



## PROPERTIES OF WATER IN OIL EMULSIONS (W/O) STABILIZED WITH MIXTURES OF PGPR AND POLYGLYCEROL FATTY ACID ESTERS

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*Water in oil (W/O) emulsions are dispersed systems which have very wide application as a carriers in the food, pharmaceutical and cosmetic industry products. The main problem with practical application of such systems is their low stability. The emulsifiers used to stabilize this type of emulsions are with low hydrophilic-lipophilic balance values. The present work examines the possibility of the application of mixtures of lipophilic emulsifiers polyglycerol polyricinoleate (PGPR) and polyglycerol fatty acid esters for stabilization of W/O emulsions. First of all, the adsorption properties of the used emulsifiers were examined by tensiometric measurements. Based on these results, two emulsifiers PGPR and decaglycerol decaoleate (Caprol 10G100) were selected for the preparation of the emulsions, as well as their mass ratios and total concentrations. The results of the investigation of the emulsions properties (dispersion analysis and sedimentation stability) showed that more stable emulsions can be obtained by decreasing the Caprol 10G100 mass ratio and increasing the total concentration of emulsifiers.*

**KEY WORDS:** lipophilic emulsifiers, PGPR, polyglycerol fatty acid esters, W/O emulsions

### INTRODUCTION

There are many products in the pharmaceutical, cosmetic and food industry that contain W/O emulsions. The most commonly utilized products in the food industry that are in the form of W/O emulsions are butter, margarine, spreads and shortenings (1, 2). W/O emulsions can also be used to encapsulate and control the release of hydrophilic active ingredients such as water-soluble vitamins, flavors, colors, proteins (3-5). One of their applications is also as the inner (primary) emulsions in double W/O/W emulsions.

The stability mechanism of these emulsions differs from the oil in water (O/W) emulsions, which can be stabilized by both, steric and electrostatic repulsion. In the case of W/O emulsions, only steric forces are expected to stabilize the emulsion, because of the low electrical conductivity of the continuous phase (6). The main problem with practical application of such systems is generally their low stability, because of the high mobility of water droplets, which can easily sediment, flocculate or coalesce. A better understanding of the interactions between water, oil and emulsifier at the interfaces would

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allow producing stable W/O emulsions and, therefore, encourage the development of new products and applications (7).

The emulsifiers used to prepare W/O emulsions have low hydrophilic-lipophilic balance (HLB) values. The development and application of food grade emulsifiers have followed the advancement in the food industry, which has increased tremendously over the last few decades. Numerous types of oil soluble emulsifiers have been used either individually or in combination to facilitate the formation and stability of W/O emulsions. Among these, polyglycerol esters of fatty acids (PGEs) are used in a number of food products. Commercial PGEs may vary considerably in composition owing to differences in the extent in polymerization of the glycerol, in the nature of the hydrophilic part, and in the degree of esterification (8). Considering the wide interval of HLB values, they may be used as a stabilizer of W/O and O/W emulsions (9). In the European Union regulation for food additives, PGEs are given the reference number E475.

Polyglycerol polyricinoleate (PGPR) has been reported as the most effective emulsifier for the stabilization of W/O emulsions (10). It is oligomeric, nonionic emulsifier produced by the esterification of castor oil fatty acids with polyglycerol. It is commonly used emulsifier in chocolate manufacture, since it has been shown to inhibit thickening of chocolate due to its excellent water binding properties (11). PGPR is given the reference number E476.

The aim of the present work was to investigate the possibility of formation stable W/O emulsions stabilized with the mixtures of PGPR and polyglycerol fatty acids esters. First of all, the adsorption properties of the surfactants and their mixtures were investigated. After this, stability parameters, such as droplet size and droplet size distribution and sedimentation index of emulsions stabilized with selected mixtures of surfactants, were measured.

## EXPERIMENTAL

### Materials

The following oil soluble surfactants were used: polyglycerol polyricinoleate (PGPR) donated by Jaffa Crvenka, Serbia; decaglycerol decaoleate (Caprol 10G10O), triglycerol monostearate (Caprol 3GS), hexaglycerol octastearate (Caprol ET), all from Abitec, USA, whereas the oil phase was medium chain triglycerides (MCT) of caprylic/capric fatty acids (Saboderm TCC) produced by Sabo Spa, Italy. Deionized water was used as aqueous phase.

### Methods

**Preparation of solutions.** The stock solutions of lipophilic emulsifiers were prepared by the dissolution of appropriate amount of emulsifier in the oil phase. Solutions of lower concentrations were obtained by diluting the stock solutions. Binary mixtures of the emulsifiers PGPR and Caprol 10G10O and PGPR and Caprol 3GS, at different mass ratios (1:1; 1:2; 1:5 and 1:10), were prepared by mixing the stock solutions and adding a



desired amount of the oil phase, to obtain the concentrations of the emulsifiers of 1; 2 and 3% (w/v). The HLB values of these mixtures were calculated as follows:

$$HLB_{mix} = x_A \cdot HLB_A + (1 - x_A) \cdot HLB_B$$

where  $HLB_A$  and  $HLB_B$  are HLB are the values of emulsifiers A and B, and  $x_A$  is the mass fraction of emulsifier A in the mixture. The calculated values are presented in Table 1.

**Table 1.** HLB values of the binary mixtures of lipophilic emulsifiers PGPR and Caprol 10G100 and PGPR and Caprol 3GS

PGPR:Caprol 3GS	HLB <sub>mix</sub>	PGPR:Caprol 10G100	HLB <sub>mix</sub>
1:1	4.75	1:1	3.25
1:2	5.28	1:2	3.30
1:5	5.91	1:5	3.42
1:10	6.19	1:10	3.46

**Tensiometric measurements.** Measurements of the interfacial tension between water and oil with various surfactant concentrations were carried out on a Sigma 703D tensiometer (KSV Instruments, Finland), using the *Du Noüy* ring method (12). Prior the measurements, the ring was immersed in water phase, then the oil phase slowly added on the top, and the interface was left for 15 min to equilibrate. The interfacial tension was measured at the point where the ring broke away from the interfacial layer between two phases. In all experiments the temperature was kept at 40 °C. The reported values of the interfacial tension were average of three measurements, at least.

**Preparation of W/O emulsions.** The emulsions were prepared at the water-oil mass ratio 20:80, while the aqueous phase was deionized water and continuous phases were 1; 2 or 3% (w/v) solutions of the binary mixtures of lipophilic emulsifiers, at the mentioned mass ratios, in MCT. The emulsions were prepared by dispersing water phase in the continuous phase at 40 °C by means of an *Ultra Turrax* T25 homogeniser (Ika, Germany) at 20,000 rpm during 10 min.

**Emulsion stability test.** For stability test, the emulsions were transferred into 10 ml graduated cylinders and stored at room temperature for 14 days. The emulsions were observed for the changes in homogeneity and phase separation during storage. The oil phase separation was visually monitored at certain time intervals. The total height of the emulsion,  $H_E$ , and the height of the oil layer,  $H_O$ , were measured. The extent of the phase separation was characterized by the sedimentation index,  $H$ , given as:

$$H = \frac{H_O}{H_E} \cdot 100(\%)$$

A higher value of the sedimentation index indicates a worse emulsion stability.



## Droplet size and polydispersity index analysis

Particle size analysis of the emulsions was performed by dynamic light scattering (DLS) measurements using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK) at 25 °C. Prior to the measurements, all samples were diluted with oil, in order to yield a suitable scattering intensity. The refractive index of MCT was 1.448. The DLS data were analyzed using the general purpose mode, thus the hydrodynamic diameter (z-average) was obtained.

### Statistical analysis

Paired *t* test at the 95% confidence level was performed to compare the difference between two measurements, and for this purpose Origin Pro 8.0 software was used.

## RESULTS AND DISCUSSION

### Interfacial tension measurements

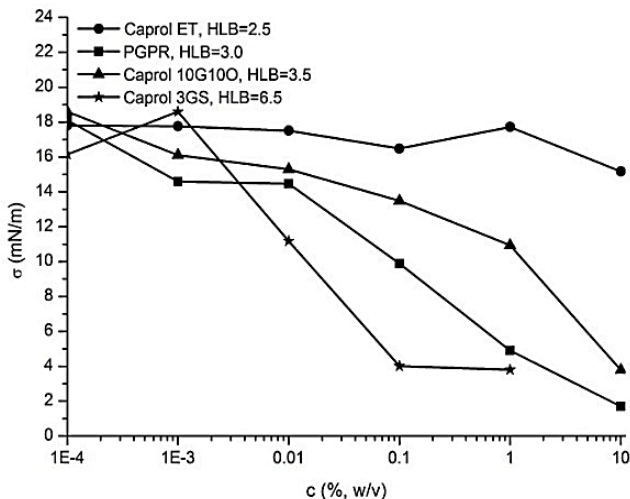
Oil and water do not mix due to the existence of the interfacial tension, which is generally about 30-35 mN/m. Addition of the surface active molecules to the system reduces interfacial tension to 1-5 mN/m, enabling dispersion one phase into the other, i.e. formation of emulsion. Except of lowering the interfacial tension, the adsorbed surface active molecules form steric and electrostatic barrier between the dispersed phase drops, preventing their coalescence.

In this work we investigated first the adsorption properties of the selected lipophilic emulsifiers. The lipophilic emulsifiers, PGPR, Caprol 10G100, Caprol ET and Caprol 3GS, differ by the fraction of glycerol and fatty acids in the molecule, as well as by the type of fatty acids. The adsorption properties were investigated by measuring the changes in the interfacial tension with the concentration of the emulsifiers in pure solutions and in their binary mixtures, which is presented in Figure 1.

As it can be observed, the interfacial tension for all investigated systems decreased with an increase in the emulsifiers concentration. Comparing the total lowering of the interfacial tension by different emulsifiers at the same concentration, it can be noticed that polyglycerol fatty acid esters significantly lower the interfacial tension by increasing their HLB values. The increase in the HLB number is due to an increase in the fraction of the hydrophilic part in the emulsifier molecule, thereby increasing its affinity to the interface at which it is oriented so that the hydrophilic part is displaced into the aqueous phase (13). On the other hand, PGPR induces higher reduction of the interfacial tension compared to Caprol 10G100, although it has a lower HLB number. This is a consequence of the branched hydrophobic chain of the PGPR molecule, which allows formation of a compact film at the interface (14, 15). Caprol ET did not show a significant effect on the interfacial tension, which is expected due to the low HLB number (2.5) and its lipophilic properties, i.e. extremely good solubility in the oil phase. Based on these results,



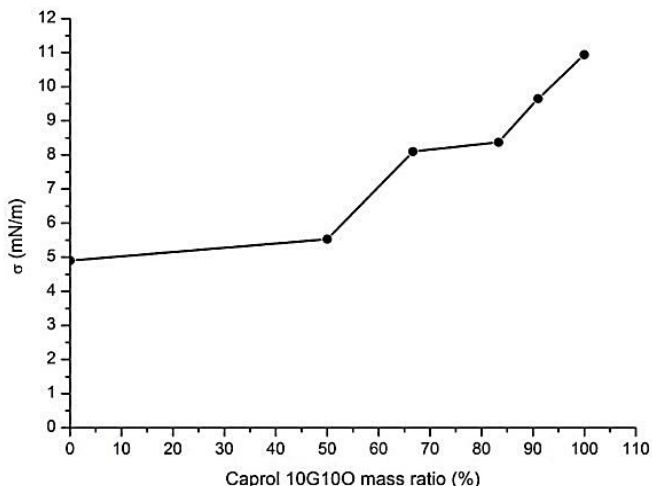
the emulsifiers which showed significant influence on the interfacial tension, i.e. PGPR, Caprol 10G100 and Caprol 3GS, were chosen for further investigations.



**Figure 1.** Changes of the interfacial tension at the O/W interface with emulsifier concentration in the oil at 40 °C.

It is known that the emulsifiers in the mixtures show different behavior compared to the pure solutions (16). In some cases, the emulsifiers in binary mixtures show a synergistic effect on the interfacial tension reduction. However, it is not always the case, because sometimes the presence of different emulsifier molecules in the system can lead to their worse packing at the adsorption layer, which depends on their properties and interactions.

Although the PGPR has a very wide application, especially in the food industry, there are increasing tendencies for reduction its concentration in some products. On the other hand, comparing the maximum allowed daily intakes (MADI) for PGPR (7.5 mg/kg bodyweight) and emulsifiers from Caprol group (25 mg/kg bodyweight) it can be seen that MADI for Caprol emulsifiers is about three times higher. For that reason, in the next experiments, the influence of the increasing mass ratio of Caprol 3GS and Caprol 10G100 in the mixture with PGPR, on interfacial tension was investigated. However, in the mixtures of PGPR and Caprol 3GS with a mass ratio of Caprol 3GS higher that 0.5, a solid film of the surfactant was formed at the interface, so the measurement of interfacial tension was not possible. For this reason, the investigations included only binary mixtures of PGPR and Caprol 10G100 (Figure 2).



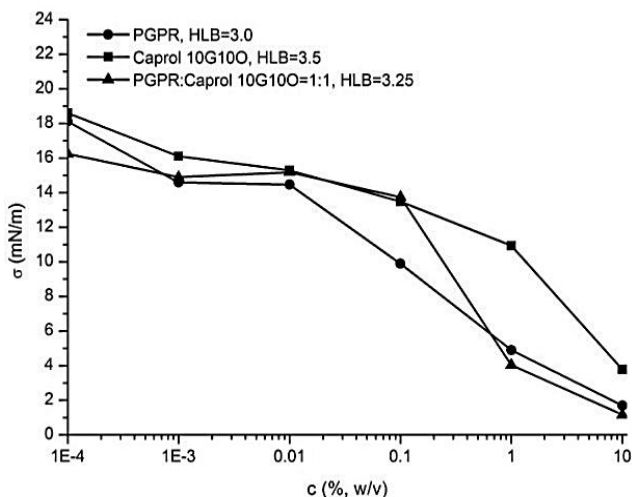
**Figure 2.** Changes of the interfacial tension at the O/W interface with increasing Caprol 10G100 mass ratio in the binary mixture with PGPR, at total emulsifier concentration of 1% (w/v) at 40 °C.

As it can be seen in Figure 2, the increase in the Caprol 10G100 mass ratio in the mixture with PGPR induces an increase in the interfacial tension at O/W interface. Although the mixtures with higher mass ratio of Caprol 10G100 have higher HLB values (Table 1), its worse emulsification characteristics are dominant here. However, the reduction of interfacial tension to a value 5.53 mN/m can be obtained with binary mixture of these two emulsifiers at their mass ratio of 1:1.

Besides the emulsifiers mass ratio in the mixture, the total concentration influences the interfacial tension and stability of the adsorption layer. For that reason, the influence of the total emulsifiers concentration, in the binary mixture of Caprol 10G100 and PGPR at their mass ratio 1:1, on interfacial tension was investigated. The results are presented on Figure 3 and Table 2, showing also the changes in the interfacial tension with the concentrations of single emulsifier solutions.

It is clear from Figure 3 and Table 2 that, as in the case of the solutions with individual emulsifiers, in their binary mixtures an increase in the total emulsifier concentration leads to lowering of the interfacial tension. On the other hand, comparing the single emulsifier solutions with their binary mixtures, it can be noticed that the emulsifiers mixtures, at concentrations higher than 0.1% (w/v), showed a greater reduction of the interfacial tension, which is a consequence of a synergistic effect of the emulsifiers molecules in the mixture. The lowering of the interfacial tension by the mixture of emulsifiers in comparison with the pure emulsifier solutions were statistically significant ( $P < 0.05$ ).

Based on these results, it is clear that a minimal emulsifier concentration required to achieve the necessary reduction of interfacial tension is 1% (w/v).



**Figure 3.** Changes of the interfacial tension at O/W interface with the emulsifier concentration in oil solutions of PGPR, Caprol 10G100 and their binary mixtures at a mass ratio 1:1, at 40 °C.

**Table 2.** Changes of the interfacial tension  $\pm$  standard deviation of PGPR, Caprol 10G100 and PGPR:Caprol 10G100=1:1 mixture with the emulsifier concentration

c (% w/v)	$\sigma$ (mN/m) $\pm$ SD <sup>a</sup>		
	PGPR	Caprol 10G100	PGPR:Caprol 10G100=1:1
0.0001	18.11 $\pm$ 0.085	18.6 $\pm$ 0.087	16.24 $\pm$ 0.108
0.001	14.59 $\pm$ 0.056	16.11 $\pm$ 0.051	14.89 $\pm$ 0.090
0.01	14.47 $\pm$ 0.064	15.3 $\pm$ 0.100	15.19 $\pm$ 0.140
0.1	9.89 $\pm$ 0.076	13.49 $\pm$ 0.056	13.73 $\pm$ 0.089
1	4.9 $\pm$ 0.080	10.94 $\pm$ 0.040	4.04 $\pm$ 0.147
10	1.69 $\pm$ 0.036	3.79 $\pm$ 0.021	1.17 $\pm$ 0.076

<sup>a</sup> - mean  $\pm$  standard deviation

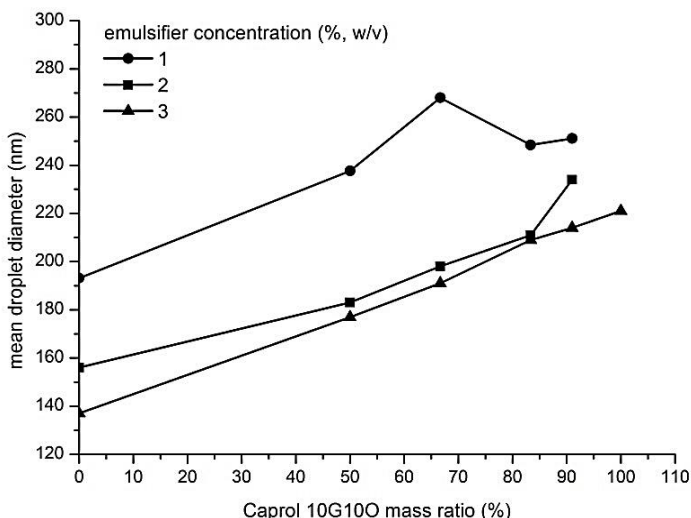


## Properties of the emulsions stabilized with PGPR and Caprol 10G100 mixtures

Previous measurements showed good surface activity of the lipophilic emulsifiers PGPR and Caprol 10G100, as well as a synergistic effect of their molecules on surface tension reduction at the O/W interface. However, the low value of interfacial tension is not the only precondition for the formation of a stable dispersed system. For this reason, the possibility of obtaining stable 20% W/O emulsions, stabilized with these mixtures of emulsifiers, was further investigated.

The dispersed phase droplets size is the parameter which shows a high influence on the emulsions stability. A lower diameter of the dispersed phase droplets and higher uniformity of droplet size distribution increase the sedimentation stability of the system, due to the reduced tendency toward coalescence and Ostwald's ripening. For this reason, in the next set of experiments the properties of the 20% W/O emulsions, stabilized with PGPR:Caprol 10G100 mixtures at their mass ratios: 1:0; 1:1; 1:2; 1:5; 1:10; 0:1, were investigated. The total concentrations of emulsifiers were 1, 2, and 3% (w/v).

By microscopic examination of the obtained emulsions, very small droplets were observed, so the method of light scattering on a Zetasizer Nano ZS apparatus was used for the dispersion analysis. The changes of droplets mean diameter with increasing Caprol 10G100 mass ratio in the mixtures with PGPR at different total emulsifier concentrations, are presented in Figure 4.



**Figure 4.** Changes of the droplets mean diameter of 20% W/O nanoemulsions with increasing Caprol 10G100 mass ratio in the mixtures with PGPR, at total emulsifiers concentrations of 1, 2, and 3% (w/v).



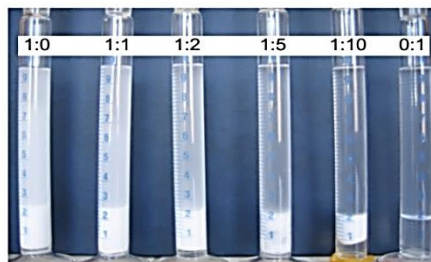
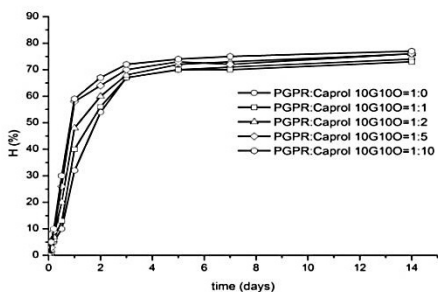


As it is shown in Figure 4, all emulsion samples have droplets with a mean diameter lower than 280 nm, i.e. the obtained systems were nanoemulsions. Also, the increase in the Caprol 10G100 mass ratio induced an increase in the in droplets mean diameter. This is in agreement with the tensiometric measurements, which showed a worse surface activity of Caprol 10G100 molecules. Also, the increase in the total emulsifiers concentration induced a reduction of the droplet mean diameters. The dispersion analysis could not be performed on the emulsion samples stabilized with 1 and 2% (w/v) of pure Caprol 10G100 because of their pronounced instability. Namely, these emulsions broke down right after the preparation.

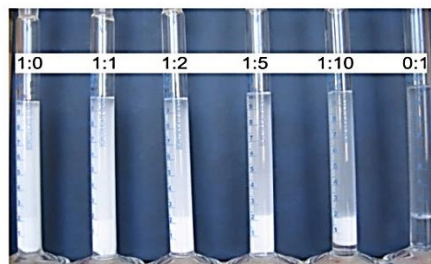
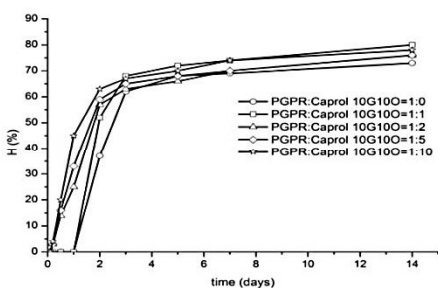
A very important characteristic of the emulsions from their application aspect, is their sedimentation stability. Sedimentation instability is a consequence of the density difference between the dispersed and the continuous phase. Namely, due to the effect of gravity force phase separation occurs, and if dispersed phase has lower density their droplets goes to the top, forming a creamy layer, i.e., if the dispersed phase has a higher density, its droplets go down and form a sediment layer (17, 18). In this sense, the stability of the investigated nanoemulsions is expressed as a change of the sedimentation index (H) over time, as shown in Figure 5. Stability was monitored for 14 days at room temperature, and the appearance of the emulsions after this time period was also shown in Figure 5.

In all the nanoemulsions stabilized with PGPR and Caprol 10G100 mixtures, at a total emulsifiers concentration of 1% (w/v), separation was observed just in the first few hours after the preparation. The sedimentation index increased gradually during the first five days, but after this period no significant change of this parameter was noticed (Figure 5A). In the nanoemulsions stabilized with the mixtures of PGPR and Caprol 10G100, at their mass ratios 1:0 and 1:1 and with total emulsifiers concentration of 2% (w/v) (Figure 5B), separation occurred two days after the preparation, while in the nanoemulsions with higher Caprol 10G100 mass ratio separation occurred just at the first few hours after preparation. A further increase in total emulsifiers concentration to 3% (w/v) (Figure 5C) led to more pronounced differences in the separation rate. Namely, at this concentration, in the nanoemulsions stabilized with PGPR and Caprol 10G100 at mass ratios of 1:0; 1:1 and 1:2 separation occurred seven days after the preparation, with a significantly lower value of sedimentation index (less than 10%), indicating their high sedimentation stability. The nanoemulsions with higher Caprol 10G100 ratios separated just at the first few hours after the preparation. In view of the fact that the separation of emulsion is a consequence of sedimentation instability, i.e. spreading of the emulsion droplets by size, the values of sedimentation index are in correlation with the results of dispersion analysis.

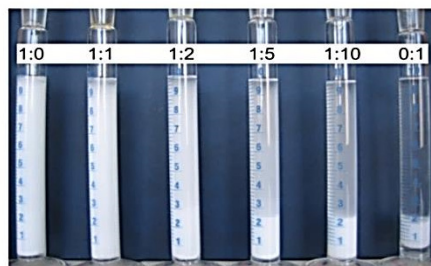
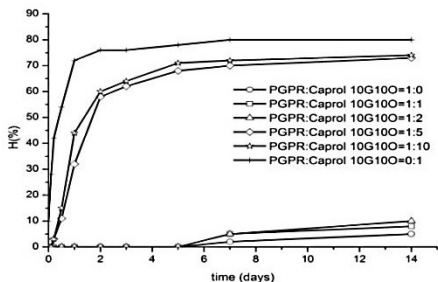
Figure 5 it clearly shows that in the nanoemulsions stabilized with PGPG and Caprol 10G100 mixtures at the emulsifiers mass ratio 1:10 and their total concentration of 1 and 2% (w/v), as well as in the nanoemulsion stabilized with pure 3% (w/v) Caprol 10G100, complete separation of a portion of the aqueous phase occurred at the bottom of the cylinder, which is a consequence of advanced coalescence and partial and/or complete break up of the emulsion, indicating a poor emulsifying properties of Caprol 10G100 molecules (18, 19).



A



B



C

**Figure 5.** Changes of the sedimentation index with time and appearance of the 20% W/O nanoemulsions stabilized with PGPR and Caprol 10G100 mixtures at different mass ratios after 14 days of storage at room temperature. Total emulsifiers concentration was 1% (A); 2% (B) and 3% (w/v) (C).

## CONCLUSION

The investigation of the interfacial behavior of the lipophilic emulsifiers PGPR, Caprol 3GS, Caprol 10G100 and Caprol ET showed that the emulsifiers from the Caprol group significantly lowered the interfacial tension with an increase in their HLB value, due to a higher hydrophilicity of the molecule and more affinity to the interface. PGPR



induced a higher reduction of the interfacial tension compared to Caprol 10G100, although it has a lower HLB number, which is a consequence of the branched hydrophobic chain of the PGPR molecule. The investigation of the interfacial behavior of the binary mixtures of PGPR and Caprol 10G100 showed a synergistic effect of their molecules on the interfacial tension at concentrations higher than 0.1% (w/v). The freshly prepared 20% W/O emulsions stabilized with PGPR and Caprol 10G100 mixtures, at their different mass ratios, had a submicron droplet diameter in the interval of 130-280 nm, so the obtained systems were nanoemulsions. An increase in the mass ratio of Caprol 10G100 induced an increase in the droplet diameter. This was confirmed also by the investigation of sedimentation stability of emulsions. An increase in the total emulsifiers concentration showed a positive effect on the emulsions stability, so the most stable emulsions were obtained at the PGPR:Caprol 10G100 mass ratios of 1:0; 1:1 and 1:2, and their total concentration of 3% (w/v).

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## ОСОБИНЕ ЕМУЛЗИЈА ВОДА У УЉУ (В/У) СТАБИЛИЗОВАНИХ СМЕШОМ ПГПР-а И ПОЛИГЛИЦЕРОЛ ЕСТАРА МАСНИХ КИСЕЛИНА

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Емулзије типа воде у уљу (В/У) су дисперзни системи са веома широком применом у прехранбеној, фармацеутској и козметичкој индустрији, као носачи активних метерија. Основни проблем код практичне примене ових система је њихова нестабилност. Емулгатори који се користе за стабилизацију овог типа емулзија су емулгатори са ниским ХЛБ бројем. У овом раду испитана је могућност примене липофилних емулгатора полиглицерол полирицинолата (ПГПР-а) и полиглицерол естара масних киселина, за стабилизацију емулзија В/У. Најпре су испитане адсорпционе особине коришћених емулгатора применом тензиометрије. На основу тих резултата одабрана су два емулгатора, ПГПР и декаглицерол дека-



олеат (*Caprol 10G100*), за припрему емулзија, при њиховим различитим масеним односима и укупним концентрацијама. Резултати испитивања особина емулзија (дисперзна анализа и седиментациона стабилност) су показали да се стабилније емулзије добијају смањењем удела *Caprol-a 10G100* у смеси, као и повећањем укупне концентрације емулгатора.

**Кључне речи:** липофилни емулгатори, ПППР, полиглицерол естри масних киселина, адсорпционе особине, В/У емулзије

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