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Studies on the formation of O/W nano-emulsions, by low-energy emulsification method, suitable for cosmeceutical applications*

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The formation of oil/water (O/W) nano-emulsions suitable for cosmeceutical application was studied. Nano-emulsions were prepared by using phase inversion composition (PIC) method, one of the low-energy emulsification methods. The process consist of stepwise water addition to oil/surfactant mixture, at T = 25°C. Caprylic/capric triglycerides (GTCC), propylene glycol dicaprylate/dicaprate (PC) and oleic acid (OA) were applied as an oil phase. Polysorbate 80 was used as the surfactant. Kinetic stability of the nano-emulsions was analyzed by measuring droplet size as a function of time for different oil/surfactant ratio. The particles size distribution was analyzed by means DLS measurement technique (Dynamic Light Scattering), using Zetasizer Nano ZS (Malvern Instruments, UK). One of triterpenoic acid, practically non-water soluble substance was selected as an active and incorporated into the stable formulation. The obtained results proved that the nanoemulsion NE-T80-GTCC-20:80 based on caprylic/capric triglycerides with the oil/surfactant ratio O/S = 2 0:80 and the droplet size r = 25 nm was the most stable one and additionally showed the highest solubilisation capacity for the triterpene.

Key words: nano-emulsions, crodamol GTCC, crodamol PC, oleic acid

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INTRODUCTION

Cosmeceuticals are cosmetic products with biologically active ingredients, showing not only cosmetic but also drug-like properties, used for the care and the improvement of skin conditions (Gao *et al*., 2008).

Nano-emulsions are one of the most promising system for transdermal delivery of active substances because of the ease of preparation, long term stability, absence of organic solvents and high solubilization capacity for both, hydrophilic and lipophilicactives (Maghraby, 2008; Peltola *et al*., 2003). Moreover, nano-sized systems improve the penetration of active ingredients into the skin (Teo *et al*., 2010). Apart from good cosmetic properties also from technological point of view the nano-emulsions offer several advantages for cosmeceutical application. Due to the small droplets sizes, they are characterized by kinetic stability against sedimentation, flocculation and coalescence. It also helps to resist the process of creaming because the droplet's Brownian motion overcoming gravity force (Tadros *et al.,* 2004).

Currently, nano-emulsions are object of interest to pharmaceutical manufacturers as SEDDS (self-emulsifying drug delivery systems), an isotropic mixtures of oils, surfactants, solvents and co-solvents/surfactants which can be used for the design of formulations in order to improve the oral absorption of highly lipophilic drug compounds (Gursoy *et al*., 2004). Thanks to their properties it was possible to increase absorption after oral administration of poorly water-soluble drugs: Sandimmune® and Sandimmun Neoral® (cyclosporin A), Norvir® (ritonavir), and Fortovase® (saquinavir) (Gursoy *et al*., 2004), Sporanox® (itraconazole) (Hong *et al*., 2006), Coenzyme Q10 (Balakrishnan *et al*., 2009) or proteins such as β-lactamase (Rao *et al.* 2008).

Terpenoids are the largest group of natural bioactive compounds exhibiting multiple nutriceutical activities, they are also known as active cosmetic ingredients (Wang *et al*., 2005). Especially the triterpene acids, (e.g. betulinic, maslinic, oleanolic, ursolic) exhibit unique biological and pharmacological activities such as: anti-inflammatory, antimicrobial, antiviral properties, cytotoxic effects, against cancer and cardiovascular diseases (Silva *et al.* 2012).

The formation of oil/water (O/W) nano-emulsions as a vehicle for triterpene acid, suitable for cosmeceutical application was studied.

MATERIAL AND METHODS

Non-ionic surfactant, Tween® 80 (INCI: Polysorbate 80) was obtained from Sigma-Aldrich. The surfactant was selected because of its good cosmetic properties and lack of irritation and toxicity to the skin. The oils used in the study: Crodamol GTCC® (INCI: Caprylic/Capric Trigliceryde, HLB_R=9.88), Crodamol PC (Propylene Glycol Dicaprylate/Dicaprate, HLB_R =10.99) were purchased from Croda Poland. Oleic Acid (HLB_R =16.00) was supplied by Chempur. As the aqueous phase of the nano-emulsions distilled water was used.

Nano-emulsions were prepared by stepwise water (W) addition to the mixture of oil (O) and surfactant (S), at room temperature (25°C). The mixture was homogenized using IKA VORTEX GENIUS 3. The compositions were considered as nano-emulsions when they

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Abbreviations: GTCC, caprylic/capric triglycerides; OA, oleic acid; O, oil; O/S, oil/surfactant; O/W, oil/water; PC, propylene glycol dicaprylate/dicaprate; S, surfactant; W, water

Table 1. Composition and characteristic of the formulations.

Name of sample	Composition				Z-Ave [r. nm]/ PDI	
	Water phase	Oil phase	Surfactant	O/S	without active	with active
NE-T80-GTCC-10:90				10/90	8/0.349	–
NE-T80-GTCC-20:80		Crodamol GTCC		20/80	25/0.500	253/0.53
NE-T80-GTCC-30:70				30/70	222/0.233	–
NE-T80-PC-10:90	Water	Crodamol PC	Polysorbate 80	10/90	22/0.220	–
NE-T80-PC-20:80				20/80	25/0.608	–
NE-T80-OA-10:90		Oleic Acid		10/90	339/0.802	–
NE-T80-OA-20:80				20/80	350/1.000	–

were transparent and translucent, showing a bluish shine or opaque. It was confirmed that they were not microemulsions as their properties depended on the preparation method and the storage temperature.

The mean droplet sizes of nano-emulsions were measured by Dynamic Light Scattering (DLS) method, using Malvern Zetasizer Nano ZS apparatus, which measures particle size (range from 0.3 nm to 10 μm) by scattering photons from a sample and determine the change in diffracted light intensity. Scattering angle was 173°. The analysis was performed three times for each sample to determine mean values.

The emulsions stability was assessed by measuring droplet size as a function of time at constant temperature (25°C). Moreover, the samples were stored, at ambient temperature, for 24 hours, one week and one month. The stability of the formulations were also assessed visually.

One of the triterpene acid was incorporated to oil/surfactant mixtures, homogenized with a vortex and placed in an ultrasound bath for 10 min. The active solubility was determined by the sample observation using optical microscopy, the appearance of crystals has indicated exceeding the limit solubility of the acid). The nano-emulsions were formed by water addition to the oil/surfactant/active mixtures, and stored in a water bath at 25°C. The stability of the formulations were examined by visual and optical observation after 24 hours of the preparation.

RESULTS AND DISCUSSION

In the first stage of the studies different types of oils were tested to obtain stable nano-emulsion systems. All formulations were prepared by low energy emulsification method, by dropwise addition of water to the mixture of oil/surfactant in varied ratios 90:10, 80:10, 70:30, 60:40 and 50:50.

An area of the occurrence of stable nano-emulsions for each of the oils depended on the ratio of the oil/surfactant. In case of Crodamol GTCC, transparent or transparent-bluish liquid dispersions appeared after addition of approximately 57 wt. % of water for oil/surfactant (O/S) mixture of ratio 10:90, 20:80 and 30:70. Nano-emulsion region could not be extended at O/S ratio higher than 40/60. For the Crodamol PC/Polysorbate 80/water system, the region of nano-emulsions was determined for oil/surfactant (O/S) ratio 10:90 and 20:80, also after addition of approximately 57 wt. % of water. The translucent emulsion area in case of oleic acid was identified, after addition of 80% of water phase to the mixture of oil/surfactant in a ratio 10:90 and 20:80. Table 1 shows the composition and characteristic of the systems.

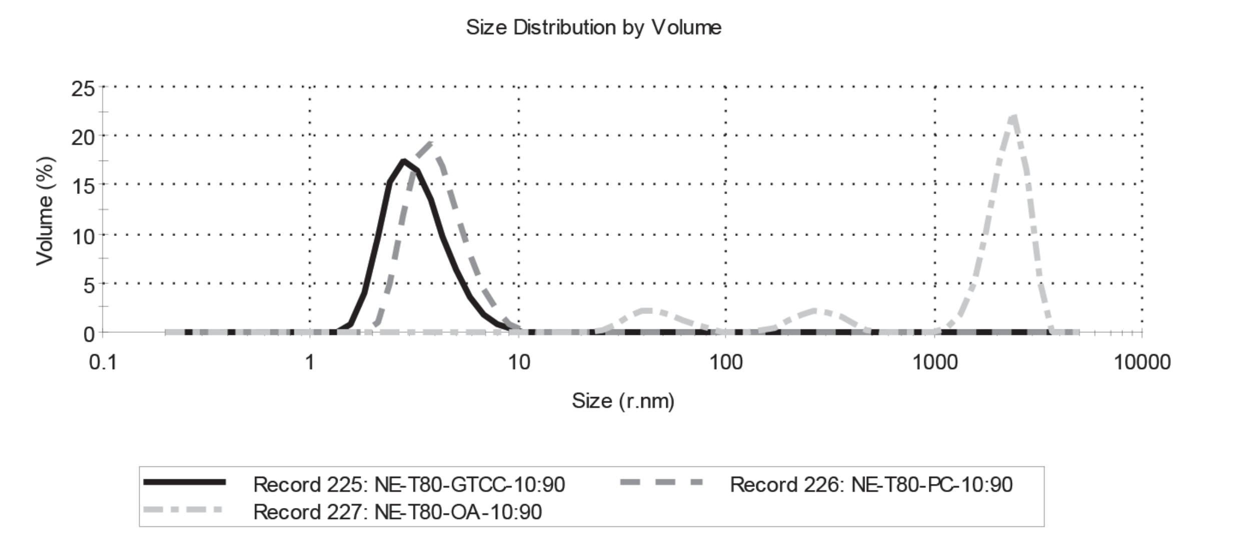

Figure 1. Size distribution of the formulations based on different oils: NE-T80-GTCC-10:90 (Crodamol GTCC), NE-T80-PC-10:90 (Crodamol PC) and NE-T80-OA-10:90 (Oleic Acid).

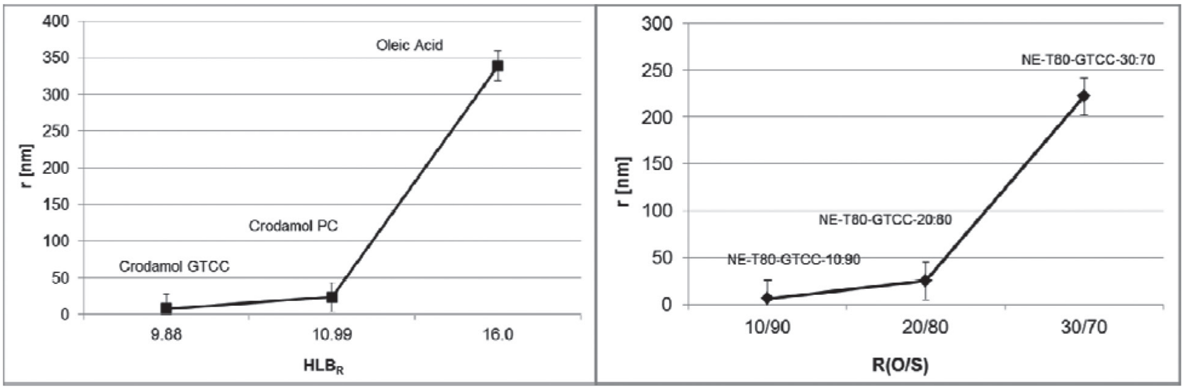

Figure 2. The droplet size dependence of hydrophilic-lipophilic balance (HLB$_R$) of oils used in studies (a) and dependence of oil/surfactant ratio R (O/S) for crodamol GTCC/Tween 80/water system (b).

The droplet size of nano-emulsions was determined by means of DLS method. The obtained result showed that the droplet size of inert phase depend on kind of the oil. The date presented in Fig. 1 shows that formulation based on crodamol GTCC is characterized by the small droplets size (r = 8 nm), similarly to crodamol PC nano-emulsion (r = 22 nm) and contrary to oleic acid-based emulsion (r = 339 nm) at 10/90 oil/surfactant ratio. Moreover emulsion based on oleic acid is characterized by very high polydispersity. The difference between those two systems is very significant. It could be explain by the fact that caprylic/capric tiglicerydes are the most hydrophobic oil among the studied. It is characterized by the lowest value of required $HLB_r = 9.88$, while the oleic acid is characterized by the highest $HLB_r = 16.00$. It is believed that lipophilicity of oil phase significant influence on the size distribution and stability of nano-emulsions. (Fig. 2a).

Apart from the kind of the oil, also oil/surfactant ratio influences the properties of the nano-emulsion. Figure 2b shows, on the bases of crodamol GTCC/polysorbate 80/water system, effect of O/S ratio on the droplet size distribution. The data presented in Fig. 2b indicates that the droplet size increases with the increase of O/S ratio. For the oil/surfactant ratio in range from 10/90 and 20/80 the difference is quite small, mean radius of droplets is respectively: r = 8 nm and r = 25 nm, while further increase in oil concentration causes significant changes in droplet size, in case of O/S ratio 30/70 the droplet size is 222 nm. It should be noticed that nano-emulsion droplet sizes of water/non-ionic surfactant oil systems studied are expectionally small compared to those reported in the literature (Sadurni *et al.*, 2005).

Effect of the incorporation of the active on nano-emulsion systems shows Table 1. Only in case of crodamol GTCC, stable, triterpene acid loaded nano-emulsion was obtained. In case of oleic acid and crodamol PC based systems crystallization effect of the active, at very low, (below therapeutic), concentrations were observed. The results are in agreement with literature data which show that medium-chain triglycerides are one of the effective solubilisers for many lipophilic actives incorporated in nano-emulsions e.g. carbamazepine (Kelmann *et al.*, 2007), β-carotene (Yuan *et al.*, 2008) or vitamin E (Saberi *et al.*, 2013).

In the next stage of the work, the stability of nano-emulsions based on crodamol CTCC was determined by measuring droplet size dependence of time, at 25°C. These systems were selected from the studied due to the best properties, i.e. lowest droplet size and low polidyspersity index (Table 1). The obtained results are showed in Fig. 3.

Crodamol GTCC-based nano-emulsions, irrespective of O/S ratio, are stable systems in time. In all cases minor changes of droplet size distribution were observed within the 24 hours observation. In addition, no phase separation took place in this period of time (after one week and one month).

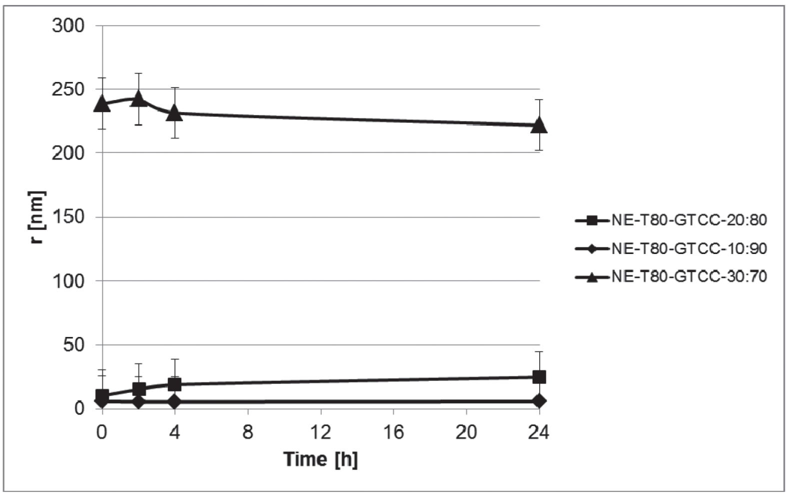

Figure 3. Stability of crodamol GTCC-based nano-emulsions.

CONCLUSIONS

The obtained results showed that both, the kind of the oil phase and the O/S ratio influenced on the nano-emulsion properties. Systems based on crodamol GTCC, are very stable formulations, characterizing by the smallest droplet size The droplet size decreases when the surfactant concentration increases in emulsion system.

Additionally, crodamol GTCC/Polisorbate 80 nano-emulsions, due to their good solubility of triterpenes, no crystallization effect of the active was observed), could be used as the self-emulsifying triterpenoic acid delivery systems, suitable for cosmeceutical applications.

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