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Regular paper

Study of O/W micro- and nano-emulsions based on propylene glycol diester as a vehicle for geranic acid

Małgorzata Jaworska, Elżbieta Sikora✉, Jan Ogonowski and Monika Konieczna

Institute of Organic Chemistry and Technology, Cracow University of Technology, Kraków, Poland

Nano- and microemulsions containing as the oil phase caprylic/capric propylene glycol diesters (Crodamol PC) were investigated as potential vehicle for controlled release of geranic acid. The influence of emulsifiers and co-surfactants on stability of the emulsions was investigated. Different kind of polysorbates (ethoxylated esters of sorbitan and fatty acids) were applied as the emulsifiers. The short-chain alcohols (ethanol, 1-propanol, 1-butanol) were used as co-surfactants. The emulsions were prepared at ambient temperature (25°C), by the phase inversion composition method (PIC). The stable O/W high dispersed emulsion systems based on Crodamol PC, of mean droplets size less than 200 nm, were prepared. Microemulsions stabilized by the mixture of Polisorbat 80 and 1-butanol were characterized by the largest degree of dispersion (137 nm) and the lowest PDI value (0.094), at surfactant/co-surfactant: oil weight ratio 90:10. The stable nano-emulsion (mean droplet size of 33 nm) was obtained for surfactant: oil (S:O) weight ratio 90:10, without co-surfactant addition. This nano-emulsion was chosen to release studies. The obtained results showed that the prepared stable nano-emulsion can be used as a carrier for controlled release of geranic acid. The active substance release from the nano-emulsion and the oil solution, after 24 hours was 22%.

Key words: nano-emulsions, microemulsion, Crodamol PC, geranic acid, release profile

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INTRODUCTION

Geranic acid is known as a fragrance in cosmetic products. Moreover, it is a promising representative in the range of natural multifunctional fragrances because, apart from floral scent, it provides a well equilibrated antimicrobial profile which is capable to effectively support the biological stabilization of natural formulations (information materials at www.dr-straetmans.de). Recently, in the literature it has been reported that geranic acid may act as a skin depigmenting agent *via* the inhibition of tyrosinase activity and expression within melanocytes (Choi, 2012). Taking into account the fact that, skin whitening/lightening has become one of the most common claims in anti-aging cosmetics, it seems interesting to find an appropriate form for cosmetic products that could deliver, in a controlled manner, geranium acid to the skin.

Among the systems with high degree of internal phase dispersion micro- and nano-emulsion are distinguished. Due to their small droplet size they are optically transparent or translucent, sometimes, in case of microemulsions, they are milky (Bouchemal *et al*., 2004; Maghraby *et al*., 2008; Peltola *et al*., 2003; Rhee *et al*., 2001). Due to their special features, both of the systems, offer several advantages for pharmaceutical and cosmetic application. They are characterized by the long term stability and high solubilization capacity for hydrophilic and lipophilic actives. Moreover, they demonstrate the ability to improve delivery of active substances (Bouchemal *et al*., 2004; Maghraby *et al*., 2008; Peltola *et al*., 2003).

Most of authors in studies concerning micro- and nano-emulsion used as an oil phase mixture of caprylic and capric triglycerides. There is not much information concerning nano-emulsion based on the mono- and diesters of caprylic and capric acids and polyhydric alcohols, such as glycerin or propylene glycol, which are popular emollients. Wais and co-workers (2012) were working on obtaining nano-emulsion system for transdermal delivery of glibenclamide. The research team has used the mixture of Labrafac®PG (propylene glycol dicaprylate/dicaprate) and Triacetin (glycerin triacetate) as the oil phase. The emulsion system was stabilized by mixture of Polysorbate 80 (as surfactant) and diethylene glycol monoethyl ether (as co-surfactant). They have obtained nano-emulsions characterized by small droplet size (<117 nm) and uniform size distribution (PDI<0.247). Nano-emulsions based on Sefsol 218 (Propylene glycol monocaprylic ester), stabilized by PEG-35 castor oil and diethylene glycol monoethyl ether as co-surfactant were obtained by Shafiq-un-Nabi and coworkers (2007). The final formulation consisted of 20% oil, 27% surfactant/co-surfactant mixture, and 53% aqueous phase. The droplet size of the dispersed phase was about 34.5 nm. Patel and coworkers (2012) were also working on developing of microemulsions based on mono-, di- and triesters of medium chain fatty acids, using PEG-35 castor oil to stabilize the systems. They have found that microemulsions with smaller particle size may be obtained from combination of propylene glycol (PG) monoester with either a PG diesters or triglycerides.

Based on these literature reports caprylic/capric propylene glycol diesters were chosen as the oil phase for the studies concerning micro- and nano-emulsions. As

✉e-mail: esikora@pk.edu.pl

Abbreviations: W, water; O, oil; S, surfactant; CoS, co-surfactant; S/CoS, surfactant/co-surfactant; S/CoS:O, surfactant/co-surfactant: oil; S:O, surfactant: oil; O/W, oil/water; W/O, water/oil; ME, microemulsion; NE, nano-emulsion; PIC, phase inversion composition; DLS, dynamic light scattering; PDI, polydispersity index; Q, released amount of geranic acid

cosmetic ingredients the mixture offers several functional benefits such as: solubilization capacity of the lipophilic substances, oxidative stability and good spreading ability.

The aim of this work was to prepare and characterize high dispersed emulsion systems, containing as the oil phase Crodamol PC, as the potential vehicle for controlled release of geranic acid.

MATERIAL AND METHODS

Materials. Applied in the studies non-ionic surfactants (Polysorbates 40, 60 and 80) were bought from Caesar&Loretz GmbH. The surfactants were selected because of their good performance, lack of irritating and toxic reactions to the skin. The oil, Crodamol PC® (Propylene Glycol Dicaprylate/Dicaprate), was kindly supplied by Croda Poland. The short-chain alcohols (ethanol, 1-propanol, 1-butanol), used as co-surfactants, were purchased from POCh Gliwice. Geranic acid was bought in Sigma-Aldrich. As the aqueous phase of the emulsions distilled water was used.

Formation of emulsion systems. The emulsion systems were prepared by gradual water (W) addition to the mixture of oil (O) and surfactant (S)/co-surfactant (CoS) or to the mixture of oil and surfactant, at ambient temperature (25°C). The mixtures were prepared by using a magnetic stirrer, IKA C-MAG HS7 equipped with speed control. On the other hand, the systems without the co-surfactant were homogenized with the use of IKA vortex shaker. The obtained compositions were considered as micro- and nano-emulsions based on visual assessment, whether they were opaque, transparent, translucent and had bluish lustre. A distinction between micro- and nano-emulsions was based upon that the system properties dependented or not, respectively, on the method of preparation and temperature of storage.

Construction of a phase diagrams. Pseudo-ternary phase diagrams were constructed in the following way: mixtures of surfactant/co-surfactant:oil (S/CoS:O), (weight ratio of surfactant/co-surfactant was kept at 1/1), or surfactant:oil (S:O) mixtures were titrated by water, at 25°C, to the solubilization limit, which was defined as the transition from monophasic region to a polyphasic region or to a birefringent phase. The weight ratios of S/CoS:O or S:O were different as 90:10, 88:12, 85:15, 80:20 and 70:30. During the process, there was a visual observation of transition from the monophasic area to a polyphasic region. The step of W/O emulsion transition into O/W was done by conductivity measurement of the samples (Metler Toledo SevenCompact™). Additionally, the inversion stadium was visually observed by the change in appearance of samples from transparent to opaque. The presence of liquid crystals phase was observed by means of optical microscop Motic B1 with polarized light (Merazet Company).

Droplet size determination. The average internal phase droplet size of the emulsions was measured by the Dynamic Light Scattering (DLS) method, using Malvern Zetasizer Nano ZS device. The measurement of particle size which ranges from 0.3 nm to 10 μm based on scattering photons from a sample and determining the change in diffracted light intensity. The scattering angle was 173°.

Preparation of geranic acid-loaded O/W nano-emulsion. Among obtained high dispersed emulsion systems, nano-emulsions due to lower surfactant concentration compared to microemulsions were chosen to release studies. As a carrier of geranic acid the nano-emulsion with the highest kinetic stability, characterized by droplet size less than 50 nm was selected. During the preparation of geranic acid-loaded O/W nano-emulsion, first the active substance was incorporated to the mixture of surfactant:oil (S:O) and homogenized by the use of vortex aparature. Subsequently, the water phase was added, drop by drop, until final formulation was formed. The concentration of geranic acid in the obtained formulation reached 0.5% w/w.

Release studies. Drug release study of geranic acid was carried out using the Spectra/Por Standard Regenerated Cellulose (RC) membrane, at the temperature T=32°C. For release experiment, about 3 g of formulation were filled in a dialysis bag and submerged in receptor solution for 24 h. The concentration of geranic acid in the receptor solution (i.e. PBS/Ethanol mixture in the volume ratio 80/20) was analyzed by means of UV-Vis spectroscopy (Machery-Nagel Company), at the wavelength of 210 nm, on the basis of calibration curve.

RESULTS AND DISCUSSION

Formation and characterization of microemulsions

Different types of Polysorbates were tested as the emulsifiers in the first stage of the studies. Additionally, the short-chain alcohols (ethanol, 1-propanol and butanol) were used as co-surfactants (Table 1).

The obtained results showed that the stable microemulsion systems with Crodamol PC was obtained only

Table 1. Composition of studied formulations.

Ingredient	Composition (% mas)								
	E-1 T80E	E-1 T80P	E-1 T80B	E-1 T60E	E-1 T60P	E-1 T60B	E-1 T40E	E-1 T40P	E-1 T40B
Crodamol PC	5	5	5	5	5	5	5	5	5
Aqua	45	45	45	45	45	45	45	45	45
Polysorbate 80	25	25	25	–	–	–	–	–	–
Polysorbate 60	–	–	–	25	25	25	–	–	–
Polysorbate 40	–	–	–	–	–	–	25	25	25
Ethanol	25	–	–	25	–	–	25	–	–
1-Propanol	–	25	–	–	25	–	–	25	–
Butanol	–	–	25	–	–	25	–	–	25
Stability	no	no	yes	no	no	yes	no	no	yes

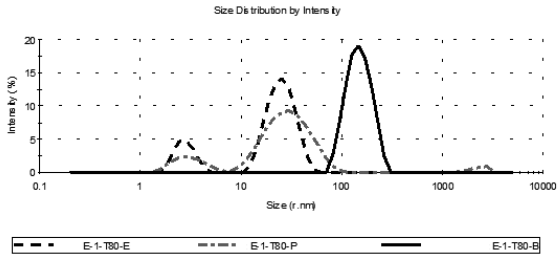

Figure 1. Comparison of particle size distribution of emulsions based on Crodamol PC with different type of co-surfactants E-1-T80-E (PDI= 0.813; ethanol), E-1-T80-P (PDI= 0.575; 1-propanol), E-1-T80-B (PDI=0.094; butanol), S/CoS: O=90:10.

when 1-butanol was used as a co-surfactant (CoS), additionally only in specific concentration area (S/CoS=1/1, S/CoS:O=90:10). Figure 1 presents the influence of co-surfactants addition on particle size distribution. It was observed that with increasing chain length of the co-surfactant the stability of the emulsions increases (polydispersity index (PDI) decreases). This observation is in accordance with the results obtained by others authors (Shevachman *et al.*, 2004) and probably it is related to the increase in hydrophobicity of the co-surfactant.

The most important role of co-surfactants in the formulation of high dispersion systems is to lower the interfacial tension, increase the fluidity and destroy the liquid crystalline and/or gel structure (Gaonkar *et al.*, 2003). Short-chain alcohols (such as ethanol) are relatively soluble in water and sparingly soluble in Crodamol PC. Medium-chain alcohols (butanol, pentanol and hexanol) have better solubility in Crodamol PC but they are less soluble in water, and thus they penetrate into hydrophobic core and decrease its hydrophobicity resulting in increase of emulsion stability. The obtained results confirmed that the kind of used surfactants influence the emulsion droplet size. As it is shown at Fig. 2, emulsions stabilized by Polysorbat 80 (E-1-T80-B) were characterized by the largest degree of dispersion, in this case the average droplet size was 137 nm while in case of emulsions containing polysorbate 40 and polysorbate 60 it was 162 nm and 196 nm, respectively.

These preliminary results allowed selecting Polysorbate 80 as the surfactant and butanol as a co-surfactant for further investigations, considering phase behavior and stability study of the emulsions based on Crodamol PC.

In the next step of the studies, the influence of oil/surfactant/co-surfactant/water (Crodamol PC/Polysorbate 80/1-butanol/Water) weight ratio on stability of the system was investigated. The attempts were made to

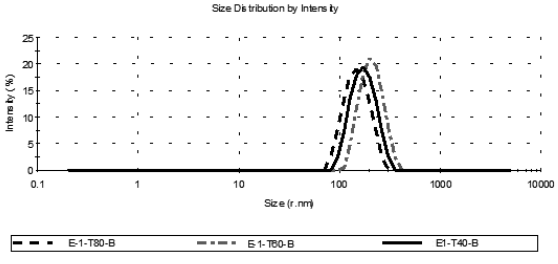

Figure 2. Comparison of particle size distribution of emulsion based on Crodamol PC with different type of the surfactant: Polysorbate 80 (E-1-T80-B), Polysorbate 60 (E-1-T60-B), Polysorbate 40 (E-1-T40-B) and 1-buthanol as a co-surfactant (S/CoS:O = 90:10).

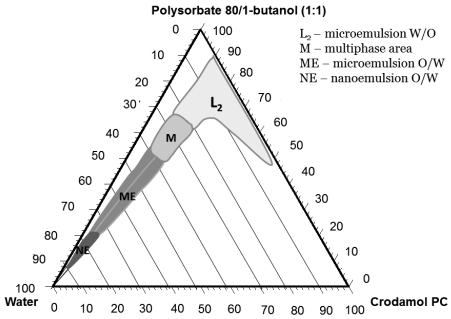

Figure 3. Pseudo-ternary phase diagram for the Crodamol PC/Polysorbate 80/butanol/Water system, at 25°C.

reduce the concentration of the surfactant/co-surfactant (S/CoS) mixture in the final formulation.

All formulations were prepared by low energy emulsification method, by dropwise addition of water to the mixture of surfactant/co-surfactant: oil (S/CoS:O). The phase diagrams of the pseudo-ternary system, Crodamol PC/Polysorbate 80/1-butanol/water were done to specify the area of stable high dispersed systems occurrence (Fig. 3). The L_2 region, which extends along the water: Polysorbate 80/1-butanol axis up to 25:75 water: surfactant/co-surfactant weight ratio corresponds to reverse micelles or W/O microemulsions. The multiphasic region extends along the water: Polysorbate 80/1-butanol axis, from water surfactant weight ratios of 25:75 to 40:60. The microemulsions region occupying most of the diagram from water/surfactant:co-surfactant weight ratios of 40:60 to 80:20. Transparent liquid dispersions appeared after addition of approximately 40 wt.% of water to surfactant/co-surfactant: oil mixture (S/CoS:O) of 90:10 ratio. These transparent colloidal dispersions are microemulsions because when the samples were prepared by mixing all the components, at the final composition, they also showed transparent appearance. It is believed that in case of microemusions their properties did not depend on the preparation method but were related to storage temperature (Sadurni *et al.*, 2005).

Figure 4 shows the effect of S/CoS:O ratio on stability of Crodamol PC-based emulsions. It is visible that the increase of the concentration of surfactant/co-surfactant mixture increases the stabilitsy of Crodamol PC-based emulsion (lower polydispersity index was observed).

The destabilization process of the emulsions was also visually observed (Fig. 5). The nano-emulsion with S/CoS:O ratio 85:15 (E-3T80-B) was more opaque than this obtained with a S/CoS:O ratio of 90:10 (E-1T80-B). A more opaque appearance usually indicates bigger oil droplet size and consequently lower stability.

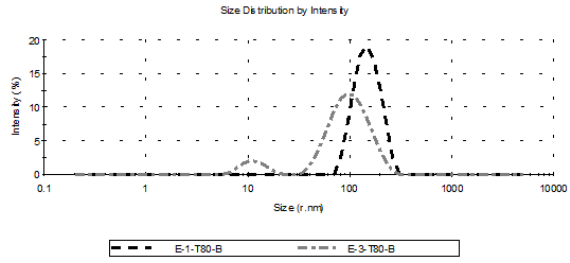

Figure 4. Comparison of particle size distribution of Crodamol PC-based emulsions with
S/Co: O ratio = 85:15 (E-3-T80B, PDI=0.346) and 90:10 (E-1-T80B, PDI=0.094).

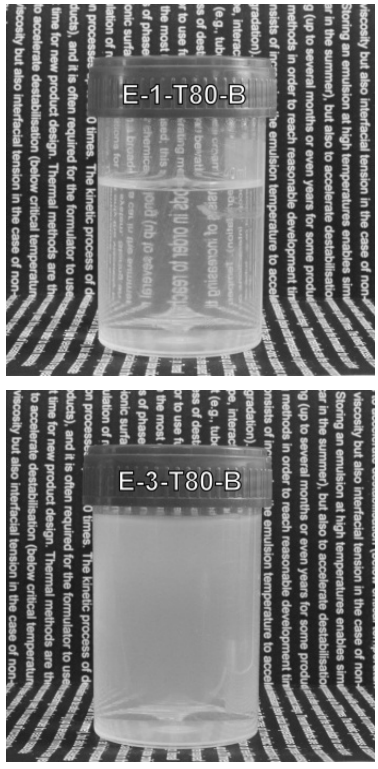

Figure 5. Macroscopic picture of emulsions E-1-T80-B (**a**) and E-3-T80-B (**b**).

Figure 6 shows changing of the E-1-T80-B emulsion conductivity with an increase of water content. It was observed that after addition of about 35% of water phase the conductivity of emulsion suddenly increased from 24.4 μS/cm to 90 μS/cm. The observed clear leap of the conductivity means a change of the emulsion type from W/O into O/W. It was also visually observed that the emulsion becomes from opaque to transparent again at 40 wt.% of water.

Nano-emulsions formation and characterization

Considering potential application of the high dispersed emulsions and irritation effect of butanol on the skin, in the next stage of the studies attempts were made to obtaining stable formulations without co-surfactants. In this case, also the phase diagram of the ternary system Crodamol PC/Polysorbate 80/water was done to specify the area of stable high dispersed systems occurrence (Fig. 7).

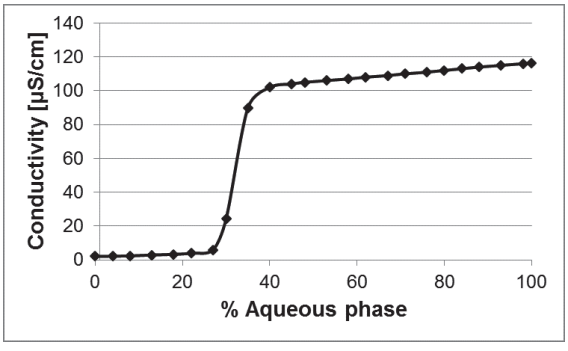

Figure 6. Changes in conductivity for the formulation E-1-T80-B.

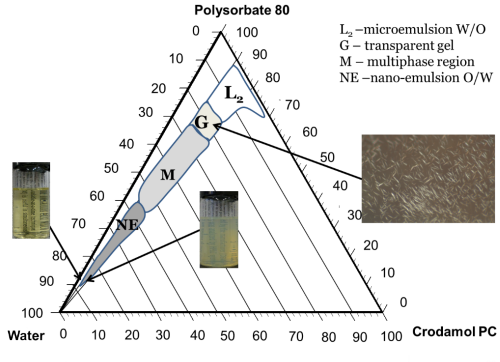

Figure 7. Ternary phase diagram for the Crodamol PC/Polysorbate 80/Water system, at 25°C.

Figure 7 shows that nano-emulsions region could not be extended at surfactant: oil (S:O) ratio higher than 80:20. These transparent colloidal dispersions are nano-emulsions because their properties depended on the preparation method and their properties did not depend on the temperature storage. The observed relatively large area of W/O ME (L_2) and a small area of O/W NE could be explain through a bulky hydrophilic head group of Polysorbate 80 and its packing more loosely at the interface resulting to allow better penetration of oil chains into the surfactant tails. Warisnoicharoen and coworkers (2000) found that length and molecular volume of the hydrophobic chains of the surfactant and oil are undoubtedly extremely important in determining whether the small or larger molecular volume oils are solubilized to the greater extent. The shorter hydrophobic chain surfactants, the smaller molecular volume oils are solubilized. The capacity of a surfactant to solubilize Crodamol PC strongly depends on the ability of the oil to penetrate into the hydrophobic part of the surfactant film. Crodamol PC has a high molecular weight (356.54 g/mol) so it could be supposed that, when compared with a simple alkane, propylene glycol diesters of caprylic and capric acids occupy a relatively large molecular volume fraction at the interface.

Characteristic of nano-emulsions

As a result of conducted research nano-emulsion systems differing in kinetic stability have been obtained. As it is shown at Fig. 8, the droplet size of nano-emulsion NE-CPC-80:20, containing 80% of water and surfactant:oil (S:O) ratio 80:20 increased with passing time (Fig. 8). The droplet size of this nano-emulsion after preparation was 33 nm and it increased up to 240 nm after 24 hours.

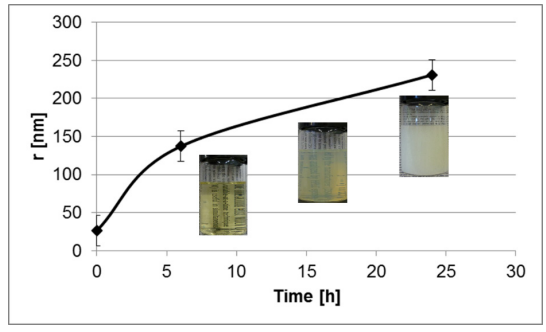

Figure 8. Droplet size of nano-emulsion NE-CPC-80:20 as a function of time.

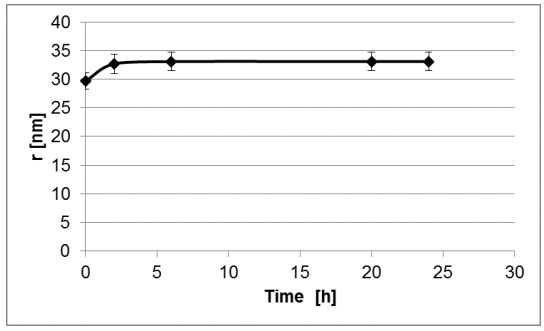

Figure 9. The droplet size of nano-emulsion NE-CPC-90:10 as a function of time, T=25°C.

The two most probable breakdown processes in this system must be coalescence and Ostwald ripening. This phenomenon indicates that the droplet size is getting bigger in time and destabilization process occurs in this system. The droplet size measurement in time confirms these observations.

The stability of NE-CPC-90:10 nano-emulsion, with 90% water concentration and surfactant: oil (S:O) ratio 90:10, was assessed as a function of droplet size versus time. The stability studies were performed only at 25°C, because the properties of nano-emulsions are independent of the storage temperature (Fernandez *et al.*, 2004; McClements, 2012; Anton & Vandamme, 2011). The data shown at Fig. 9 indicated that no significant destabilization process, due to creaming or sedimentation, or due to droplet size changes take place in this period of time.

Release results

Figure 10 shows the release profiles of geranic acid from the nano-emulsion (NE-CPC-90:10) and to compare from the oil (Crodamol PC). Both of the profiles are similar and the released amounts of the active after 24 hours, in both cases, are comparable (up to 22%). However, from the application point of view, as a form of cosmetics, nano-emulsions show better user properties in comparison to the oils. The obtained results have shown that the nano-emulsions based on Crodamol PC, stabilized by Polysorbate 80, can be used in cosmetic products as the carrier for controlled release of geranic acid.

CONCLUSIONS

The stable, transparent, O/W high dispersed emulsions, containing Crodamol PC as an oil phase were obtained using Polysorbate 80 as the emulsifier with or without co-surfactant. The prepared emulsions were characterized by droplets size less than 200 nm. The obtained results confirmed that the kind and the concentration of the co-surfactant significantly influence the stability the systems. It was observed that with increasing length of the co-surfactant chain the stability of the microemulsions increased (low polydispersity index PDI was observed). The stable systems were obtained only with application of 1-butanol as the co-surfactant. Contrary to Crodamol PC based microemulsions, nano-emulsions containing the diesters as the oil phase were formed without co-surfactant. Moreover, the results have shown that the obtained stable nano-emulsion can be used as a carrier for controlled release of geranic acid.

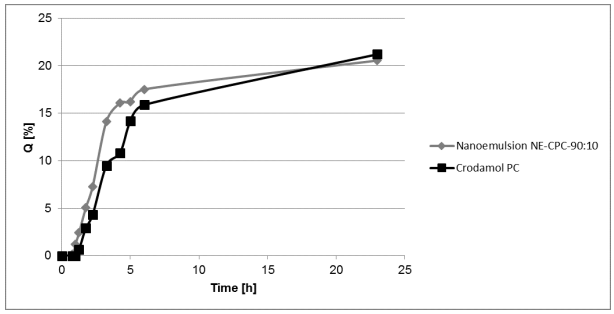

Figure 10. Release profiles of geranic acid as a function of time, at 32°C, form nano-emulsion NE-CPC-90:10 and from Crodamol PC, (Q — released amount of geranic acid, expressed as % of initial value).

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