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MICROEMULSIONS AS POTENTIAL OCULAR DRUG DELIVERY SYSTEMS: PHASE DIAGRAMS AND PHYSICAL PROPERTIES DEPENDING ON INGREDIENTS

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Abstract: The microemulsion systems, both o/w and w/o, composed of isopropyl myristate, soybean lecithin (Epikuron 200), Polysorbate 80, Cremophor EL, n-butanol and triacetine, taken at various amounts and in various combinations, were tested in order to assess their physical-chemical properties. Some ingredients of the microemulsions were physiologically acceptable, except one formulation containing n-butanol used as a model reference system. Phase diagrams were made for all microemulsions studied in order to test the influence of components on the boundaries of the stable microemulsion domains. The greatest area of stable microemulsion system was obtained for the formulation in which n-butanol was used, while the smallest area was obtained when soybean lecithin (Epikuron 200) was used as a surfactant. The following physical parameters were analyzed: osmotic tension, density, viscosity, refractive index, pH and particle size. Microemulsions stored at 25°C for the period up to 12 months, showed physical changes depending on ingredients. The study made it possible to select the most stable microemulsion system meeting the requirements of eye drops.

Keywords: Microemulsion; Eye drops, Physical stability; Soybean lecithin; Phase diagram

Microemulsions have unique physical properties. They are composed of water, oil and a mixture of surfactants making a homogeneous, optically isotropic and thermodynamically stable solution. Microemulsions can be sterilized by filtration and their production is relatively simple and inexpensive. Because of these properties, they have attracted a great interest as drug delivery vehicles (1,2). Microemulsions can be applied as liquid membrane carriers to transport lipophilic substances through an aqueous medium or to carry hydrophilic substances across lipoidal medium. They are proposed for oral, topical, dermal, transdermal, parentenal and pulmonary administration of drugs (3). Although microemulsions have been known for a long period, their potential as vehicles for topical ocular drug delivery has been investigated only within the last decade (4).

The main problem in a microemulsion application is a high concentration and a narrow range of physiologically acceptable surfactants and co-surfactants (5, 6). On the other hand, the large surfactant concentration determines their stability. Several authors have reported preparation of microemulsions using alcohols of short or medium length chains (e.g., butanol, heptanol or pentanol) as co-surfactants (7). These substances limit the potential application of microemulsion due to their toxic and irritant properties. A selection of components for microemulsions suitable for pharmaceutical use involves a consideration of their toxicity and, if the systems are to be used topically, their irritation and sensitivity properties (8). The ionic surfactants are generally too toxic to be used for preparation of lipid emulsions; therefore, non ionic surfactants, such as the poloxamers, polysorbates, polyethylene glycol are preferred. Polysorbate 80 is widely applied to pharmaceutical preparations, including ophthalmic preparations, due to its history of usefulness and safety, and it is listed in the United States Pharmacopoeia-National Formulary, the European Pharmacopoeia and the Japanese Pharmacopoeia (9).

With the recent improvements in aseptic processing and the availability of new well-tolerated emulsifieres (polysorbate 80), emulsion technology is currently under evaluation for topical cyclosporine A delivery. Ding (10) developed a castor oil in water microemulsion. This microemulsion is stabilized by polysorbate 80 where the active substance cyclosporine A remains stable over 9 months and causes only mild discomfort and slight hyperemia on the rabbit eyes applied 8 times per day during 7 days**.** This encouraging result allowed the formula-

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tions to undergo clinical trials of phase II and III in dry eye disease. The II phase trial performed on 162 patients demonstrated good tolerance of the emulsion (12).

It is known that most of the substances which are physiologically acceptable are chemically unstable. In our study, we prepared stable and pharmaceutically approved microemulsion systems for potential applications as ophthalmic drug vehicles.

In the first part of the study, we report the pseudo-ternary phase diagrams of water / isopropyl myristate (oil phase) and lecithin, Cremophor EL, triacetin, Tween 80 as surfactants. Lecithin is an attractive component of microemulsion due to its non-toxicity (11). Some microemulsions were prepared with n-butanol as a co-surfactant. N-butanol was used in order to compare the influence of this short-chain alcohol on the physical parameters and phase diagram of microemulsion with the same parameters of formulations containing non-toxic ingredients. Stability of all physical parameters suitable for eye drops, are described in the second part of the paper. The aim of the present *in vitro* study is to examine possible changes of physical parameters (i.e., density, viscosity, particle size, pH, osmotic tension) during long term incubation and to assess the existence range of microemulsions reported in phase diagrams. The values of the physical parameters presented below refer to the systems obtained with the smallest surfactants concentration, which were

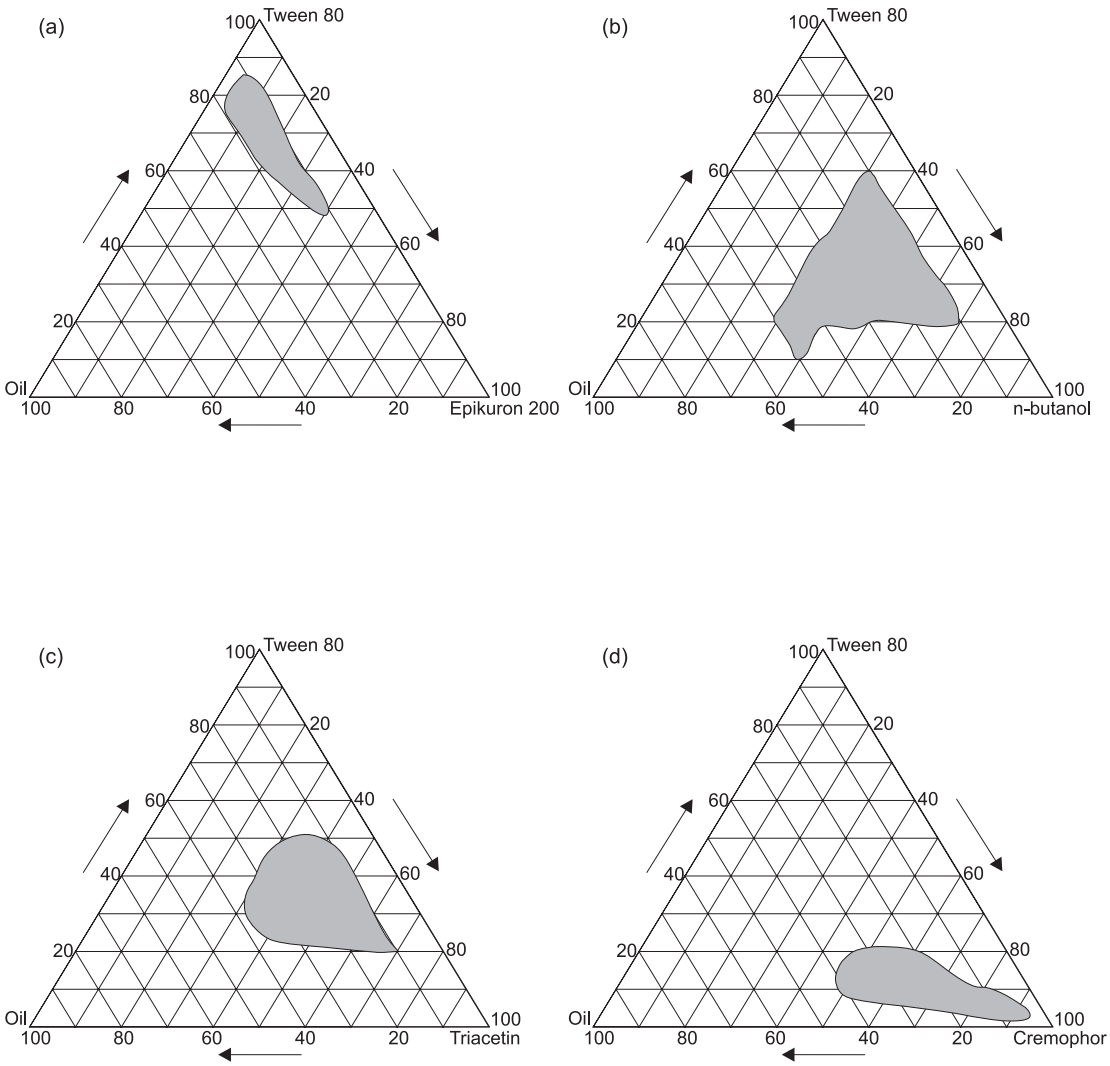

Figure 1. Pseudo-ternary phase diagram of the microemulsion containing isopropyl myristate/water, Tween 80 and :
– (A) Epikuron 200; – (B) n-butanol; – (C) Triacetin; – (D) Cremophor EL

transparent and single-phase for at least 12 months at the temperature 25°C.

EXPERIMENTAL

Materials

Soybean lecithin (Epikuron 200) with phosphatidylcholine content > 93% was purchased from Degussa GmbH&Co. KG (Germany) and used without further purification. Isopropyl myristate (98% pure), triacetine and n-butanol were supplied from Sigma-Aldrich Chemicals (Poland). Tween 80 (polyoxyethylene 20 sorbitan monooleate, Polysorbate 80) was from Merck Co. (Germany), Cremophor EL (castor oil polyoxyl) was from BASF GmbH (Germany). Bidistilled water from a well-seasoned all-glass system was used throughout the study.

Preparation of microemulsions

Appropriate amounts of the surfactants and co-surfactants or mixture of two surfactants were dissolved in the oily phase (isopropyl myristate). The samples were stirred during a sufficient time (20 min. at 25°C), and then bidistilled water was added. In the presence of n-butanol, at first a mixture consisting of oily phase, surfactant and bidistilled water was obtained, then n-butanol was added up to the final concentration of the microemulsion. The compositions of microemulsions used in the study are given in Table 1.

The systems obtained were classified as microemulsions on the basis of the criterion of transparency. After preparation, all formulations were left to equilibrate at 25°C for 24 h prior to measurements.

METHODS

Particle size analyses of the oil droplets or water droplets were determined by photon correlation spectroscopy (PCS) with an N4 Plus apparatus (Coulter Electronics GmbH, Coulter Scientific Instruments, Great Britain). The light source was a helium-neon laser (632.8 nm). The intensity of the scattered light was measured at the angle of 90°, and at least three runs for each sample were done. The viscosity of microemulsions was measured with rotational viscometer Dv-1 P Anthon Paar (Anthon Paar, Austria) equipped with a low viscosity adapter (LV Adapter). We used the controlled shear rate method at a constant shear rate of 1.32 rpm/s at the temperature of 25°C. The density of microemulsions was measured with the DA-110 M density meter (Mettler Toledo, Germany). A thermostat controlled the temperature at 25 ± 0.01°C. The osmotic tension was indicated for all o/w microemulsion systems by Osmometer 800 (Trident, Poland). For measuring refractive index, an Abbe refractometer (Analytik Jena, GmbH, Germany) was used. For all formulation systems, the pH changes were measured by pH meter CG 842 (Schott Glass, Germany) with a special electrode for emulsion systems – Electrode Blue Line 13. All measurements were made at 25°C.

Prior to the measurements, the microemulsions were filtered through 0.22 μm membrane filters (Millipore, UK). The formulations were incubated at 25°C, and the physical stability parameters were determined at the time of 0, 3, 9 and 12 months (Table 2).

Construction of ternary phase diagram

To investigate the microemulsion regions, phase diagrams were constructed by a progressive titration of series of surfactant/co-surfactant mixtures in the oily phase with bi-distilled water at the room temperature. Each series contained from 5% to 100% of water. When a sample exhibited birefringence, the titration was continued so as to determine the endpoint of the liquid crystalline area and to es-

Table 1. Compositions of the microemulsion systems [% w/w].

Components	Microemulsion systems			
	A	***B***	***C***	***D***
Isopropyl myristate	59.5	18.0	36.0	1.2
Aqua bidistilled	3.3	40.0	10.0	86.0
Epikuron 200	28.9			
n-Butanol		12.0		
Triacetin			18.0	
Tween 80	8.3	30.0	36.0	7.9
Cremophor EL				3.7
Glicerol				1.2

tablish a second isotropic region. The phase behavior of the systems was mapped on the phase diagrams with the top apex representing by Tween 80 and the other apices represented by oil and a surfactant (Cremophor EL, triacetine, n-butanol or Epicuron 200). The samples were assessed visually to determine the regions of transparency (13, 14, 15). All formulations prepared with compositions within transparent microemulsion regions were stable for at least 3 months, when they were stored at 25°C. The results are presented in Figure 1.

RESULTS AND DISCUSSION

Preparation of microemulsions requires determination of the existence range of microemulsions which depend on mixtures of surfactants, co-surfactants, oily phase and aqueous phase reported in a phase diagram (4). Thus, knowledge of a phase diagram of a multicomponent system is a necessary step for understanding of physics of the system (1,16). The diagrams of the systems show the dismixing limit and the stable single-phase mixtures. We did not investigate any other forms of surfactant systems, e.g., mesophases.

At first, the phase diagrams were made in order to establish the ranges of the microemulsion phase and to find the most stable systems containing the least amount of surfactants.

One of the factors which affects the phase behavior of the microemulsions systems is the hydrocarbon chain length of various oils and surfactants. Phase behavior of the microemulsion, e.g. with isopropyl miristate, has larger microemulsion area than that one with isopropyl palmitate (using the same surfactants systems). The same situation is observed using such surfactants as Tween 80 and Tween 20. Tween 20 has shorter hydrocarbon chain than Tween 80 and decreases the microemulsion area (17).

Also, the phase behavior is strongly influenced by the size of molecule of the oil used (18). Isopropyl myristate is small in size compared to medium chain triglycerides. Depending on the chain length and on the volume of the molecule, penetration of the surfactant into the hydrocarbon tails will change the hydrocarbon chain volume of the surfactant molecule and thus the effective geometric packing parameter (19). It is one of the reasons why in our study we used isopropyl myristate and Tween 80 in microemulsion formulations.

The main disadvantage of using microemulsions for pharmaceutical application is that they usually contain a large amount of surfactants which can cause, e.g., irritation of the eye ball. In the systems

Table 2. Values of the physical parameters characterizing the microemulsion systems, measured at the time = 0 months and at the time = 12 months.

Parameters	MICROEMULSION							
	A		B		C		D	
Time [months]	**0**	**12**	**0**	**12**	**0**	**12**	**0**	**12**
Density [g/cm^3]	0.901±0.005	0.911±0.001	0.961±0.0120	0.965±0.007	0.912±0.011	0.928±0.020	1.011±0.008	1.012±0.009
Viscosity [mPa s]	20.35±0.25	46.76±0.34	55.89±0.62	60.85±0.72	66.71±0.12	67.23±0.28	2.82±0.87	3.41±0.69
pH	6.28±0.89	4.46±0.57	6.90±0.29	4.60±0.76	8.26±0.87	6.67±0.66	6.86±0.98	6.44±1.20
Refractive index	1.446±0.020	1.447±0.016	1.405±0.035	1.405±0.011	1.439±0.078	1.456±0.087	1.368±0.015	1.370±0.020
Osmotic tension [mOsm/l]	-	-	1090±0.98	1315±1.25	1520±0.88	1640±0.78	330±0.25	348±0.32
Particle size [nm]	43.59±0.19	67.13±0.12	3.67±0.12	6.73±0.18	3.59±0.20	6.21±0.16	32.6±0.21	39.9±0.17

investigated here, the concentration of surfactants varied from 11.6 to 54.0% (Figure 1). The surfactants used included Tween 80, Cremophor EL, soybean lecithin in the form of Epikuron 200, triacetin and the co-surfactant – n-butanol. Although n-butanol is highly toxic, it is commonly used as a model co-surfactant (3, 20).

As expected, the greatest range of the microemulsion phase was found for the systems with n--butanol. The medium and short chain alcohol surfactants are such alcohols which are capable of swelling the chain volume allowing substantial oil uptake (21) (Figure 1 B). Similar results were obtained in the systems with triacetin. The smallest range of the microemulsion phase occurrence was found for the system with soybean lecithin (Figure 1 A). Because of being too lipophilic, lecithin is not capable of producing isotropic solutions of water and oil without a second surfactant. It is known to form highly rigid films (22).

Due to addition of higher amounts of non ionic surfactant, the lecithin's hydrophilicity increases what favors the interfacial film curvature and leads to the microemulsion formation (23).

Due to its surface activity, Tween 80 is capable to reduce the interfacial tension of the system and to increase the hydrophilic-lipophilic balance of lecithin. Lecithin also contributes to the higher solubilization of oil in the internal phase of the microemulsion due to its amphiphilic nature (24).

The system containing 37% of the mixture of lecithin and Tween 80 gave a stable microemulsion whose parameters met the requirements of eye drops. The systems containing either less than 35% or more than 40% of the mixture of the surfactants, did not meet these requirements. The lowest concentration of surfactants (11.6%) was obtained when Cremophor EL was used in the mixture with Tween 80 (Figure 1D).

For the system with triacetin and Tween 80, a stable microemulsion was obtained already for 54% concentration of the surfactants (Figure 1C).

The creation of the phase diagrams made it possible to select the stable microemulsion systems with the lowest concentration of surfactant.

On the basis of values of the physical parameters determined (viscosity, density, osmotic tension and particle size), the microemulsions meeting the requirements of eye drops (fully or in part) were selected (Table 2). The system with n-butanol as a co--surfactant was a reference system for all other biologically accepted microemulsions.

The therapeutic substances applied as eye drops must be isotonic and isohydric; their pH can vary from 3.5 to 8.5 (25). As expected, the lowest pH after 12 months was measured for the microemulsions with soybean lecithin (Epikuron 200) because of small chemical stability of this substance (26). During incubation, the oxidation of the acyl chains at unsaturated sites or hydrolysis at the polar head can be fast, especially in the presence of water. The smallest difference in pH over a one year period of storage was obtained for the microemulsion with Cremophor EL. It should be emphasized that in all systems the value of pH decreased over a year of storage. This was caused by the appearance of acidic decomposition products in the microemulsions. However, this decrease was never below pH=3.5, and all systems met the requirements for eye drops (Table 2). As far as viscosity is concerned, it is known that jump changes in its value occur upon phase inversion and during critical phenomena (27). In general, in all microemulsions studied an increase of viscosity was observed over a year of storage by 0.8 – 46.0%. The smallest increase was noted for the microemulsions containing triacetin and Cremophor EL, and the largest increase was observed for systems containing Epikuron 200. The high increase of viscosity in microemulsion A (Table 2) was probably caused by polymerization process in this formulation induced by autooxidation of soybean lecithin (Epikuron 200). The growth of particle size in microemulsion A indicates beginning of the polymerization process. The high growth of viscosity in microemulsion type B (Table 2), where butanol was used as a surfactant is probably caused by slow evaporation of this substance. It was also demonstrated by growth of the particle size. Our results showed that after one year of incubation, viscosity in all investigated microemulsions did not meet the requirements for eye drops (required viscosity for eye drops cannot be higher than 20.0 mPa s) except one system with Cremophor EL, where viscosity achieved the value of 3.41 mPa s.

It was found that the light refraction index underwent rapid changes when a multi-component system was transformed into a multiphase system. The light refraction index of the tear fluid is 1.340. It was recommended that the eye preparations should have the refraction index not higher than 1.476, with an optimum value equal to 1.404 (28). For the microemulsions studied the refraction index varied in the range of 1.368-1.446.

The values of the refraction index remained unchanged over a year of storage. The values of density also did not change (Table 2). The absence of changes of these parameters, confirmed a good selection of surfactants which stabilized the systems

physically. The osmotic tension of the eye preparations should be about 340 mOsm/L. The value of this parameter in microemulsions could be controlled by changing the composition, e.g., when it was too low, a small addition of glycerol caused its increase. For microemulsion systems B and C, the osmotic tension was too high (Table 2). It was caused by the presence of butanol and triacetin which themselves produced high osmotic tension. This disqualified such microemulsions as potential eye drops. The osmotic tension for the system with Cremophor EL was biologically accepted, and the smallest increase in this parameter after one year of storage was noted. In the system with Epicuron 200 (as a microemulsion of w/o type) the osmotic tension was not measured.

Another parameter tested was the size of the particles in the microemulsions. The smallest particles were noted for the microemulsions containing n-butanol, what proved its stabilizing influence. The largest particles after preparation were observed in the systems with soybean lecithin. However, their diameter did not exceed the value of 140 nm and all systems remained monophasic and transparent.

After one year of storage the size of particles doubled almost in each system. The greatest increase in the size of particles was noted in the systems with Epikuron 200 (soybean lecithin), what means that it had the poorest stabilizing influence and showed tendency to form aggregates. However, this increase was not large enough to break the microemulsion phase (Table 2).

The effect of temperature on stability of the microemulsions was also investigated. After determination of the initial values of the particular physical parameters at the room temperature, the preparations were kept frozen at –20^{o}C for 10 days, and then heated again up to the room temperature (13). The systems subjected to this procedure were still monophasic. A similar result was obtained when the procedure was reversed, and the systems were heated to 50^{o}C and then cooled to the room temperature. The systems' parameters measured after this procedure at the room temperature had the values equal to the initial ones.

The abovementioned results showed that after one year of storage the changes in the physical parameters of the microemulsions depended on ingredients. In contrast to the microemulsions with biological acceptable ingredients, the microemulsions containing medium-length aliphatic chains, e.g., n--butanol as a co-surfactant, are good model systems for investigations of structure and properties of microemulsions. However, they cannot be used for medical therapy because of their toxicity. All the others ingredients were biologically acceptable. Lecithin (Epicuron 200) is a major component of membrane lipids. Tween 80 and Cremophor EL as non ionic surfactants are widely applied to pharmaceutical preparations including ophthalmic preparations (9).

We concluded that the microemulsion systems containing Epikuron 200 and Cremophor EL, partly or fully met the requirements of eye drops. Thus, they should be the subjects of further study. The systems obtained were characterized by considerable thermodynamic stability which was typical for microemulsions.

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