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NATURE AND STABILITY OF LIQUID-LIQUID COLLOIDAL SYSTEMS CONTAINING SAFFLOWER OIL

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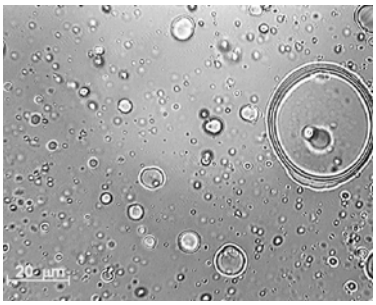
The liquid-liquid colloidal systems with safflower oil have many applications, due to their reduced toxicity. These colloidal systems are very important, especially for the medical studies and for the food industry. The aim of this research is to study the stability of the liquid-liquid colloidal systems prepared by dispersing the safflower oil in a sodium chloride solution, in the presence of a hydrophobic surfactant – Span 80. The obtained colloidal systems were examined by optical microscopy and the size distribution of the vesicles by dynamic light scattering (DLS) technique. The images were captured with a video sensor attached to the computer (VEM). As a result of dispersion process in the colloidal liquid-liquid systems vesicles are obtained. The nature of these vesicles is dependent on the concentration of salt in the aqueous phase and the dispersion process parameters. These systems are destroyed during two stages, having different durations. The first step is shorter than the second step. The emulsion that is finally obtained shows a very good stability, more than a month.



INTRODUCTION

In recent years the studies of liquid-liquid colloidal systems made with edible oils have taken a considerable advance. These colloidal systems which present a low toxicity are very important for the medical studies (tracking changes in blood lipids concentrations) and the pharmaceutical industry (preparation of encapsulated drugs). In most cases, they appear as dispersed systems with an extremely complex structure. It is due to the very complex composition of edible oils which form the disperse systems.

The safflower oil is part of semi-drying oils.[1] It is considered as dietary oil,[2] recommended in gastritis, in liver disease, in early forms of cardiovascular disease.[3]

The safflower oil is considered as the most valiant existing dietary oil on the world market. In the last years, traffic safflower oil on the market has doubled. This is a result of great content of unsaturated fatty acids from safflower oil.[2]

The studies conducted so far with emulsions containing safflower oil concerns the direct emulsions. Emulsions containing safflower oil were administered to different patients: newborns[4] and adults[5-7] by parenteral way, they constitute a source of energy for malnourished organisms.

These emulsions containing safflower oil shows no toxicity. Subsequently the direct emulsions with safflower oil were considered standard in the parenteral administration of other direct emulsions. In all cases the variation of fatty substances concentration in the blood plasma and energy intake were studied. It should be noted that in all these works only the direct emulsions with safflower oil were prepared and only hydrophilic surfactants were used. [4-7]

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So far there are no studies regarding the emulsions/colloids with safflower oil containing hydrophobic surfactants.

Besides emulsions, in particular conditions, the presence of phospholipids and soaps traces in the oil-water system can lead to obtain liposomes or vesicles. The obtaining of double emulsions requires the use of two emulsifiers, which are disposed on both separation surfaces: first, between the internal droplets of the dispersed phase and the internally dispersion medium (to form the primary emulsion) and second, between the globules of the primary emulsion and the external dispersion medium (which form the double emulsion),[8] so the two interfaces have different curvatures and their elasticity is different.[9] However, the realization of the liposomes or vesicles involves using a surfactant (or mixture of surfactants) which has a curvature around zero.[10]

A net hydrophilic emulsifier or a net hydrophobic emulsifier is unable to form vesicles or liposomes.[9] Only the use of the surfactant mixtures can generate, depending on their concentrations, at zero interfacial tension (null curvature of the interface) vesicles into the oil-water mixtures.[11] Any imbalance in this report leads to the realization of a double emulsion whose type is determined by the HLB value of the surfactant mixture.

Liposomal systems and vesicles are characterized by large fluctuations of the bilayer, which can be easily deformed and sheared.[11] This explains the destruction of the dispersed system by strong shearing.

Also noteworthy is the differential behavior of the colloidal systems according to the NaCl concentration of the solution. The formation of a greater number of layers with increasing saline concentration reminds of increased stability of simple inverse emulsion. Taking into account that methods for making vesicles and liposomes, producing them only with phospholipids (such as surfactants), it is expected that the presence of Span 80, a hydrophobic emulsifier at a high enough concentration, relative to the oily phase will have a big influence on the type and stability of such systems.

The safflower oil is a complex system with mixed content originated from the plant material and it contains, amongst others, phospholipids, which are hydrophilic surfactants.[2] Previous studies have shown that by dispersion of edible oil in a saline solution, in the presence of a hydrophobic surfactant a dispersed system with complex structure is obtained, not a simply reversed emulsion.[12, 13] This structure is due to the presence of different types of surfactants in the same medium.

The aim of this work is to highlight the type of vesicles that appear in the liquid-liquid colloidal systems prepared by dispersing the safflower oil in the solution of sodium chloride in the presence of a hydrophobic surfactant, the sorbitan monooleate – Span 80.

We propose to prepare the colloidal systems which contain safflower oil in the presence of a hydrophobic surfactant (Span 80), and we want to prove the influence of hydrophilic surfactants derived from plant material on the behaviour of the system. In this way we want to emphasize the role of these emulsifiers to form complex colloidal systems and the type of these colloids.

RESULTS AND DISCUSSION

Observed by optical microscopy, the premix has a relatively monodisperse appearance regardless of the saline solution concentration with which it was prepared (Fig. 1).

By the dispersion of the premix in the oil, at a moderate speed (1000 r.pm.), double and even multiple emulsions are obtained, although their proportion is low. The characteristics of these emulsions are: relatively large dimensions of globules and the irregular walls of these globules (Fig. 2).

Fig. 2 shows the presence of the vesicles rather than the globules of the double emulsion, which has been explained in vision of elasticity of the bilayers theory.[14] This assertion is based on the non-uniform appearance of the walls. The behavior is due to the walls fluctuation phenomenon of dispersed droplets, which is characteristic to the vesicles and the liposomes.[9-11] As a result of zero interfacial tension, at these droplets is found an occurrence of “corners” which indicate the existence in regions immediately surrounding of areas with positive, negative, and even zero curvature. Such a phenomenon occurs at the contact between the two emulsions drops when generating an orifice which leads to the droplets coalescence, so to the emulsion destruction; this phenomenon is very fast, the droplets of dispersed phase in emulsions consistently showing one type of interface curvature (due to the surfactant type). In the case of vesicles and liposomes, the walls are elastic and can present as a result of thermal agitation, small oscillations around the equilibrium position (to the change of curvature, which is either positive or negative), without destroying the system.[9-11]

a b

Fig. 1 – Microphotographs of premix (1090×10x) containing: a) 0.6 M NaCl; b) 0.4 M NaCl.

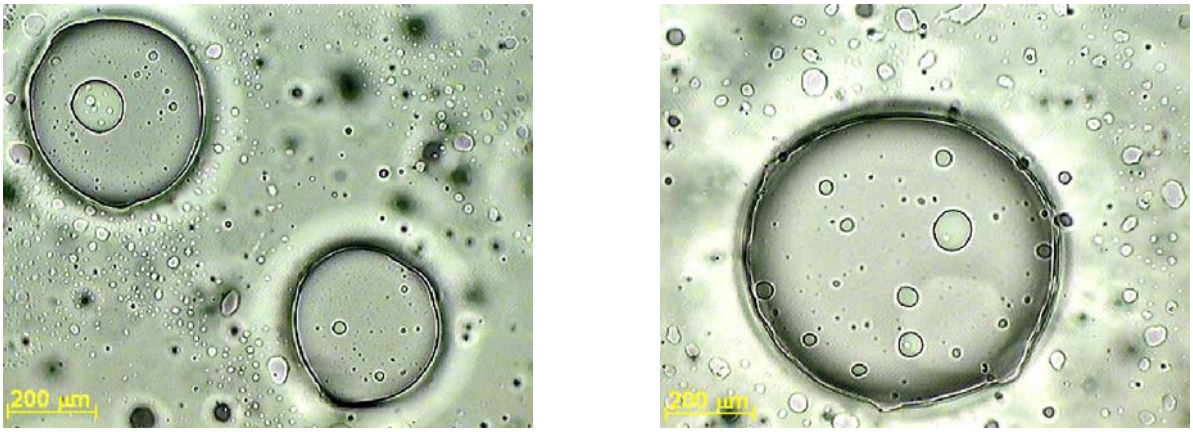

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Fig. 2 – Appearance of dispersed systems safflower oil – NaCl solution obtained using a moderate speed (1000 r.p.m.), observed under the microscope with 100×10x, realized with: a) 0.8 M NaCl; b) 1 M NaCl.

Another indicator of the vesicles presence in the dispersed systems is the wall thickness of the dispersed droplets. In the case of the emulsion droplets, the separation interface is carried out by means of a surfactant layer, having the polar part oriented to aqueous phase and non-polar part oriented to the oily phase. The formation of vesicles or liposomes involves making at the interface of one or more bilayers of surfactant arranged in parallel layers. As a result, the boundary surface becomes thicker, which is equal to the thickness of parallel superimposed layers of surfactant. Such a phenomenon can be seen in Fig. 2, where the distinction between internal and external phase of the droplets is extremely clear.

If the colloidal systems are prepared by moderate agitation (by dispersing the premix in the oil at 1000 r.p.m.) and the concentration of salt in the aqueous phase is low (0.2 ÷ 0.4 M) the mono-layer vesicles are obtained (see Fig. 3 a).

When in the aqueous phase a high concentrations of salt (0.8 ÷ 1.0 M) is added, the multi-layered vesicles are formed (Fig. 3 b).

The formation of vesicles occurs because the edible oils are complex systems, which contain not only non-polar compounds (fatty acids and triglycerides), but also various substances from the plant material that may be surface active. From this point of view, a special effect is produced by the phospholipids, which can operate as direct emulsifiers. Since in the vegetable oil refining process concentrated solutions of NaOH were used, the presence of some small traces of formed soap is sufficient to alter the properties of the oil-water interface. As a result of the presence of these substances, the behavior of the colloidal systems prepared by dispersion of edible oil in an aqueous solution using a hydrophobic surfactant is very complex.

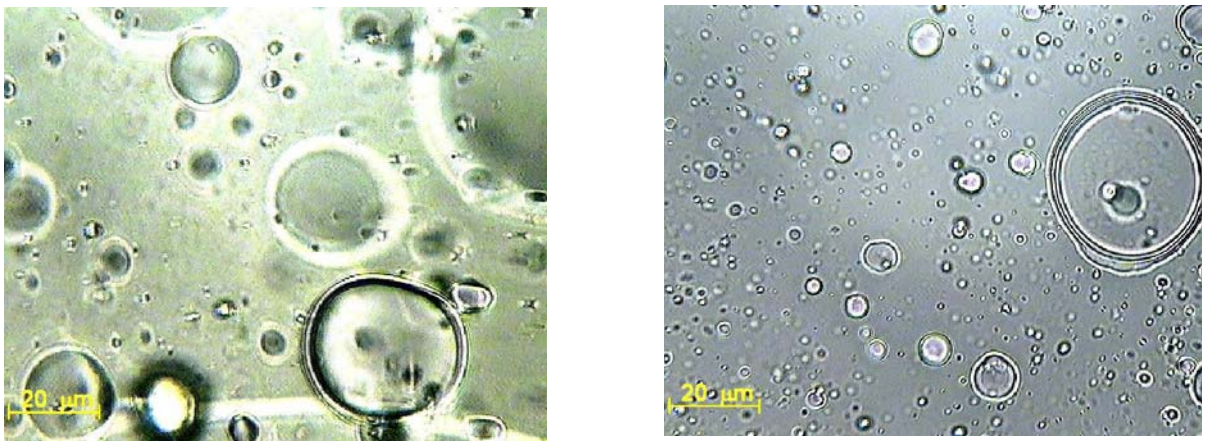

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Fig. 3 – Photos of dispersed systems safflower oil-NaCl solution obtained using a moderate speed (1000 r.p.m.), observed under the microscope with 1090×10x, realized with: a) 0.2 M NaCl; b) 0.8 M NaCl.

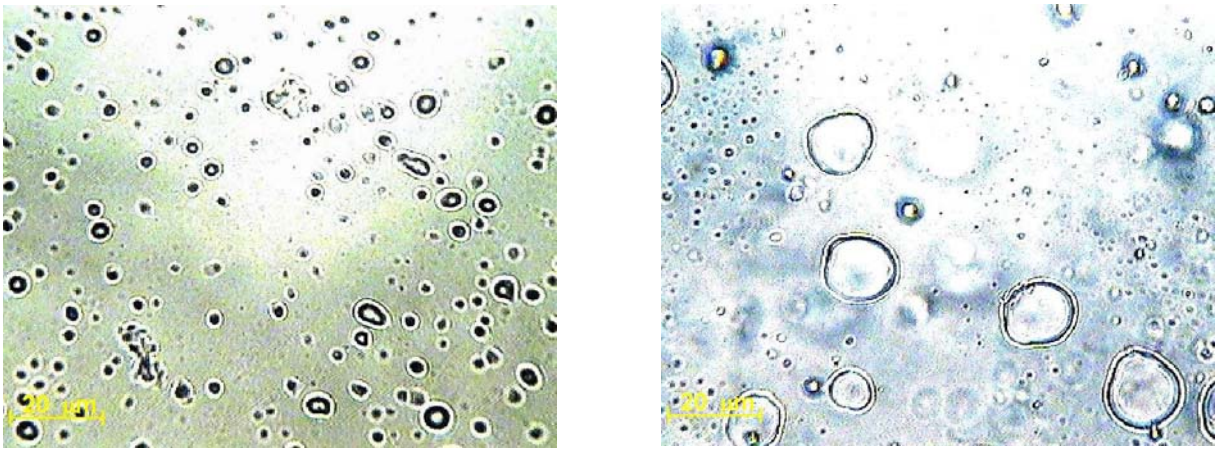

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Fig. 4 – Appearance of dispersed systems safflower oil – NaCl solution obtained using a vigorous speed (3500 r.p.m.), observed under the microscope with 1090×10x, realized with: a) 0.4 M NaCl; b) 1 M NaCl.

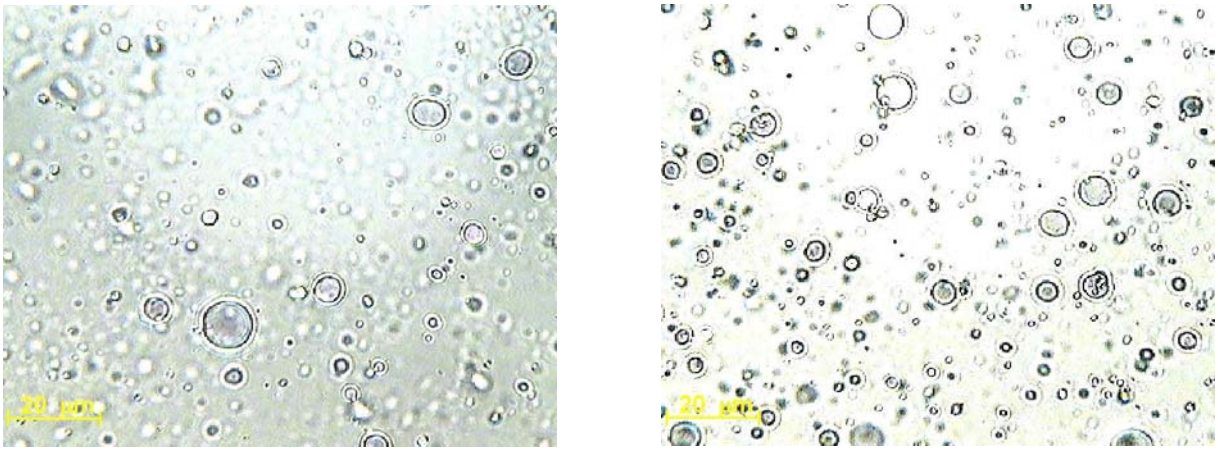

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Fig. 5 – Photos of dispersed systems safflower oil – NaCl solution 0.6 M, observed under the microscope with 1090×10x, obtained: a) using a moderate speed (1000 r.p.m.); b) using a vigorous speed (3500 r.p.m.).

If the dispersion of the premix in the oil is carried out by vigorous stirring (3500 r.p.m.), mono-layer vesicles are obtained irrespective of the NaCl concentration in the aqueous phase

(Fig. 4). In this case, even if the salt concentration is high, the dispersed vesicles are smaller than the colloids obtained by mild agitation.

The intensity of the shearing process has no influence on the appearance of colloidal systems made with intermediate concentrations of NaCl solutions (0.6 M), such as the vesicles size of the dispersed phase (see Fig. 5).

When aqueous phase contains intermediate NaCl concentration (0.6 M), the formation of vesicles is less likely. The size of the vesicles obtained in this case is reduced. This observation can be correlated with results of others authors obtained for similar systems, prepared with edible oils, when a maximum stability of the double emulsions was observed for the same concentration of NaCl in the aqueous phase.[12, 13]

The intensity of the shearing process has also an impact on how the colloidal systems evolve, both in terms of systems type that are obtained, and from the point of view of the rate of destruction of the colloidal systems.

In the disperse systems obtained using moderate shear, the colloidal droplets change their appearance in time. In the disperse systems containing a higher concentration of NaCl solutions, the vesicles keep about their size, but the poly-layer vesicles become the mono-layer vesicles (Fig. 6). It is interesting that in this case is not a profound change of the vesicles size, but the redistribution of surfactants at the interface predominates.

Microscopically, in systems containing dilute solutions of NaCl growth of the vesicles can be observed in time (Fig. 7); the phenomenon can be explained both by the coalescence process occurring at moderate speed (the existence of both types of surfactants on the same interface, or the cancellation of the interface tension and the interfacial tension fluctuations causes the easier formation of the holes and channels in the liquid film, resulting in the phenomenon of coalescence), and through Ostwald ripening process (which takes place as a result of the high degree of the droplet polydispersity).

The mono-layer vesicles undergo a destabilization processes. Subsequently, the colloidal system is converted into an emulsion, fact attested by the vesicles size analysis, performed three days after preparation (Fig. 8). It is noticed that the dispersed droplets have, in this case, a small size (average diameters is about 5 μm). This emulsion is very stable (more than a month).

At the end of this process, during which the dispersed droplets change their appearance (either increase by coalescence or the surfactants redistribution occurs, with the formation of vesicle mono-layer), which takes about two days, a slow coalescence occurs. Separation of phases appears slowly (in more than one month) resulting in clear aqueous phase and an oily phase.

In the disperse systems obtained by high speed stirring, rapid destabilization of the dispersed system takes place with immediate separation of the phases, in less than one day (see Fig. 9 b).

Particle size analysis of the complex obtained at high shear rates, performed nine hours after preparation, indicating an average diameter of dispersed vesicles of about 1 nm, which is close to detection limit of the apparatus (see Fig. 10).

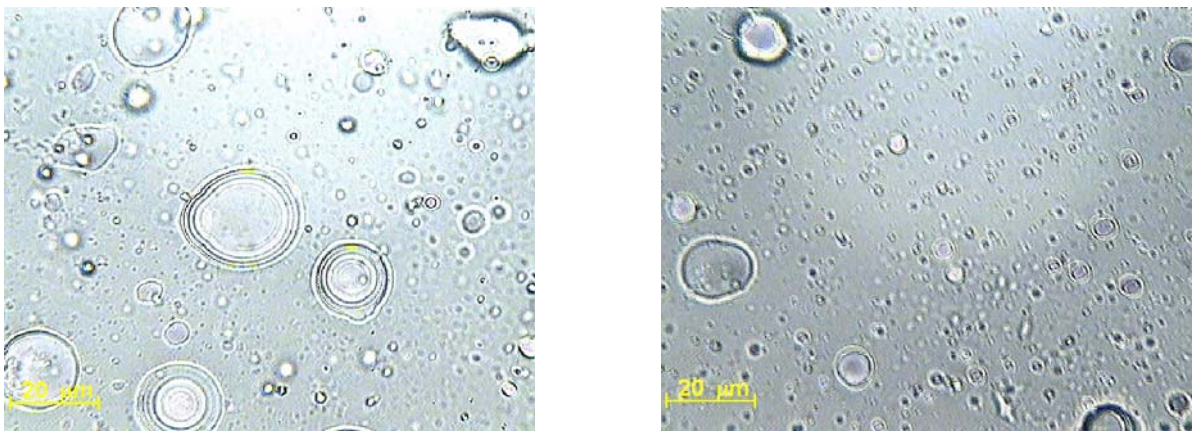

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Fig. 6 – Appearance of dispersed systems safflower oil – 1 M NaCl, observed under the microscope with 1090×10x, obtained using a moderate speed (1000 r.p.m.): a) immediately after preparation; b) one day after preparation.

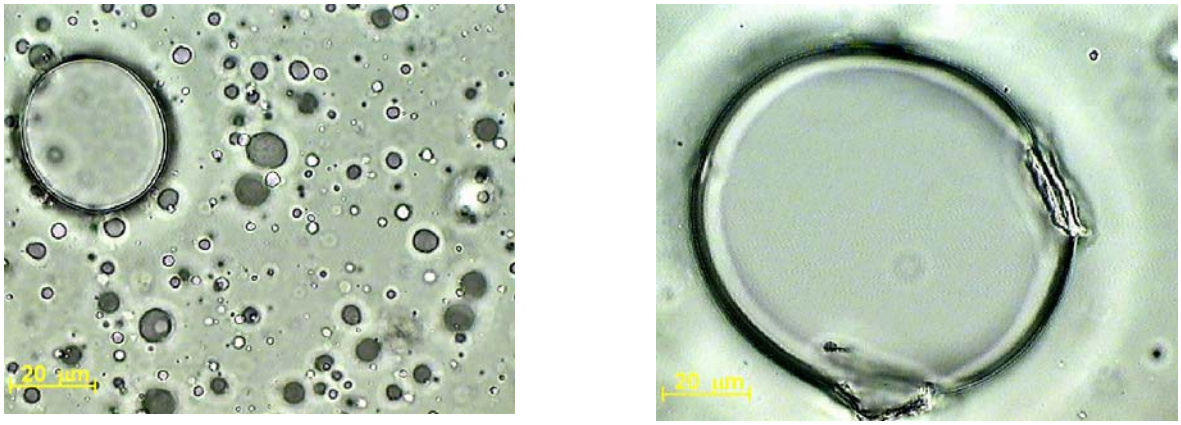

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Fig. 7 – Photos of dispersed systems safflower oil – 0.2 M NaCl, observed under the microscope with 1090×10x, obtained using a moderate speed (1000 r.p.m.): a) immediately after preparation; b) one day after preparation.

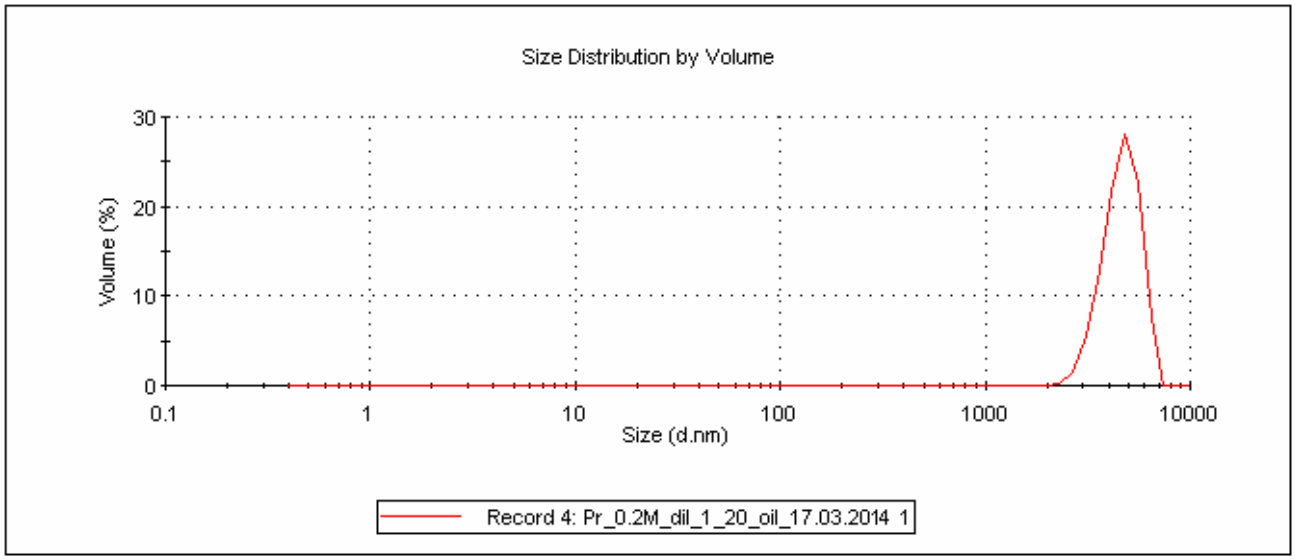

Fig. 8 – Size analysis of the droplets of the colloidal system with NaCl solution 0.2 M, obtained using a moderate speed (1000 r.p.m.), three days after preparation.

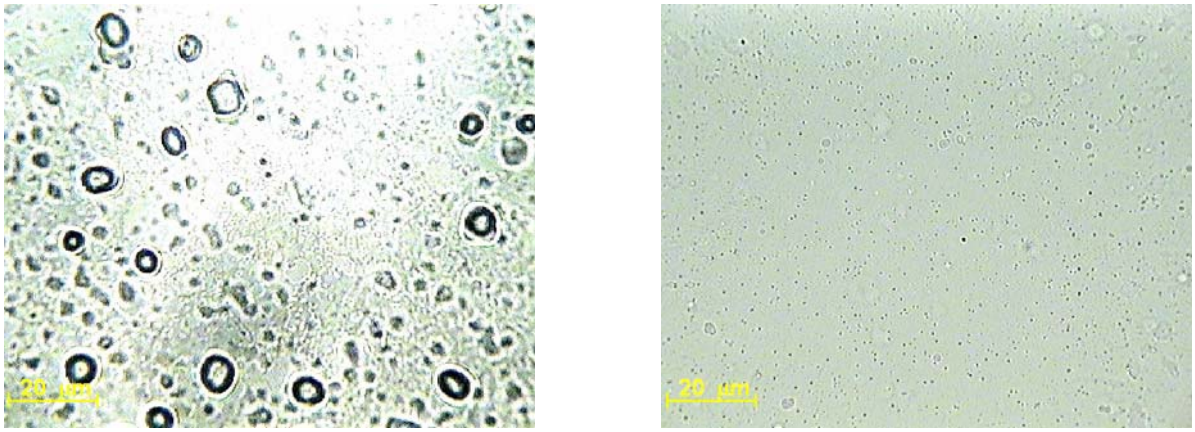

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Fig. 9 – Appearance of dispersed systems safflower oil – 0.4 M NaCl, observed under the microscope with 1090×10x, obtained using an intense speed (3500 r.p.m.): a) immediately after preparation; b) one day after preparation.

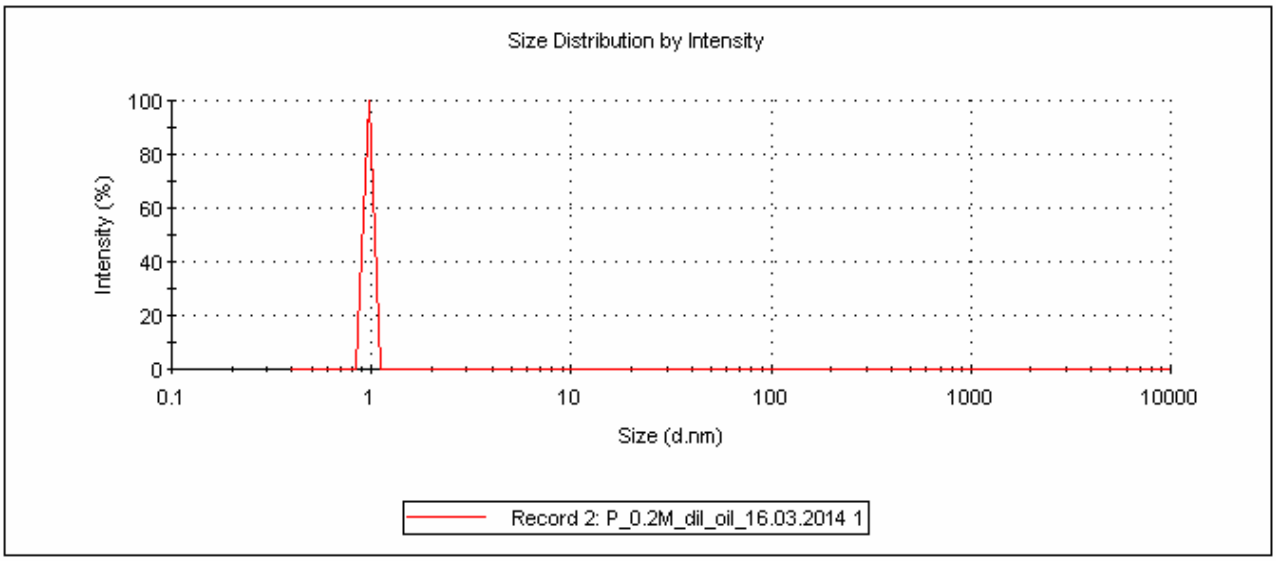

Fig. 10 – Size analysis of the particle of the colloidal system with NaCl solution 0.2 M, obtained using an intense speed (3500 r.p.m.), nine hours after preparation.

This very low size of the drops indicates that in the system only spherical surfactant micelles and free molecules surfactant (dispersed in the continuous volume phase), having nanometric dimensions are present.

Therefore, the particle size analysis confirms that the systems obtained by high shear are less stable. These colloidal systems are destroyed in less than nine hours.

After only one day a rapid phase separation occurs in these disperse systems (made by high speed stirring), resulting a clear aqueous phase and an oily phase.

EXPERIMENTAL

1. Materials

Safflower oil obtained by cold pressing without using solvents was supplied by Herbavita BVBA (Belgium). In this research the sorbitan monooleate (Span 80) was used as surfactant from Aldrich (Germany). As aqueous phase were used sodium chloride solutions at different concentrations (0.2 ÷ 1 M), made from 99.9% NaCl (Fluka-Germany). In all the experiments of this work bidistilled water was used.

2. Obtaining of the colloids

The dispersed systems with the composition shown in Table 1 were prepared. The NaCl solutions of various concentrations were used as aqueous phase and the safflower oil was used as oily phase. The sorbitan monooleate (Span 80) was used as surfactant; its concentration was 5% (wt/v) in the oil mass, in all the cases.

To prepare the colloidal systems, in the first step a premix was made by introducing the NaCl solution in the surfactant. Then, the premix was dispersed in the same amount of oil, using a mechanical stirrer with a speed of 1000 r.p.m. for 5 min. Similar dispersions were also obtain using a speed of 3500 r.p.m. for 5 min.

3. Characterization of the vesicles

The obtained dispersed systems were followed through video enhanced microscopy by using a videocamera (VEM). The size and morphology of the vesicles were determined by using a Motic microscope (China) having objectives of 100x and 1090x (Optirom-Roumania). Using the program attached to the Motic microscope we noticed the approximate dimensions of the vesicles. Measurements of the mean diameter and size distribution of the vesicles by dynamic light scattering (DLS) were performed using a granulometer Zetasizer Nano from MALVERN.

Table 1

Composition of colloidal systems with safflower oil and NaCl solution

Sample	NaCl solution		Safflower oil
	Concentration (M)	Volume fraction (%)	Volume fraction (%)
1	0.2	20	80
2	0.4	20	80
3	0.6	20	80
4	0.8	20	80
5	1.0	20	80

CONCLUSIONS

Liquid-liquid colloidal systems consisting of safflower oil, Span 80 and sodium chloride solutions were prepared.

The systems obtained at a stirring speed of 1000 r.p.m. and 0.2 ÷ 0.4M NaCl appear as single-layer vesicles, whereas for 0.8 ÷ 1.0 M NaCl multi-layer vesicles are produced. One major difference between the two categories of the colloidal systems mentioned above is the concentration of NaCl solution when vesicles are producing.

The use of high speed agitation, namely 3500 r.p.m., leads to formation of mono-layer vesicles even at high concentrations of NaCl 1.0 M. Most stable colloidal systems are produced by moderate agitation. At concentrations of 0.6 M arise small and very unstable vesicles, regardless of shear rate.

In the colloidal system of safflower oil-NaCl solution the obtaining of vesicles is due to a balance between the hydrophobic surfactant (Span 80) and the hydrophilic surfactant (traces of soap and phospholipids), which ensures the stability of the system.

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