsification, high-pressure homogenization, in particular, microfluidics and membrane emulsification. The most widely used low-energy methods include the PIT method, the emulsion inversion point (EIP) method and the spontaneous emulsification in nonequilibrium systems. The combined methods include high-shear stirring to obtain macroemulsions containing special substances (so-called nano-emulsifiers) as the first step. Subsequent diffusion of nano-emulsifiers through the

interphase boundary results in the formation of NE droplets.

In the early stage of studies on NEs, researchers tried to prepare them by the high-energy methods only, high-energy stirring and ultrasonic emulsification being most widely used. Then, apparatuses for preparation of NEs under high-pressure treatment were produced.

Recently, interest in the low-energy methods has grown considerably because they are 'soft', non-destructive and cause no damage to encapsulated molecules. In addition, these methods are more energy-saving and therefore more attractive for large-scale production.

1. High-energy emulsification methods

When NEs are prepared by the high-energy methods (Fig. 3), the droplet size of the internal phase often exceeds the nanometre range. This is first of all due to high energy consumption necessary for dispersion, especially in the case of viscous systems. Also, an increase in the droplet size can be due to insufficient amount of surfactant to achieve the complete adsorption of all droplets formed upon dispersion on the interface. In this case, coalescence occurs in the system, and the average size of droplets increases.

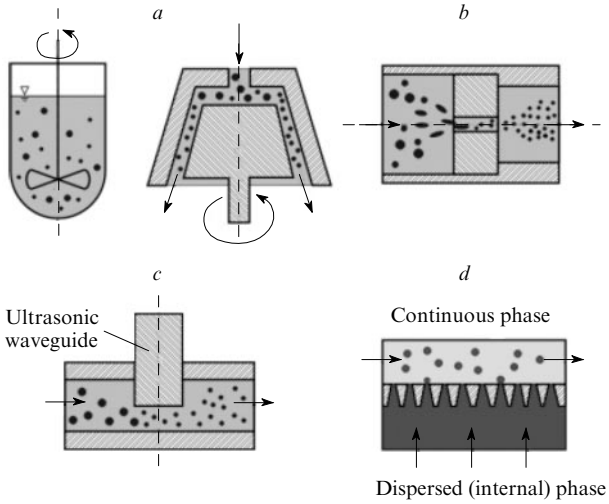


Figure 3. Schematic representation of the main high-energy emulsification methods:[22] rotor–stator systems (*a*), high-pressure homogenization (*b*), ultrasonic emulsification (*c*), and membrane dispersion (*d*).

An important factor affecting the final size of droplets is the adsorption velocity of the surfactant. If it is lower than the coalescence velocity, the droplets in the emulsion will grow after completion of the dispersion process even if nanometre-size droplets have been formed and there is sufficient amount of surfactant. This factor is particularly important for reverse emulsions which are stabilized using oil-soluble surfactants, because the velocity of surfactant diffusion towards the interface in the organic phase is much lower than in the aqueous phase.[23]

a. High-shear stirring

Initially, high-energy mixers and rotor-stator systems were used for the preparation of NEs. By increasing the mixing intensity in these devices one can significantly decrease the droplet size of the internal phase, but preparation of emulsions with the average droplet size less than 200–300 nm is rather difficult.

The process can be carried out in the batch regime in conventional mixers. To realize a continuous mode and to increase the shear stress upon dispersion, colloid mills are employed. Among them, the most popular are Silverson flow mixers in which rotors and stators have different configurations to achieve more efficient emulsification.[24]

At high rotor speed, a high rarefaction is created inside the disintegrating head, and the emulsion components are sucked in the rotor–stator unit. Under the action of centrifugal force, the emulsion is thrown away to peripheral areas, and intense dispersion occurs in the gap between the rotor and the inner wall of the stator. Then, the emulsion passes through the outer orifice of the stator at high speed and exits the apparatus. Modern apparatuses are designed in such a manner that the degree of aeration during emulsification be very low. Often, the maximum degree of dispersion possible for the system is not attained in the single-pass regime; therefore, the multi-pass regime is used conventionally.

When viscous media are used, the efficiency of high-shear stirring considerably decreases[25,26] and emulsions with a high fraction of the internal phase and a droplet size exceeding 1 μm are formed.

b. Ultrasonic emulsification

Nanometre-size emulsion droplets are formed under sonication due to cavitation bubble collapse and local release of a substantial amount of energy.[27,28] An increase in ultrasonic power to a certain limit results in generation of smaller droplets of the internal phase.[29] Further increase in the ultrasonic power leads to insignificant changes in the droplet size (Fig. 4).

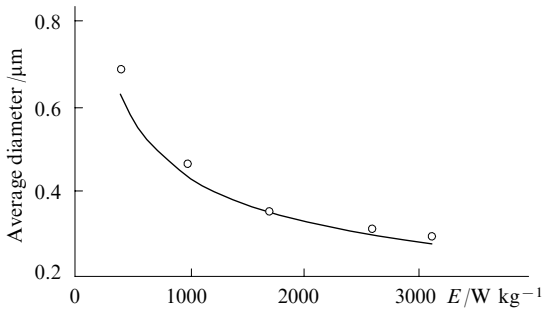


Figure 4. Average diameter of droplets of the internal phase plotted *vs.* specific energy of sonication (per unit mass of emulsion).[29]

The use of high-energy sonication is undesirable because it can result in thermal decomposition of water into radicals H$^{\bullet}$ and $^{\bullet}$OH whose action may cause decomposition of surfactant molecules collected on the surface of cavitation bubbles.[30,31]

Effective emulsification occurs in the immediate vicinity of the waveguide radiator only. Emulsification of large volumes requires mechanical stirring of the mixture. Therefore, it is most appropriate to employ this method for the

preparation of NEs for research purposes or small batches of NEs.

O/W emulsions called NEs[32] were prepared under sonication. The aqueous phase of the emulsions consisted of aqueous solution of serum protein and maltodextrin while the organic phase was formed by *d*-limonene. It was shown that the average droplet size decreased with the emulsification time, the minimum average droplet diameter of the internal phase being 243 nm.

Preparation of O/W NEs consisting of 15 vol.% flaxseed oil, 5.6 vol.% polyoxyethylene(20)sorbitan monopalmitate (Tween 40) and 79.4 vol.% deionized water under sonication was described.[33] Preliminary emulsification of the mixture was carried out in a mechanical mixer at 13 500 rpm and then the mixture was sonicated; as a result, droplets with an average diameter of 135 ± 5 nm were formed.

A O/W NE with a droplet size of 60–70 nm was prepared under sonication.[34] The NE droplets consisted of the inner core (of hexadecane), stabilizing shell (multilayer lamellar structures comprising phospholipids, cationic and nonionic surfactants) and hexadecane solubilized between the lamellar bilayers.

The degrees of dispersion of direct emulsions prepared by high-energy stirring and under sonication using the Ultra-Turrax (10 000 rpm) and Misonix Sonicator XL 2020 (20 kHz, 130 W) apparatuses, respectively, were compared.[35] The organic phase was kerosene and the stabilizer was polyoxyethylene(20)sorbitan monostearate (Tween 60) or its analogue (Montanox 60). It was shown that at equal duration of the emulsification process, direct emulsions with smaller droplets of the internal phase are formed under sonication (Fig. 5).

Thus, ultrasonic emulsification allows one to prepare emulsions with relatively small average droplet diameters of the internal phase; however, larger droplets (more than 100 nm) are also formed quite often.

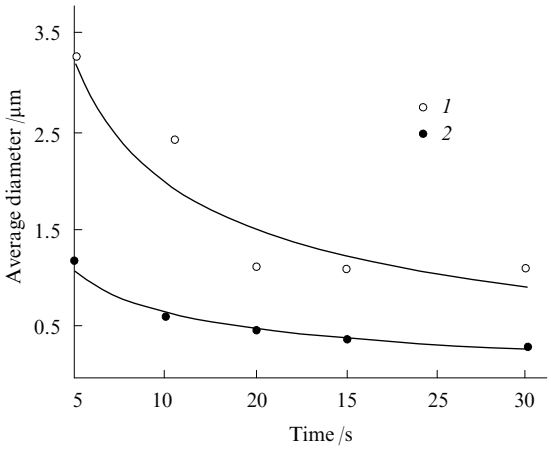


Figure 5. Average diameter of droplets of the internal phase plotted *vs.* the time of mechanical (*1*) and ultrasonic (*2*) dispersion ($\varphi = 0.25$, the surfactant concentration is 10 g litre^{-1}).[36]

c. High-pressure homogenization

High-pressure homogenization is most often used for emulsification of systems of low and medium viscosity. High-pressure systems are continuously operated. With respect to the type of flow directions in the dispersion cell, the homogenizers are divided into radial diffusers, jet dispersers and nozzle devices.

In a conventional radial diffuser, the emulsion flow deviates by 90° from the starting direction. A mobile valve makes it possible to vary the slit width and thereby to control the flow rate of the emulsified liquids and the applied pressure. Homogenizers with flat valve are most often used in the industrial manufacture of emulsions (Fig. 6*a*). Homogenizers of various designs are created for laboratory studies, particularly, with tooth-valves of various configurations, with inclined seats deviating the flow to different angles (Fig. 6*b*), with cutting-edge valves for an additional deviation of flows (Fig. 6*c*), *etc.*[36]

The operation principle of the capillary-jet homogeniz-

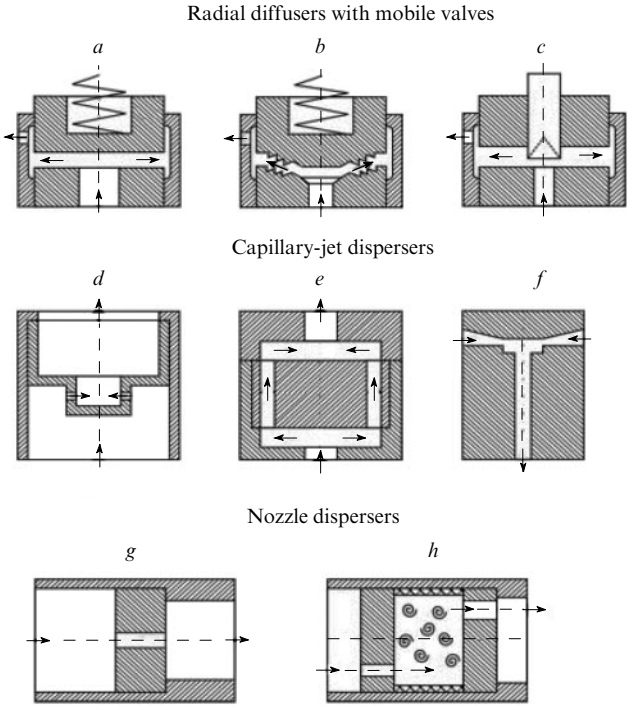


Figure 6. Schematic representation of different types of high-pressure homogenization systems.[22]
For notations (*a*–*h*), see text.

ers is based on collision of two or more flows moving at high velocity. Of this type of devices, the best known are a jet disperser (Fig. 6*d*),[37] various designs of microfluidizers from Microfluidics Corp. (Fig. 6*e*),[38] and the 'Nanojet' device (Fig. 6*f*).[39] At present, apparatuses from Microfluidics Corp. are often used for industrial and laboratory preparation of emulsions (for details, see below).

In nozzle aggregates, flow streams usually move either codirectionally (Fig. 6*g*) or along different axes (Fig. 6*h*). Recently, combined orifice valves have been designed; they include elements of nozzle aggregates and a mobile baffle valve (as in radial diffusers); as a result, dispersion is intensified.[40]

The jet and nozzle dispersers have no moving parts; this allows one to produce higher pressures compared with radial diffusers.

Under high-pressure homogenization conditions, the system is simultaneously affected by shear forces, turbulence and cavitation. The droplet size in emulsions is determined by the fluid flow regime (it depends on the valve and outlet nozzle design), the fluid viscosity and the pressure produced in the homogenizer. There are homogenizers operating in a wide pressure range from 10 to 350 MPa. The width of the slit between the plunger and the outlet nozzle is 10 – 100 μm; therefore, the flow velocity of the fluid is very high and amounts to hundreds of metres per second. The residence time of the emulsion droplets in the slit is of the order of a few milliseconds.[24]

The time necessary to attain equilibrium adsorption of surfactants is usually longer than the time of formation of new droplets under such dispersion conditions;[41] therefore, surfactants characterized by a very high rate of adsorption at the interface should be used for preparation of NEs. The overall retention time of emulsions in the dispersion zone is short and the droplets formed rapidly exit the zone of turbulent flow and enter the zone of laminar flow where insufficiently stabilized droplets coalesce. Coalescence of droplets in high-pressure homogenizers can be made considerably slower using surfactants with high adsorption rate, but it cannot be stopped. That is why high-pressure homogenizers operate in the multipass regime.

If there is a sufficient amount of surfactant in the system, the high-pressure homogenization results in emulsions with the droplet size of the internal phase from 50 to 350 nm.[42 – 44]

Figure 7 shows a photomicrograph of a O/W NE with a droplet size of about 50 nm.[21] Such NEs consisted of aqueous glycol solution, various cosmetic oils (triglycerides, ethers, hydrocarbons, silicones) and were stabilized by a mixture of PEG-8 isostearate and disodium stearoyl glutamate. Dispersion was carried out in a homogenizer operating at a pressure of 120 MPa.

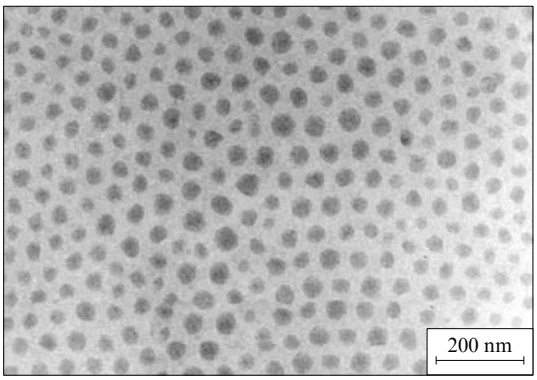


Figure 7. Photomicrograph of direct emulsion prepared in a high-pressure homogenizer.[21]

Direct emulsions containing medium-chain triacylglycerides or tripalmitins (10%), Tween 80 (4%) and water (86%) prepared using an 'EmulsiFlex-C5' high-pressure homogenizer have been studied.[45] Figure 8 shows the average droplet diameter plotted *vs.* pressure in the homogenizer at a constant number of passes through the homogenizer. The smallest droplet size was 89 nm for the emulsion of

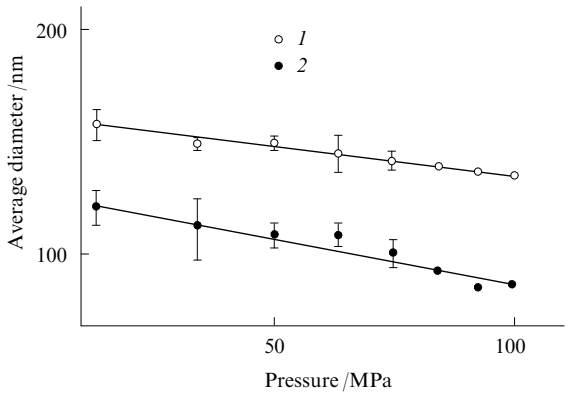


Figure 8. Average diameter of droplets of the internal phase plotted *vs.* pressure in homogenizer.[45]
The organic phase of the emulsion: tripalmitins (*1*) and triacylglycerides (*2*).

triglycerides in water and 126 nm for the emulsion of tripalmitins.

The average droplet diameter in NEs is plotted *vs.* number of passes through the homogenizer at a pressure of 50 MPa in Fig. 9. The average retention time of droplets in the apparatus during one pass was 0.001 – 0.01 s. As can be seen, the average droplet diameters remained practically unchanged after 5 and more passes.

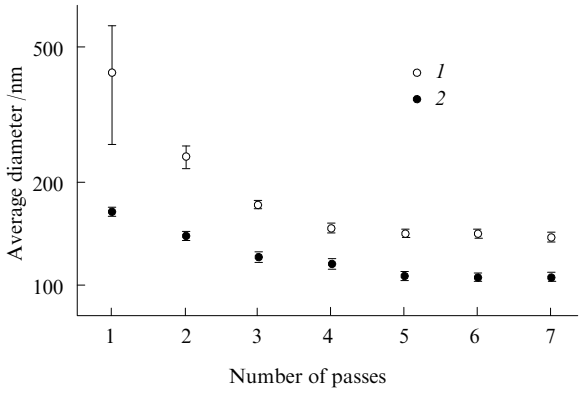


Figure 9. Average diameter of droplets of the internal phase plotted *vs.* number of passes through a homogenizer at 50 MPa.[45]
The organic phase of the emulsion: tripalmitins (*1*) and triacylglycerides (*2*).

It was also found[46] that the average droplet diameter in an O/W emulsion also decreases with increasing the number of passes through the homogenizer operating at 50 and 90 MPa; the droplet size was a few hundreds of nanometres.

It should be noted that an increase in pressure in the homogenizer does not alway lead to a decrease in the droplet size in the emulsion formed. An example is provided by the results of a study[47] of direct emulsions consisting of 20 vol.% sunflower oil, 0.75 vol.% methylcellulose and 79.25 vol.% water. Preliminary emulsification was carried out in an Ultra-Turrax colloid mill to obtain rough emulsions. Subsequent dispersion was performed in a Stansted homogenizer operating at pressures up to 350 MPa. The

average droplet size of the internal phase in the initial coarse emulsion was about 30 μm. It decreased after high-pressure homogenization, and emulsions with the droplets size of the internal phase of < 1 μm and small polydispersity were obtained at a pressure of 150 MPa. Further increase in pressure up to 300 MPa led to an increase in the droplet size (Fig. 10).

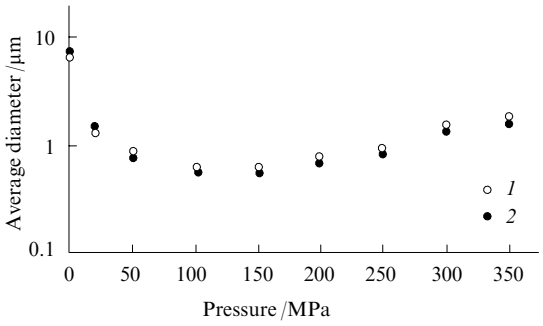


Figure 10. Average diameter of droplets of the internal phase plotted *vs.* homogenizer pressure.[47]
The initial temperature of the dispersion: 20 (*1*) or 5 °C (*2*).

The fact that the average droplet size in the emulsions prepared in the Stansted homogenizer did not exceed a value of 400 nm[47] was explained by the laminar regime of the fluid flow in the course of dispersion. The increase in the average droplet size of the internal phase could be due to destruction of methylcellulose molecules under the action of high pressure and to a local increase in temperature in the homogenizer. Direct emulsions stabilized by whey proteins, which were prepared in the same homogenizer, showed that an increase in the fraction of the internal phase in the emulsion led to an abrupt increase in the average diameter from about 400 nm at $\varphi = 0.1$ to 1.5–2.5 μm at $\varphi = 0.5$.[48]

High-pressure emulsification can also be used to prepare[49] reverse emulsions, in particular, W/O emulsions containing aqueous insulin solution in the internal phase whose fraction was nearly 30 vol.%. The emulsions were stabilized by a mixture of C_8–C_{12} triglycerides and soy lecithin. Homogenization was initially carried out under mechanical stirring at 6000 rpm for 30 s and then in the Micron Lab 40 high-pressure homogenizer at 50 MPa. This treatment gave stable reverse emulsions with the droplet sizes from 30 to 800 nm. The sizes of some droplets of the internal phase corresponded to the range typical of NEs; however, the fraction of larger droplets was rather high.

Thus, NEs can be prepared using high-pressure homogenizers. However, it is necessary to take into account that the droplet size abruptly increases when the fraction of the internal phase or the viscosity of the system being emulsified is high.

d. Microfluidic and membrane methods

Microfluidic methods make it possible to carry out controlled formation of liquid droplets and gas bubbles and to transport them in microchannels. Recent extensive development of microfluidics methods is connected with the possibility to create a lab-on-a-chip.[50, 51] Such devices make it possible to miniaturize chemical and biochemical processes by transporting microquantities of a liquid in the form of drops and bubbles through a system of microchannels to a specified place and to perform, for instance, a quick analysis of the drop content then. Studies on realizing such devices for isolation of proteins,[52] analysis of DNA,[53] enzymes,[54] for encapsulation of cells,[55] making of biosensors[56] are now in progress.

In microfluidizers, emulsification occurs through collision of the flow streams of two immiscible fluids moving in microchannels under high pressure.[57] The stability of the emulsification regime depends on the wetting of the channel walls by the emulsion components. Usually, W/O and O/W emulsions are produced in devices with hydrophobic and hydrophilic surfaces, respectively.[58, 59] The wetting angle can be controlled by choosing the microchannel material or using various surface modifiers including surfactants.

Dispersion in these homogenizers occurs under the action of shear deformation, impact stress and cavitation.[60]

There are three basic options for arrangement of flow streams in microfluidizers, namely, co-directional, in opposite directions and their T-shaped intersection.

Co-directional flow stream motion is implemented in the devices with coaxial arrangement of a capillary and a tube (Fig. 11 *a*). This efflux geometry is called co-flow. If the efflux velocity of the fluid is low, single almost monodisperse droplets are formed. With increasing the efflux velocity, a jet is formed at the capillary outlet; in this case, the droplet size distribution is wider.

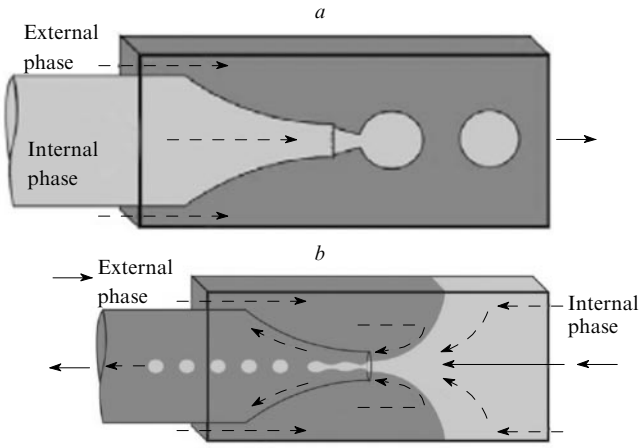


Figure 11. Schematic representation of microfluidizers with codirectional (*a*) and oppositely directed (*b*) flow streams.[61, 62]

Flow stream motion in opposite directions is realized in the devices called 'flow-focusing'. In this case, two liquids are fed from different sides, the external phase captures the internal phase and enters a narrow capillary (Fig. 11 *b*). Smaller droplets are formed with this geometry.[63]

T-Shaped intersection of microchannels was used in the first microfluidizers.[64–67] In this case, two fluid flow patterns are possible, *viz.*, the continuous phase flows in the horizontal channel while the internal phase flows in the vertical channel or *vice versa* (Fig. 12). In the first case, droplets with a narrower size distribution are formed.

Intersecting flow geometry was implemented in the microfluidizers used in more recent studies (Fig. 13).[69]

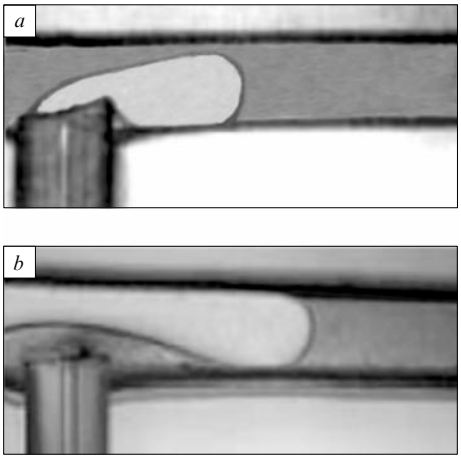


Figure 12. Fluid flow patterns in T-shaped microfluidizers.[68]
The dispersion medium flows in the horizontal channel and the disperse (internal) phase flows in the vertical channel (*a*) and *vice versa* (*b*).

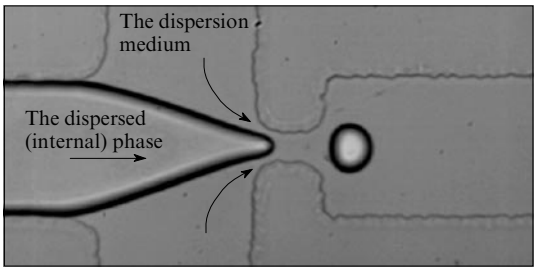


Figure 13. Formation of droplets in the crossing flow streams.[69]

By and large, microfluidizers are characterized by high performance and allow one to prepare almost monodisperse emulsions with controlled droplet size of the internal phase.[70] For instance, the standard deviation of the droplet diameter in the internal phase from the average value does not exceed 2% for the devices with T-shaped geometry and is less than 5% for the oppositely directed flows [71] and less than 3% for the co-directional flow stream motion.[72]

The droplet size depends on the properties of the liquids being emulsified, the flow regime, channel geometry and on the properties of the channel surfaces. The droplet size decreases with decreasing the flow velocity and the viscosity of the continuous phase and increases with increasing the flow velocity of the internal phase.[73–75] For co-directional channels, it was established that if the viscosity of the internal phase is lower than some threshold value (100 mPa s), the droplet size decreases with increasing viscosity; if the viscosity of the internal phase is higher than the threshold value, the droplet size significantly increases with increasing viscosity.[76]

To reduce the droplet size, various flow intersection designs with different junction angles are used.[77] However, preparation of NEs using such microfluidizers is impossible because the droplet size in the emulsions is of the order of a few micrometres.

Nanoemulsions are prepared in microfluidizers with a bifurcation zone.[77–84] Initially, droplets are formed in, for example, microfluidizers with T-shaped intersection of channels. The droplets move along the channel and reach the bifurcation point where they are divided into two daughter droplets. If the flow rates in two channels are equal, two droplets of the same volume are formed. By varying the flow rate ratio in the channels one can substantially reduce the droplet size in one flow stream as compared with the droplet size in the other flow stream. This occurs, for instance, when the pressure increases at one of the two outlets,[79] under the action of electric current,[80] under the mechanical action of a valve,[81] upon changes in the microchannel geometry.[82]

A fairly simple method of preparation of almost monodisperse small droplets consists in control of the fluid flow rates using a 'tuning flow' of the continuous phase fed into one of the two channels.[78] A schematic diagram and photomicrographs of the droplets formed in such a device are shown in Fig. 14.

When no 'tuning flow' was used, the flow rates in both channels were equal, and droplets of the same size formed (Fig. 14 *a*). If the continuous phase was introduced into one channel, the volumetric flow rates in both channels changed, and droplets of different volume formed (Fig. 14 *b*,*c*). By varying the flow rates of the liquids in the channels one can produce droplets of a desired size.

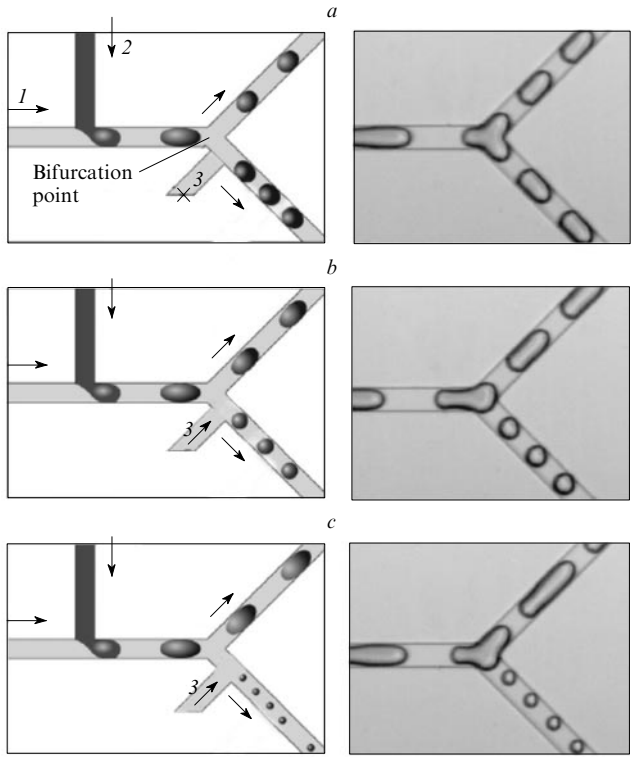


Figure 14. Schematic diagrams showing the control of droplet division at the bifurcation point of microfluidic channels (on the left) and photomicrographs showing droplet division (on the right).[78]
The tuning flow rate of the continuous phase is 0 (*a*), 15 (*b*) and 30 μl h^{-1} (*c*). Shown are the channels for supply of the continuous (*1*) and disperse (*2*) phases and the 'tuning flow' channel (*3*).

The devices produced by Microfluidics Corp. use multiple separation and mixing of flow streams (Fig. 15); this makes it possible to significantly reduce the size of droplets formed.

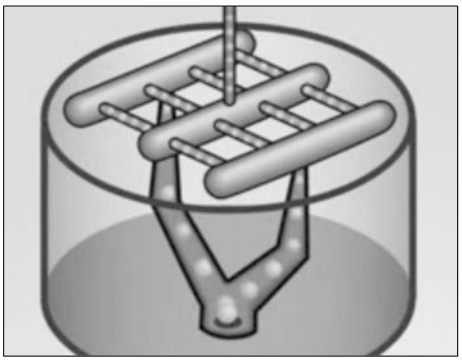


Figure 15. Schematic diagram of channels in 'Microfluidizer® Processor' (see http:\\www.microfluidicscorp.com).

On its website, Microfluidics Corp. presents data, according to which the particle size of liposomes processed using an Avestin high-pressure homogenizer is twice as large as that obtained using a 'Microfluidizer® Processor'. In the latter case, the particle size equal to 113 nm after one pass decreased to 95 nm after two passes and to 72 nm after three passes.

Direct emulsions were obtained using a Model M-110 L (Microfluidics, USA) microfluidizer.[32,85] The aqueous phase of the emulsions was a solution of whey protein concentrate and maltodextrin while *d*-limonene was used as the organic phase. It was shown that the average droplet diameter changed insignificantly with an increase in the pressure in the microfluidizer. At low pressures, an increase in the number of passes through the microfluidizer led to reduction of the average droplet diameter, for instance, from 387 to 125 nm after three passes at 35 MPa.

Preparation of O/W NEs with high concentration of the internal phase in a microfluidizers was reported.[86] The emulsions were prepared from polydimethylsiloxane, sodium dodecyl sulfate and water. First, coarse direct emulsions with an average droplet diameter of 10 μm were prepared by high-shear stirring. Then, they were passed through a microfluidizer. The influence of various parameters on the droplet size in the emulsions prepared was studied. Figure 16 *a* shows the dependence of the average droplet diameter on the number of passes through the microfluidizer. After the first pass, the droplet diameter decreased approximately by two orders of magnitude. With increasing the number of passes, the average droplet diameter continued to decrease, but to a lesser extent.

In a single-pass case, the NE droplet diameter depends on the operating pressure in the microfluidizer. The higher the pressure the higher the shear rates produced in the device; this led to formation of droplets with smaller diameters (Fig. 16 *b*). An increase in the fraction of the internal phase to about 0.4 resulted in a slight increase in the average droplet diameter (Fig. 16 *c*). Further increase in φ was accompanied by abrupt enlargement of droplets of the internal phase. Attempts to prepare NEs with the

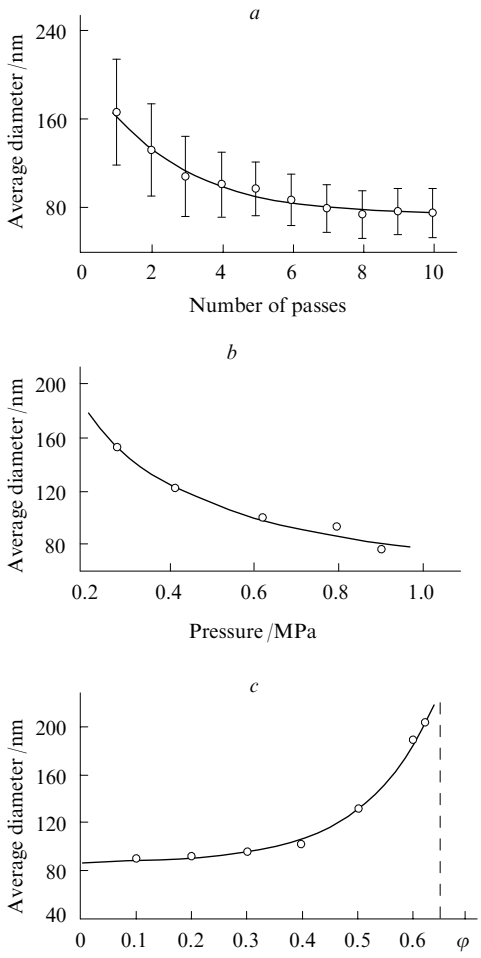


Figure 16. Average diameter of droplets of the internal phase in emulsions plotted *vs.* number of passes through a microfluidizer (*a*), pressure in the microfluidizer (*b*) and fraction of the internal phase in the emulsion (*c*).[86]
The viscosity of the organic phase is 10 mPa s, the fraction of the internal phase is 0.2 (*a*, *b*), the number of passes through the microfluidizer is 1 (*b*, *c*) and the pressure is 0.28 MPa (*a*) or 0.62 MPa (*c*). Dashed line indicates the limiting φ value; at higher φ values, the phase inversion occurred.

fraction of the internal phase higher than 0.64 failed because of the phase inversion.

It was shown that the average droplet diameter decreased logarithmically with increasing pressure in the apparatus.[87] Also, the droplet size depended on the type of emulsifying agent and increased in the following series:

sodium dodecyl sulfate < Tween 20 < β-lactoglobulin < < sodium caseinate,

where Tween 20 is polyoxyethylene(20)sorbitan monooleate. This fact was explained by faster absorption of smaller surfactant molecules.

A comparison of direct emulsions prepared using a microfluidizer and by ultrasonic emulsification was reported.[33] The emulsions consisted of flaxseed oil, Tween 40 and water. Coarse emulsions with a droplet size of 8.37 μm prepared using a Silverson mixer were then passed through a Microfluidics M-110Y microfluidizer at a

pressure of 100 MPa. The second type of emulsion with a droplet size of 6.4 μm was also prepared in the Silverson mixer. Then, it was passed through a 330-W ultrasonic flow cell at a flow rate of 20 ml min^{-1}. The dependences of the average droplet diameter on the number of passes through the devices are shown in Fig. 17. It is seen that the average droplet diameter decreased after a single pass through the ultrasonic cell and changed only slightly after subsequent passes. On the contrary, the average diameter of droplets that passed through the microfluidizer decreased after each pass and became equal to nearly 118 nm after six passes.

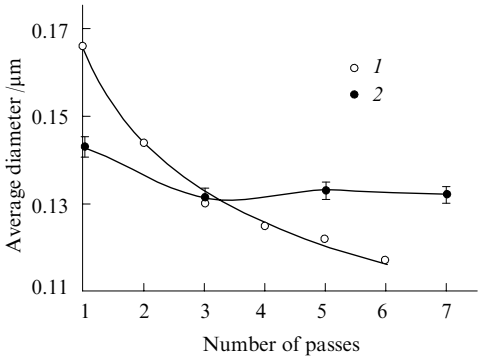


Figure 17. Average droplet diameter plotted *vs*. number of passes through microfluidizer (*1*) or ultrasonic disperser (*2*).[33]

Microfluidizers can also be used for the preparation of multiple emulsions.[50, 58, 88, 89]

Although microfluidizers allow one to prepare almost monodisperse droplets, they have some disadvantages. Their manufacturing is expensive compared to other high-energy devices. They are less handy in practical use because channels can be blocked by solid particles and gas bubbles.[90] Also, problems arise concerning sterilization of microfluidizers when they are used to prepare emulsions for medicine and pharmacology.

Membrane methods of emulsification, like the microfluidic methods, belong to methods of formation of individual droplets unlike emulsification in turbulent regime under discontinuity of the fluid flow.[91] In the membrane methods, droplets of the internal phase are formed by extrusion of a fluid through many pores or microchannels in a membrane (Fig. 18).[92, 93]

In one-step emulsification, droplets are formed at the membrane/continuous phase interface when the internal phase is extruded through the membrane. To prepare emulsions with smaller droplets of the internal phase, membrane emulsification is carried out after preliminary dispersion, *i.e*., a coarse emulsion is extruded through a membrane whose pore size is smaller than the droplet size of the coarse emulsion. Pumping the coarse direct emulsion through a hydrophilic membrane leads to decrease in the droplet size. If the coarse direct emulsion is pumped through a hydrophobic membrane, the phase inversion occurs resulting in the formation of a fine reverse emulsion.[94]

If the membrane is fixed in a device, usually, either recirculation or stirring of the formed emulsion is carried out for faster detachment of droplets from the surface. Devices with rotating or vibrating membranes are used to accelerate the detachment of droplets; however, this significantly complicates the designs of apparatuses.

Usually, the average diameter of droplets in the prepared emulsions is approximately equal to the pore diameter[95] or 1.5–3.0 times larger.[94, 96] Theoretical calculations[97] revealed a linear correlation between the droplet diameter and the pore size including the nanometre range. Emulsions with droplet sizes of 200–300 nm were prepared using planar cellulose acetate[98] and polycarbonate[99] membranes. The preparation of NEs using a tubular membrane made of hydrophilic microporous glass SPG (Shirasu porous glass membrane) was reported.[100] Despite the fact that the average pore diameter in the membrane was 2.5 μm, the authors succeeded to prepare NEs in which droplets of the internal phase were 50–70 nm in diameter.

The major disadvantage of the membrane emulsification methods is their moderate productivity because the extrusion rate of the internal phase should be sufficiently low to prevent the formation of continuous jets flowing from the membrane pores.[93, 101] One-step membrane emulsification is most efficient for preparation of emulsions with the fraction of the internal phase up to 30 vol.%. In this case, emulsions have narrow droplet size distributions. The polydispersity of droplets increases with an increase in the fraction of the internal phase.

Preliminary emulsification makes it possible to increase the efficiency of the process by an order of magnitude and more; in addition, emulsions with smaller droplets are formed. If membrane emulsification with the phase inversion is carried out, the fraction of the internal phase can be

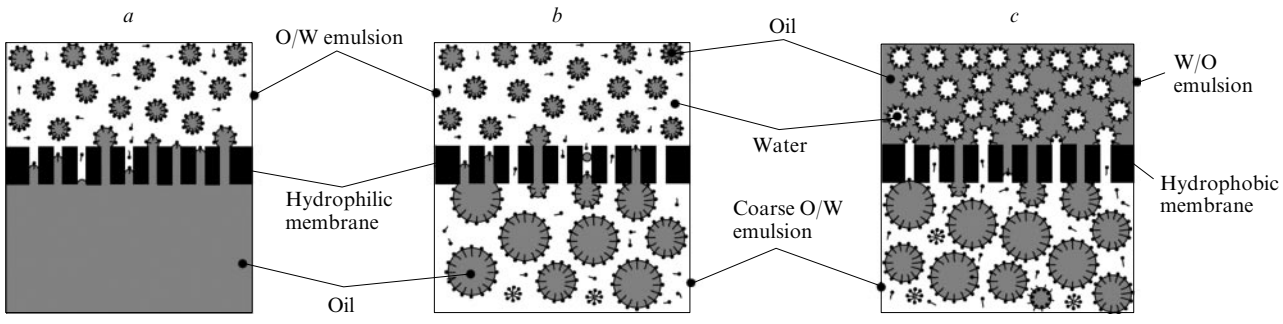


Figure 18. Various designs of membrane emulsification.[92]
One-step emulsification (*a*), with pre-emulsification (no phase inversion) (*b*) and with pre-emulsification and phase inversion (*c*).

increased substantially. Both direct and reverse emulsions with the fractions of the internal phase of 0.90 and 0.84, respectively, were obtained in this way.[96]

In this Section, we considered the high-energy methods, which can be used to prepare emulsions with the droplet size in the nanometre range. Several parameters of various types of devices are presented in Table 2; these parameters make it possible to compare different methods in the degree of polydispersity of NEs formed, energy consumption and organization of the emulsification process.

Table 2. Qualitative characteristics of devices used in high-energy methods of emulsification.[102]

Device type	Droplet size distribution	Specific energy consumption	Process type
Conventional mixers	broad	low to moderate	batch/ continuous
Colloid mills	moderately broad	moderate to high	continuous
Ultrasonic	"	the same	batch/ continuous
High-pressure valve homogenizers	"	"	continuous
Microfluidizers	practically monodisperse droplets	low	"
Membrane devices	narrow	"	batch/ continuous

Undoubtedly, one of the most interesting methods is the microfluidic method, which provides almost monodisperse droplets and is characterized by relatively low energy consumption. This method is being developed intensively at present. However, O/W NEs for large-scale applications are most often prepared using high-output valve homogenizers.

2. Low-energy emulsification methods

The low-energy methods of preparation of NEs were developed much later than the high-energy ones. These methods are based on the phase inversion in emulsion upon a change in the composition or temperature. The principles suggested by Japanese researchers [103, 104] in the 1960s form the basis for the low-energy methods. The curvature of the surfactant adsorbed layer changes with temperature. At a certain temperature, the system reaches the state characterized by zero curvature of the surfactant monolayer and low interfacial tension (10^{-2}–10^{-5} mN m^{-1}).[105, 106] The phase inversion occurs under these conditions (Fig. 19).

A spontaneous change in the radius of curvature can also occur upon change in the composition of the system, for example, when the fraction of the aqueous phase or the concentration of other components changes. This corresponds to catastrophic phase inversion (see Fig. 19).

a. The phase inversion temperature method

The phase inversion temperature (PIT) method is based on the specific property of surfactants (especially, typical of nonionic ethoxylated surfactants), namely, the ability to change the water and oil affinity depending on temperature. The molecules of ethoxylated surfactants are hydrophilic at low temperatures due to hydration of polar groups. The surface area occupied by the hydrated polar groups is larger than that occupied by hydrophobic hydrocarbon chains. Therefore, the surfactant monolayer bends and O/W emulsions are formed. As the temperature increases, oxyethylene groups are easily dehydrated and the molecules become lipophilic. In this case, the surface area occupied by the hydrocarbon chains becomes larger than that occupied by the polar groups and the formation of W/O emulsions becomes more favourable.[108]

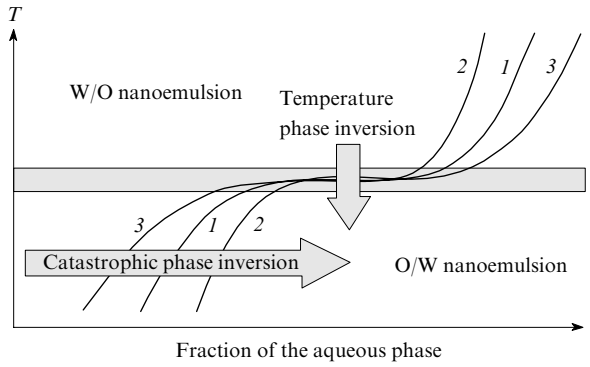


Figure 19. Schematic representation of temperature and catastrophic phase inversion in preparation of nanoemulsions.[107]
Curve (*1*) corresponds to the phase inversion conditions in the emulsions; curves (*2*) and (*3*) limit the hysteresis region of curve (*1*).

If a W/O emulsion stabilized by ethoxylated surfactant is prepared at elevated temperature and then cooled, the phase inversion occurs at a certain temperature, and an O/W emulsion forms (Fig. 20).

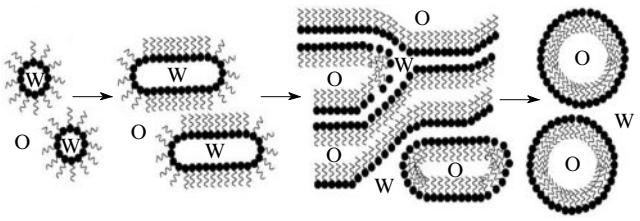


Figure 20. Transformations of droplets during the formation of direct nanoemulsion.[107]
'O' is oil and 'W' is water.

Ultralow interfacial tensions at temperatures close to the PIT facilitate the formation of small droplets, although their coalescence is also enhanced under these conditions.[108] Therefore, fast cooling of the system is necessary for the preparation of kinetically stable NEs with narrow droplet size distribution.[1]

Many researchers believe that NEs with smaller droplets form if the system represents a bicontinuous microemulsion or a conventional microemulsion (D) existing in equilibrium with isotropic aqueous phase or with the liquid-crystalline phase (L_α) at the temperature at which the emulsification begins.[109–113]

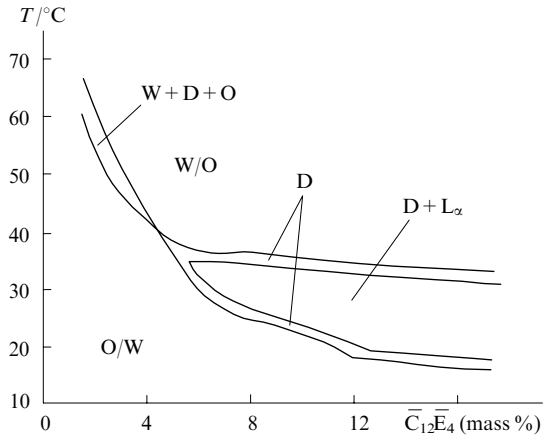


Figure 21. Phase diagram of the water – $\overline{C}_{12}\overline{E}_4$ – isohexadecane system.[113]
The concentration of isohexadecane is 20 mass %.
'W' is the isotropic aqueous phase, 'D' is microemulsion, 'L_α' is the lamellar liquid-crystalline phase and 'O' is the isotropic organic phase.

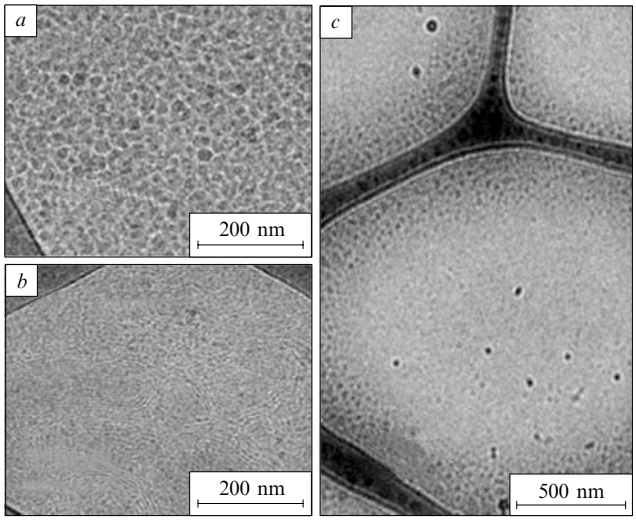


Figure 22. Photomicrographs of the microemulsion (*a*) and lamellar liquid-crystalline (*b*) phases at a temperature above the PIT and a nanoemulsion formed upon the phase inversion (*c*).[115]

The phase diagram of the water – $\overline{C}_{12}\overline{E}_4$ – isohexadecane [where $\overline{C}_{12}\overline{E}_4$ is polyoxyethylene(4)lauryl ether] system is shown in Fig. 21. Studies [113, 114] showed the following: if the process starts in the D, D + L_α or B + D regions, smaller droplets are formed. At lower surfactant concentrations in the system, emulsification begins in the B + D + M or B + D + L_α region, and larger droplets are formed.

Nanoemulsions consisting of lauryl acrylate, polyoxyethylene(10)oleyl alcohol (Brij 96V) and 10 mM aqueous NaCl solution were prepared.[115] If the system represented a microemulsion or a lamellar liquid-crystalline phase at a temperature above the PIT, its cooling led to formation of an NE with 25 – 50 nm droplets (Fig. 22).

Reduction of the polydispersity of droplets at higher concentrations of ethoxylated surfactants was revealed.[116] Other researchers believe that the formation of NE droplets is determined by the surfactant diffusion rather than is related to the structure the system has at the moment of emulsion formation.[117] An abrupt decrease in temperature causes the distribution coefficients of the surfactant between the organic and aqueous phases to change and spontaneous emulsification occurs upon surfactant diffusion to the aqueous phase.

It is believed [118] that the phase inversion due to the change in the curvature of the surfactant adsorption layer is not a necessary condition for NE formation in the PIT process, the existence of hydrodynamic shear strain is required. This idea is confirmed by the fact that preparation of NEs requires slow stirring (coarse emulsions are formed without stirring). It has been also shown that, to prepare NEs, it is sufficient to heat the system to the state with minimum turbidity (*i.e.*, up to temperatures 1 – 2 °C lower than the PIT) and then to cool it abruptly. The NEs of the same composition prepared by the conventional PIT method and by heating only to the state with the lowest turbidity were characterized by equal droplet sizes.

Nanoemulsions stabilized by ethoxylated surfactants were prepared in most PIT emulsification studies because the hydrophilic-lipophilic balance of these surfactants is most sensitive to temperature variations. However, NEs stabilized by other surfactants were also prepared by this method. For instance, these are NEs stabilized by a mixture of polyoxyethylene(20)sorbitan monooleate (Tween 80) and sorbitan monooleate (Span 80) taken in a 0.56 : 0.44 ratio by mass.[119] It was shown that the PIT decreased from 85 to 40 – 50 °C with increasing the electrolyte concentration in the aqueous phase. With respect to their efficiency in reducing the phase inversion temperature, electrolytes can be arranged as follows:

$$AlCl_3 \approx NaCl > KCl > CaCl_2 .$$

The droplet size in NEs depends on the temperature to which the system was cooled. For example, NEs with smaller droplets and lower degree of polydispersity were formed when the system was rapidly cooled to a temperature 25 – 30 °C lower than the PIT.[118, 120]

To increase the PIT in the system after preparation of NE and, therefore, to get more ultrafine NEs, it was suggested [109, 121] to introduce ionic or nonionic surfactants into NEs immediately after their preparation. For example, a NE stabilized by $\overline{C}_{12}\overline{E}_4$ was prepared by the PIT method and then an aqueous solution of Tween 80 or sodium dodecyl sulfate was added to the emulsion.[121] The surfactant added partially displaced $\overline{C}_{12}\overline{E}_4$ from the interface, and NEs more stable against coalescence formed. The amount of Tween 80 and sodium dodecyl sulfate should not exceed a few percent of the content of the main emulsifier. At higher concentration of the added surfactant, the rate of the increase in the average droplet diameter increased to the starting level.

The PIT method has been best explored at present. It is successfully used for the preparation of NEs stabilized by ethoxylated surfactants. However, this method is inapplicable when using ionic and nonionic surfactants whose hydrophilic – lipophilic balance is substantially less sensitive to changes in temperature. Some complications can emerge upon the use of the PIT method in industry because commercially available ethoxylated surfactants represent mixtures of various oligomers; this requires correction of

the concentration ranges and the temperature regime of emulsification.[122]

b. The emulsion inversion point (or the phase inversion composition) method

Another method of preparation of NE is based on the phase inversion upon a change in the composition of an emulsion system. It involves the introduction of substances changing the hydrophilic–lipophilic balance of the system at constant temperature. This results in spontaneous changes in the radius of curvature of the surfactant monolayers and in catastrophic phase inversion in the emulsion (see Fig. 19). For instance, the formation of water droplets in oil occurs upon gradual addition of the aqueous phase to the organic phase. An increase in the amount of the aqueous phase above a certain threshold value results in phase inversion, and droplets of the organic phase in water are formed.[123] The phase inversion can also occur upon the addition of electrolytes, other surfactants, alcohols, *etc*. to the system.[122] In the literature, this method is usually called the emulsion inversion point (EIP) or the phase inversion composition (PIC); sometimes, it is called 'titration'. This method was used[124] to prepare O/W emulsions and to measure the minimum amount of the aqueous phase required for the phase inversion in systems containing various mixtures of surfactants.

As in the case of the PIT emulsification, the formation of the smallest droplets by the EIP method requires that interfacial tension in the system be low upon the phase inversion, *i.e*., that the system be a microemulsion or a liquid-crystalline lamellar phase in the beginning of the process.[1,2] The degree of hydration of the surfactant molecules and, therefore, their affinity toward the aqueous phase increases with increasing the water/oil ratio in the microemulsion; this leads to instability of the microemulsion and its transformation to a nanoemulsion.

Diffusion of surfactants also plays an important role in the formation of nanodroplets. When direct emulsions are prepared by this method, the aqueous phase is added to a mixture of oil and a surfactant poorly soluble in the organic phase. The addition of the aqueous phase causes diffusion of the surfactant from the organic phase to the aqueous phase; this favours the formation of nanodroplets at low interfacial tension.[125]

The EIP method makes it possible to prepare NEs not only with ethoxylated but also with other types of surfactants. Nanoemulsions were stabilized by mixtures of ethoxylated surfactants including polyethoxylated castor oil (Cremophor EL) and poly(ethylene glycol) (15) hydroxystearate (Solutol HS 15);[126] by mixtures of nonionic surfactants such as Surfom R-400 and Span 80,[127] various Tween and Span;[128–131] by anionic sodium dodecyl sulfate;[132] and by cationic surfactants including tetracetyltrimethylammonium bromide and two ethoxylated amine derivatives, Noramox S2,[133,134] Noxamium S11M.[134]

Using contrast-variation small-angle neutron scattering, it was shown[135] that the internal phase of NEs composed of decane, polyoxyethylene (5) lauryl ether ($\overline{C}_{12}\overline{E}_5$) and water is represented by spherical droplets 20–25 nm in diameter. The thickness of the shells surrounding oil droplets was calculated using the core–shell model for decane–$\overline{C}_{12}\overline{E}_5$–$D_2O$ nanoemulsions; it appeared to be equal to 1.1 nm, which corresponds to the thickness of the adsorbed surfactant layer.

O/W NEs were obtained in the system consisting of 20 vol.% propylene glycol monocaprylic ester (Sefsol 218), 27 vol.% mixture of a surfactant Cremophor EL and a co-surfactant diethylene glycol monoethyl ether (Carbitol), and 53 vol.% water.[136] The droplet size of the internal phase in this NE was equal to 35–45 nm (Fig. 23).

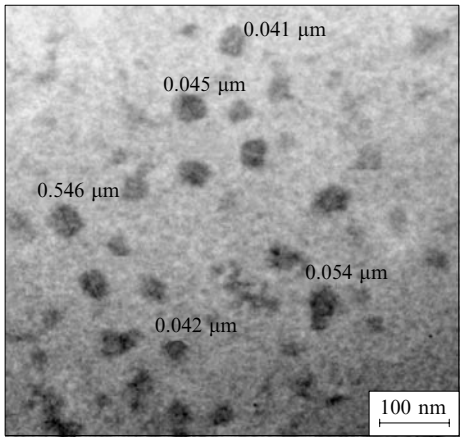


Figure 23. Electron microscope image of a nanoemulsion containing Sefsol 218 (20 vol.%), Cremophor EL/Carbitol mixture (27 vol.%) and water (53 vol.%).[136]

It should be noted that the NE in this system was formed in the presence of rather high concentrations of the surfactant and co-surfactant; this is atypical of NEs of other composition.

A comparison of the phase inversion temperature and phase inversion composition methods reveals the following advantages of the EIP method. First of all, this is the possibility to utilize a wider range of surfactants whose hydrophilic–lipophilic balance is less dependent on temperature. Next, there is no need to use temperature gradients, which is important when encapsulating thermally unstable substances.

However, the EIP method is very sensitive to changes in the composition of the mixture being emulsified. Therefore, the emulsification conditions should be rechecked when additional substances are introduced, for example, when drug encapsulation is carried out.

The PIT process can be repeated several times by increasing and decreasing the temperatures. The composition of the emulsion system can be changed only once (provided no additional steps considerably complicating the production process), for example, upon increase in the electrolyte concentration or the fraction of the aqueous phase in the emulsion.

c. Spontaneous nanoemulsification

Consider two liquid phases that are not in equilibrium with each other. In some cases, bringing them into contact with each other is followed by spontaneous emulsification.[137] Spontaneous emulsification was for the first time reported in 1878.[138] There are three principal mechanisms of this process:[139]

— the interphase instability (the Marangoni effect) induced by the surface tension gradient upon diffusion of

substances through the interface results in dispersion and formation of individual droplets;

— dispersion occurs as a result of a short-time decrease in the interfacial tension to almost zero; this is accompanied by spontaneous increase in the surface area of the interface;[140] and

— emulsification occurs when a new phase condenses in the local supersaturation areas.

The first two mechanisms are related to dispersion, *i.e.*, mechanical rupture of the existing interface. The third mechanism is due to the formation of a heterogeneous system from homogeneous one; a classical example is provided by toluene emulsification.[141] If a mixture of toluene and methanol is brought into contact with water, the solution of toluene in methanol diffuses into the aqueous phase. When a substantial amount of methanol has dissolved, toluene condensation occurs at some distance from the water surface and droplets are formed. This emulsification mechanism is called diffusive.[139]

An analogous situation was observed during diffusion of n-propanol from 2,3-dimethylpentane.[142] A photomicrograph of droplets formed at some distance from the interface, obtained using an optical microscope, is shown in Fig. 24.

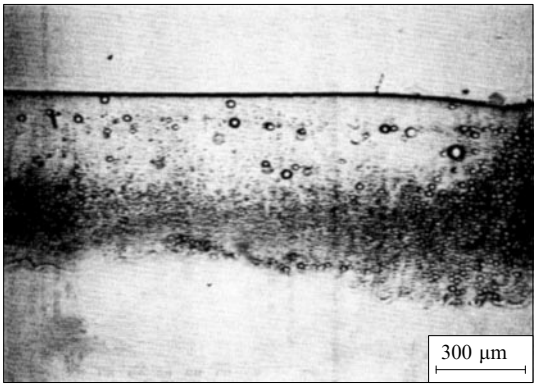


Figure 24. Photomicrograph of emulsion formed upon spontaneous emulsification at the interface between water and n-propanol (65%) – 2,3-dimethylpentane (35%) mixture at 35 °C.[142]

The mechanisms listed above are interrelated. Although mass transfer through the interface can be governed by one mechanism, other mechanisms also contribute to spontaneous emulsification. For instance, if the Marangoni effect takes place, the amplitude of waves on the interface depends on the interfacial tension (a decrease in the interfacial tension facilitates detachment of droplets).[139] If the third mechanism is realized in the case of diffusion through the interface, the interfacial instability does not result in spontaneous emulsification but accelerates the process if self-emulsification occurs.[142]

An interfacial instability due to a local decrease in the interfacial tension can be induced by nonuniform surfactant adsorption upon transfer of surfactant molecules through the interface[143, 144] if chemical reactions occur at the interface, especially, if the reactions result in the formation of surfactants.[145, 146] The type of an emulsion formed upon transfer of surfactant molecules depends on the direction of diffusion. If the surfactant is transferred from the organic to aqueous phase, a direct emulsion is formed, if it diffuses from the aqueous to organic phase, a reverse emulsion is produced.[147]

Ultralow interfacial tension are due to co-adsorption of two types of surfactants, for example, if one is well soluble in oil while the other is well soluble in the aqueous phase.[148]

The droplet size and the droplet size distribution in spontaneous emulsification depend not only on the interfacial tension, but also on the interfacial viscosity and on the viscosity of the dispersed phase, and the surfactant structure and concentration.[137]

A study of the strength properties of adsorption layers, interfacial structures and conditions for emulsification at the monomer/water boundary in the presence of surfactants of different nature and various emulsifying additives (alcohols with the alkyl chain of different length) showed[149] that, depending on the introduction conditions, the additives can either reduce the strength of the interfacial layers in microemulsions and suppress the emulsification process or, on the contrary, increase the strength of the adsorption layer and accelerate the emulsification.

The lower the interfacial tension and the viscosity of the organic phase the more intense emulsification of oil in the aqueous phase.[150] If the viscosity of the organic phase is high, detachment of droplets is more difficult. In addition, an increase in the viscosity causes a decrease in the diffusion rate of surfactant molecules toward and through the interfacial boundary and reduces the interfacial instability. Therefore, the formation of a gel-like or liquid-crystalline phase (especially, of the inverse type) resulted in enlargement of droplets formed when using self-emulsifying concentrates due to the reduction of the rate of penetration of the aqueous phase into the concentrate.[151]

The formation of an intermediate liquid-crystalline phase was necessary for spontaneous emulsification in other systems.[142] For instance, spontaneous emulsification occurred when a mixture of hydrocarbons and a surfactant were added to the aqueous phase.[152] The surfactant was transferred to the aqueous phase, which led to formation of oil droplets several tens of micrometres in size. The author of that study believes that the smallest droplets formed when the surfactant formed lamellar micelles (lamellar bilayers) in the organic phase. When the phases were in contact, water molecules rapidly penetrated into the space between the surfactant layers near the interface and then diffused into the bulk of the organic phase. As a result, the organic phase divided into fragments. Then, the surfactant bilayers disintegrated, and oil droplets formed.

Bringing a droplet consisting of decane and a short-chain alcohol into contact with aqueous solution of amine oxide led to formation of myelin figures (structures resembling concentric myelin layers) on the droplet surface due to the formation of the liquid-crystalline phase.[153] The myelin figures immediately decayed with the formation of a lot of small droplets.

Spontaneous emulsification of a mixture of hexadecane, oleyl alcohol and polyoxyethylene (6) lauryl ether ($\overline{C}_{12}\overline{E}_6$) in water was studied.[154] Emulsification of the entire organic phase with the formation of oil droplets about 1 μm in diameter occurred only if diffusion of water to the organic phase was accompanied by transformation of the organic phase into a lamellar liquid-crystalline phase or microemulsion. Nucleation and growth of oil droplets occurred under supersaturation of the liquid-crystalline or micro-

emulsion phase. The most stable emulsion was formed when droplets of the organic phase were covered with a layer of the lamellar phase.

Swelling of a reverse microemulsion followed by phase inversion was observed.[155] The starting microemulsion contained methyl ether of partially hydrogenated rosin, potassium salts of partially hydrogenated rosin acids and water. Diffusion of water into the microemulsion droplets proceeded upon contact with the aqueous phase. Growth of droplets led to their coalescence and subsequent phase inversion. As a result, a direct emulsion with an average droplet size of more than 150 nm formed (Fig. 25).

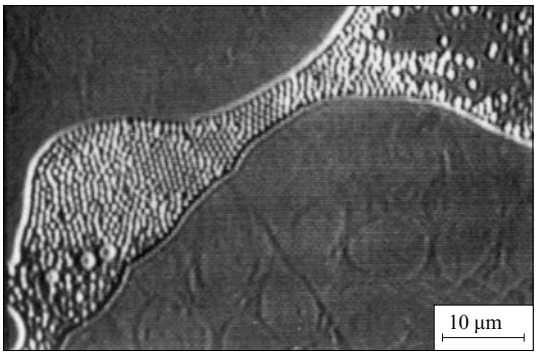


Figure 25. Emulsion formation at the interface.[155]
For the composition of the system, see text.

Recently, spontaneous emulsification proceeding by the diffusion mechanism known as the solvent displacement method or the ouzo effect has attracted considerable interest from the standpoint of production of NEs. Ouzo is the Greek anise alcoholic beverage. Analogous beverages are widely spread in Mediterranean countries, namely, pastis in the south of France, raki in Turkey, sambuca in Italy. When preparing an aperitif, such beverages are diluted with water and a milky emulsion forms (Fig. 26). The major components of the concentrate are anethole (~0.2 vol.%), ethanol (~45.0 vol.%) and water (~54.8 vol.%). Anethole is almost insoluble in water, but soluble in a mixture of ethanol with water. When a large amount of water is added (usually, in a 1 : 5 ratio), condensation of anethole occurs and a direct emulsion of anethole in aqueous solution of ethanol is formed.[156, 157]

The region of the ouzo effect is shown in the phase diagram of the ternary system (Fig. 27). The binodal characterizes the limiting solubility at a given temperature and separates the regions where single-phase systems and two-phase systems exist. The spinodal characterizes the limit of thermodynamic stability of the system. Within the spinodal curve, a system under study may undergo separation into two phases with the compositions corresponding to the binodal curve. Between the binodal and spinodal curves, the system may exist in a metastable state for quite a long time.

Using NMR spectroscopy, it was demonstrated[159] that rapid transition of a ternary solution to a metastable state upon the addition of water is followed by homogeneous nucleation and growth of droplets of the organic phase. The initially formed small anethole droplets about 2 nm in

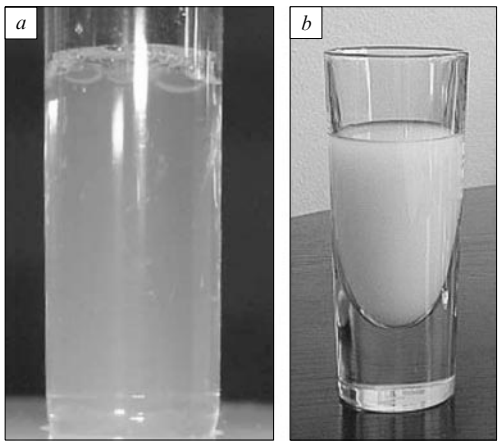


Figure 26. Emulsion formed upon preparation of pastis (*a*)[156] and the view of the beverage (*b*).

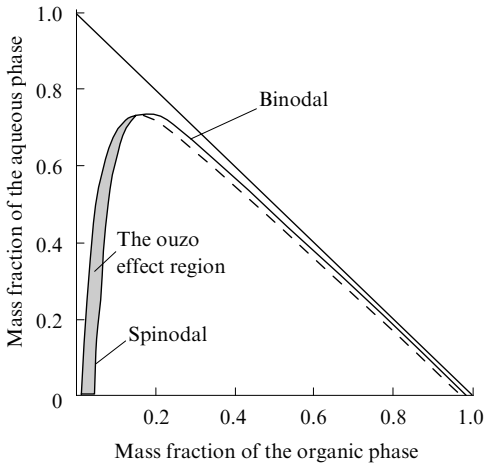


Figure 27. Phase diagram of a ternary system at constant temperature and pressure.[158]
The region of the ouzo effect corresponds to formation of metastable O/W emulsion.

diameter aggregate within 40–60 min after emulsification, and emulsion with a droplet size of nearly 1 μm is formed.

Using the ouzo effect, relatively stable direct emulsions can be prepared without adding surfactants and mechanical treatment.[158] The addition of surfactants into the system makes it possible to prepare emulsions stable for more than 6 months.[160] The size and polydispersity of droplets in the emulsion increase with increasing the oil/solvent ratio in the starting concentrate.[158] The emulsification is more intense if oils with higher solubility in the aqueous phase are utilized.[161]

Emulsions prepared from a concentrate containing soybean oil, soy-bean lecithin, glycerol, pharmaceuticals (muramyltripeptide-cholesterol, diazepam or amphotericin B), and ethanol or methanol as a solvent were studied.[162] Emulsification using the ouzo effect gave W/O emulsions with a droplet size of 200–300 nm.

Direct emulsions (droplet size 200 nm) stabilized by a mixture of a nonionic surfactant Synperonic NPE 1800

(block copolymer of nonylphenol, propylene and ethylene) and a cationic surfactant Ethoduomeen T20 (oxyethylated diamine) were prepared.[163]

The effect of the type of surfactant on the average droplet size in the emulsion was investigated.[164] The concentrate contained a lipophilic surfactant and the aqueous phase contained a hydrophilic surfactant. Emulsions with the smallest droplets (124 ± 46 nm) were formed using a mixture of surfactants Lipoïd S75 (soy-bean lecithin) and Pluronic F68 (block copolymer of ethylene oxide and propylene oxide). The other two emulsions were prepared using a mixture of Span 80 and Tween 80 and a mixture of sorbitan oleate (Span 85) and Tween 20. They were characterized by droplet diameters of 516 ± 71 nm and 725 ± 198 nm, respectively. The formation of the finest emulsions using Pluronic F68 as stabilizer was also confirmed elsewhere.[165, 166]

Emulsification using the ouzo effect at elevated temperatures (50–60 °C) followed by cooling of the system to room temperature gives direct emulsions with narrower droplet size distribution.[159]

This emulsification technique is most often used for fabrication of polymer particles and nanoparticles by emulsion polymerization. For instance, nanoparticles of a copolymer of methacrylic acid and ethyl acrylate (Eudragit) with a particle size of 50 nm and larger,[167] as well as polylactide and polycaprolactone nanoparticles 50 nm in size and larger [168, 169] and various branched polyester nanoparticles 59–191 nm in size [170] were synthesized by this method.

The ouzo effect can be used for preparation of liposomes less than 100 nm in diameter. Dilution of an ethanolic solution of monoolein with water can be used as an example.[171] Both liposomes and cubosomes, nanoparticles with a cubic liquid-crystalline structure, formed in this case (Fig. 28). The cubosome size was 150–200 nm, whereas that of liposomes was about 50 nm.

The method of spontaneous emulsification is quite simple in practical implementation. Emulsification proceeds in the whole volume of the mixture; therefore, it can be scaled with ease. The major disadvantage of the method is that it is suitable for preparing emulsions with a low fraction of the internal phase, *viz.*, about 1% immediately after dispersion and less than 5% after evaporation of the solvent.[159]

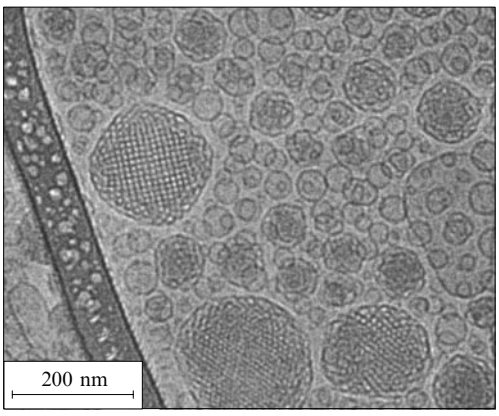


Figure 28. Photomicrograph of cubosomes and liposomes obtained using the ouzo effect.[171]

When using high-energy methods, the total energy consumption and the time of the process depend on the amount of NE produced. When low-energy methods are used, there is no such dependence because emulsification occurs in the entire volume and almost simultaneously. Only the PIT method, which requires heating of the system up to a specified temperature, is characterized by some energy consumption depending on the volume of the mixture being dispersed.

One of the most important advantages of the high-energy methods consist in the possibility to introduce various compounds into droplets of the internal phase. In the case of low-energy methods, the composition of the NE formed upon spontaneous emulsification is exclusively governed by the physicochemical properties of the system (mainly by the surfactant properties). If the addition of a substance leads to a significant shift of the phase inversion point, the use of the low-energy emulsification methods becomes impossible.

Application of the high-energy methods is undesired if it is required to encapsulate unstable substances like peptides, proteins and nucleic acids, which are often used for pharmaceutical and medical purposes because emulsification may cause destruction, denaturation or the loss of activity of the pharmaceuticals.

3. Combining high- and low-energy methods for preparation of reverse nanoemulsions

The high- and low-energy methods described in the previous Sections are widely used for preparation of O/W NEs. Reverse emulsions, especially those containing large fractions of the internal phase, are highly viscous systems, thus being much more difficult to prepare. When using high-energy methods, an increase in the fraction of the internal phase leads to abrupt increase in the droplet size due to an increase in the viscosity of the emulsion. Low-energy methods allow one to obtain reverse NEs with smaller droplet sizes, but the fraction of the internal phase in these emulsions is rather low.

For instance, reverse NEs containing 20 mass % of the aqueous phase were prepared by stirring followed by sonication.[172] The emulsions were stabilized by Span 80 or KLE3729 [poly(ethylene-*co*-butylene)-*b*-poly(ethylene oxide)], the average droplet size varied from 80 to 200 nm depending on the composition of the phases and on the concentration of the emulsifier.

Reverse NEs consisting of physiological saline dispersed in fluorocarbons were prepared by high-pressure homogenization.[173] The droplet size in the NEs was 30–100 nm and the fraction of the aqueous phase was less than 5 mass %.

Reverse NEs containing water, isopropyl myristate and a Cremophor EL–Cremophor WO7 surfactant mixture were obtained by the method of phase inversion upon composition change.[174] The average droplet diameter varied from 60 to 160 nm and the fraction of the aqueous phase was at most 4.5 mass %.

W/O nanoemulsions formed by stirring and stabilized by mixtures of surfactants, such as sorbitan monolaurate (Span 20), Span 80, Tween 20 and Tween 80B, were reported.[175, 176] The organic phase of the emulsions was decane and the aqueous phase was distilled water. The smallest droplets (20 nm) were obtained when the content

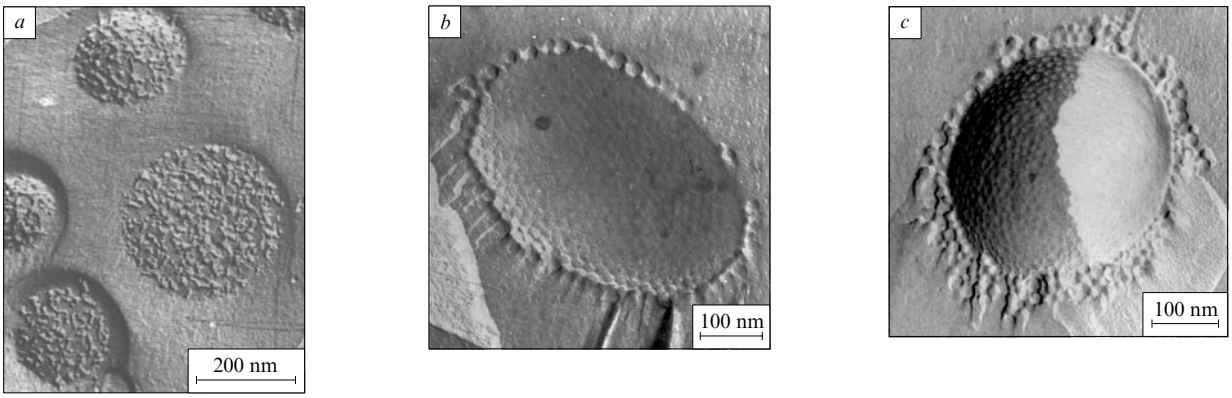


Figure 29. Photomicrographs of a macroemulsion obtained in the first step by high-shear stirring (*a*), and a nanoemulsion formed upon low-energy emulsification (*b*) and (*c*).
The ethanol concentration difference (Δc) between the internal phase of the emulsion and the external aqueous phase is 1.5 (*b*) and 2.5 mol litre^{-1} (*c*).

of the internal phase was 5 mass % and the surfactant : decane ratio was 15 : 85. An increase in the fraction of the internal phase to 25 mass % was accompanied by an increase in the droplet size to 80 – 110 nm.

For practical use of emulsions in preventive and curative medicine it is desired that the droplet size of the internal phase be much less than 100 nm. If NE is considered as a means for transdermal drug delivery, the droplets of the internal phase should be 20 to 40 nm in size for efficient penetration through the skin *stratum corneum*. In some cases, *e.g.*, when surfactants are extracted from biological fluids, it is necessary that the specific surface area of the interface be very large.[177] This can be attained by simultaneous reduction of the droplet size of the internal phase and an increase in their concentration in the emulsion up to 60% – 70% (and higher).

None of the modern methods of formation of NEs gives reverse NEs with such a high content of the internal phase.

A combined method of preparation of NEs was proposed.[178 – 180] In the first step, reverse macroemulsions with an average droplet size of about 250 nm were prepared by high-energy stirring. Subsequent low-energy emulsification led to formation of a large number of water droplets 15 – 25 nm in size.

Nanoemulsifiers (for example, ethanol) were introduced into the internal phase of the macroemulsions. During low-energy emulsification, the macroemulsions contacted the aqueous phase of the same composition as that of the internal phase of the macroemulsions except the nanoemulsifiers. As a result, diffusion of substances through the interfacial surface of the macroemulsion resulted in the formation of NE droplets.

Photomicrographs of Pt/C replicas of the cleavages of reverse emulsions including the macroemulsion obtained in the first step of the process and the emulsions with nanometre-size droplets formed in the second step of dispersion are shown in Fig. 29. The NE droplets were concentrated on the surface of droplets of the starting macroemulsion. Depending on the number of the NE droplets formed, they produced either a monolayer surrounding larger droplets or a multilayer coating consisting of droplet clusters.

The combined method led to emulsions with a bimodal droplet size distribution (Fig. 30). In the starting macroemulsion, the droplet size varied from 50 to 700 nm, the average diameter being 250 nm. Diffusion of ethanol through the organic phase resulted in the formation of droplets 17 – 25 nm in diameter, and the second maximum appeared in the size distribution curves. The average diameter of the starting droplets decreased to nearly 225 nm after contact with the outer aqueous phase because of the formation of NE droplets from them and due to transfer of a fraction of ethanol from the internal aqueous phase into the external phase. As Δc increased from 1.5 to 2.5 mol litre^{-1}, the amount of nanodroplets abruptly increased.

This method does not allow one to produce only small droplets, but the size of large droplets decreased to 200 – 230 nm in the second step of the process (during low-energy emulsification). The number of nanodroplets considerably exceeded the number of large droplets, and thus the specific surface area of the interface in such emulsions significantly increased.

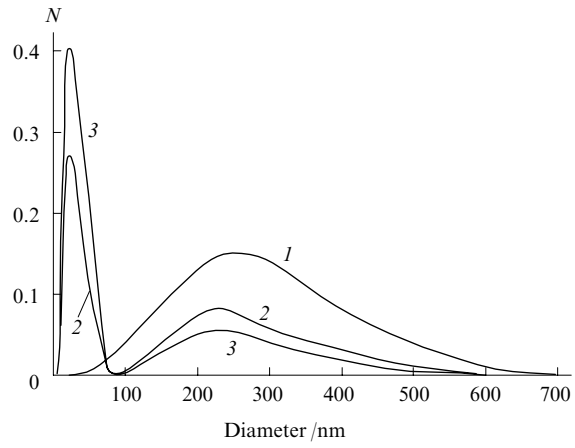


Figure 30. The droplet size distribution curves for a macroemulsion prepared by high-energy emulsification (*1*) and a macroemulsion containing nanodroplets after the second step of emulsification at Δc = 1.5 (*2*) and 2.5 mol litre^{-1} (*3*).

IV. Applications of nanoemulsions

Due to the small size of droplets of the internal phase, relatively high kinetic stability and optical transparency in comparison with conventional macroemulsions, NEs attract increased attention of many researchers. The authors of almost all studies on the preparation and investigation of NEs emphasize great prospects for practical applications of these systems. However, only a few studies on particular applications of NEs have been reported during the last fifteen years. This is first of all due to the fact that NEs undergo breakdown with time. Although some authors emphasize that NEs can be stable for months and even years, the Ostwald ripening results in growth of droplets of the internal phase, which limits the number of practical applications of NEs. This disadvantage can be eliminated by using NEs as a basis for production of other nanomaterials, *e.g.*, nanometre-size latexes, polymeric nanospheres, solid lipid nanoparticles, *etc.*

Major studies on applications of NEs are related to the prospects of their use (or the use of other carriers based on NEs) for encapsulation of medicines and their controlled delivery into the human body. There are also publications on possible utilization of NEs in agrochemical and petroleum industries, in production of printer's ink, *etc.*

1. The use of nanoemulsions for controlled drug delivery

The possibility of using NEs for transportation of medicines into the human organism has attracted the major attention of many researchers. It is suggested to use O/W NEs for therapy of cancer diseases,[181–183] for creation of vaccines against flu,[184,185] medicines for combined therapy of HIV-infected patients,[186] antiviral drugs and disinfectants, including those against Ebola virus,[187] for the delivery of genetic material,[188] *etc.*

It has been emphasized repeatedly that the penetration rate of the medicines using NEs is much higher than when utilizing macroemulsions, micellar solutions, gels, suspensions and other carriers.[181,189–192]

Studies of transdermal transport using NEs were performed for various pharmaceuticals including aceclofenac,[189] tocopherol,[190] nimesulide.[193] Transfollicular transport of inulin dissolved in water droplets of reverse NEs was investigated.[191] The rate and degree of inulin transfer increased with a decrease of the hydrophilic–lipophilic balance of the surfactants used to stabilize NEs. The transfer rates of inulin and tranexamic acid having different molecular masses were practically equal. This indicates that the water droplets of the NE are the carriers of the substances.

Different generations of emulsions and NEs used for parenteral introduction into the organism during, *e.g.*, chemotherapy, anesthesia, *etc.*, have been considered in a review.[194]

It was proposed[195] to use O/W NEs with a droplet size of 15.5–16.7 nm for intranasal controlled delivery of risperidone in brain tissue. The introduction of chitosan into such NEs allowed one to significantly improve the mucoadhesive properties (the ability to adhere to mucous tunics) and to increase the ratio of the risperidone concentrations in blood and brain tissue up to 0.948 (*cf.* a value of 0.054 for intravenous administration).

Both NEs themselves and various structures based on them, *e.g.*, nanospheres and nanocapsules, can be used for controlled drug delivery.

2. Preparation of polymeric nanoparticles, nanocapsules and nanocomposites by the miniemulsion polymerization method

A large number of publications on the application of NEs concern emulsion polymerization which in this case is called the nanoemulsion or miniemulsion polymerization method.[196–198] This method allows one to synthesize polymeric nanoparticles, hollow nanospheres, polymeric nanocomposites whose sizes are determined by the droplet size of the starting NE.[197,199]

When preparing polymeric nanoparticles, the internal phase of the NE represents a monomer. If a high-energy method is used and the monomer exhibits the properties of a surfactant, no surfactants are added to the system. If an NE is formed by the phase inversion method, surfactants should be added to the system but they are not involved in the polymerization. The initiator is usually introduced into the continuous phase of the NE; miniemulsion polymerization can also be initiated by exposing the system to UV or γ-irradiation, ultrasonic treatment, *etc.*

If the emulsification time is shorter than the polymerization time, the initiator can be introduced into the droplets of the internal phase of the NE. In this case, the formation of the NE may be followed by polymerization upon, for example, an increase in the system temperature.[200]

Polymerization in water droplets using reverse NEs makes it possible to synthesize nanoparticles with encapsulated hydrophilic components.[201,202]

Nanoemulsions for subsequent polymerization are mainly prepared by high-energy methods; numerous examples are well documented in reviews.[196,197] The low-energy methods are more sensitive to changes in the system composition are therefore more rarely used for the synthesis of polymeric nanoparticles. A method for the preparation of polymeric nanospheres using the PIT method was proposed.[203]

Nanoemulsions can be used for the synthesis of polymeric nanocapsules consisting of a liquid hydrophilic or hydrophobic core surrounded by a polymeric shell. Application of these nanocapsules for controlled drug delivery has been described in a review.[204] Nanocapsules can be formed by, *e.g.*, adding a monomer to the dispersion medium after formation of NE using all methods described above and subsequent polymerization. For instance, the synthesis of nanocapsules with water core in the polymerization of alkyl cyanoacrylate on the surface of nanodroplets in reverse NE was reported.[205,206]

In most studies, monomers were added into droplets of the internal phase of NEs. For example, a O/W NE containing a monomer, an initiator and an oil in droplets of the internal phase was prepared under sonication.[207] The polymerization was initiated by increasing temperature. The resulting polymer precipitated on the interface and formed nanocapsules. Polymerization at the interface upon introduction of the initiator into the dispersion medium of NE and formation of the nanocapsule shells from polyurea was reported.[208]

The nanoemulsion polymerization is a promising method of synthesis of nanoparticles representing various

polymeric nanocomposites. For instance, fabrication of hybrid polyurethane–polyacrylate latexes was studied.[209] Polymerization in a O/W NE resulting in the formation of nanospheres with encapsulated magnetite particles was reported.[210] Polymerization in a reverse NE allowed one to obtain magnetite nanoparticles encapsulated in polyacrylamide nanoparticles.[211] Polymerization in a W/O/W multiple miniemulsion resulted in nanocapsules containing magnetite nanoparticles surrounded by a polymeric shell.[212]

Core–shell nanocomposite particles have been described, including those with a SiO_2 core and a polystyrene shell[213] as well as those with polystyrene core and a TiO_2 shell.[214]

3. Preparation of polymeric nanoparticles, nanocapsules and nanocomposites from pre-synthesized polymers

Nanoemulsions can be used as matrices for the preparation of nanoparticles from pre-synthesized polymers dissolved in droplets of the internal phase of NEs. The solvent is removed from the droplets of the internal phase either by evaporation[215] or by the solvent exchange technique.[167,216–219] In the latter case, the solvent diffuses into the aqueous phase, which leads to precipitation of the polymer inside the droplets. This method makes it possible to produce particles of not only synthetic, but also natural polymers like chitosan, polysaccharides, alginates, gelatine, *etc*.

Nanocapsules formed from a pre-synthesized polymer can also be prepared based on NEs. In this case, NEs are prepared by a high-energy or low-energy method, a polymer is introduced into the dispersion medium and then its precipitation on the surface of NE droplets is carried out by, for example, evaporation of the dispersion medium or coacervation. For instance, it was proposed to encapsulate a chlorhexidine gluconate antiseptic solution in water droplets of W/O nanoemulsions under sonication.[220] Poly(methyl methacrylate), polymethacrylate or polycaprolactone were dissolved in dichloromethane and gradually added to the dispersion medium of a NE. When dichloromethane was evaporated, polymers precipitated on the surface of NE droplets, and nanocapsules were formed.

Nanocapsules with oil cores and poly(ethylene oxide)-*b*-poly(ε-caprolactone) shells were prepared.[221] The block copolymer was dissolved in oil at 70–80 °C and a O/W NE formed. On cooling the system, the polymer precipitated and was cured on the surface of nanodroplets.

4. Preparation of solid lipid nanoparticles based on nanoemulsions

A significant number of publications is devoted to preparation of solid lipid nanoparticles from NEs and their applications as drug carriers[222–226] and in cosmetics.[227,228] As a rule, such particles consist of a biocompatible and biodegradable lipid and are stabilized by surfactants; they preserve a solid-like structure at a physiological temperature.

To produce lipid nanoparticles, the system is warmed up to a temperature 5–10 °C higher than the melting point of the lipid, and a NE is formed by high-pressure homogenization,[223,227,229] ultrasonic dispersion,[224–226] low-energy phase inversion composition emulsification[230,231] or by other methods. Then, the NE formed is cooled to room temperature which causes crystallization of the lipid.

A method for preparation of lipid nanoparticles in the course of spontaneous emulsification was reported.[232–236] In study,[232] glyceryl monostearate was dissolved in ethanol/acetone mixture at 50 °C and then diluted with an aqueous solution. As a result, particles 50 to 500 nm in size depending on the type of the encapsulated drug were obtained.

Porous lipid nanoparticles can be prepared by adding an oil with low solidification temperature to the initial lipid mixture.[236] By varying the oil content one can form matrices of different porosity after crystallization of lipids with high solidification point.

Nanoemulsions can also be used for preparation of lipid nanocapsules comprising a liquid organic core and a solid shell formed by surfactant molecules.[237,238] The liquid cores in the nanocapsules for medical applications is usually formed by moderate-long-chain triacylglyceride oils, while the shells are formed by polyoxyethylene stearates. Lipid nanoparticles with tripalmitin and propylene glycol monostearate shells were reported.[45]

The PIT method with repeated temperature cycling is most often used to obtain lipid nanoparticles; this allows one to prepare smaller nanocapsules with more uniform size distribution.[3,239]

5. The use of nanoemulsions in cosmetic industry

The possibility to use O/W NEs with the organic phase representing a mixture of oils with different molecular masses (for optimal effect on the human skin) as cosmetic products is of particular interest.[21] An increase in the fraction of high-molecular-mass oils results in increasing stability of O/W NEs against the Ostwald ripening. However, the high-molecular-mass oils are worse absorbed and leave oily films on the skin surface. If an emulsion contains low-molecular-mass oils, it faster penetrates into the surface layers of skin, but its stability against the Ostwald ripening decreases. Rather stable NEs can be obtained using a mixture of oils with a certain ratio of the low- and high-molecular-mass components. As shown above (see Section II), the kinetic stabilization of NEs against the Ostwald ripening is due to different solubility of such oils in the aqueous phase.

It was proposed to utilize NEs in various creams, lotions, gels, remedies for hair care, *etc*.[21] The compositions and methods of preparation of NEs containing vitamins A, D, C, E and their derivatives[240] and phospholipids,[241,242] as well as NEs for restoration of the epidermis[243] are patented. In particular, the L'Oréal Group took out several tens of patents (see, *e.g*., Refs 244–248).

6. Other applications of nanoemulsions

The possibilities of using NEs in other fields are under study. Nanoemulsions can be used in agrochemistry for preparation of products containing pesticides and herbicides. As a rule, these are emulsion concentrates which give NEs upon dilution with water owing to spontaneous emulsification.[151]

Microemulsions capable of transforming to O/W NEs with a droplet size of 50–100 nm upon dilution with water or an electrolyte solution were reported[249] and their possible application as washing agents is demonstrated.

In the synthesis of nanoparticles of controlled size, droplets of the internal phase serve as a matrix restricting the particle growth. For instance, the formation of SiO_2 and TiO_2 nanoparticles 30–230 nm in size in reverse NEs prepared by dispersion of an aqueous solution in an oil/surfactant mixture was reported.[250] Also, $3\,Y-ZrO_2$ nano-

particles about 30 nm in size,[251] indium and tin oxide nanoparticles nearly 38 nm in size,[252] chitosan nanoparticles 0 – 150 nm in size [253] and barium sulfate nanoparticles 15 – 100 nm in size [254] were also prepared in reverse NEs. The preparation of hydroxyapatite nanoparticles and nanospheres 10 – 30 nm in size was reported.[255] However, it should be emphasized that nanoparticles are most often synthesized using microemulsions because they are thermodynamically stable, form spontaneously and their droplets are characterized by a narrower size distribution.

It was proposed [129] to use paraffin-in-water NEs as cold accumulators. It was also proposed to use O/W NEs instead of viscous reverse emulsions for lifting of heavy crude oil.[122]

Thus, recent publications outline a rather wide range of promising fields of NE application.

V. Conclusion

Nanoemulsions considered in this review represent a special class of liquid disperse systems. Their droplet sizes are less than 100 nm but the systems are kinetically stable. Despite thermodynamic instability, they can exist for a rather long time (a few months or even years).

The main mechanism of decrease in the free energy of the system during the growth of droplets in O/W NEs is the Ostwald ripening. Reverse NEs are unstable against flocculation of droplets of the internal phase and subsequent sedimentation. The best way to ensure the kinetic stabilization of O/W nanoemulsions against the Ostwald ripening is to add a second component which is less soluble in the continuous phase than the main component. To decelerate flocculation of droplets of the internal phase of NEs, one should choose appropriate surfactants and polymeric additives preventing approach of droplets.

It should be noted that almost all practically used NEs have rather complicated chemical compositions. Various thickeners for the dispersion medium and disperse phase make emulsions more stable.

At present, NEs are prepared by high-energy and low-energy methods and by the combined method. The most popular high-energy methods include high-pressure homogenization in valve apparatuses and microfluidizers. However, these methods allow one to obtain emulsions with nanometre-size droplets only at low and moderate fractions of the internal phase. The droplet size increases abruptly with increasing the viscosity of the system. The high-energy devices are energy consuming and have complicated design. Nevertheless, they offer the unquestionable advantage consisting in that the use of these devices enables encapsulation of unlimited range of compounds and pharmaceuticals provided that they preserve their chemical stability and biological activity at high pressures and high shear rates.

The low-energy methods based on the phase inversion upon changes in the temperature or composition of the system as well as spontaneous emulsification attract increased interest of researchers. The phase inversion methods make it possible to prepare NEs with droplets less than 50 nm in sizes and rather narrow size distributions. Emulsification occurs almost simultaneously in the entire volume, thus requiring much less energy consumption in large-scale production. The main disadvantage of these methods is their dependence on the physicochemical properties of the system and on the type of the surfactant. It is reasonable to use these methods for encapsulation of unstable substances that decompose in high-energy devices.

Almost all publications devoted to NEs emphasize the advantages of their applications in various areas as compared to macroemulsions and other disperse systems. Most publications are related to the possibility of using NEs for encapsulation of pharmaceuticals and their controlled delivery into human organism. This can be done using both NEs and various structures based on them, *e.g.*, polymeric nanospheres, nanocapsules and lipid nanoparticles. It was proposed to create anticancer agents, virus vaccines, vectors for gene delivery and other products on the basis of NEs. Due to the small size of droplets of the internal phase, NEs are promising for transdermal transport of drugs and biologically active substances. This is of special interest for specialists in pharmaceutical and cosmetic industries. In addition to medicine, the most promising fields of application of NEs include agrochemical, paint and varnish and petroleum industries.

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