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Research paper

Formulation of ascorbic acid microemulsions with alkyl polyglycosides

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ABSTRACT

Ascorbic acid microemulsions for topical application were developed. In this study, microemulsions were prepared using HLD (hydrophilic lipophilic deviation) concept to optimise the formulation. From this optimal formulation, the realisation of dilution ternary diagrams leads to obtain microemulsion zones. In addition, the effects of composition variable on the physicochemical characteristics of each system were investigated. After optimisation of the microemulsion systems, ascorbic acid was loaded in the formulations. Surface tension and small angle neutron scattering were used to characterise the surface properties and the structure of the microemulsions. Bicontinuous structure microemulsions were identified, and the influence of ascorbic acid localisation at the interface leading to modifications of the microemulsion structure was pointed out. The solubilisation of ascorbic acid, the stabilisation and *in vitro* transdermal penetration "Frantz cells" of ascorbic acid microemulsions were studied. Three different microemulsions were envisaged. The results confirmed that these microemulsion systems present a real interest for formulation and protection of ascorbic acid. Regarding their transcutaneous penetration behaviour, the different microemulsions studied could be useful for different topical applications. A major location of ascorbic acid found in the epidermis where the decomposition of melanin occurred indicates that microemulsion could be considered as a suitable carrier system for application of ascorbic acid as a whitening agent. In addition, a good passage of the drug in the dermis could be interesting for the relative oxygen matrix damage.

1. Introduction

The "Microemulsions" were first introduced by Hoar and Schulman in 1943. The original use of the term by these authors was to describe the transparent systems obtained, when normal emulsions were titrated to clarity with hexanol [1]. However, the microemulsion definition provided by Danielsson and Lindman in 1981 will be used as the point of reference. Microemulsion is defined as "a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution" [2]. Normally, microemulsions are quaternary systems composed of an oil phase, a water phase, surfactant and co-surfactant [3,4].

Microemulsions seem to be ideal liquid vehicles for drug delivery because of their several advantages such as thermodynamic stability (long shelf-life), very small droplet size (5–100 nm), easy formulation (low interfacial tension and almost spontaneous formation), low viscosity (with Newtonian behaviour) and high surface area (high solubilisation capacity) [5].

The application of microemulsion vehicles for transdermal drug delivery is becoming increasingly popular due to their high solubilisation potential for both lipophilic and hydrophilic drugs. It was demonstrated that permeation rates from microemulsions were significantly higher than those from conventional emulsions [6,7].

For some years, microemulsions based on alkyl polyglycosides have been investigated [8–10]. This new class of nonionic surfactants is made from renewable raw materials such as glucose and fatty alcohols [11]. These surfactants have outstanding biodegradability [11,12], excellent dermatological properties [13,14] and good surface active properties [15].

Many studies have found that phase behaviour and properties of alkyl polyglycosides are less influenced by temperature, unlike the case of ethoxylates surfactants [8,15,16]. A microemulsion with very low interfacial tension can be formed if an optimum ratio between alkyl polyglycoside and a suitable co-surfactant is selected [9,10]. Microemulsion formation by alkyl polyglycosides is very largely electrolyte and temperature independent, and also proceeds very rapidly compared with other surfactants, which is interesting for many technical applications [15]. Recently, some authors proposed alkyl polyglycosides in colloidal structures as prospective topical vehicles for a pharmaceutical drug [17,18].

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Ascorbic acid (AA) is a major protector from reactive oxygen skin damage. It could improve the morphogenesis of dermal epidermal junction, and is also known for its skin lightening properties [19–21]. However, ascorbic acid is a very unstable molecule, which remains highly vulnerable to oxidation. Several derivatives have been studied but they must first be absorbed into the skin and then transformed to ascorbic acid. Because of their structure, they weakly penetrate the skin, therefore, must be used at high concentration. That is why ascorbic acid remains the most interesting molecule to have biological effects [22,23]. Its stability and percutaneous penetration have been studied by several authors. They pointed out several conditions for stabilising AA in formulations such as the control of different physicochemical parameters (pH and electrolyte concentration) [24]. The mechanism of penetration of this high hydrophilic molecule has also been envisaged, and diffusivity, partition coefficient and solubility of AA in stratum corneum have been related to skin permeation [25,26].

The objective of the present work is the development of an alkyl polyglycoside microemulsion system, which presents a good stability, can protect AA from degradation and promote an adequate penetration of AA into the skin for topical application. In our study, microemulsions were prepared using HLD (hydrophilic lipophilic deviation) concept, which permit to optimise the formulation. Then, the realisation of dilution ternary diagrams led to obtain microemulsion zones. The effects of composition (various surfactant/co-surfactant associations and two different oils) on the physicochemical characteristics of each system were investigated. After optimisation of the microemulsion systems, ascorbic acid was loaded into the formulations. The solubilisation of ascorbic acid, the stability and *in vitro* transdermal penetration (Franz cells) of ascorbic acid microemulsions were studied.

2. Materials and methods

2.1. Materials

The components for microemulsions were used as follows: decylglucoside (Cognis, D-Düsseldorf) as a surfactant (S), sorbitan monolaurate (Seppic, F-Paris) as a co-surfactant (CoS). The lipophilic components were dioctylcyclohexane (Cognis, D-Düsseldorf) and mineral oil (Schell, F-Paris). Distilled water was used as hydrophilic component. Ascorbic acid is of European Pharmacopoeia grade (Prolabo, F-Paris).

2.2. Preparation of microemulsions by HLD concept

The HLD concept has been widely described by Salager et al. [27,28]. It concerns an equilibrated surfactant–oil–water system and is associated with the achievement of an ultra-low tension, which permits the maximum solubilisation of oil and water in a microemulsion phase. In fact, depending on the formulation and surfactant concentration, a balanced surfactant–oil–water system may produce one phase or several phases. When the system presents three phases with a microemulsion in equilibrium with both an oil phase and an aqueous phase (Winsor III), the formulation is defined as optimum if the microemulsion contains equal amounts of oil and water.

The HLD concept is a useful tool to compare quantitatively the relative influence of the different formulation factors or to establish an experimental scale for the classification of oils, surfactants and alcohols [29]. The present work uses the HLD concept to formulate microemulsions. Formulations scans were carried out by altering the surfactant/co-surfactant ratio with two different cosmetic oils in order to find the optimum formulation.

2.2.1. Determination of optimum formulation by varied surfactant/co-surfactant ratio (S/CoS)

The oil/water ratio (50/50), the total amount of surfactant and co-surfactant (S/CoS) (1%) and the nature of oil (dioctylcyclohexane or mineral oil) were fixed. The surfactant/co-surfactant ratio was varied (from 1/0 to 0/1 with a step of 0.1).

The protocol is composed of two phases mixed in trial tubes; phase A: consisted of solution of *x*% CoS in oil, phase B: solution of *y*% S in water (containing 3% NaCl). $(x + y)$ represents 1% (S/CoS). After mixing of two phases, the tubes were slowly turned and equilibrated in the water bath (70 °C). NaCl and 70 °C temperature were used to accelerate phase equilibrium and to obtain clear Winsor systems.

2.2.2. Observation of microemulsions zones

From the optimal surfactant/co-surfactant ratio, pseudo-ternary phase diagrams by dilution were performed. Surfactant was mixed with the co-surfactant at a fixed ratio. The binary mixtures (S + CoS)/oil was prepared and gradually diluted by the aqueous phase under stirring by magnetic agitator at room temperature (25 °C). After equilibrium, the balanced samples were assessed visually. When determined as being clear and transparent, they correspond to the entry in the zone of microemulsions. The aqueous phase is added until the disappearance of the transparency corresponds to the exit of the microemulsion zone.

Initially, the S/CoS ratio corresponding to the optimal formulation is considered. Then, the study of other ratios, more lipophilic, and more hydrophilic, makes it possible to increase the zone of microemulsion.

2.3. Microemulsion characterisations

All preparations were visually observed to evaluate their transparency and were microscopically observed under polarised light to notice the absence of liquid crystalline organisation (optical polarising microscope Zeiss, Axiostar Plus). The structure of the microemulsion systems in a nanometer scale was investigated by Small Angle Neutron Scattering (SANS). The experiment has been carried out on the spectrometer D22 at the Laue Langevin Institut (ILL). Three instrument configurations were chosen to cover a wide q-range from 3×10^{-3} to 0.4 $Å^{-1}$. (λ = 6 Å, sample to detector distance D = 1.4, 5 and 17 m with a collimation length of 17.6 m). This q-range gives access in the direct space to distances from 16 to 2000 Å. The samples were held in rectangular Hellma quartz cells and thermostatted at 25 °C. The raw data were corrected for electronic background and empty cell signal and normalised to the water signal, following the standard ILL procedures in order to obtain normalised intensities $I(q)$ in cm^{-1}.

SANS offered the unique feature of contrast variation and molecule labelling. The replacement of H_2O by D_2O does not modify the structure of the microemulsions but increases considerably the contrast between the hydrophilic and hydrophobic domains in the sample (see Table 1). Moreover, the use of D_2O allows one to reduce the incoherent background. The scattering length densities (SLDs) of the different molecules, which characterise the interaction strength between atoms and neutrons beam, are given in Table 1.

2.4. Preparation of microemulsions containing ascorbic acid: solubility and stability of ascorbic acid

The incorporation of AA into the microemulsions that was previously obtained leads to destabilisation of the preparations. Consequently, the new zones of microemulsions containing AA should be determined by realisation of additional pseudo-ternary diagrams with solutions of ascorbic acid instead of distilled water. The physical stability of the AA microemulsions was estimated

Table 1
Scattering length densities of the different molecules.

	M (g/mol)	d	v (cm^3)[a]	ρ (cm^{-2})
H_2O	18	1	2.99×10^{-23}	-5.608×10^9
D_2O	20	1.104	3.01×10^{-23}	6.367×10^{10}
Cetiol	305	0.83	6.104×10^{-22}	-6.979×10^8
Ascorbic acid	176.25	1	2.927×10^{-22}	1.529×10^{10}
Decylglucoside	≈500	1.25	1.302×10^{-22}	4.160×10^9
Polar head			1.302×10^{-22}	3.089×10^{10}
Aliphatic chains			2.964×10^{-22}[b]	-4.076×10^9
Sorbitan laurate	365	0.98	5.877×10^{-22}	4.645×10^9
Polar head			2.639×10^{-22}	1.524×10^{10}
Aliphatic chains			3.233×10^{-22}	-3.995×10^9

[a] Molecular volume.
[b] Calculated with the Tanford equation v (Å^3) = 27.4 + 26.9i, where i is the number of carbon atoms.

over 2 months by visual observation of macroscopic aspect (cloudiness or formation of two distinct layers). Ascorbic acid stability in microemulsion was performed by HPLC (Shimadzu Corporation, J-Kyoto). The stationary phase was PRONTOSIL® 120-3-C8 HS 3.0 μm (150 × 4.6 mm, Bischoff chromatography, D-Leonberg). The mobile phase was phosphate buffer pH 2.5 ± 0.05. UV detection was carried out at 245 nm; injection volume was 40 μl and flow rate was 0.6 ml min^{-1} (USP 29Ed.) [30].

In order to obtain information on the structure of the AA microemulsions, several of them were also evaluated by SANS and the interfacial activity of the S/CoS mixtures against oil was evaluated with and without ascorbic acid. The instrument used for interfacial measurements was a Krüss K100 tensiometer with a platinum-iridium ring. The oily phase was poured over the surfactant aqueous solution, and the equilibrium between the two phases is obtained after 15 min. Interfacial tension was calculated from the apparent interfacial tensions (average of ten measurements) using a correction factor dependent on the densities of the two phases and the ring dimensions [31].

2.5. In vitro transdermal penetration study

Drug release was determined through ear pig skin by Franz diffusion cell (diffusion area cell 0.785 cm^2), and the receptor medium was phosphate buffer with pH 2.5 ± 0.05 at 37 ± 0.5 °C. In order to control skin integrity, transepidermal waterloss (TEWL) of each skin sample was measured on the cells after 30 min equilibrium with receptor medium. TEWL examinations were performed with a Tewameter 210 (Courage and Khazaka Electronic GmbH, Cologne, Germany) and only the pieces that had TEWL values less than 10 g/m^2/h were used for the test. Temperature was also verified on each skin sample.

The concentration of ascorbic acid in the microemulsions was 4 wt.% and 50 μl sample was dropped onto the skin. After 1, 3, 5, 7, 10 and 14 h, the skin was separated for three parts: stratum corneum, epidermis and dermis: after rinsing the skin and donor chamber with 10 ml of phosphate buffer, the stratum corneum was obtained by stripping with 10 pieces of adhesive tape. The epidermis was heat separated from dermis (60 °C for exactly 150 s).The ascorbic acid in each skin part and in the receptor medium was assayed by HPLC (Shimadzu Corporation, J-Kyoto). All experiments were repeated six times, and the average was calculated.

3. Results and discussion

3.1. Preparation of microemulsions

3.1.1. Determination of optimum formulation by varied surfactant/co-surfactant ratio (S/CoS)

After preparing the series of tubes with surfactant/co-surfactant ratios that vary from 1/0 to 0/1 with a step of 0.1, the systems were balanced at constant temperature (70 °C) during one or two days, sometimes more if the liquid was viscous. Then, if the systems were perfectly transparent and present three phases, the phase containing the microemulsion was detected using a pocket laser whose beam becomes visible by the Tyndall effect. The "optimal formulation" (HLD = 0) corresponded to the tube (S/CoS ratio), where microemulsion phase was present in between the oil and aqueous phase with equal volume (Fig. 1).

For the first system containing dioctylcyclohexane as oily phase, the S/CoS ratio which performs the optimal formulation was (0.26/0.74). For the second system containing mineral oil the S/CoS ratio was (0.20/0.80). These ratios are quite similar, but it can be observed that more lipophilic surfactant is needed to achieve optimal formulation with mineral oil. As described by von Rybinski et al. [9], dioctylcyclohexane is less polar than mineral oil (dielectric constant of 1.2 and 2.4, respectively), and so it can be expected that it plays the role of a co-surfactant at the interface layer.

3.1.2. Formulation of microemulsion zones

Phase studies were performed to investigate the effect of surfactant/co-surfactant ratio on the microemulsion regions. For system containing dioctylcyclohexane, the decylglucoside/sorbitan monolaurate ratio used for studying the microemulsion zone was at first, 0.26/0.74. Then, other ratios were studied such as 0.20/0.80, 0.28/0.72, 0.30/0.70 and 0.325/0.675 in the order to increase the microemulsion zone (Fig. 2). The mixtures obtained in the present study formed spontaneously at ambient temperature when their components were brought into contact. They are very fluid, isotropic, completely transparent and sensible to laser beam.

It can be observed that when the S/CoS ratio becomes more lipophilic (increasing CoS, SML value), the microemulsion zone moved to the lipophilic side of the diagram, permitting incorporation of more oily phase into the microemulsions. On the contrary, when the S/CoS ratio becomes more hydrophilic (increasing S = decylglucoside value), the microemulsion zones are moved to the hydrophilic region of the diagram, permitting the incorporation of more aqueous phase into the formulations and also using less surfactants to perform the microemulsions.

For the system containing decylglucoside, sorbitan monolaurate and mineral oil, primarily, the (0.20/0.80) S/CoS ratio was envisaged, then other ratios were studied (0.25/0.75 and 0.30/0.70). For this system, two narrow zones of transparent and isotropic system only can be obtained for the S/CoS ratio (0.20/0.80) (Fig. 3). This result can be explained by the interfacial tension of dioctylcyclohexane, which is lower than that of mineral oil. This can

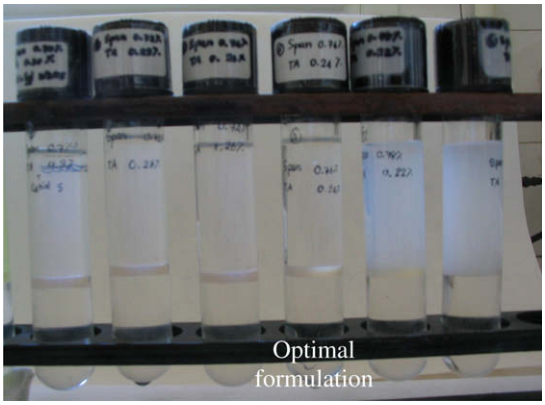

Fig. 1. Formulation scans of equilibrated (decylglucoside/sorbitan monolaurate)–dioctylcyclohexane–water systems at different (S/CoS) ratios. The three phase system with a microemulsion in equilibrium with equal quantities of oily phase and aqueous phase corresponds to optimum formulation.

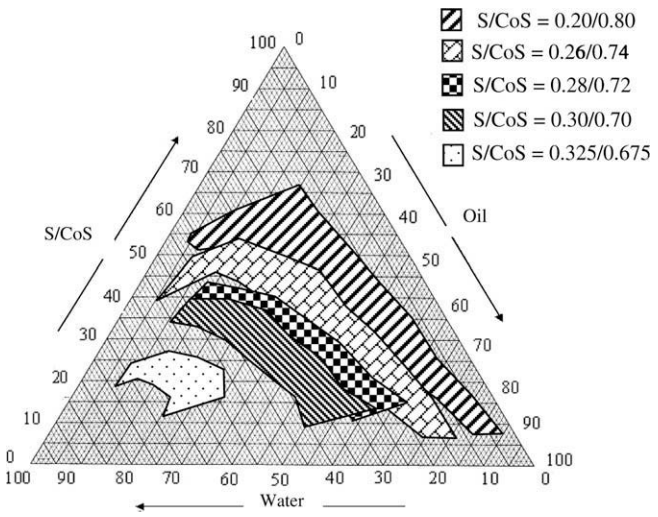

Fig. 2. Pseudo-ternary phase diagrams of decylglucoside/sorbitan monolaurate/dioctylcyclohexane/water microemulsion systems. Microemulsions zones at different S/CoS ratios (0.20/0.80, 0.26/0.74, 0.28/0.72, 0.30/0.70 and 0.325/0.675).

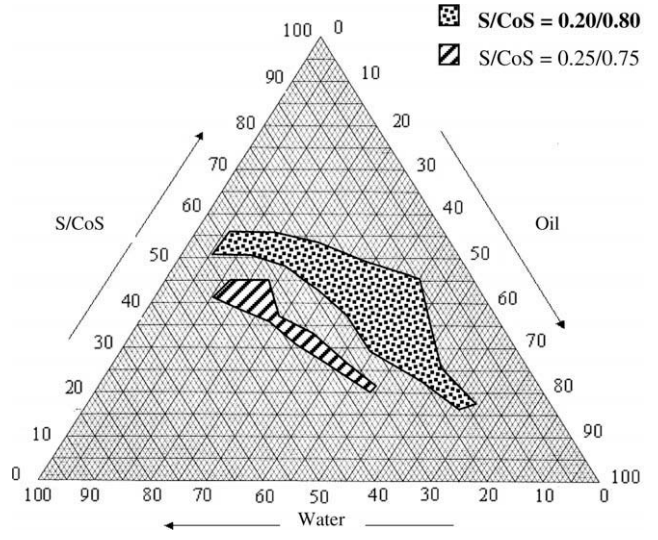

Fig. 3. Pseudo-ternary phase diagrams of decyl glucoside/sorbitan monolaurate/mineral oil/water microemulsion systems. Microemulsions zones at different S/CoS ratios (0.20/0.80, 0.25/0.75).

facilitate the spontaneous emulsification, as described by Chai et al. [32]. They explained the fact that less surfactant and co-surfactant are necessary to balance the dioctylcyclohexane microemulsion systems. This system is less interesting than the dioctylcyclohexane one, because the area of systems potentially useful for an application is reduced.

3.2. Incorporation of ascorbic acid

Ascorbic acid has been studied in both dioctylcyclohexane and mineral oil systems. For dioctylcyclohexane systems: two ratios S/CoS were chosen for the preparation of ascorbic acid microemulsions, with the lowest quantities of surfactant. Incorporation of ascorbic acid in formulated microemulsions leads to microemulsion structure destabilisation and do not allow to obtain transparent isotropic systems what about the protocol envisaged. Therefore, ascorbic acid microemulsions zones were searched by realisation of pseudo-ternary diagrams with solution of ascorbic acid instead of distilled water. Fig. 4(A) shows the displacement of the microemulsion zones to the lipophilic region of ternary diagram.

For mineral oil microemulsion systems, the (0.20/0.80) S/CoS ratio was used because it gives the broadest zone of microemulsions and therefore, must be the most robust to the incorporation of ascorbic acid. We can also observe here a displacement of the systems to the lipophilic region of the diagram (Fig. 4(B)).

3.3. Solubility and stability study

Some points of each zone were chosen to formulate microemulsions. Table 2 gives the composition of these systems. We chose systems that would point out the influence of different composition variables. First the oily phase nature: dioctylcyclohexane or mineral oil. Then, for each oily phase, the ratio surfactant/co-surfactant has been fixed: (0.30/0.70) for dioctylcyclohexane and (0.20/0.80) for mineral oil. The second composition variable studied is the oil/water (O/W) ratio, which vary between 0.5 and 3 for each oily phase. Finally, we could note also the influence of the surfactant quantities which vary from 20% to 57% according to the formulations. Solubility and stability of ascorbic acid in these systems were evaluated.

Concerning solubility study (Table 3), some differences can be observed according to the formulations. ME 1 can solubilise 7% of AA against 2% for ME 4. ME 2 and 3 show intermediate solubilisation values of 5% and 4%, respectively. The influence of the oily phase seems to be preponderant. Effectively, ME 1 and ME 3 that have similar formulations with comparable O/W ratios and surfactant quantities can be compared; the presence of dioctylcyclohexane in ME 1 formulation permits to solubilise much more AA. The same comparison can be achieved with ME 2 and ME 4 where ME 2 solubilises more than two-fold AA quantity. For both oily phases, we can note that the microemulsions containing the highest quantity of water and also the highest quantity of surfactant (ME 1 and ME 3) are the ones where AA is the most dissolved.

Regarding the AA stability in the microemulsion systems (Table 3), 93–97% of AA at $t = 0$ can be recovered. The slight loss of AA may be due to the process of incorporation of AA into the microemulsions, which involves the dissolution of the component in water during a few seconds before mixing with other ingredients. Therefore, after 1 month, more than 80% of AA can be recovered in the microemulsions. This can be correlated with Farahmand et al.'s study of AA stability in multiple emulsions [33]. He supposed that stability of AA in oily continuous phase systems should be improved due to prevention of ionization and oxidation. Effectively, the formation of microemulsion structure, which could entrap the molecule inside the hydrophilic heads of the surfactants, may play an important role in stabilising AA.

3.4. Interfacial activity of the S/CoS mixture

S/CoS mixture was solubilised in water at different concentrations and interfacial tensions against dioctylcyclohexane with and without ascorbic acid were studied at 25 °C. Gibbs equation leads to determine several parameters of molecules interfacial adsorption. $C\gamma_{min}$ which represents the minimal concentration leading to minimal surface tension, the effectiveness or surface pressure which is the difference between solvent surface tension and minimal surface tension of the surfactant system, the surface excess and area occupied by one molecule at the interface, were the parameters envisaged (Table 4). Here, an equivalent area was determined, considering the area of S and CoS in a combine film, which gives information about the interfacial film composition, taking into account the interaction between both the molecules. Minimal interfacial tension is very low (zero order, with the precision of K100 tensiometer), and effectiveness is similar for both the

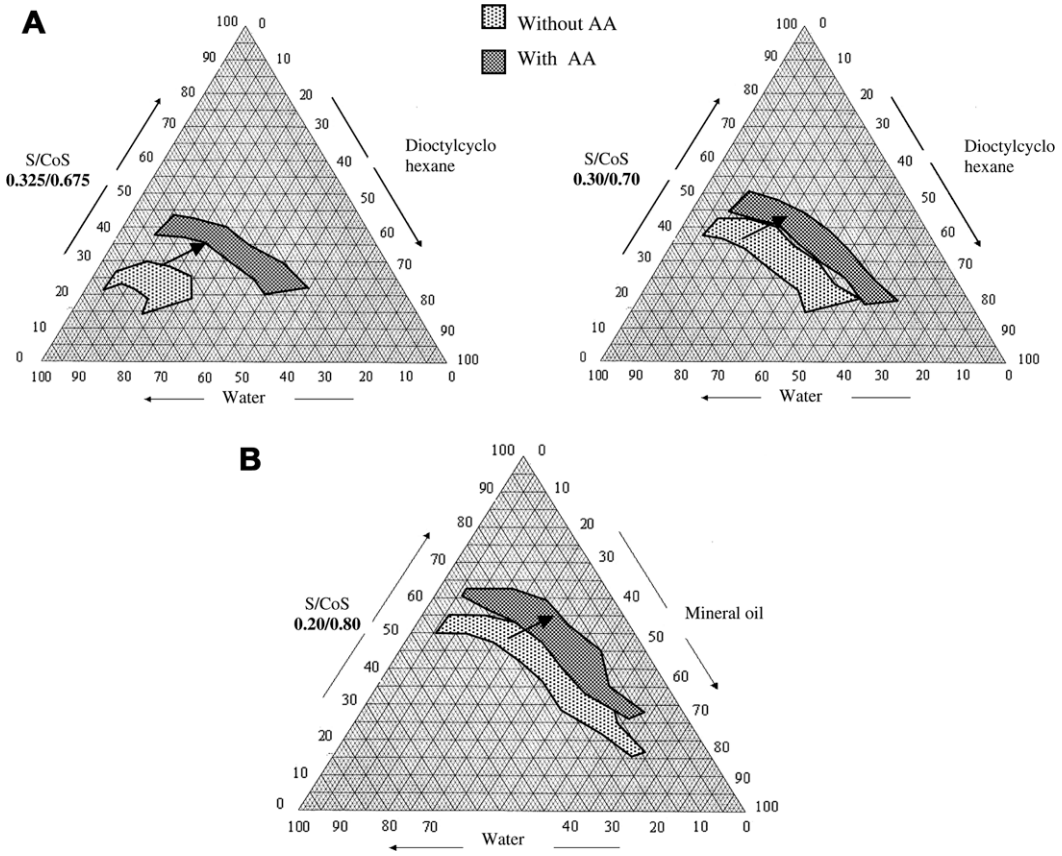

Fig. 4. Pseudo-ternary phase diagrams of microemulsion systems with and without ascorbic acid: (A) for decylglucoside/sorbitan monolaurate/dioctylcyclohexane/water at S/CoS ratios is (0.325/0.675) and (0.30/0.70), (B) for decylglucoside/sorbitan monolaurate/mineral oil/water at (0.20/0.80) S/CoS ratios. Arrows show the displacement of microemulsion zone with AA.

Table 2
Composition of the four microemulsions studied.

	ME 1	ME 2	ME 3	ME 4
Dioctylcyclohexane (%)	20	60	–	–
Mineral oil (%)	–	–	20	50
S/CoS ratio	0.30/0.70	0.30/0.70	0.20/0.80	0.20/0.80
S/CoS (%)	45	20	57	35
Water (%)	35	20	23	15

Table 3
Solubility and stability of ascorbic acid (AA) in microemulsion systems after 1 month.

	ME 1	ME 2	ME 3	ME 4
maximum of AA solubilised (%)	7	5	4	2
AA recovered (%)				
t = 0 days	93	97	94	96
t = 30 days	82	80	82	84

Table 4
Interfacial activity of surfactant mixture with and without ascorbic acid against dioctylcyclohexane at 25 °C.

	$C\gamma_{min}$ (mol L^{-1})	γ_{min} (mN/m)	Effectiveness (mN/m)	Surface excess ($\times 10^{-4}$)	Area at the interface ($Å^2$)
S/CoS/water/oil	2×10^{-4}	0	31	36.9	44.9
S/CoS/water/oil Ascorbic acid 4%	1×10^{-4}	0	31	53.5	31.03

systems. $C\gamma_{min}$ is two fold lowest in the presence of ascorbic acid and area at the interface is also decreased (surface excess increased). These results show that ascorbic acid has a weak influence on the interfacial tension and point out the location at the interface of AA molecules.

Small Angle Neutron Scattering studies were performed to obtain more information on the influence of ascorbic acid on microemulsion behaviour.

3.5. SANS characterisation of microemulsions

ME 1 and ME 2 were chosen to perform Small Angle Neutron Scattering studies in comparison with two microemulsion systems without ascorbic acid, $S(a)$ which contain important concentration of oily phase and $S(b)$ which is rather similar to ME 2. They were chosen in the aim to evaluate the influence of the presence of ascorbic acid and also the influence of the formulation parameters (surfactant concentration and oil/water content). The detailed sample compositions are summarised in Table 5.

For the SANS experiments, distilled water is replaced by D_2O during the sample preparation. The remaining H_2O comes from the stock solutions of decylglucoside and sorbitan monolaurate. The replacement of H_2O by D_2O increases the difference in scattering length densities (SLDs) between the oil phase and the aqueous phase by a factor of 10 and reduces the incoherent background coming from hydrogen molecules.

The scattered intensity from a suspension of randomly oriented particles can be written as

$$I(q) = \Phi V_p (\Delta\rho)^2 P(q,R) S(q) \quad (1)$$

Table 5
Sample composition.

	S(a)	S(b)	ME 1	ME 2
V Decylglucoside (cm^3)	2.4	4.8	10.8	4.8
V Sorbitan laurate (cm^3)	11.54	13.46	30.28	13.46
V Dioctylcyclohexane (cm^3)	96.38	54.21	24.09	72.29
V Ascorbic acid (cm^3)	0	0	4	4
V D_2O (cm^3)	2	29	17.5	10
V H_2O (cm^3)	3	6	13.5	6
Φ Aqueous phase[a]	0.04	0.33	0.35	0.18
Φ TA and co-TA	0.12	0.17	0.41	0.17
Φ Cetiol	0.84	0.50	0.24	0.65
Volume ratio surfactif/ cosurfactif	0.208	0.356	0.356	0.356
ρ_{eau} (aqueous phase) (cm^{-2})	2.21×10^{10}	5.18×10^{10}	3.14×10^{10}	3.32×10^{10}
$\Delta\rho$ ($\rho_{aqueous} - \rho_{cetiol}$) ($cm^{-2}$)	2.28×10^{10}	5.25×10^{10}	3.21×10^{10}	3.39×10^{10}

[a] The aqueous phase is made up of H_2O, D_2O and ascorbic acid.

where Φ is the volume fraction of scatterers, V_p is the volume of the particle, $\Delta\rho = \rho_p - \rho_s$ is the difference between the SLD of the particle ρ_p and the solvent ρ_s. $P(q,R)$ is the form factor of one particle and describes the shape and size of the individual particle. $S(q)$ is the structure factor and describe the particle organisation in volume; for dilute system without interaction, $S(q) = 1$ in the whole q-range.

For a homogeneous sphere of radius R, the form factor is given by

$$P(q,R) = \left[3\frac{\sin(qR) - (qR)\cos(qR)}{(qR)^3}\right]^2 \quad (2)$$

The theoretic intensity suffers from smearing by the polydispersity in size of the particles and also by the instrument resolution due to the final size of the direct beam and of the wavelength spread of 10%. Eq. (1) becomes

$$I_{model}(q) = \int_0^\infty R(q,\Delta q,q')\int_0^\infty G(r_{ext},\sigma,r')I(r',q')\,dr'\,dq' \quad (3)$$

The polydispersity in size is represented by a log-normal function

$$G_{LN}(R_0,\sigma,r) = \frac{1}{r\sigma\sqrt{2\pi}}\exp\left(-\frac{1}{2\sigma^2}\left(\ln\frac{R_0}{r}\right)^2\right) \quad (4)$$

where σ is the standard mean deviation, related to the fw_{hm} by $\Delta r_0 = \sigma r_0$. The mean radius of the particle obtained by this function is $\langle r\rangle = r_0\exp(\sigma^2/2)$.

The resolution function is described with a Gaussian function $R(q,\Delta q,q')$

$$R(q,\Delta q,q') = \frac{1}{\Delta q\sqrt{2\pi}}\exp\left(-\frac{(q'-q)^2}{2(\Delta q)^2}\right) \quad (5)$$

where Δq is the resolution term, the calculation details are available in [34].

Bicontinuous microemulsion structures can be modelled according to the Teubner–Strey equation [35]

$$I(q) = \frac{I_0}{a_2 + c_1q^2 + c_2q^4} \quad (6)$$

where a_2, c_1 and c_2 are variables related to the Landau free-energy expansion. The previous equation can be re-written as follows:

$$I(q) = \frac{I_0}{\left(1 - \frac{I_0}{I_{max}}\right)\left(\frac{q^2}{q_{max}^2} - 1\right)^2 + \frac{I_0}{I_{max}}} \quad (7)$$

where I_0 is the absolute intensity when q tends to zero, q_{max} and I_{max} are the value of the wave vector and the intensity at the peak position. From the Eq. (6), the two physical parameters d_m and ξ representing the domain size (periodicity) and the correlation length, respectively, can be calculated

$$\xi = \left[\frac{1}{2}\left(\frac{a_2}{c_2}\right)^{1/2} + \frac{1}{4}\frac{c_1}{c_2}\right]^{-1/2} \quad \text{and}$$

$$d_m = \left[\frac{1}{2}\left(\frac{a_2}{c_2}\right)^{1/2} - \frac{1}{4}\frac{c_1}{c_2}\right]^{-1/2} \quad (8)$$

At high q, the intensity is sensitive to the interface. The specific surface Σ in m^2/m^3 developed by the surfactant layer at the interface between oil and water can be analysed using the Porod equation [36]

$$\Sigma = \frac{1}{2\pi(\Delta\rho^2)}\lim_{q\to\infty} I(q)q^4\Delta\rho = \rho_{water} - \rho_{oil} \quad (9)$$

The limit can be obtained from the experimental data or from the Teubner–Strey equation

$$\lim_{q\to\infty} I(q)q^4 = \frac{I_0}{c_2} \quad (10)$$

Finally, the area per head group σ is obtained by dividing the specific surface by the surfactant and co-surfactant concentration at the water/oil interface. $\sigma = \frac{\Sigma}{c_{TA}}$

The Teubner–Strey parameters are reported in Table 6.

The results obtained clearly show two different behaviours:

First, Fig. 5 shows scattering spectrum of the system without AA and containing the most important quantity of oil, noted $S(a)$. The curve fits very well with the model of polydisperse spheres. In that case, the emulsion can be considered as the dispersion of small water globules in a continuous oily phase. The mean radius of the particles is of 50 Å with a polydispersity in size σ equivalent to 29%. The preparations in that zone of the diagram can be considered as oily isotropic, with inverse micelles. There are no bicontinuous microemulsion systems and they will not be taken into account for the following experiments.

The other three samples can be analysed using the Teubner–Strey approach and show typical spectra of bicontinuous microemulsion (Fig. 6). This analysis yields values for the domain size (d_m), the correlation length (ξ) and the amphiphilicity factor (fa) (Table 6).

The scattered intensity at low q depends on the size of the domains, on the sample composition and difference in length-scattering densities between the aqueous and oil phases. We observe an increase of $I(0)$ by a factor of 100 between $S(b)$ and ME 1. The correlation peak shifts to a higher q value for ME 1, an indication of the presence of smallest domains. It is interesting to note that the scattering intensities of $S(b)$ and ME 2 are superimposed at large q, in a region that is sensitive to the interface.

$S(b)$ and ME 2 microemulsions have nearly the same composition. They contain the same volume of S/CoS mixture (18.26 cm^3). The oil fraction is quite similar and they differ by

Table 6
Teubner–Strey parameters.

	S(b)	ME 1	ME 2
I_0 (cm^{-1})	588.4	42.3	184.1
a_2	1.0	1.0	1.0
c_1 (Å^2)	−2660.3	−581.2	−1700.6
c_2 (Å^4)	3.42×10^6	1.32×10^5	1.81×10^6
ξ (Å)	114.7	60.3	85.5
d_m (Å)	291.6	126.2	255.1
fa	−0.72	−0.80	−0.63
I_0/c_2 (cm^{-5}) (Porod limit)	1.72×10^{28}	3.20×10^{28}	1.02×10^{28}
Σ (cm^2/cm^3)	9.92×10^5	4.94×10^6	1.41×10^6
Average surface per head group (Å^2)	40.5	58.6	49.7

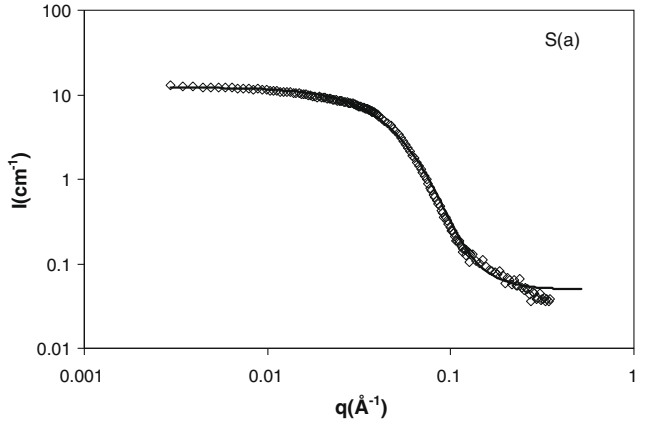

Fig. 5. SANS scattered intensity for the *S*(*a*) system. The ◇ symbols are experimental points. The full line is calculated with the model of polydisperse spheres.

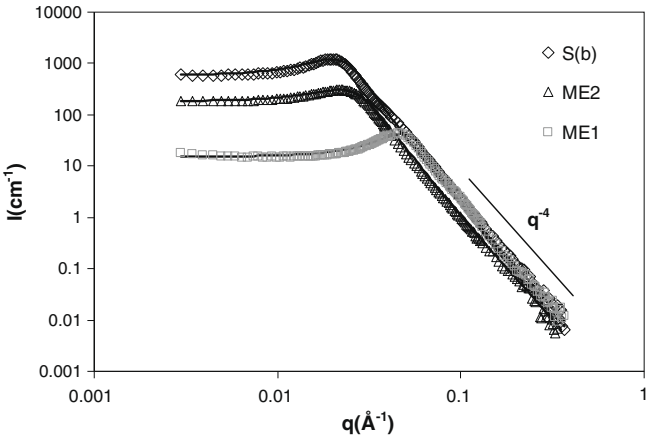

Fig. 6. SANS scattered intensity for *S*(*b*), ME 1 and ME 2 samples. The full lines are calculated with the Teubner–Strey model.

the presence of ascorbic acid (exactly the same content could not be assessed because of the displacement of the microemulsion zone with AA). Analysing the Treubner-Strey parameters shows that both the domain size and the correlation length decrease with the presence of ascorbic acid in the system. The amphiphilic factor for ME 2 becomes less negative, meaning that the sample is becoming less structured [37]. Specific surfaces (Σ) obtained are in the order of 10^6 cm^2/cm^3, which is in conformity with the literature.

ME 1 structure seems to be different from the other ones. The domain size is two fold smaller than that of ME 2 (126 Å versus 255 Å). This can be related to the highest amount of total surfactant in the microemulsion. The amphiphilic factor also decreases, showing the progression to more strongly structured mixture. The results of specific surface and surface per head group obtained with the model are in accordance with those obtained by interfacial measurements (31 Å^2). With high concentration of surfactant, the surfactant layer becomes more rigid and slightly tighter.

The influence of ascorbic acid localisation at the interface, leading to some modifications in the microemulsion structure for low surfactant content, should be less effective with high surfactant content. These SANS results confirm the pertinence of neutron for characterisation of these microemulsions, but a better understanding of the influence of AA will require more systematic studies along the several dilution lines of the ternary diagram.

3.6. In vitro transdermal penetration of ascorbic acid

ME 1, ME 2 and ME 3 were chosen to evaluate *in vitro* transdermal penetration of ascorbic acid. These formulations are the most interesting for the quantity of AA solubilised and permit the analysis of the influence of various parameters on the percutaneous penetration study: oily phase nature, particle size and composition of the microemulsions. In the aim of homogenising the experimental conditions, the three microemulsions were formulated with 4 wt.% ascorbic acid.

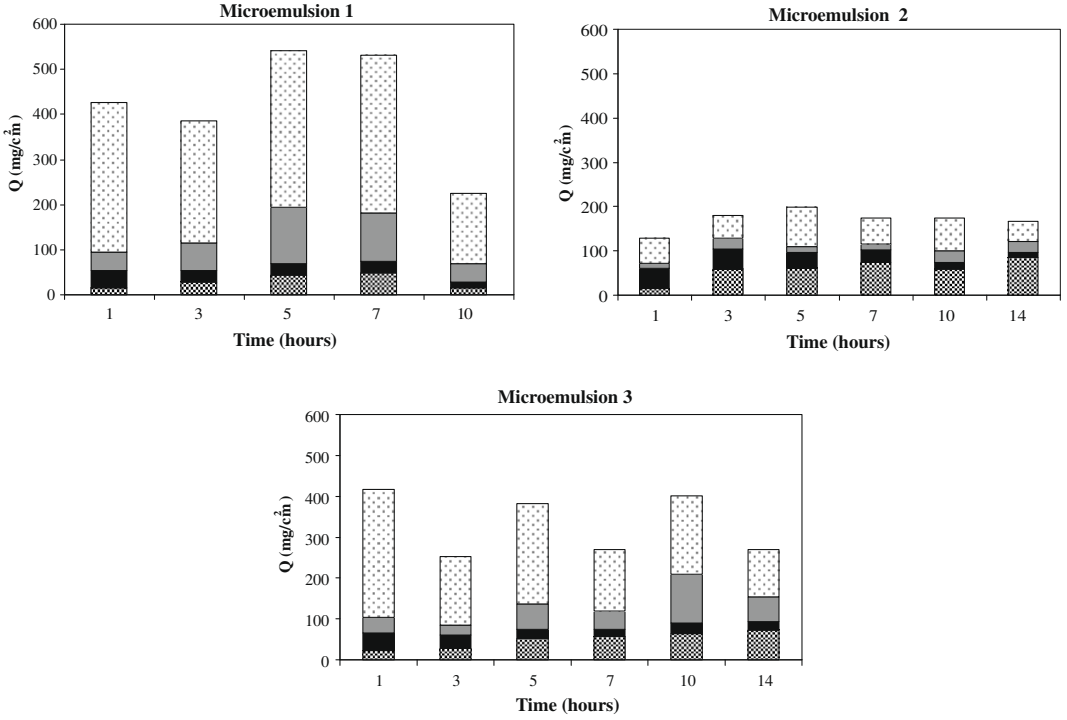

Fig. 7. *In vitro* transdermal penetration of ascorbic acid from three microemulsions; stratum corneum, epidermis, dermis and receptor medium.

Fig. 7 shows the cumulative amount of AA located in the different parts of the skin for the three microemulsions. Schematically, we can see that ME 1 permit the accumulation of the major quantity of AA in the stratum corneum and epidermis, with a total quantity in the different compartments representing 21.65% of the AA applied. ME 3 has a similar behaviour with a preponderant amount of AA in the stratum corneum and the epidermis but totalise 15.4% of the AA deposited on the skin. Concerning ME 2, we can notice that the major part of AA is located in the dermis and the receptor medium, which show a better capacity of penetration, but the total amount in the different compartments represents only 7.8% of the AA applied on the skin.

For more detailed analysis, Fig. 8 shows the amount of drug located in the receptor medium (A), epidermis (B) and dermis (C) for the three microemulsions. The concentration of AA was plotted as a function of time. Considering the concentration-time profiles of AA in the receptor medium (Fig. 8(A)), the permeation rates through the skin were determined. The three microemulsions provided a two-stage zero order release profile for AA during the 4- or 5-h study period with a good linearity. The initial AA flux for ME 1, ME 2 and ME 3 was 6.91 μg/cm^2/h, 21.34 μg/cm^2/h and 9.10 μg/cm^2/h, respectively. Concerning ME 1, it seems that after a maximum penetration at 7 h, AA is degraded and remains in a few quantity in the different parts of the skin. For ME 2 and ME 3, slowest flux of 2.30 μg/cm^2/h and 2.20 μg/cm^2/h, respectively, has been found. These results are quite interesting, if compared with what was found in the literature. Much slowest rates of 0.077–0.108 μg/cm^2/h have been described by Farahmand et al. [33] with multiple emulsions, and Lee and Tojo [26] presented flux of AA in glycerine/water (50/50) solvent, through whole skin near to 3.43 μg/cm^2/h. In order to show the effect of particle size and oil phase amount on the AA passage through the skin, we can compare ME 1 to ME 2. For the same oil phase (dioctylcyclohexane), ME 2 differs from ME 1 by its particle size (256 nm versus 126 nm) and its oil amount (60% versus 20%). ME 2 is the microemulsion that presents the best penetration rate. As ME 2 presents the largest particle size, the influence of the particle size of the vehicle on drug bioavailability, as described by several authors [38–40], does not seems to be the preponderant parameter. Generally, the authors suggested that nanometer particles penetrated to a significantly greater extent and provided a larger surface area for absorption than the micrometer particles, thereby providing a greater efficacy as a delivery system. Here, both the microemulsions are nano-scaled, so the difference in penetration behaviour can be attributed to the oil content. The important oil content of ME 2 can perturb the skin barrier and favour the AA penetration.

By comparing ME 1 and ME 3 (the same oil concentration, but different oil phase nature, surfactant and water concentrations), the amount of AA in the receptor medium shows that both the microemulsions have a similar initial flux, but ME 3 seems to protect AA from degradation. As a matter of fact, AA is still present in the diverse compartments of skin with ME 3, while it practically disappears with ME 1. Effectively, ME 3 contains less water phase and more surfactant, so this will lead to smallest amount of aqueous phase in the globules, which will permit a better protection of AA molecules by the hydrophilic heads of surfactant.

In another way, the amount of drug located in the epidermis and dermis can be compared. Regarding the epidermis location of AA (Fig. 8(B)), it is quite significant in ME 1 and ME 3. The major quantity of AA in the epidermis can be found with ME 1 after 5 h (>120 μg/cm^2) and shift to 10 h for ME 3 (100 μg/cm^2). ME 2 that has a rapid penetration did not have an important location in the epidermis (30 μg/cm^2). The amount of ascorbic acid in the dermis was rather weak for the three systems, but logically regarding their penetration behaviour, the AA amount was preponderant for ME 2 at the beginning and later for ME 3 (Fig. 8(C)).

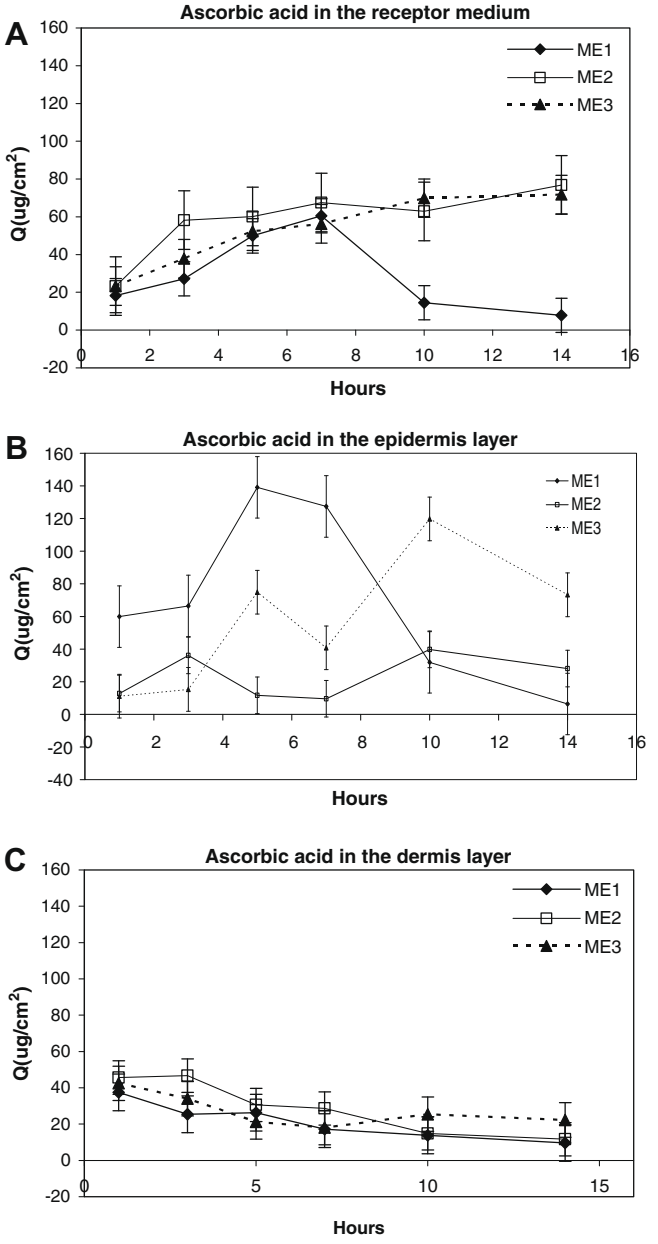

Fig. 8. The amount of drug located in receptor medium (A), epidermis (B) and dermis (C) for the three microemulsions.

These results confirmed that these microemulsion systems present a real interest for formulation and protection of ascorbic acid. Regarding their transcutaneous penetration behaviour, the different microemulsions studied could be useful for different topical applications. A major location in the epidermis where the site of action by the decomposition of melanin could be a suitable carrier system for the application of ascorbic acid as the whitening agent and a good passage in the dermis could be interesting for the protection of reactive oxygen matrix damage. In conclusion, we can say that the association of HLD concept and dilution pseudo-ternary diagram is a successful tool to characterise surfactant–oil–water systems and to perform stable cosmetic microemulsions. In this work, the study of these diagrams point out the interest of dioctylcyclohexane for obtaining a largest zone of microemulsions, which type and composition can be adapted to several cosmetic applications.

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