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In vitro release of caffeine from concentrated W/O emulsions: effect of formulation parameters

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Abstract

Concentrated water in oil emulsions have been obtained with four different emulsifiers to study the effect of formulation parameters on the in vitro release of caffeine. The in vitro release was studied on polysulfone membranes. Among the four emulsifiers, only one gave a statistically higher release of caffeine after 15 h (at a fixed percentage of dispersed phase). The concentration of the emulsifier does not have a significant effect on the release of caffeine. In contrast, diffusion of caffeine from concentrated W/O emulsions has been found to be highly dependent on the internal phase volume. The flux of caffeine increases with the percentage internal water phase. The droplet diameter decreases and the apparent viscosity increases with the percentage of the dispersed phase. And, the shape of the droplets goes from spherical to polyhedral as the percentage dispersed phase is increased. However, the flux could be correlated neither with the apparent viscosity nor with the droplet diameter at a fixed percentage of the dispersed phase. Results suggest that the shape factor may have an influence on the release of caffeine from concentrated emulsions. All the release profiles followed a zero-order kinetic.

Keywords: W/O Concentrated emulsions; Membrane diffusion; Controlled release; Formulation effects; Caffeine

1. Introduction

Concentrated emulsions (CE) are a peculiar type of emulsion where the volume fraction (percentage of the dispersed phase) is more than 74%. The droplets of these emulsions cannot be spherical and assume some transitional form between spheres and polyhedra (Lissant, 1966). This type of emulsion, because of its high volume fraction, has the aspect and consistency of gels. Water in oil concentrated emulsions can be formulated with a very high amount of water (up to $\phi = 0.99$), and very low surfactant concentration (as

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low as 0.5% w/w) (Kunieda et al., 1987; Solans et al., 1993). These aspects make them attractive for economical, environmental and toxicological reasons. Concentrated emulsions can be used in many diverse applications like formulation of pharmaceutical, culinary and cosmetic products (Lissant, 1975; Aronson and Petko, 1986).

The structure of some W/O concentrated emulsions can be complex with an internal/water phase surrounded by a continuous phase composed of a W/O microemulsion (Kunieda et al., 1987; Solans et al., 1988a,b; Ravey and Stébé, 1990; Pons et al., 1993; Solans et al., 1993). They are obtained with very little mechanical energy, or simply by heating the system which contains all components in one step (Pons et al., 1994). The existence of transparent W/O gels has also been reported for a few hydrophobic hydrogenated ethoxylated alcohols with the highest water concentrations (98–99%) and with mixtures of fluorinated nonionic surfactants and fluorocarbons (Kunieda et al., 1987; Ravey and Stébé, 1990; Pons et al., 1993; Rocca et al., 1998).

Numerous rheological studies have been conducted on concentrated emulsions (Princen and Kiss, 1986; Princen, 1989; Pons et al., 1995; Jager-Lezer et al., 1998). The structure of concentrated emulsions results in solid-like responses such as elastic behaviour at low strains and yield stresses.

The stability of these systems can be influenced by many factors and it has been observed that electrolytes dissolved in the aqueous phase of concentrated W/O emulsion dramatically increased emulsion stability (Kunieda et al., 1987; Solans et al., 1988a; Pons et al., 1992; Aronson and Petko, 1993; Aronson et al., 1994; Caldero et al., 1997). The electrolytes appeared to enhance the stability of these water-in-oil emulsions by increasing the resistance of the water droplets to coalescence (Aronson and Petko, 1993) and by the prevention of ice crystallisation at low temperature (Aronson et al., 1994).

The factors affecting the transport properties of these systems have been recently studied with various membranes (Solans et al., 1993; Caldero et al., 1997; Clément et al., 2000) and without membrane (Pons et al., 1996; Caldero et al., 1998; Rocca et al., 1998, 1999). Results from previous studies on hydrogenated and fluorinated concentrated emulsions indicate that the release rate is strongly system dependent. The release of a hydrophilic probe was found to be higher in hydrogenated systems than in fluorinated ones (Caldero et al., 1998) whereas, when the probe molecule is lipophilic, the contrary have been observed (Rocca et al., 1998). These latest studies also showed the possibility of modulating the release of a probe by mixing hydrogenated and fluorinated surfactants in various proportions (Caldero et al., 1998; Rocca et al., 1998). The in-vitro tests have limited applicability for estimating the complex process of percutaneous absorption but are useful as screening tools for drug release (Shah et al., 1989; Smith and Haigh, 1989; Dias et al., 1999). Recently, a study of the release of caffeine from concentrated emulsion on different synthetic membranes showed the importance of the choice of the membrane (Clément et al., 2000).

From a cosmetic point of view, these systems have great potential since they allow for very high concentrations of internal phase components while still retaining the feel of the external phase. In addition, they were shown to act as a controlled release vehicle for the release of caffeine (Clément et al., 1998).

The objective of this paper is to study the effect of formulation parameters of cosmetic concentrated W/O emulsions on the release profile of caffeine. The effect of water content, emulsifier type and its concentration will be discussed. Various physico-chemical factors which may affect drug release from topical cream were evaluated including droplet size, viscosity, stability and structure. Caffeine was selected as a model molecule since it is frequently used in cosmetic products (Dias et al., 1999).

2. Materials and methods

2.1. Materials

Raw materials of cosmetic grades and deionised water were used for the fabrication of the concentrated emulsions. The commercial emul-

sifiers employed were sorbitan sesquioleate, (Arlacel 83, ICI), cetyl dimethicone copolyol (Abil EM90, Goldsmith), Methyl glucose dioleate (Isolan DO, Goldsmith) and two emulsifiers coupled: a mixture of sorbitan oleate, beeswax, hydrogenated castor oil, stearic acid (Montane 481, Seppic) and PEG-7 hydrogenated castor oil (Simulsol 989, Seppic). Heptanesulfonic acid (Na salt) and the phosphate buffer pH 7.4 were purchased from Sigma (St-Quentin Fallavier, France). The HPLC-solvents methanol and glacial acetic acid were obtained from Carlo Erba (Val de Reuil, France). Ethomeen S 12 (*N*,*N*-bishydroxyethyl ollylamine) used as a wetting agent for the polysulfone membrane was obtained from Akzo Nobel(Stenungsund, Sweden).

2.2. Preparation of emulsions

Concentrated W/O emulsions containing 5% caffeine were prepared by slow addition of the internal phase to the external phase under continuous mechanical stirring. The aqueous phase contained caffeine, a moisturiser, electrolytes and preservatives, all in concentrations allowed by the legislation concerning cosmetic. The oil phase is composed of isohexadecane and the emulsifier. After the water addition was completed, the emulsion was mixed for another 30 min. The composition of the emulsions under study is presented in Table 1. The emulsions were stored in HDPE (high density polyethylene) jars and inox beakers for the Arlacel 83 products. The characteristics of these emulsions can be reproduced from one batch to another as verified by their droplet diameter and apparent viscosity.

2.3. Characteristics of emulsions

The stability of the concentrated emulsions was observed at four temperatures (-20, 4, 42 and 50°C). At definite time intervals (1, 8, 15 days, 1, 2, 3 and 6 months) the emulsions were allowed to come back to room temperature and the stability was assessed by visual observation of phase separation of the emulsion. At the same intervals, the apparent viscosity was taken at room temperature with a Brookfield viscometer set at a speed of 5 rpm for 60 s (Brookfield DV-II model, USA). Particle size distribution was determined by laser diffractometry using a Malvern Mastersizer μplus (Malvern Instruments, UK). The sample was diluted 1:4 in the oil of the continuous phase. Fresh dilutions were made for all measurements. This dilution did not affect the size of the droplets as confirmed by the electronic micrographs if it is done just prior to the measurement. A high resolution cryo scanning electron microscope (VG Polaron LT 7400 SEM cryo preparation unit coupled with a Philips XL 40 FEG SEM) was used to obtain images of the concentrated emulsions. The samples were frozen in subcooled nitrogen, fractured at 110 K, coated with 4 nm Pt and observed at 110 K.

2.4. Membrane diffusion experiments

Membrane diffusion was performed on hydrophilic polysulfone membrane (HT Tuffryn, pore size 45 μm, 165 μm thick, Gelman Science, Champs sur Marne, France) with wetting agent (15% Ethomeen in isopropyl myristate). An infinite dose (300 mg) was applied and the cells were

Table 1
Concentrated emulsions composition

Emulsifier	Type	Emulsifier (%)	% Dispersed phase (w/w)
Arlacel 83	Sorbitan	3	83, 87, 91
Montane 481/Simulsol 989	Mixed	2.2/0.8	83, 87, 91
Abil EM90	Silicone	3	83, 87, 90
Isolan DO	Glucoester	3	83, 87, 91
Isolan DO	Glucoester	1, 2, 4	91

occluded throughout the experiment. Test material was applied to the membrane with a glass rod, and the dose was determined by weighting the rod before and after application. Membrane diffusion was measured using static diffusion Franz cells (donor area 1.76 cm^2, receptor volume 6.5 ml). The receptor fluid was an isotonic phosphate buffer pH 7.4 stirred constantly (300 rpm) and maintained at 32°C. Membranes were allowed to equilibrate with the receptor phase for 2 h before charging each donor compartment with the formulations. Four application times were investigated (3, 6, 9 and 15 h). Samples 1.2 ml of receptor phase were withdrawn and each sample removed was replaced by an equal volume of fresh receptor phase using an automated system (Microette, Hanson Research Corporation, USA). Afterwards, the membrane surface was washed with 1 ml of 50:50 MeOH:H_2O and the excess liquid was absorbed on cotton swabs. Caffeine was obtained from the washing and membrane by dissolution in 50:50 MeOH:H_2O for 24 h. The diffusion experiment was performed six times for each vehicle except if noted differently.

2.5. HPLC analysis

The HPLC analysis method used is a modified version of a previously described method (Potard et al., 1999). A linear standard curve was constructed using caffeine concentrations ranging from 2.0 to 50 mg/l, and the unknown concentrations were determined by using the standard curve as reference. Analysis was performed on a HPLC system equipped with a pump (Beckman 110B), an UV detector (Jasco UV-975) set at 273 nm, an integrator (Merck D-2500), an injector (Merck AS-200A), and a RP-18 column and guard column (5 μm 250 × 4 mm Lichrosorb column, Lichrospher RP-18E guard column, Merck). Separation was carried out at room temperature using 34:66 methanol: 7.5 mM Heptanesulfonic acid aqueous solution containing 1% glacial acetic acid. The flux rate was 0.9 ml/min and the injection volume 20 μl.

2.6. Statistical analysis

Analysis of variance (ANOVA) or *t*-test (when only two groups had to be compared) were used to test significant difference between the means. The tests were carried out at a level $P < 0.05$.

3. Results

The authors selected four different emulsifiers, a sorbitan ester (Sorbitan), a silicone polymer (Silicone), a Glucoester (Glucoester) and two emulsifiers coupled (Mixed) for studying the influence of the formulation parameters of concentrated emulsions on the release of caffeine. This selection was made to cover the major groups of surfactants used in the cosmetic industry. Three emulsions of dispersed phase 83, 87 and 91% (w/w) were prepared with 3% of each surfactant while for Silicone the most concentrated emulsion produced could only be 90%. The range of % dispersed phase w/w (DP) was selected to be as large as possible for these concentrated emulsions. The lowest concentration of water phase selected was the one that allows the production of stable products. DP (91%) was the highest concentration that could be achieved with most emulsifiers and was therefore selected as the maximum DP. A study of the effect of the surfactant concentration (1–4%) was also conducted with one emulsifier system (Glucoester) at a fixed percentage of dispersed phase (91%). Table 1 shows the composition of the emulsions.

3.1. Characterisation of the concentrated emulsions

The emulsions prepared were characterised in terms of their droplet diameter, apparent viscosity, their stability at various temperatures (accelerated ageing) and their structure observed by electronic microscopy. The results are presented in Table 2 Tables 3 and 4 and Figs. 1 and 2. No rheograms will be presented since rheology of concentrated emulsions is not the subject of this study.

Table 2
Droplet diameters in μm (D50%, $n = 2$) after 1 and 6 months

Emulsifier		83%		87%		91%	
		1M	6M	1M	6M	1M	6M
Sorbitan	3%	1.70	1.67	1.38	1.33	1.13	1.11
Silicone	3%	2.73	2.58	2.04	1.90	1.74[a]	1.63[a]
Glucoester	3%	2.20	2.28	1.56	1.72	1.09	1.11
Glucoester	1%					2.78	13.6[b]
Glucoester	2%					1.45	1.43[b]
Glucoester	4%					0.82	0.97[b]

[a] 90% dispersed phase.
[b] 3M instead of 6M.

3.2. Influence of the emulsifier type

A difference in stability is observed with the different emulsifiers used. Table 4 presents the results of accelerated aging (50°C) and low temperature (-20°C) stability for the systems under study. The data obtained showed a clear superiority of the Silicone emulsifier. In addition, the apparent viscosity and the droplet diameter of these emulsions are the same 6 months after the fabrication (Tables 2 and 3).

The mixed emulsifier is also very efficient for the production of concentrated emulsions. The constant apparent viscosity after one and 6 months confirmed the good stability observed with the accelerated ageing experiments. The only problem with the emulsions produced with this system is their instability at very low temperature (-20°C). At low temperature the water internal phase volume of W/O CE expands and the oil film has to deform to compensate this increase (Aronson et al., 1994). The waxes present in the surfactant mixture renders the oil film very rigid and probably hindered a deformation. The consequence could be that the oil film breaks. No particle size distribution measurement was possible for this system because of the interfering presence of waxes. Observation under the optical microscope and the electronic micrographs tells us that the droplet diameter of these emulsions is similar to the ones obtained with the Sorbitan and Glucoester systems.

Sorbitan and Glucoester are emulsifiers that are less efficient than the two previous ones but still allow the production of stable concentrated emulsions, at least for the duration of the study. It was noted that the 91% emulsions produced with Glucoester all show a drastic reduction in their apparent viscosity in the 30 days following their preparation (results not shown), whatever the surfactant concentration used. The droplet diameter also changed during the first month but stayed stable afterwards except for the 1% Glucoester where the emulsion showed signs of coalescence after 3 months. The emulsions produced with Sorbitan show constant droplet diameter and apparent viscosity after six months. It can be noted that although Sorbitan and Glucoester are very different surfactants due to their structural characteristics, the most concentrated emulsions produced with them (91% DP) have similar apparent viscosity (135 Pa.s. for Sorbitan and 112 Pa.s. for

Table 3
Apparent viscosity in Pa.s. after 1 and 6 months

Emulsifier		83%		87%		91%	
		1M	6M	1M	6M	1M	6M
Sorbitan	3%	16	15	59	45	135	132
Mixed	3%	46	38	114	94	260	246
Silicone	3%	65	54	220	240	700[a]	614[a]
Glucoester	3%	10	8	25	23	112	87
Glucoester	1%					30	28[b]
Glucoester	2%					74	67[b]
Glucoester	4%					116	107[b]

[a] 90% dispersed phase.
[b] 3M instead of 6M.

Table 4
Stability results (in days, D, or months, M)

	−20°C			50°C		
	83%	87%	91%	83%	87%	91%
3% Sorbitan	<2 M	<2 M	<3 M	<2 M	<2 M	<1 M
3% Mixed	<8 D	<8 D	<8 D	>6 M	>6 M	>6 M
3% Silicone	<3 M[a]	<6 M	<6 M[a]	>6 M	>6 M	>6 M[a]
3% Glucoester	<1 M	<8 D	<8 D	<1 M	<1 M	<1 M
1% Glucoester			<8 D			<8 D
2% Glucoester			<8 D			<8 D
4% Glucoester			<8 D			<1 M

[a] 90% dispersed phase.

Glucoester) and droplet diameter (1.13 μm for Sorbitan and 1.09 μm for Glucoester).

The emulsifier type can have an influence on the droplet diameter and the apparent viscosity. However, the difference in the droplet diameter among the different surfactants is not as marked as the difference in the viscosity. In effect, the diameter for all the emulsions produced vary between approximately 1 and 3 μm (Table 2), whatever the surfactant and the percentage of the dispersed phase. In contrast, the apparent viscosity can vary as much as by a factor of 10 (Table 3). For example, a 87% dispersed phase emulsion Glucoester has an apparent viscosity of 23 Pa.s. and the same emulsion with Silicone has an apparent viscosity of 240 Pa.s.

3.3. Influence of the percentage dispersed phase

In addition to the differences in stability of the various emulsifiers, the phase volume ratio can also play a role in the variation of the apparent viscosity and the droplet diameter. For all the emulsions under study, an increase in the percentage of dispersed phase causes a reduction in the droplet diameter and an increase in the apparent viscosity (Tables 2 and 3).

Normal microscopic examination lacks sufficient resolution and depth of focus to reveal the details of the microstructure of the most concentrated emulsions. We therefore used electronic microscopy to observe the structure of these emulsions. The electronic micrographs of the 91% DP Glucoester and mixed emulsions are presented in Fig. 1. It can be noticed that they have a structure composed of polyhedral water domains surrounded by a thin layer of oil and surfactant. This was expected since the volume fraction in these cases is superior to 74%. The less concentrated (83%) emulsions have a structure composed of spherical droplets adjacent to each other (Fig. 2). As the percentage of the dispersed phase is increased, the droplet structure deforms from a spherical to a polyhedral geometry.

It is well known that formulation parameters can play a role in the release of an active from an emulsion (Kundu et al., 1993). This is why the authors studied the characteristics of the systems evaluated. This characterisation will allow us to make correlation between the formulation parameters of CE and the diffusion profiles of caffeine. Viscosity, stability, droplet diameter and shape can be important parameters controlling the release of caffeine from CE.

3.4. Diffusion studies

The caffeine solubility in the receptor fluid was previously verified (Clément et al., 2000) and was shown not to be a limiting factor for conducting experiments in sink conditions. Most of the diffusion studies were undertaken 24 h after the emulsion fabrication. Additional studies were made only after a granulometric measurement con-

firmed the stability of the sample. Emulsions studied were never older than two months.

The following results present the influence of the formulation parameters of concentrated emulsions on the in vitro release of caffeine. In general the recovery of caffeine at the end of the experiment was always higher than $92 \pm -3.2\%$.

3.5. Influence of the emulsifier system

Four different emulsifier systems were selected (Sorbitan, Silicone, Mixed and Glucoester). Table 5 presents the release characteristics of the concentrated emulsions obtained with 3% emulsifier. The steady state fluxes have been estimated by

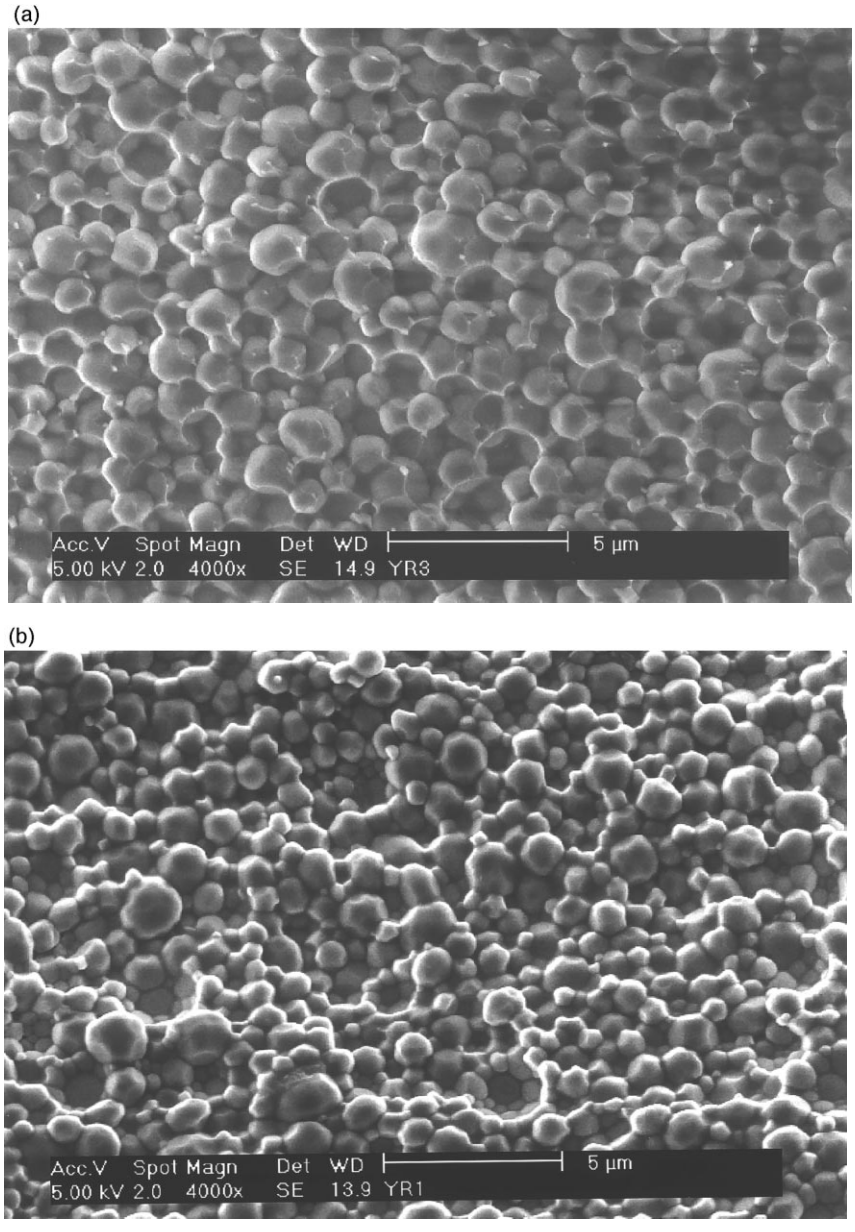

Fig. 1. Electronic micrographs of 91% (w/w) w/o concentrated emulsions obtained with 3% Glucoester emulsifier (a) and 3% Mixed emulsifiers (b).

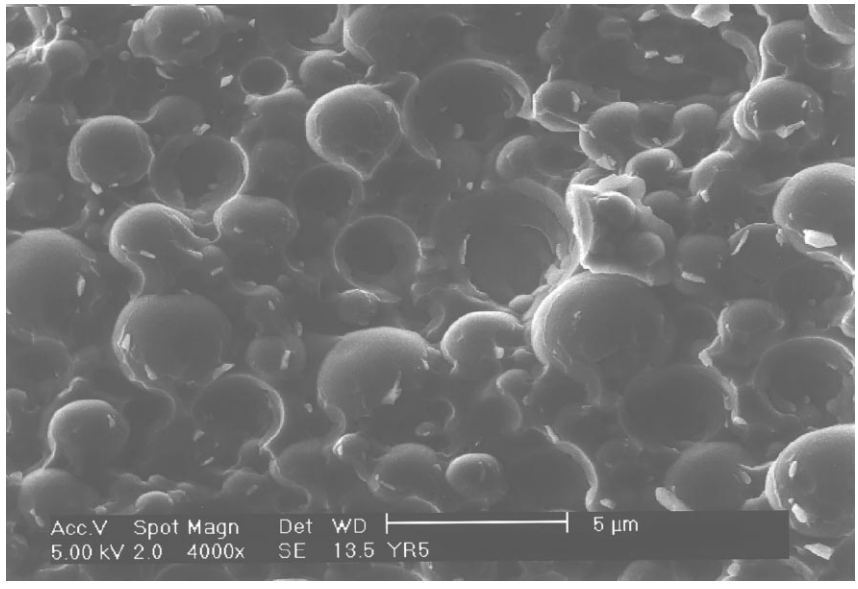

Fig. 2. Electronic micrograph of 83% (w/w) w/o concentrated emulsion obtained with 3% Silicone emulsifier.

Table 5
Release characteristics from concentrated emulsions obtained with 3% emulsifier ($n = 6$)[a]

Formulations	83% dispersed phase (w/w)		87% dispersed phase (w/w)		91% dispersed phase (w/w)	
	Flux µg/cm²/h	Q_{15} µg/cm² (SD)	Flux µg/cm²/h	Q_{15} µg/cm² (SD)	Flux µg/cm²/h	Q_{15} µg/cm² (SD)
Sorbitan	130.1 $R^2 = 0.999$	2123.99 (286.6)	181.8 $R^2 = 1.000$	2848.8 (305.8)	232.3 $R^2 = 0.999$	3669.7 (253.7)
Silicone	122.1 $R^2 = 0.995$	2002.9 (174.4)	169.6 $R^2 = 0.95$	2735.1 (220.2)	174,7 $R^2 = 0.990$	2897.6 (276.7)
Mixed	161.2 $R^2 = 1.000$	2560.6 (267.5)	199.6 $R^2 = 1.000$	3103.9 (277.8)	254.3 $R^2 = 0.995$	3991.0 (480.1)
Glucoester[a]	133.6 $R^2 = 0.999$	2123 (132.3)	174.2 $R^2 = 1.000$	2655.9 (110.6)	223,1 $R^2 = 0.997$	3358.0 (225.8)

[a] $n = 4$.

linear regression of the release profiles. For example the flux for the 91% emulsions are 232.3, 254.3 and 223.1 µg/cm² per h for the Sorbitan, Mixed and Glucoester emulsifiers respectively.

The quantity of caffeine released after 15 h (Q_{15}) obtained is significantly different when the 4 emulsifiers are compared with an analysis of variance (ANOVA) test (comparison of only three emulsifiers for the 91% DP emulsions). However, this difference is not very pronounced except for the 83% emulsions ($P = 0.2$ for 83% emulsions, $P = 0.46$ for 87% and $P = 0.047$ for 91% emulsions). A look on the Q_{15} in Table 5 tell us that only the Mixed emulsifier is different among the group. The emulsions made with the Mixed emulsifier release caffeine faster than the other systems. This is observed at the 83, 87, and 91% dispersed phase. If one compares Sorbitan, Silicone and Glucoester, no significant difference is obtained anymore ($P = 0.57$ for 83% emulsions, $P = 0.46$ for the 87% emulsions and $P = 0.08$ for the 91% emulsions). Therefore, in general, the

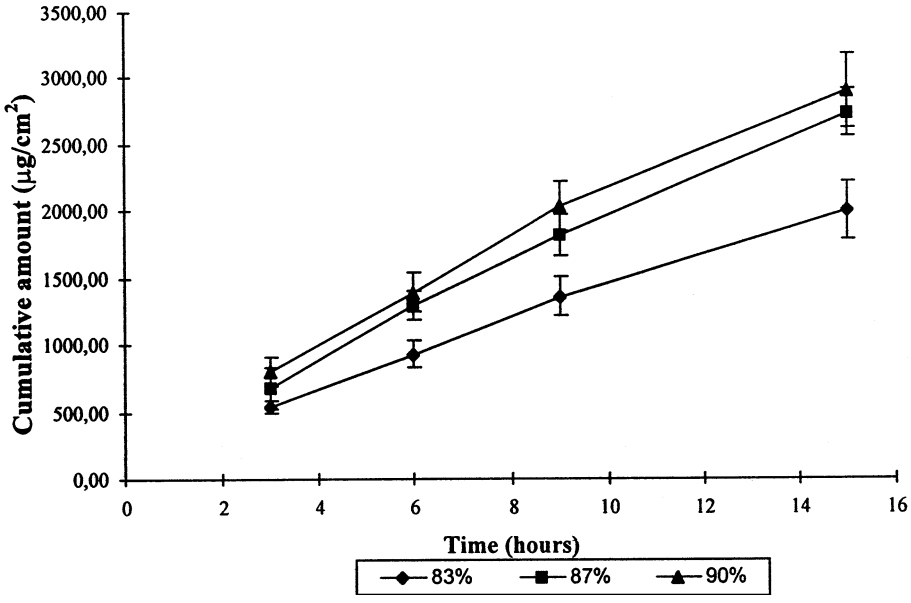

Fig. 3. Effect of the% dispersed phase (w/w) on the in vitro release profile of caffeine from concentrated emulsions obtained with 3% Silicone emulsifier. (mean ± S.D.).

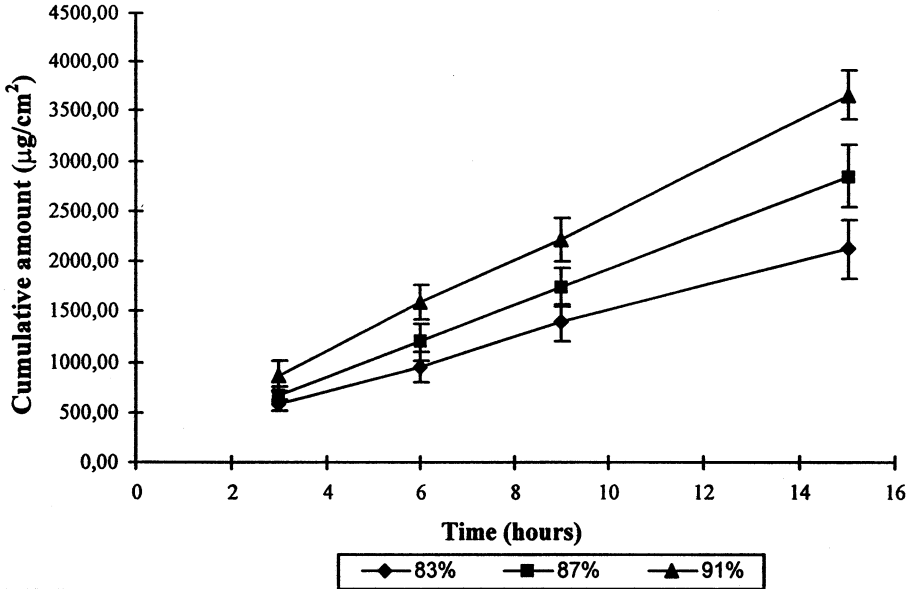

Fig. 4. Effect of the % dispersed phase (w/w) on the in vitro release profile of caffeine from concentrated emulsions obtained with 3% Sorbitan emulsifier. (mean ± S.D.).

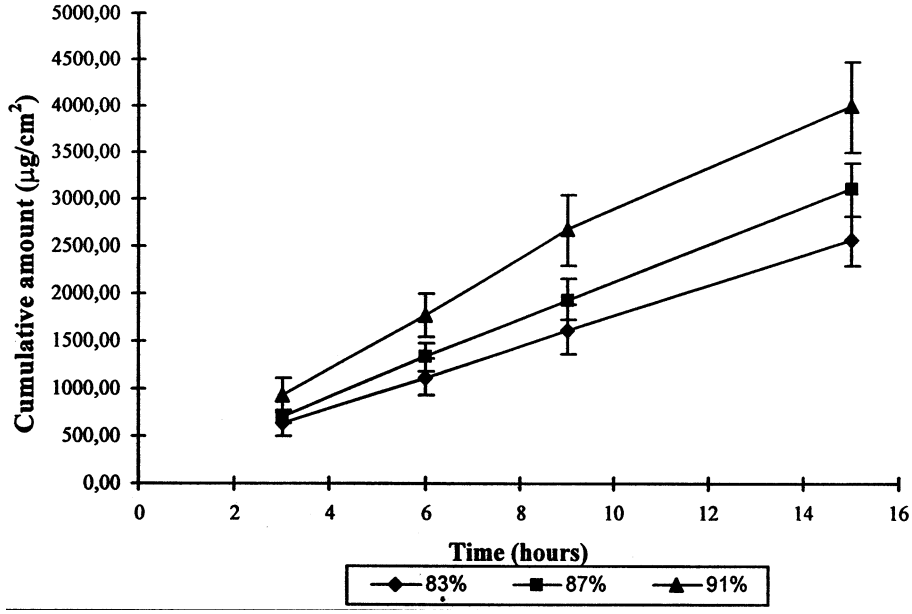

Fig. 5. Effect of the % dispersed phase (w/w) on the in vitro release profile of caffeine from concentrated emulsions obtained with 3% Coupled emulsifiers. (mean ± S.D.).

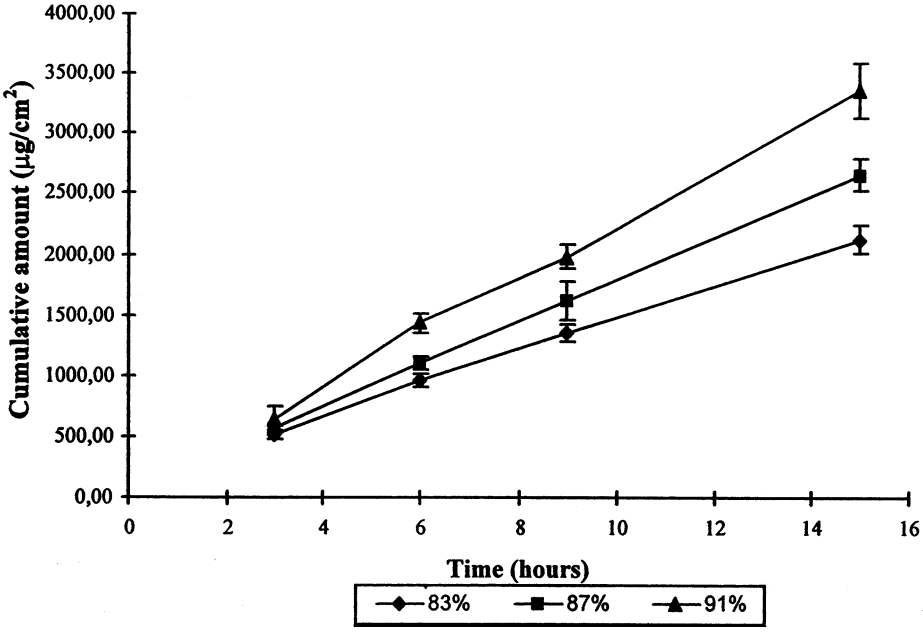

Fig. 6. Effect of the % dispersed phase (w/w) on the in vitro release profile of caffeine from concentrated emulsions obtained with 3% Glucoester emulsifier. (mean ± S.D.).

emulsifier type does not always have an effect on the release profile of caffeine from concentrated emulsions.

3.6. Role of the percentage dispersed phase of concentrated emulsions

The diffusion of caffeine from the different dispersed phase emulsions is shown in Figs. 3–6. (ANOVA) on the 15 h data points shows that there is a significant difference ($P < 0.001$) between 83, 87 and 91% dispersed phase emulsions for all the emulsifiers under study. The steady-state flux and quantity released after 15 h are summarised in Table 5. These graphs also show that all the release profiles from concentrated emulsions follow a zero order kinetic. The regression made on the liberation profile always shows a regression coefficient around 0.99. The flux increases from about 140 to 230 μg/cm^2 per h when the percentage dispersed phase of the emulsion increases from 83 to 91%.

3.7. Influence of the percentage of emulsifier

Only one emulsifier was selected for this part of the study since the emulsifier type does not have a major role in the control of the liberation of caffeine. The Glucoester emulsifier was selected and emulsions of 91% (w/w) dispersed phase with emulsifier concentration of 1, 2 and 4% were prepared and compared with the results obtained with the 3% Glucoester. The characterisation of these emulsions (Tables 2 and 3) showed a variation in the apparent viscosity (30–116 Pa.s.) and in the droplet diameter (0.82–2.8 μm). Table 6 shows the release characteristics obtained. No statistical difference has been observed on the 15 h data points ($P = 0.317$) even if the apparent viscosity and droplet diameter of these products are different. A tendency of faster release for higher emulsifier concentration is suggested by the data.

4. Discussion

The stability of concentrated emulsions is influenced by factors like water content, emulsifier type, the oil, the presence of additives, the temperature and the method of preparation (Kunieda et al., 1989; Chen and Ruckenstein, 1991; Pons et al., 1992). In the present study, differences in the stability, as well as in viscosity, droplet diameter and shape were observed for the emulsions produced with the four emulsifier types. The Silicone emulsifier gave the more stable products. This emulsifier is a silicone polymer, which produces an emulsion with a very strong interfacial film due to steric crowding. The Mixed system also allows the production of very stable products, probably helped by the presence of stearic acid and beeswax in the emulsifier commercial mixture. The two other emulsifiers used (Sorbitan and Glucoester) are small molecules, which allow the production of elastic interfacial films. The cosmetic qualities of these products are superior to the two previous ones but due to a lack of rigidity of their interfaces, these systems are less stable. Emulsion stability or interfacial film properties could play an important role in the release process (Solans et al., 1993; Caldero et al., 1997). They could give explanations for the differences observed in the release

Table 6
Influence of the Glucoester concentration (91% DP emulsions) on the release characteristics ($n = 6$)

Emulsifier concentration (%)	Flux μg/cm^2 per h	Release Rate μg/cm^2 per h$^{0.5}$	Q_{15} μg/cm^2 (S.D.)
1	202.6	1145.4	3132.0 (565)
2	223.8	1273.4	3425.7 (410)
3[a]	223.1	1258.8	3358.0 (226)
4	231.6	1319.3	3568.4 (216)

[a] $n = 4$.

profiles of caffeine from the concentrated emulsions under study.

Among the four emulsifiers under study, only the Mixed emulsifier gave a statistically higher amount of caffeine released after 15 h. This faster release could be attributed to the presence of waxes, which give a more rigid oil film. This rigidity could be responsible for an earlier breakage of the film upon application thereby causing a more rapid release of caffeine. However, this hypothesis needs to be verified. The three other emulsifiers gave the same release rate for a fixed percentage of dispersed phase.

These results are surprising since the concentrated emulsions made with these four emulsifier systems are very different when one looks at their apparent viscosity, and to a lesser extent, their droplet diameter. A difference in the flux was expected, at least between the emulsions, which have very different apparent viscosities. However, it was demonstrated with the 91% DP emulsions Glucoester with 1–4% of emulsifier, that differences in viscosity and droplet diameter do not change the flux of caffeine from concentrated emulsions. In the case of classic oil in water emulsions, some authors observed that the droplet diameter and the viscosity of an emulsion had no direct influence on the release kinetic of an active (Kundu et al., 1993).

Differences in the release profile from these CE were expected since the surfactants used do not have the same structure and it has already been demonstrated that this can have an effect on the release rate of actives (Caldero et al., 1997, 1998; Rocca et al., 1999). They could act differently in terms of contact with the membrane (Ferreira et al., 1994) or at the level of the partition coefficient of caffeine. The authors showed in a previous study that the partition coefficient of caffeine is similar in concentrated emulsions obtained with Sorbitan and Silicone (Clément et al., 2000). The partition coefficient of caffeine from the Mixed emulsifiers emulsions could not be measured. It was not possible to separate these emulsions after their formation even with a strong centrifugal force. Caldero et al. (1997) also observed that the partition coefficient of mandelic acid in two concentrated W/O emulsions was not influenced by the surfactant type (different hydrocarbon chain length). However, diffusion of the active was faster with the shorter surfactant emulsion.

The amount of surfactant in the CE does not seem to play a role in the release of caffeine. Recently, some authors made this observation with the release of coumarin from a CE obtained with a fluorinated surfactant (Rocca et al., 1999). The range of surfactant's concentration selected in our study was not very large but the emulsions obtained had different droplet size and apparent viscosities. It is probable that there was already an excess of surfactant even at the lowest concentration studied. In the absence of interaction between the surfactant and caffeine, additional emulsifier probably has no effect on the partition coefficient of caffeine.

The results obtained in this study showed a marked influence of the percentage of the dispersed phase on the release profile of caffeine. The flux of caffeine from the concentrated emulsions increases with the percentage of the internal water phase. The volume of the dispersed phase is known to be a very important factor controlling the flux of actives from concentrated emulsions (Solans et al., 1993; Pons et al., 1996; Caldero et al., 1997; Rocca et al., 1999). Caldero et al. (1997) argued that higher volume fraction decreases the stability of concentrated emulsion and increases the release rates. They also found that emulsifier type and electrolytes have an influence on the release rate. The emulsions studied in our experiment were all stable at the time of the diffusion studies. Therefore, a difference in stability could not explain the increase in the release rate observed when the percentage of the internal phase is increased. In addition the influence of the viscosity and the droplet diameter on the release rate has been found to be negligible for a fixed % DP. However, other parameters vary between these emulsions like the amount of water (which can affect the amount of solubilised caffeine) and the shape of the droplets. Some authors suggest that as the internal phase volume of a W/O emulsion is increased, the surface area of the internal phase becomes more important and the volume of the

continuous phase is considerably reduced (Kundu et al., 1993; Solans et al., 1993; Rocca et al., 1999). This decreases the diffusion pathway and the consequence is an increase of the release rates.

The structure of the droplet probably plays a role in the diffusion of caffeine inside a concentrated emulsion. For a fixed percentage of dispersed phase the structure is similar for all the emulsions studied and the data obtained showed only small differences in the flux. However, as the percentage of the internal phase increase, structure and the flux of caffeine change. The electronic micrographs (Figs. 1 and 2) showed the polyhedral droplets of the most concentrated emulsions (91% DP), which can be compared with the spherical droplets of a 83% DP emulsion. The 91% DP emulsions have a thin interfacial film due to the shape of the droplets (higher surface area), which, therefore, could be a smaller barrier for the passage of caffeine in comparison with the 83% DP emulsions. The importance of shape factor in drug delivery has already been observed with niosomes (Arunothayanun et al., 1999). Polyhedral niosomes slow the release of a peptide when compared with a solution but accelerate it in comparison to spherical niosomes.

5. Conclusion

Concentrated emulsions can be an interesting vehicle for the controlled delivery of actives. The flux of caffeine from these emulsions is mostly influenced by the percentage of the dispersed phase but not directly by viscosity, droplet diameter, surfactant type or its concentration. The structure of the droplets, which change from spherical to polyhedral as the % DP is increased, seemed to play an important role in the release process, the polyhedral shape increasing the flux of caffeine. The linear profile of liberation obtained with all the emulsions under study is an interesting property. Further studies have to be done to verify that these properties still exist when the emulsion is applied on skin (instead of synthetic membranes), as well as with other actives.

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