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Effect of camphor/cyclodextrin complexation on the stability of O/W/O multiple emulsions

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Abstract

Camphor (CA) encapsulation in oil/water/oil multiple emulsions prepared with cyclodextrin disturbs the emulsifier potential of α- and β-natural cyclodextrins (CD). It was suggested that the size and geometrical fit between the CD cavity and CA could induce CD/CA complex formation in place of emulsifier formation leading to perturbation of emulsion stability. The complexation between CA and α-, β- or γ-CD in solution in the presence of oil phase are confirmed by phase-solubility diagrams, circular dichroism and ^{1}H NMR. Furthermore, in order to mimic the emulsion system, CD/CA/soybean oil ternary dispersions were prepared to observe the complexation behavior of α-, β- or γ-CD/CA by circular dichroism. X-ray diffraction on emulsion samples prepared with α- and β-CD confirms that the precipitates observed in emulsions are probably composed of crystals of CD/CA complexes. A preliminary study of the interaction between drug and CD before the formulation seems indispensable to prevent the risk of incompatibility.

Keywords: Camphor; Complexation; Cyclodextrin; Multiple emulsion

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1. Introduction

Cyclodextrins (CD), which are cyclic oligosaccharides consisting of α-1,4-linked D-glucose residues, have a cavity in the centre of the molecule allowing formation of inclusion complexes with a range of organic compounds. The inclusion complex with a triglyceride which comprises the hydrophilic CD molecule with the hydrophobic fatty acid residues protruding from the cavity, was used as an emulsifier to prepare oil in water emulsions (Shimada et al., 1991) and oil in water in oil multiple emulsions (Yu et al., 1999). The structure of α- and β-CD emulsifiers was proposed by Shimada et al. (1992). It was supposed that the CD-triglyceride inclusion complex might act as a surfactant at the oil/water interface, with CD oriented to the aqueous phase and the fatty acid residues of the triglycerides, not included within the CD, oriented to the oil phase. It is of interest to know if hydrophobic molecules, which are able to be included in the CD cavity, can be introduced into the

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emulsion formulation without modification of emulsion stability. In this research, complex formation between camphor (CA), a model compound and small molecule and α-, β- and γ-CD was studied by different physicochemical methods. Finally, the stability of O/W/O emulsions prepared with CA added in the oil phase and α-, β- or γ-CD as emulsifier was evaluated.

2. Materials and methods

2.1. Materials

α-, γ-CD (gift from Wacker-Chemie, Germany) and β-CD (gift from Roquette Frères, France) and soybean oil purchased from Bertin Michel (France) were used for the preparation of emulsions. Natural CA (1R-(+)-CA, Sigma, France) was chosen to be a model of hydrophobic molecule introduced in emulsions. Candelilla wax (gift from Kahl, Germany) was used as a thickening agent. The super-refined soybean oil employed in all physicochemical tests, was donated by Croda (France). Milli-Q water was used for the preparation of emulsions as well as all of the solutions used in the physicochemical studies.

2.2. Phase-solubility diagrams

The phase-solubility technique permits the evaluation of the affinity between CD and CA in water. Solubility diagrams were obtained according to the Higuchi and Connors (1965) method, in water. An excess of CA was added to vials containing increasing concentrations of CD (0–150 mM for α- and γ-CD and 0–16 mM for β-CD). Vials were agitated at 200 rpm for 7 days at room temperature using a rotary agitator (Bioblock Scientific, France). After agitation, the contents of each vial were filtered through an ester cellulose filter (Millex GS pore size 0.22 μm). The concentration of CA in each filtration medium was measured at 287 nm using an UV spectrometer (Perkin-Elmer, Germany).

The apparent association constants K (M^{-1}) according the hypothesis of 1:1 stoichiometric ratio of complexes were calculated from the phase-solubility diagrams using the following Eq. (1) (Higuchi and Connors, 1965).

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \qquad (1)$$

The slope is obtained from the initial straight-line portion of the plot of CA concentration against CD concentration, and S_0 (M) is the equilibrium solubility of CA in water.

2.3. Preparation of CD/CA complexes

The preparation of CD/CA complexes was performed by the recrystallization method (Palmieri et al., 1998). One part unsaturated CA in ethanol solution (164 mM) was mixed with two parts unsaturated α-, β- and γ-CD in water (18.5, 11.9 and 60.7 mM, respectively), for 24 h, to form the complexes. After precipitation, complexes were filtered, then dried at 37 °C for 3 days.

2.4. ^{1}H NMR study

The D_2O solutions of CD/CA complexes (α-, β- and γ-CD/CA) were prepared with the complexes described before (all with 2 mM CD concentration). Solutions of 2 mM α-, β- and γ-CD were used for reference. The ^{1}H NMR experiments were performed at 400 MHz using a Bruker ARX 400 spectrometer. The probe temperature was regulated to 300 ± 0.1 K. All chemical shifts are given relative to external TSP (sodium 2,2,3,3-$^2H_4$-trimethyl-silyl propionate) at 0 ppm.

2.5. Circular dichroism study

At first, the complexation of α-, β- or γ-CD (10 mM) with CA (10 mM) in aqueous solution was analyzed by circular dichroism (Dichrograph, Jobin Yvon Mark V, France). CA solution (10 mM) was used as a reference.

After stirring for 20 days at room temperature, the solutions were filtered through an ester cellulose filter (Millex GS pores size 0.22 μm) then analyzed at different wavelengths. The maximum dichroic coefficients $\Delta\varepsilon$ ($M^{-1}\,cm^{-1}$) of filtrates were measured at 291 nm for CA solution and α-CD/CA solution but at 295 nm for β- and γ-CD/CA solutions with a quartz cell (Suprasil, Hellma, France) of 1 cm.

In a second step, a ternary system containing CA, CD and soybean oil was prepared to evaluate the competition between CD/CA and CD/lipid complex formation.

95.4 μl of super-refined soybean oil was added in 10 ml of water to prepare CA/CD/super-refined soybean oil dispersion with an equal concentration of 10 mM. Super-refined soybean oil was added in 10 ml of CA solution (10 mM) as a control in order to observe the interaction of CA with the oil phase. After stirring for 20 days at room temperature, the filtrates of CA/oil dispersion and α-CD/CA/oil dispersion were measured at 291 nm and those of β- and γ-CD/CA/oil dispersions were measured at 295 nm as described before.

2.6. Preparation of O/W/O multiple emulsions

O/W/O multiple emulsions were prepared by a two-step emulsification procedure (Jager-Lezer et al., 1997) using a homogenizer (Rayneri, Germany). 0.25 g of CA was dissolved in soybean oil q.s. 30 g to get a concentration of 47 mM. The oil phase with CA was mixed with 20 g of an aqueous solution of 0.1 M of α-, β- or γ-CD at room temperature to prepare the primary emulsion by mechanical stirring at 3000 rpm for 20 min. The semi-solid waxy outer phase was prepared by dissolving candelilla wax (5 g) in soybean oil (45 g). To obtain the O/W/O multiple emulsions, 50 g of primary emulsion was mixed with 50 g of waxy outer phase at 2000 rpm for 1 min. In addition, O/W/O multiple emulsions without CA were prepared as a control.

2.6.1. Characterization and evaluation of the multiple emulsion stability

The macroscopic appearance of these multiple emulsions was evaluated at 20 ± 1 °C, 40 ± 1 °C and 4 ± 1 °C after storage over 20 days.

The emulsions were centrifuged at 2000 × g, at 20 °C for 10 min. The stability of the emulsions was determined as the percentage of waxy outer phase separation.

2.6.2. X-ray diffraction analysis of emulsion samples

The experiment was carried out on D43 ($\lambda = 0.145$ nm) bench of DCI synchrotron of L.U.R.E. (Laboratoire pour l'Utilisation de Rayonnement Electromagnétique, Orsay, France). Wide-angle diffraction analysis was performed on O/W/O multiple emulsions and O/W primary emulsions with or without CA. The emulsions were stored at 20 °C and studied for more than 20 days after manufacture. The diffraction pattern of candelilla wax was also analyzed. A two-dimensional detection system with image plates was used (Molecular Dynamics scanner). Intensity profiles $I(s)$ were extracted ($s = 2\sin\theta/\lambda$ where 2θ is the scattering angle) and the s values can be converted into distances (Å) by the relationship $d = 1/s$.

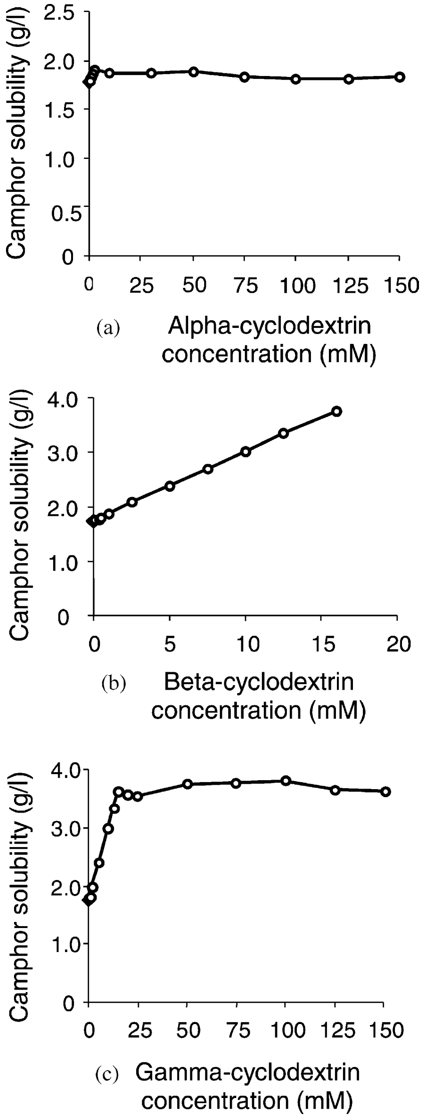

Fig. 1. The solubility diagrams of CA with α-CD (a), β-CD (b) or γ-CD (c).

3. Results

3.1. Phase-solubility diagrams

The phase-solubility diagrams of CA in CD aqueous solutions are presented in Fig. 1. A B_S-type curve is noted for α- and γ-CD (Fig. 1(a) and (c)) indicating the formation of an insoluble inclusion complex with a solubility in water of 0.8 and 13 mM, respectively. From the linear segment of these diagrams, the association constants are calculated: 80 and 370 M^{-1} for α- and γ-CD, respectively.

A linear relationship is observed between CA solubility and β-CD concentration (Fig. 1(b)). The A_L diagram suggests a typical soluble inclusion complex with a solubility in water of 13 mM at 16 mM of β-CD. The association constant calculated is 430 M^{-1}.

3.2. ^{1}H NMR analysis

The complexation behavior between CD and hydrophobic molecules in aqueous solution (D_2O) can be studied by comparison of ^{1}H NMR spectra between the free CD in solution and CD/hydrophobe complex in solution (Djedaïni et al., 1990; Djedaïni and Perly, 1990, 1991; Djedaïni-Pilard et al., 1993). As a consequence of inclusion, the chemical shift change of protons on CD can be induced. Located in the interior of the cavity, H3 and H5, protons attached to C-3 and C-5 of the glucose subunits of CD, show an upfield shift (negative chemical shift change $\Delta\delta = \delta_{\text{free}} - \delta_{\text{complex}}$) of the signals while inclusion is achieved (Fig. 2(a) and (b)). This upfield shift is mainly due to the anisotropic effects of double bonds and carbonyl group of guest molecule. The free CD state (δ_0), the complexed state (δ_c) and the chemical shifts change $\Delta\delta$ (ppm) ($\Delta\delta = (\delta_0 - \delta_c)$) of H3 and H5 CD protons are reported in Table 1. Generally, the inclusion behavior of CD induces negative chemical shift changes which are more pronounced for the H5 (the hydroxyl group located inside of CD cavity) than for the H3 (the hydroxyl group near the open of CD cavity) of the glucopyranose units of CD (Djedaïni and Perly, 1990).

In this study, a significant negative chemical shift change is observed with all of α-, β- and γ-CD/CA solutions. These quantitative results seem to indicate

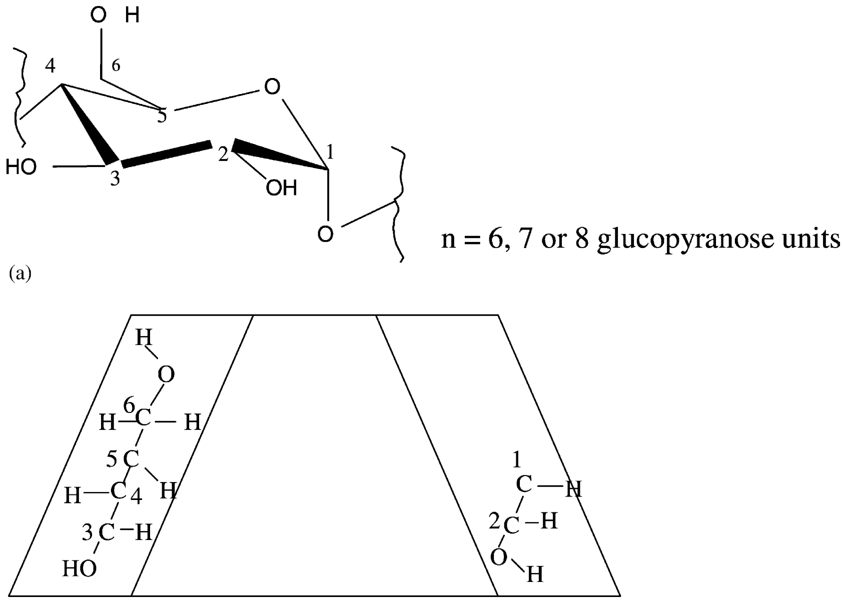

Fig. 2. Chemical representation of a glucopyranose unit of a CD molecule. The secondary hydroxyls at the 2- and 3-positions exist on the secondary face, and the primary hydroxyls at the 6-position exist on the primary face (a). Schematic representation of a CD cross-section with carbon atom numerotation (b).

Table 1
The chemical shift changes $\Delta\delta = (\delta_0 - \delta_c)$ (ppm) of H3 and H5 CD protons between the free δ_0 and complexed state δ_c of CD/CA complex solution

	α-CD (±CA)		β-CD (±CA)		γ-CD (±CA)	
Proton	H3	H5	H3	H5	H3	H5
δ_0	4.038	3.935	4.027	3.925	3.995	3.923
δ_c	4.041	3.867	3.978	3.839	3.941	3.849
$\Delta\delta$	0.00	−0.07	−0.05	−0.09	−0.05	−0.07

Table 2
Dichroic coefficient ($\Delta\varepsilon$) measured by circular dichroism of CA solution with or without super-refined soybean oil and complex solutions of α-, β- and γ-CD/CA with or without soybean oil

Dichroic coefficient $\Delta\varepsilon$ ($M^{-1}\,cm^{-1}$)	CA solution	α-CD/CA solution	β-CD/CA solution	γ-CD/CA solution
Without oil	1.47	0.77	1.25	1.35
With oil	0.64	0.40	1.16	1.22

the inclusion of CA in the cavity of α-, β- and γ-CD in these solutions.

3.3. Circular dichroism study

The circular dichroism spectrum allows the measurement of dichroic coefficient $\Delta\varepsilon$ ($M^{-1}\,cm^{-1}$) of a chiral substance having its own optical activity or an induced optical activity at a specific wavelength. However, in the case of complexation by a CD, the dichroic signal of molecule can be modified.

Before filtration at day 20, the CA solution, β- and γ-CD/CA solutions are translucent but some precipitates were observed in α-CD/CA solution. The $\Delta\varepsilon$ values of filtrates from CA only, CD/CA, CD/CA/oil and CA/oil solutions are presented in Table 2. As a chiral substance, CA possesses an optical activity with maximum dichroic coefficient $\Delta\varepsilon = 1.47\,M^{-1}\,cm^{-1}$ at 291 nm. The complexation of CA by CD seems to diminish its dichroic optical activity. In the case of α-, β- or γ-CD/CA solutions, the CD decreases CA dichroic signal of about 50, 20 and 10%, respectively.

The influence of super-refined soybean oil on the $\Delta\varepsilon$ values of CA/CD solutions was studied. The addition of super-refined soybean oil appears to decrease by 50% dichroic signals of CA solution without CD probably by diffusion of CA to the soybean oil phase. The addition of super-refined soybean oil decreases in a more important way the dichroic signal of CA in the α-CD solution (about 70%) than in the β- or γ-CD solution (about 20%).

3.4. Evaluation of multiple emulsion stability

3.4.1. Macroscopic observations of multiple emulsions at different temperatures

All multiple emulsions with or without CA are stable after storage over 20 days at 4 °C. Storage at 20 °C, however, induces exudation in β- and γ-CD multiple emulsions with or without CA but 3% oil phase was found to be exuded in α-CD multiple emulsions with CA (Table 3). At 40 °C, the waxy phase separations represent the instability of multiple emulsions prepared with α- and β-CD in this temperature, which are amplified by the presence of CA. However, it seems that the presence of CA does not change the instability of emulsions prepared with γ-CD (Table 3).

3.4.2. Centrifugation test

The stability of multiple emulsions at day 20 were also evaluated by the waxy outer phase separation (v/v %) after centrifugation. Only the α- and β-CD

Table 3
The multiple emulsion stability after storage during 20 days at 4, 20 and 40 °C

	α-CD multiple emulsion			β-CD multiple emulsion			γ-CD multiple emulsion		
	4 °C	20 °C	40 °C	4 °C	20 °C	40 °C	4 °C	20 °C	40 °C
Multiple emulsion without CA	Stable	Stable	3% WPS	Stable	Some oil exuded	5% WPS	Stable	Some oil exuded	2% WPS
Multiple emulsion with CA	Stable	3% oil phase exuded	11% WPS	Stable	Some oil exuded	9% WPS	Stable	Some oil exuded	2% WPS

WPS, waxy phase separation (liquid state at 40 °C but becomes solid at 20 °C) of total multiple emulsions.

Table 4

Waxy outer phase separation expressed as a percentage (v/v %) after centrifugation of multiple emulsions at day 20 ($n = 3$) (the theoretical waxy outer phase is about 50% v/v of total emulsion)

	α-CD multiple emulsion (%)	β-CD multiple emulsion (%)	γ-CD multiple emulsion (%)
Multiple emulsion without CA	3 ± 1	2 ± 0	54 ± 1
Multiple emulsion with CA	73 ± 1	63 ± 14	56 ± 2

emulsions without CA are stable. On the contrary, these multiple emulsions are destabilized by the presence of CA (Table 4).

A large waxy outer phase separation was observed with the γ-CD emulsion without CA indicating the fragility of this preparation. However, the incorporation of CA has no significant influence on the γ-CD emulsion instability (Table 4).

3.4.3. X-ray diffraction

X-ray diffraction has been used to determine crystal structure of CD complexes (Le Bas et al., 1984). This method provides information on the presence of complexes and allows the crystalline structures of complexes within the emulsion systems to be identified.

In wide-angle diffraction analysis, the same intensity profile for the primary and multiple emulsions is acquired, from the emulsions prepared with α-, β- or γ-CD with or without CA, except some additional peaks more especially those around 4 Å are found in the multiple emulsion diffraction patterns (Fig. 3). These additional peaks correspond to which in diffraction pattern of candelilla wax used to prepare the outer phase of multiple emulsions. It indicates that no modification of candelilla wax structure can be observed.

α-CD primary and multiple emulsions with CA do not display the same diffraction curve as that of α-CD emulsions without CA (Fig. 3(a)). The introduction of CA resulted in a disappearance of the peaks at 12 and 18 Å and the appearance of a peak at 16 Å.

The diffraction curve for β-CD emulsion without CA is different to that of α- or γ-CD emulsions, suggesting that the α-, β- or γ-CD emulsions without CA possess their own and different structured organization. The presence of CA modifies the diffraction curve of β-CD emulsions with some peaks around 7 Å slightly shifted and extended (Fig. 3(b)) and a broad peak around 16 Å.

There are no differences between the diffraction curves of the γ-CD emulsions with or without CA

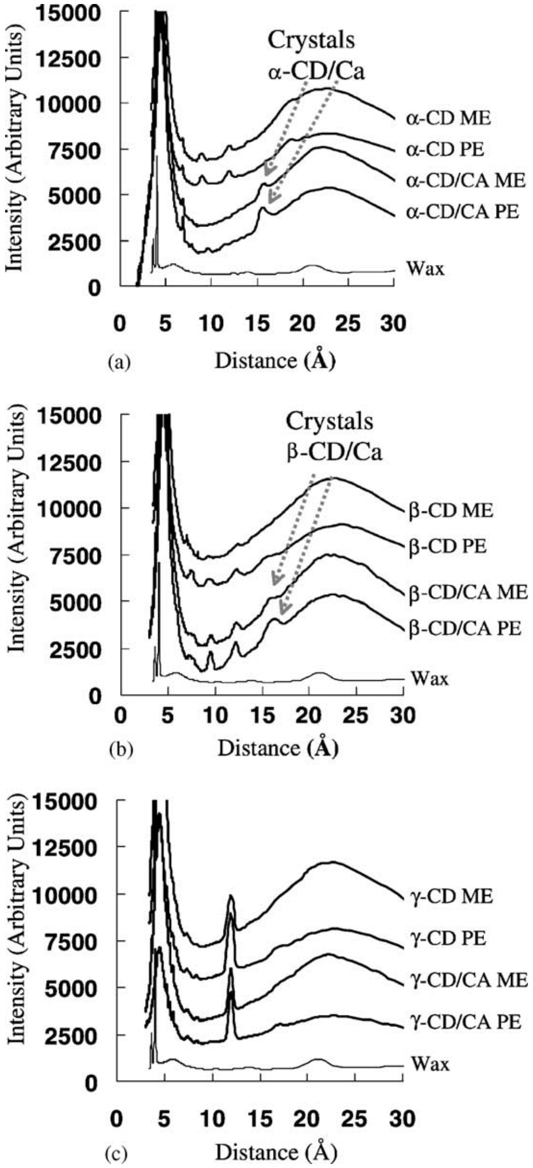

Fig. 3. Analysis of the effect of CA introduction in primary emulsions (PE) and multiple emulsions (ME) prepared with α-CD (a), β-CD (b) and γ-CD (c) by wide-angle X-ray diffraction.

(Fig. 3(c)), probably because both the γ-CD/oil component and the γ-CD/CA complex powders have the same diffraction curve (data not shown).

4. Discussion

In the literature, the complexation between CA and CD has been analyzed with energy values or separation factors, by several techniques such as gas–liquid chromatography and reversed-phase-high-performance liquid chromatography (Sybilska et al., 1995; Bielejewska et al., 1999; Dodziuk et al., 2000; Asztemborska et al., 2000; Bielejewska et al., 2001). A stability constant ($K_{1:1}$) of α-CD/CA complexes of $147 \pm 44\,\mathrm{M}^{-1}$ was obtained by Bielejewska et al. (2001).

In this study, different physicochemical methods such as phase-solubility diagrams, ^{1}H NMR analysis and circular dichroism, were employed to study the complexation between α-, β- and γ-CD and CA in aqueous solution. To avoid the interference of solvent on complexation behavior, all of our physicochemical analyses were performed in aqueous solution.

Phase-solubility studies indicate notable complexations only between β-, γ-CD and CA and a very weak solubility of the α-CD/CA complex. However, significant inclusion phenomena of CA are shown with α-CD as with β- and γ-CD, by the ^{1}H NMR analysis.

In the circular dichroism study, the large signal decrease of CA (about 50%) with the presence of α-CD probably resulted from complex precipitation due to the poor aqueous solubility of α-CD/CA complex of 0.8 mM (see Fig. 1(a)). Conversely, both β- and γ-CD/CA complexes have a water solubility of more than 10 mM, as indicated in the solubility diagrams (Fig. 1(b) and (c)). With β- and γ-CD, the decrease of the dichroic signals of CA is certainly due to formation of soluble β- and γ-CD/CA complexes.

The signal of CA in solution decreases with the presence of soybean oil probably due to the diffusion of CA molecule from the water to the oil phase.

Circular dichroism study in the CD/CA/oil ternary dispersion provides a better understanding of the correlation between CD/CA complexation and emulsion stability modification. The results show that multiple phenomena coexist in this system: CA complexation by CD, CA diffusion to the oil phase, and lipid oil component complexation by CD. There is probably a competition between CA and oil components for complexation with CD, resulting in less CD/CA complex formation.

Multiple emulsion stability was evaluated by macroscopic observation at different temperatures and by centrifugation. X-ray diffraction demonstrated the presence of crystallized complexes in the emulsions.

After storage of 20 days at 20 °C, only the α-CD emulsion without CA was perfectly stable. Some oil exudation on the surface of β- and γ-CD emulsions without CA and of α-, β- and γ-CD emulsions with CA was observed. At low temperature (4 °C) the viscosity of the emulsion may be increased, thus preventing the diffusion of the oil components, conserving the emulsion stability. Conversely, storage at 40 °C for 20 days induces waxy phase separation in all emulsions, probably as a result of several factors. The high temperature can reduce the CD emulsifier potential as shown by Shimada et al. (1991), can alter the thickening function of candelilla wax (the fusion of wax by heating) and can accelerate oil component diffusion.

In the centrifugation test, even if a total sedimentation had occurred, theoretically only 50% v/v waxy outer phase separation could be observed. This significant phase separation of more than half of the emulsion volume (Table 4) is observed indicating the destruction of α- or β-CD multiple emulsions by the presence of CA. This destruction of multiple emulsions probably results from the CD emulsifier alteration, which facilitates the fusion of inner oil phase to the waxy outer phase under centrifugation. Whereas large outer phase separation observed in γ-CD multiple emulsion with and without CA indicates that γ-CD itself is an inefficient emulsifier, it was difficult to evaluate the influence of introduction of CA in the γ-CD emulsion on the instability of these preparations.

A correlation between emulsion stability evaluation and circular dichroism effect is shown. The α- and β-CD emulsions without CA are considered stable but their stability can be disturbed by CA addition. The CA introduced in the oil phase of emulsions can diffuse to aqueous phase to be complexed by CD. In these formulations, the interaction between CA and α- or β-CD, as shown in physicochemical study, is strong enough to induce the destabilization of emulsions, whereas the complexation of the fatty acid residues of triglycerides of soybean oil by CD is insufficient to stabilize these emulsions.

In the X-ray diffraction study, the diffraction curves of both α- and β-CD primary or multiple emulsions changes with CA. In these instable emulsions, the repetitive distance of 16 Å probably indicates the presence of α- and β-CD/CA complex crystals which were previously observed in simple emulsion by microscopic observation (Yu et al., 2001), with respectively, a bilayer organization of pseudohexagonal structure and a dimer organization.

In conclusion, it was showed that multiple emulsions can be prepared in the absence of classical surfactants, and by using appropriate CD (α- and β-CD). The conditions of emulsion stability in the presence of lipophilic active ingredient, or any additive, are explained and are depending on the possible competition between the lipophilic molecule and the fatty acid residues of triglycerides to enter the CD cavity.

From this standpoint, we can imagine that high molecular weight active ingredients will not destabilise emulsions prepared with α-CD due to its small cavity.

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