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Influence of Surfactant on the Thermal Behavior of Marigold Oil Emulsions with Liquid Crystal Phases

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ABSTRACT Vegetable oils have been largely consumed owing to the interest of pharmaceutical and cosmetic industries in using natural raw materials. The production of stable emulsions with vegetable oils challenges formulators due to its variability in composition and fatty acids constitution within batches produced. In the present work, it was studied that the influence of the size of carbon chain and the number of ethylene oxide moieties of the surfactant on the thermal behavior of eight emulsions prepared with marigold oil stabilized by liquid crystal phases. Differential scanning calorimetry (DSC) was used to determine the thermal behavior of the emulsions. The ratio of bound water was calculated, being between 29.0 and 42.0%, confirming the extension of the liquid-crystalline net in the external phase. Changing the lipophilic surfactant from Ceteth-2 to Steareth-2, there was an increase in the temperature of phase transition of the liquid crystal influencing the system stability. Calorimetric study is very useful in understanding the performance of liquid crystals with the increase of temperature and to estimate emulsions stability.

KEYWORDS Lamellar liquid crystal, DSC analysis, Marigold oil, Vegetable oils, Emulsion, Phase transition, Free water

INTRODUCTION

Fluid and semisolid emulsions are widely used in pharmaceutical and cosmetic products due to their therapeutic properties and as vehicles for cosmetic agents and drugs for delivery into skin. In the past, this type of formulation was often considered as a simple and elegant structure for drug transport. However, currently it is known that the colloidal properties of this type of vehicle can influence the bioavailability of the active ingredients in different ways, being able to interact with them, influencing the solubility and diffusion of product into the skin (Eccleston, 1997; Santos et al., 2004).

Cosmetic and pharmaceutical emulsions are rarely simple two-phase oil/water systems. Such preparations often contain several interacting excipients and may be composed of additional phases to oil and water. In aqueous systems containing surfactant/fatty alcohol combinations, the additional phase

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usually form when the emulsifier, in excess of that required to form a monomolecular film at the oil droplet interface, interacts with continuous phase water to form a gel network of vastly swollen bilayer structures (Ribeiro et al., 2004).

Calendula officinalis (Marigold) is an original plant from Egypt and subspontaneous in the Mediterranean region, that is a popular and very versatile plant for cosmetic and pharmaceutical use (Cetkovic et al., 2004). Several derivatives of this vegetable have been used for many years in cosmetic products due to the belief that natural products present therapeutic activity without providing adverse effect, different from synthetic products. The oil is obtained by extraction from flowers and seeds, which are very rich in liposoluble active components with outstanding intereces like carotenoids, flavonoids, and some triterpendiols which have an anti-inflammatory action (Zitterl-Eglsser, 1997).

The influence of liquid crystals (LCs) on the stability of emulsions was early introduced by Friberg et al. (1969), who evaluated the fundamental factors in a series of publications (Friberg, 1974, 1992; Friberg et al., 1976) and described the advantages of the formation of these phases for the emulsion stability and function. They are known as the third phase of the emulsion, since they cannot be defined as internal or external phase. Emulsions containing liquid crystals are considered complex drug delivery systems for cutaneous surfaces. These emulsions are normally found in systems where lecithin is used as emulsifying agent, being easily detected by polarized light microscopy due to its birefringency property (Friberg et al., 1987). Its presence in emulsions provides some functional advantages such as increased stability. The most common type of LC found in emulsions is lyotropic, in which the structural arrangement has been assigned to lamellar phases and is responsible for emulsions stabilization since it enhances their viscosity (Klein, 2002; Santos et al., 2005).

LCs stabilize the emulsions by two basic mechanisms: by forming a physical barrier and a gel phase, which acts preventing the occurrence of coalescence among the globules of emulsion (Klein, 2002; Eccleston, 1990). Another important role played by LCs is the reduction of the van der Waals forces owing to an increase on the interfacial distance between the globules (Madani & Friberg, 1978). However, this stability is intrinsically related to the temperature to which the product is submitted. According to Klein (2002), the lamellar structures can be destroyed if the emulsion is subjected to extreme temperatures, as a consequence, it will not act anymore as barrier against coalescence.

Differential scanning calorimetry (DSC) is a thermal analytical technique, which provides detailed information about physical and energetic properties of some substances (Clas et al., 1999). Therefore, DSC has been considered to be a valuable tool to study the interaction among substances in dispersed systems such as emulsions.

The stability analysis of traditional emulsions composed by two liquids is focused on two main factors: the viscosity of the continuous phase and the energetic barrier between the dispersed droplets, which are modified by the surfactant absorbed at the droplet surface. Besides the range of thermodynamic stability, the most fundamental question concerns to the phase structure (Labes-Carrier et al., 1995).

The aim of this work was to study the influence of the length of the carbon chain and number of ethylene oxide moieties of the surfactant on the transition temperature of liquid crystal phases and on the stability of emulsions prepared with marigold oil.

EXPERIMENTAL

Materials

Oily Phase

The marigold oil (*Calendula officinalis*) used was produced by compression and later percolation of seeds and flowers of marigold and was acquired from Beraca Co., São Paulo, Brazil.

Surfactants

Different polyoxyethylene (POE) alkyl ethers were used as surfactants, class of which presents as general chemical structure: $CH_3(CH_2)_n{-}O{-}(C_2H_4O)_mH$, where n is the size of carbon chain and m is the number of ethylene oxide. Polyoxyethylene (Santos et al., 2004) cetyl ether (Ceteth-2, HLB number 5.1) and polyoxyethylene (Santos et al., 2004) stearyl ether (Steareth-2, HLB number 4.7) were used as lipophilic and hydrophilic surfactants. The various alkyl ethers used were polyoxyethylene (Friberg et al., 1987) cetyl ether (Ceteth-10, HLB number 11.0), polyoxyethylene (Wendland, 1986) stearyl ether (Steareth-20, HLB number 15.3), polyoxyethylene (Zitterl-Eglsser et al.,

1997) cetyl/stearyl ether (Ceteareth-5, HLB number 9.2), polyoxyethylene (Wendland, 1986) cetyl/stearyl ether (Ceteareth-20, HLB number 15.4).

Water Phase

Water phase used was the freshly distilled water. The ingredients employed in experiments possessed pharmaceutical degree and were used as received, without further purification.

Methods

Preparation of Emulsions

The blends of surfactants (10.0% w/w), previously mixed to reach the critical HLB of marigold oil (Table 1) [6.0 according to Santos et al. (2005)], were weighted with the oil (10.0% w/w). Oil and aqueous phases were separately heated up to 75 ± 2°C and the water phase (80.0% w/w) was slowly added into the oil phase, being the stirrer speed set at 600 rpm (Mechanical Mixer Fisaton–702R) in order to provide better mixing conditions. The emulsions were kept under mixing until reaching room temperature 25 ± 2°C.

Emulsions Analyses

The analyses were performed 24 hr after emulsions preparation according to some parameters that identify the formation of liquid crystal, which are based on physical stability and thermal behavior of these systems. Such analyses were performed to understand the influence of the change of surfactant in emulsions characteristics and stability. The methods used were: polarized light microscopy, preliminary stability test (centrifugation and thermal stress) and DSC assay.

TABLE 1 Ratio of Surfactants on the Mixture to Reach HLB Value of 6.0

Surfactant pair	% (w/w)	% (w/w)
Steareth-2/Steareth-20	87.74	12.26
Steareth-2/Ceteth-10	79.37	20.63
Steareth-2/Ceteareth-20	87.85	12.15
Steareth-2/Ceteareth-5	71.11	28.89
Ceteth-2/Steareth-20	91.18	8.82
Ceteth-2/Ceteth-10	84.75	15.25
Ceteth-2/Ceteareth-20	91.26	8.74
Ceteth-2/Ceteareth-5	78.05	21.95

Ceteth-2 and Steareth-2 lipophilics; Ceteth-10, Ceteareth-5, Ceteareth-20, Steareth-20 hydrophilics.

Polarized Light Microscopy

Emulsions were submitted to a microscopic analysis performed by polarized light view microscope (Olympus BX50), where anisotropy areas were observed indicating liquid crystal presence.

Preliminary Stability Test

Preliminary stability test consisted of two different tests such as the centrifugation and thermal stress test.

At the centrifugation test, samples of each emulsion were weighted in graduated vials and submitted to centrifugation cycles in three speeds (70, 440, and 863 g) for 15 min in each. At the end of each cycle, a macroscopic evaluation was performed in order to verify a possible phase separation.

At the thermal stress test, samples were weighed in closed vials and placed on a thermostatic water bath. The emulsions were submitted to a range of temperatures (40 up to 80°C) increasing in steps of 5°C staying during 30 min in each temperature. Instability signs like flocculation and phase separation were observed just before changing temperature.

DSC Measurements

Calorimetric analysis (DSC) was performed on a DSC-50 (Shimadzu, Tokyo, Japan). Samples were placed (20 ± 3 mg) in a closed aluminum crucible and submitted to heating between –20.0 and 100.0°C, being the scanning rate 5°C/min. Results were collected and plotted in graphics and according to them, phase transition temperatures and ratio of bounded and bulk water were calculated.

The evaluation of the phase transition of the water provided the determination of structurally bounded water (SBW) ratio, which was calculated according to the following equation (Gao et al., 2003):

$$\mathrm{SBW}(\%) = [1 - (H_1 / H_0)] \times 100$$

where

H_1 = melting enthalpy of water determinate from the sample (Joule/g),

H_0 = standard melting enthalpy of water (–333.6 J/g).

SBW ratio in a factor that indicates the extension of the LC formation through the external phase of the emulsion. As higher is the SBW ratio, higher is the size of the lamellar phase around the globules, because as far it increases it adsorb water in the interlamellar spaces, decreasing free water.

RESULTS AND DISCUSSION

All emulsions presented liquid crystal formation under polarized light microscopy view (Fig. 1), as previously reported by Santos et al. (2005). They were all classified as lamellar lipotropic liquid crystal, which normally exists in a concentration range between the isotropic solution and the crystalline surfactant (Hiltrop, 1994). Usually they can be found at three different states according to their organization: the lamellar or neat phase consisting in double layers of surfactants molecules with water at the interface of polar groups; the hexagonal or middle phase consisting in cylindrical structures; and the cubic phase in which the molecules pack in a spherical pattern (Ferrari et al., 2004). Each state presents the ability of entrapping water from the external phase and interacting with the oil droplet in a different manner, influencing on system stability at a specific way. The lamellar structure found in our emulsions surrounds the oil droplet increasing stability against coalescence process.

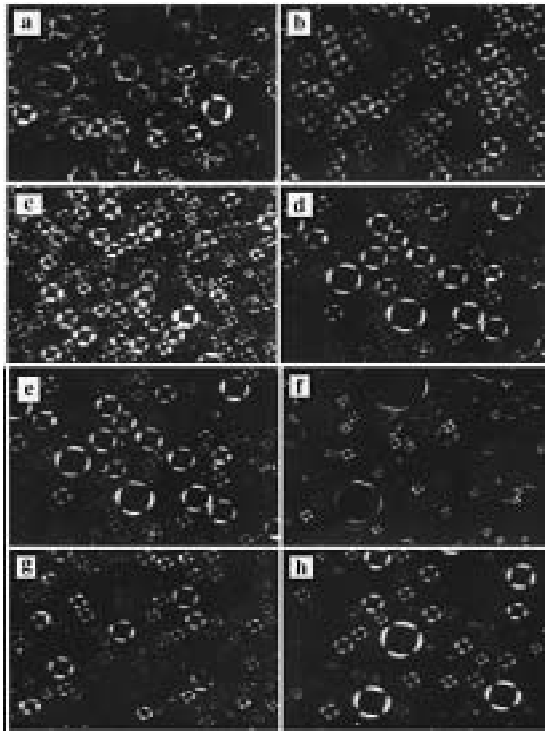

FIGURE 1 Photomicrography Under Polarized Light of Emulsions According With Surfactant Pair. (a) Steareth-2/Steareth-20; (b) Steareth-2/Ceteth-10; (c) Steareth-2/Ceteareth-20; (d) Steareth-2/Ceteareth-5; (e) Ceteth-2/Steareth-20; (f) Ceteth-2/Ceteth-10; (g) Ceteth-2/Ceteareth-20; (h) Ceteth-2/Ceteareth 5.

All the emulsions resisted to the three centrifugation cycles at the preliminary stability test, however, after the thermal stress tests, at 60°C for 30 min, all samples presented phase separation, which was related to coalescence process. These results indicate that emulsions are stable, but susceptible to temperature increase.

Thermal analysis can be defined as a group of techniques where physical properties are studied in function of temperature to which the samples are submitted (Wendland, 1986). Therefore, this procedure can provide us useful knowledge about the handling temperature of substances and products or their physics characteristics in function of temperature. Two major phase transitions that occurred in all the samples were observed in the basic thermogram illustrated in Fig. 2, being the temperature and the heat for each phase transition individually calculated from these thermograms.

The DSC is an analytical method where the ordinate, in any time or temperature, is directly proportional to the differential flux of heat between the sample and a reference material. The thermal events registered by DSC are related to structure and to the sample composition, appearing as variation on the baseline into endo or exothermic directions (Privalov & Tiktopulo, 1970).

Several types of water can be detected in surfactant-based liquid-crystalline phases. These types of water have different enthalpies and temperature of melting, as well as anomalously low supercooling temperatures

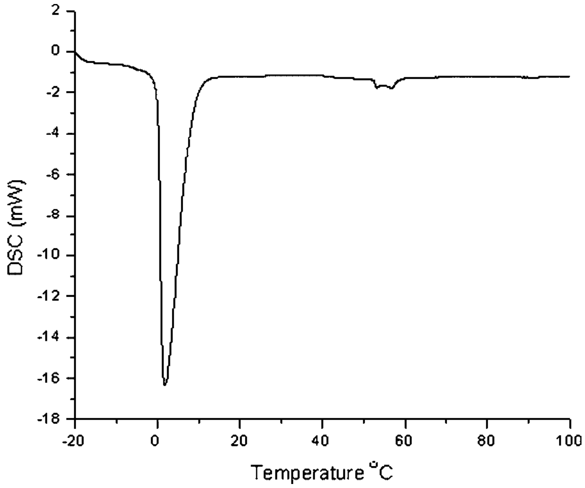

FIGURE 2 Typical Thermogram of Studied Emulsions.

associated with nonbulk states of water (Schulz et al., 1994). The water type that melts closely to 0°C corresponds to “free water”, whose properties are the same of pure water, does not associate with surfactant molecules and other emulsion components (Schulz & Puig, 1993). The melting enthalpies of free water diminish as the ratio of free water decreases, being the same as pure water when the system does not presents other types of water (Schulz et al., 1994; Schulz & Puig, 1993).

Results for the first phase transition that begins between –2.44 and –0.44°C corresponds to the melting of the free water of the emulsion (Table 2, Figure 3), not being observed a specific variation related to the change of surfactant. The structurally bounded and free water amounts present in the external phases can be calculated through the temperature and energy of its physical transition (Gao et al., 2003), being this data important to estimate the emulsion physical stability and the emulsified system structure (Gao et al., 2003; Kodama et al., 2001). When a water molecule is bounded to other molecules in the emulsion, they become less available to suffer processes, such as evaporation and migration, which modify the product characteristics, consequently promoting instability. Moreover, the presence of this free water in emulsions allows the growth of some microorganisms, resulting in undesirable changes in emulsion microbiological quality (Gao et al., 2003). In addition, the presence of high amounts of bounded water in emulsions made with nonionic surfactants is directly related to the lamellar liquid crystal phases that during formation adsorb water in the interlamellar spaces, making possible the correlation between the expansion of this phases and the decrease of free water.

TABLE 2 **Results of the First Thermal Transition, Related With Water Melting**

Sample	Peak (°C)	Onset (°C)	End (°C)	Heat J	Heat J/g
1	1.91	−2.23	9.63	−1.10	−193.11
2	2.52	−2.20	10.62	−1.25	−211.7
3	1.64	−0.44	8.69	−1.26	−221.54
4	2.32	−1.42	10.89	−1.51	−221.6
5	1.80	−2.44	10.94	−1.61	−233.81
6	2.39	−1.41	10.51	−1.46	−214.85
7	2.46	−1.83	10.24	−1.17	−191.25
8	2.41	−2.00	8.4	−1.11	−194.49

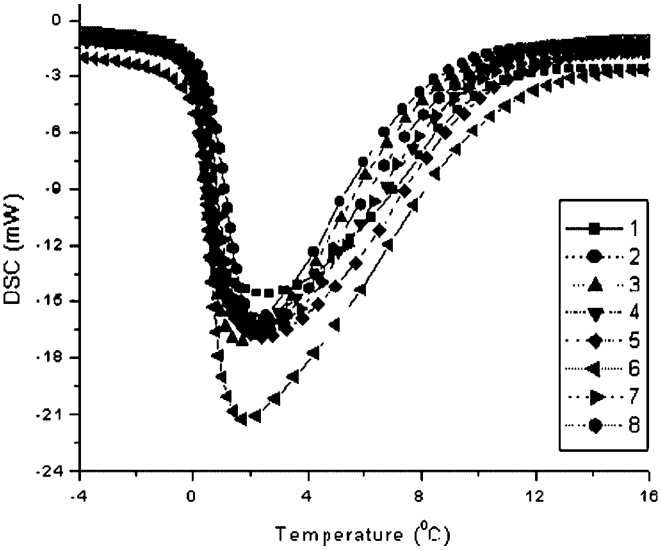

FIGURE 3 **Profile of the First Thermal Transition, Related With Water Melting. Endothermic Transitions are Down. (a) Steareth-2/Steareth-20; (b) Steareth-2/Ceteth-10; (c) Steareth-2/Ceteareth-20; (d) Steareth-2/Ceteareth-5; (e) Ceteth-2/Steareth-20; (f) Ceteth-2/Ceteth-10; (g) Ceteth-2/Ceteareth-20; (h) Ceteth-2/Ceteareth 5.**

The SBW ratio was situated between 30 and 43% (Table 3) indicating that the liquid crystalline phases had formed and decreased the amount of free water. This data can be also correlated with system stability, were the ones with higher SBW ratio presented a huge number of layers of the lamellar structure which is responsible for water adsorption and is, in this system, the main responsible for stabilization.

The second thermal transition was related to the melting of liquid crystals phases. It was observed that all the samples presented phase transition between 39 and 54°C (Table 4, Figure 4), resulting in thermograms, which shape, is characteristic from emulsions prepared with surfactants derived from POE (Kónya et al., 2002; Ribeiro et al., 2004). All the samples showed double peaks in this region, being the first

TABLE 3 **Ratio of Structurally Bounded Water (SBW) Calculated Through DSC**

Sample	SBW (%)
1	42.11
2	36.54
3	33.59
4	33.57
5	29.91
6	35.60
7	42.67
8	41.70

TABLE 4 Results of the Second Thermal Transition, Related With the Melting of Liquid-Crystalline Phases

Sample	Peak (°C)	Onset (°C)	End (°C)	Heat mJ	Heat mJ/g
1	56.71	51.46	59.15	−29.69	−5.21
2	54.19	49.57	56.6	−36.6	−6.17
3	62.42	53.73	76.26	−7310	−1280
4	52.09	50.07	55.80	−45.85	−6.74
5	47.44	39.03	50.37	−54.95	−7.96
6	46.18	43.10	48.69	−47.9	−7.04
7	47.12	45.21	49.8	−32.82	−5.38
8	46.10	42.29	48.69	−35.81	−6.28

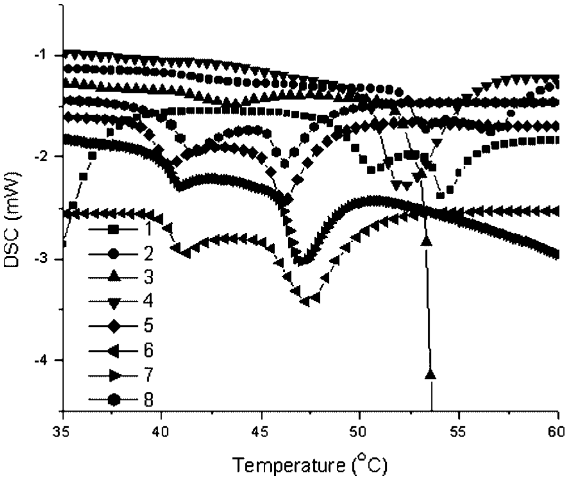

FIGURE 4 Profile of the Second Thermal Transition, Related With the Melting of Liquid-Crystalline Phases. Endothermic Transitions are Down. (a) Steareth-2/Steareth-20; (b) Steareth-2/Ceteth-10; (c) Steareth-2/Ceteareth-20; (d) Steareth-2/Ceteareth-5; (e) Ceteth-2/Steareth-20; (f) Ceteth-2/Ceteth-10; (g) Ceteth-2/Ceteareth-20; (h) Ceteth-2/Ceteareth 5.

peak probably the melting of β and γ crystals and the second representing the endothermic to α crystal melting (Ribeiro et al., 2004; Eccleston, 1990).

In a liquid crystal system, the phase transition at T_m (melting temperature) occurs between the so-called gel phase and the lamellar LC phase. In the latter, usually denoted as the L phase, the chains are in a liquid-like state with considerable conformational disorder. After cooling, the formation of a gel phase can eventually occur in the chains, where the bilayer are in a quasi-crystalline state (i.e., fully extended in all-trans conformation) and experience much slower dynamic motions (rotation, lateral diffusion). If the area of the surfactant headgroup is proportional to the hydrocarbon cross-section area, theoretically a simple "lamellar" gel phase (L) is formed, however, in cases of higher mismatch, tilted (L), rippled (P) or interdigitated gel structures may form. For some lipids, that present Tm under the main gel-to-LC transition temperature, a gel–gel phase transition may occur, involving much lower enthalpy changes. Tm is shown to be dependent on factors such as the headgroup structure, hydrocarbon chain length of the lipid, pH and ion binding (Marques et al., 2002).

These characteristics are also related to the type of surfactant used, such as those derived of fatty alcohols and POE. In this surfactant group usually occurs the formation of swelled lamellar phases, where the transition at lower temperatures represents the crystalline phase and the transition of α-hydrated gel phases occurs at higher temperatures (Ribeiro et al., 2004). The emulsions prepared with Steareth-2, as lipophilic surfactant, presented phase transition at higher temperature in comparison to those formulated with Ceteth-2 as the lipophilic. This variation probably happens due to the size of the surfactant fatty chain, which is bigger in the Steareth-2. For hydrophilic surfactants, the emulsion prepared with Ceteareth-20 also showed phases transition at greater temperature values, although this influence was not as significant as for lipophilic surfactant, since its concentration in the emulsion is smaller. The fatty chain is important to determine the melting point of the surfactants, being linear for all surfactants used in this study, consequently their melting point is directly proportional to its size or number of carbons. This also leads to a bigger resistance of the liquid-crystalline phases, formed by these surfactants, in relation to the phase transition, being useful to maintain the complete structures for a longer time.

The results presented by phase transition of the liquid crystal can explain the occurrence of an instability process at 60°C, on the thermal stress test. Below this temperature, it was observed the presence of a liquid-crystalline network, which is major responsible for the system stability since it forms a physical barrier against the oil droplets coalescence. However, the liquid crystal melted upon 60°C and owing to this, emulsions became more susceptible to instability events, such as coalescence.

The formulation 3, prepared with Steareth-2/Ceteareth-20, presented a huge melting energy when compared to the other samples, the values were about 200 times higher. This melting energy was probably related to some kind of process, occurring at the same temperature of the liquid-crystalline phase melting point and consequently consuming the system energy. At a DSC assay, the energy consumption is associated to changes on the physical state of the matter, such as melting or evaporation, or even to the occurrence of any physical-chemical event, that needs heat to occur, like displacement of surfactant molecules at the oil droplet interface. Probably, both events took place at the same temperature, indicating that not only the liquid-crystalline phase was responsible for the emulsion stability, but also another mechanism exists on this sample that is not present on the other emulsions.

CONCLUSION

In this article we tried to correlate the length of carbon chain and number of ethylene oxide moieties of non ionic surfactants with the stability of marigold oil emulsion with previously identified lamellar liquid crystal formation. Formation of liquid crystalline phases is responsible for the high amount of bounded water, indicating system structuration and increasing stability. We found that for the liquid-crystalline phase was the main responsible to the stability because after the phase transition temperature of these phases, emulsions presented a quick phase separation. Size of surfactant carbon chain influence on the phase transition temperature of the liquid crystal formed, in the same manner as occur for the melting point of pure surfactant, were as higher is its length, higher is the transition temperature. Apparently, the number of ethylene oxide moieties did not have an important influence as occurred with the carbon chain.

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