



## CHARACTERISTICS OF W/O EMULSIONS CONTAINING POLYMERIC EMULSIFIER PEG 30-DIPOLYHYDROXYSTEARATE

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*Water-in-oil (W/O) emulsions are dispersed systems which are often used in the pharmaceutical, cosmetic and food industries as products, or as carriers of active substances. It is well known that they are very unstable, so that selection of the emulsifier and properties of the oil and water phase are main factors affecting their stability. The aim of this paper was to examine the possibility of application of a lipophilic, polymeric emulsifier, PEG 30-dipolyhydroxystearate (Cithrol™ DPHS), for stabilization of W/O emulsions. Behaviour of the emulsifier at W/O interfaces was determined by means of tensiometry. A series of emulsions were prepared with 20% (w/w) of water and different types of oil. Droplet size, droplet size distribution, viscosity, and sedimentation stability during 30 days of storage at room temperature of the emulsions prepared with paraffin oil, olive oil, grape seed oil, and medium-chain triglycerides, stabilized with 1% Cithrol™ DPHS, were determined. All investigated emulsions were stable for 30 days, except the one prepared with paraffin oil. The results of this study confirmed that PEG 30-dipolyhydroxylstearate is a good emulsifier and stabilizer of W/O emulsions which contain different types of oil.*

**KEY WORDS:** water-in-oil emulsion, polymeric emulsifier, droplet size analysis, emulsion stability

### INTRODUCTION

Water-in-oil (W/O) emulsions have different applications in the food, pharmaceutical and cosmetic industries (1, 2). A number of studies about emulsions focus on oil-in-water (O/W) systems, but there are few papers about liquid W/O emulsions. The reason for this is low stability of liquid W/O emulsions, which can easily sediment, flocculate or coalescence because of the high mobility of water droplets. A better understanding of interactions between water, oil and emulsifier at the interface and factors that affect emulsion stability would allow production of stable liquid W/O emulsions and, therefore, encourage the development of new products and applications (3, 4).

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The stability mechanism of W/O emulsions differs from the 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, due to low electrical conductivity of the continuous phase (5). For that reason, W/O emulsions generally present worse stability than O/W emulsions. Common destabilization mechanisms of emulsions are flocculation, coalescence, sedimentation and Ostwald ripening (2, 6). In general, emulsions are thermodynamically metastable systems, and therefore the main challenge for W/O emulsion production is to control the system stability, as well as to provide protection against destabilization (7 - 10). Selection of emulsifier is certainly the most important step in emulsions preparation (11, 12). Moreover, the bulk physicochemical characteristics are important for formation of W/O emulsions and their stability. For example, the density of continuous phase is decisive for stability against sedimentation, viscosity of the inner phase has influence on droplets breakup and their mobility, while the oil polarity can affect the interfacial tension and partitioning of the components at the interface (3). Therefore, in the last decade, better understanding of emulsion stabilization and destabilization mechanisms has become an increasingly interesting area for research (13, 14).

In addition, chemical structure of the oil, such as fatty acids chains length, degree of unsaturation and molecule configuration, influence on emulsion stability and its use. The grape seed oil is considered to be high quality dietary oil with a high concentration of unsaturated linoleic acid, vitamin E and phytosterols (15), which are associated with promotion of cardiovascular health by down-regulating low-density lipoprotein cholesterol production (16).

Olive oil, as main active components includes oleic acid, phenolic constituents, and squalene. The main phenolics include hydroxytyrosol, tyrosol and oleuropein, which have antioxidant activity (17).

Medium-chain triglycerides are liquid lipid (oil) of low viscosity at room temperature. Fatty acid composition is usually dominated by C8 fatty acids, followed by C6, C10 and C12. Due to its low toxicity to skin and mucous membrane, medium-chain triglycerides are commonly used in cosmetic and dermatological products, as emollient and drug solvent in peroral preparations (18).

Paraffin oil (mineral oil) is a purified mixture of liquid saturated hydrocarbons obtained from petroleum (19). As it can be concluded from the above, all of these oils and their emulsions have a great potential for application in pharmaceutical, cosmetic, and food industry.

The objective of the present study was a better understanding of the specific interactions between selected oils and surfactant and their effect on W/O emulsion stability. PEG 30-dipolyhydroxystearate (Cithrol™ DPHS), a non-ionic, polymeric emulsifier suitable for double W/O/W emulsions (20) was selected as a surfactant and its interfacial properties were determined by means of tensiometry. Characteristics of the 20% (w/w) W/O emulsions such as droplet mean diameter, droplet size distribution, viscosity and sedimentation stability within 30 days of storage at room temperature were determined by means of dynamic light scattering measurement, optical microscopy, rheology and visual observation of the phase separation.



## EXPERIMENTAL

### Materials

Different oils were used as continuous phase of the W/O emulsions: olive oil (Comcen, Zemun), medium-chain triglycerides or caprylic/capric triglyceride (Saboderm TCC, Comcen, Zemun), paraffin oil (Centrohem, Stara Pazova), grape seed oil (Olitalia, Forli (FC) Italy). Oil-soluble emulsifier Cithrol™ DPHS, which is PHS/PEO/PHS block copolymer i.e. PEG 30-dipolyhydroxystearate (Macrogol 30-dipolyhydroxystearate; Ph. Eur. 8), was donated by Croda, Belgium. Deionized water was used as aqueous phase.

### Methods

**Interfacial tension.** 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 (21). Prior to the measurements, the ring was immersed in deionized water (below the surface), then the oil phase, slowly added on top and the interface, was left for 15 min to equilibrate. The interfacial tension of each system was measured at the point where the ring broke away from the interfacial layer between the two phases. In all measurements, the temperature was kept at 40°C. The reported values of the interfacial tension were average of three measurements, at least. Concentrations of Cithrol™ DPHS were varied from 0.00001% (w/w) up to 1% (w/w).

**Preparation of W/O emulsions.** The emulsions were prepared at the water – oil mass ratio 20:80, with different types of oil, while the aqueous phase was deionized water. Continuous phase of emulsions were prepared at room temperature by dissolving the emulsifier (1% w/w) in selected oil. Emulsions were prepared by dispersing a desired amount of water in the continuous phase at 40°C by means of the homogenizer Ultra Turrax T-25 (IKA, Germany) at 20000 rpm during 10 min.

**Rheological measurements.** Viscosities of continuous phases and W/O emulsions were determined using an RS600 rheometer (Thermo Electron, GmbH, Germany). The cone-and-plate geometry was used (plate diameter,  $d = 60$  mm, and cone angle  $\theta = 1^\circ$ , gap 0.052 mm). Measurements were carried out at the temperature of 25°C. The shear rates were increased from zero to 200  $s^{-1}$  and reversely. Viscosities of the emulsions were measured immediately after preparation.

**Droplet size and polydispersity index analysis.** Particle size analysis of freshly prepared emulsions and emulsions after 7 days of storage 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 that was used as the emulsion continuous phase, in order to yield a suitable scattering intensity. Refractive indexes of olive oil, medium-chain triglycerides, paraffin oil and grape seed oil were 1.475, 1.448, 1.462 and 1.475 respectively. DLS data were analysed using the general purpose mode, thus the hydrodynamic diameter (z-average) and the polydispersity index (PI) were obtained.



After 30 days of storage, the droplet size of the emulsions was over the measuring range of DLS, so the determination was carried out by photomicrography, using of Leica QWin software. Photomicrographs were taken on an optical microscope, Biooptica BEL-3000, Germany at 40x magnification. Particle mean diameter, expressed as volume-surface mean value,  $d_{vs}$  ( $\mu\text{m}$ ) and standard deviation  $\sigma$  ( $\mu\text{m}$ ) were calculated from the experimental data:

$$d_{vs} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad [1]$$

$$\sigma = \left( \frac{\sum n_i (d_i - d_{vs})^2}{\sum n_i} \right)^{1/2} \quad [2]$$

where  $d_i$  is droplet diameter and  $n_i$  is number of droplets.

**Emulsions stability test.** For stability test, the emulsions were transferred into 10 ml graduated cylinders and stored at room temperatures for 30 days. The emulsions were observed for the changes in consistency, homogeneity and phase separation during storage. The oil phase separation from the emulsions was visually monitored at regular time intervals (immediately after preparation, after 7 days, and after 30 days). The total height of the emulsion,  $H_e$ , and the height of the oil layer,  $H_o$ , were measured with time laps. The extent of phase separation was characterized by the sedimentation index,  $H$ , given as:

$$H = 100 H_o/H_e (\%) \quad [3]$$

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

## RESULTS AND DISCUSSION

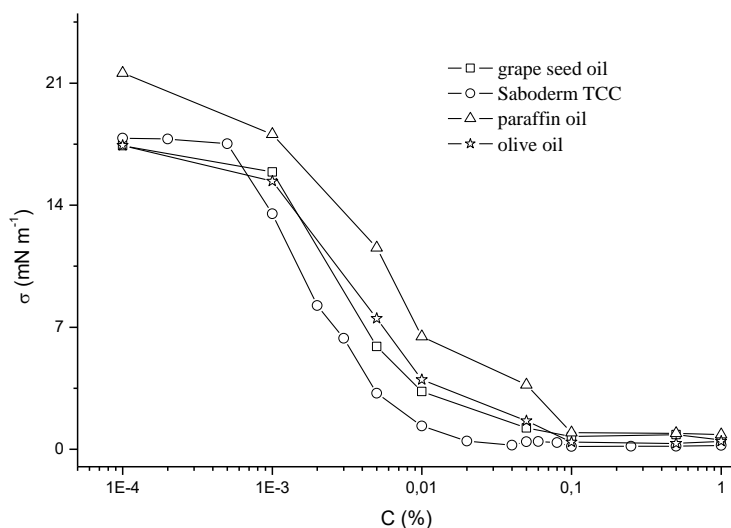
### Interfacial tension measurements

Determination of the interfacial tension of the two-phase system can offer valuable information about stability of the emulsion prepared of the two phases. Furthermore, the rapid decrease in the interfacial tension, with the emulsifier addition, is important for formation of small droplets, which results in higher stability against the gravity force (2).

The behaviour of the emulsifier Cithrol<sup>TM</sup> DPHS at the W/O interface was determined by tensiometry. The surface tension at the water-grape seed oil, water-medium-chain triglycerides, water-paraffin oil and water-olive oil interface was 19.22, 18.36, 25.49 and 18.44 mN/m, respectively. The changes in the interfacial tension for different oils, with various Cithrol<sup>TM</sup> DPHS concentrations are presented in Fig. 1. As it can be observed, the interfacial tension for all investigated systems progressively decreased with an increase in the emulsifier concentration, reaching the lowest value at 0.1% Cithrol<sup>TM</sup> DPHS. The lowest value of the interface tension (0.16 mN/m) was obtained for the water – medium-chain triglycerides system. Above this concentration, the interfacial tension remained constant, i.e. the W/O interface was saturated with surfactant molecules, and micelles



were formed in the bulk. As the values of interfacial tension were very low (from 0.16 to 0.95 mN/m), close to zero, it could be expected that Cithrol™ DPHS is suitable for obtaining W/O emulsions of all oils used. For further examination of Cithrol™ DPHS emulsifying properties, the W/O emulsions containing 20% of water, stabilized with 1% (w/w) of emulsifier, were prepared. Viscosities of the continuous phases and corresponding emulsions, droplet size, droplet size distribution, and sedimentation stability of the emulsions were studied in order to better understand the stabilization mechanism and characteristics of the water-in-oil systems.



**Figure 1.** Interfacial tension as a function of Cithrol™ DPHS concentration at 40°C

### Rheological investigations

Rheology of the continuous phases (containing emulsifier) and corresponding W/O emulsions, immediately after their preparation, was investigated at 25°C. All emulsions and their continuous phases showed a Newtonian type of flow, and their viscosities are presented in Table 1. The low values of viscosity obtained for continuous phases, that contain Cithrol™ DPHS in concentration of 1% (w/w) indicate that the addition of this polymeric emulsifier did not affect the viscosity of the oil. The obtained emulsions, as expected, were of low viscosities (from 40.4 to 115.2 mPas).



**Table 1.** Viscosities of continuous phases and W/O emulsions at 25°C

Type of oil	Viscosity (mPas)		
	Oil	Continuous phase	Emulsion
Paraffin oil	33.93	36.82	40.8
Olive oil	67.42	69.37	115.2
Saboderm TCC	23.79	23.87	40.4
Grape seed oil	40.02	46.1	81.6

### Emulsions droplet size and droplet size distribution

Besides the impact on the stability of emulsion, the size and size distribution of emulsion droplets are the parameters that greatly characterize the applied emulsifier. For this reasons, the determination of droplet size in W/O emulsions was performed immediately after the preparation and after 7 and 30 days of storage at room temperature. The droplets in all investigated emulsions, immediately after the preparation, were of nano dimensions, so that DLS was used for size determination. The results are presented in Table 2.

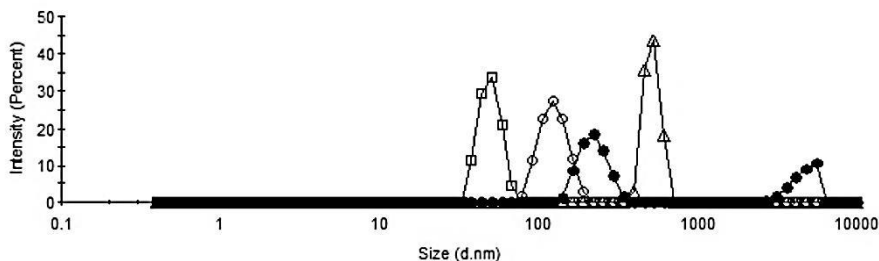
**Table 2.** Droplet size and PI with standard deviation for W/O emulsions with different type of oil, during storage at room temperature: z-average,  $d_{vs}$ -mean diameter,  $\sigma$ -standard deviation

Time (days)	0		7		30
	Z-average $\pm\sigma$ (nm)	PI $\pm\sigma$	Z-average $\pm\sigma$ (nm)	PI $\pm\sigma$	
Type of oil					$d_{vs}\pm\sigma$ ( $\mu\text{m}$ )
Paraffin oil	980.6 $\pm$ 44.94	0.26 $\pm$ 0.21	1275 $\pm$ 211.1	0.6 $\pm$ 0.14	-
Olive oil	369.6 $\pm$ 35.84	0.63 $\pm$ 0.06	393.9 $\pm$ 110.7	0.5 $\pm$ 0.1	5.14 $\pm$ 0.41
Medium-chain triglycerides	115.3 $\pm$ 10.56	0.16 $\pm$ 0.02	139 $\pm$ 20.66	0.2 $\pm$ 0.06	4.45 $\pm$ 0.31
Grape seed oil	68.40 $\pm$ 13.54	0.22 $\pm$ 0.07	78.9 $\pm$ 2.9	0.1 $\pm$ 0.03	5.15 $\pm$ 0.36

As it can be seen from Table 2, the smallest droplet diameter (z-average) was obtained in the emulsions prepared with grape seed oil and medium-chain triglycerides (68.4 and 115.3 nm, respectively), while the droplet mean diameter of emulsion with paraffin oil was 980.6 nm. The PI was in the range of 0.16-0.63, which indicates the differences in size distribution. Namely, the particle populations with very narrow size distribution have PI values in the range of 0.02–0.05, while a PI higher than 0.5 indicates very wide droplet size distribution that can lead to layering of the emulsion (22). The emulsions prepared with medium-chain triglycerides, grape seed oil and paraffin oil showed a narrow, mono-

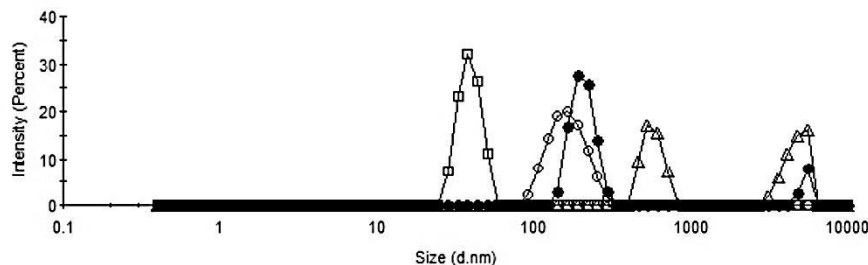


modal droplet distribution (PI from 0.16 to 0.26), as it can be seen from Fig. 2. In the case of emulsion with olive oil, a bimodal size distribution was observed (PI = 0.63), with first peak at 201 nm and a second one around 4295 nm. Such finding indicates that the emulsion with olive oil stabilized by Cithrol<sup>TM</sup> DPHS could be of lowest stability, which can lead to phase separation.



**Figure 2.** Droplets size distribution for W/O emulsions with medium-chain triglycerides (-○-), paraffin oil (-Δ-), grape seed oil (-□-) and olive oil (-●-), after preparation

The light microscope observation of emulsions after 7 days of storage at room temperature, showed the existence of small individual droplets. However, according to DLS analysis (Table 2), the mean droplets diameter increased in all investigated samples but still remaining in a nano range, except for the emulsion with paraffin oil. The emulsions with medium-chain triglycerides and grape seed oil showed a monomodal droplet size distribution with low PI (Fig. 3). Bimodal size distribution was observed for emulsions with olive oil (first peak at 190 nm and a second one near 5560 nm) and paraffin oil (first peak at 531 nm and at 5560 nm). Formation of larger droplets occurred as the result of coalescence due to a weak steric repulsion of the adsorbed layer at the interface.

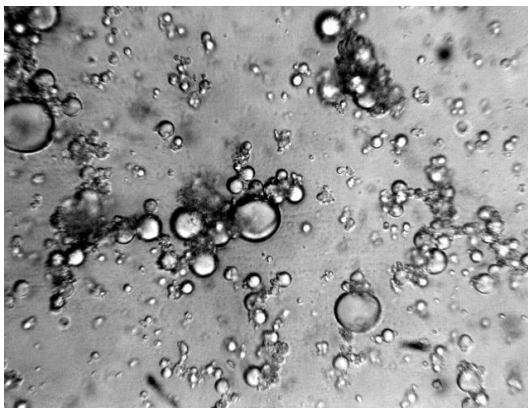


**Figure 3.** Droplets size distribution for W/O emulsions in medium-chain triglycerides (-○-), paraffin oil (-Δ-), grape seed oil (-□-) and olive oil (-●-), after 7 days

After 30 days of storage at room temperature, coalescence of droplets was enhanced in all emulsions, so that their size was determined by photomicrography. The mean droplets diameters ( $d_{vs}$ ) and standard deviation are presented in Table 2. As it can be observed, the mean droplet diameter in all emulsions was around 5  $\mu\text{m}$ . The existence of droplet aggregates was not observed, except for the emulsion with paraffin oil (Fig. 4). Deter-



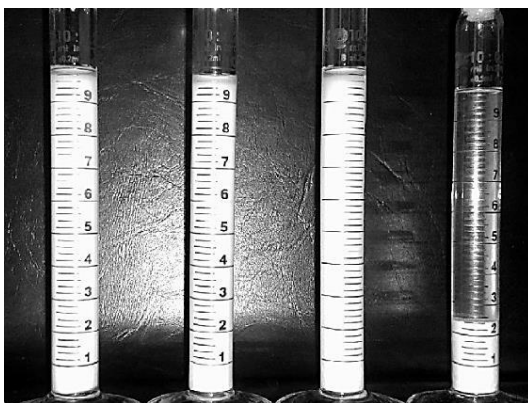
mination of droplet size in this emulsion was not possible due to the presence of closely packed aggregates of fine droplets around the large ones.



**Figure 4.** Photomicrograph of the W/O emulsion with paraffin oil after 30 days of storage at 25°C

### Sedimentation stability of emulsions

The stability of the W/O emulsions was investigated during 30 days of storage at room temperature. The cylinders filled with emulsions, after 30 days of storage, are shown in Fig. 5.



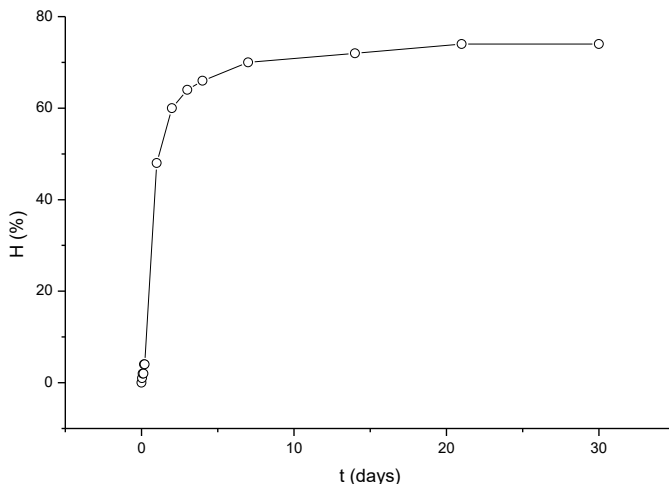
**Figure 5.** W/O emulsions prepared with different kinds of oil, from left to right: olive oil, grape seed oil, medium-chain triglycerides, paraffin oil, after 30 days of storage at 25°C





It can be noticed that the emulsions obtained with olive oil, grape seed oil and medium-chain triglycerides were stable during 30 days, i.e. no separation of oil layer was observed. Considering that the coalescence of droplets with time in these emulsions was confirmed (Table 2.), the sedimentation stability could be due to the existence of single droplets and a small differences in viscosity of the oil and water phase.

In the paraffin oil emulsion phase separation appears with time, pointing to a decrease in its stability. The change in sedimentation index H with time is presented in Fig. 6.



**Figure 6.** Sedimentation index (H) for the paraffin oil emulsion as a function of time

As it can be seen, the sedimentation index changes rapidly during the first 7 days of storage, reaching high value of 70%, and then remains almost unchanged. A bimodal distribution of droplets diameter, accompanied with formation of aggregates (Fig. 4) and very low viscosity of paraffin oil, could be a reason for such sedimentation instability, i.e. phase separation.

## CONCLUSION

The investigation of the interfacial behaviour of PEG 30-dipolyhydroxystearate (Cithrol™ DPHS) showed that its addition decreases the surface tension at the water-oil interface, for all examined types of oil, reaching the lowest value at a concentration of 0.1%. Freshly prepared W/O emulsions with 20% water and paraffin oil, olive oil, grape seed oil and medium-chain triglycerides, which were stabilized with 1% of Cithrol™ DPHS, had submicron droplet size, low viscosity and showed Newtonian type of flow. The emulsions made with natural and medium-chain triglycerides oils were stable during 30 days of storage at room temperature. Namely, regardless of the increase in droplets



mean diameter of these emulsions with time, i.e. progressive coalescence, there was no phase separation. Formation of droplet aggregates was observed in the paraffin oil emulsion, thus phase separation occurred with time. The results of this study showed that Cithrol™ DPHS, polymeric emulsifier could be used for obtaining stable W/O emulsions with natural and medium-chain triglycerides oils that could be suitable for pharmaceutical, cosmetic and food application.

### Acknowledgement

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## КАРАКТЕРИЗАЦИЈА В/У ЕМУЛЗИЈА КОЈЕ САДРЖЕ ПОЛИМЕРНИ ЕМУЛГАТОР РЕГ 30-ДИПОЛИХИДРОКСИ-СТЕАРАТ

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Емулзије типа вода у уљу (В/У) су дисперзни системи, који се као готови производи, или носачи активних материја, често користе у козметичкој и прехранбеној индустрији. Познато је да се ради о изузетно нестабилним системима, код којих често долази до сепарације фаза и других облика нестабилности, тако да избор емулгатора и особине уљне фазе представљају веома важне факторе који дефинишу њихову стабилност. Циљ овог рада био је испитивање могућности примене липофилног, полимерног емулгатора, Cithrol DPHS-а за стабилизацију емулзија В/У. Спроведена испитивања обухватила су дефинисање међуфазног понашања емулга-



тора применом тензиометрије. Припремане су серије 20% (м/м) емулзија воде у различитим типовима уља. Испитиване су величина и расподела величина капи, вискозитет и седиментациона стабилност у току 30 дана емулзија припреманих у парафинском уљу, маслиновом уљу, уљу коштица грожђа и триглицеридима средње дужине ланца, стабилованих са 1% Cithrol DPHS. Све испитиване емулзије, осим емулзије у парафинском уљу, су биле стабилне у току 30 дана. Резултати овог истраживања указују да Cithrol DPHS може бити веома добар емулгатор и стабилизатор В/О емулзија припреманих у различитим уљима.

**Кључне речи:** емулзија вода-у-уљу, полимерни емулгатор, анализа величине капи, стабилност емулзија

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