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Cyclodextrins and emulsions

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Abstract

This paper synthesises the literature on interactions between cyclodextrins (CD) and fatty acids and glycerides, and explains how these interactions allow the use of cyclodextrins to stabilise emulsions. An example of formulation with cyclodextrins is given which discusses the preparation of simple o/w emulsions, the addition of a model active ingredient, and the preparation of multiple emulsions in the absence of preformed surface active agents.

Keywords: Cyclodextrins; Emulsions; Multiple emulsions; Fatty acids; Glycerides

1. Introduction

Cyclodextrins (CD), which are ring molecules presenting the remarkable ability to include guest molecule within their internal cavity, are the subject of a dramatically high number of patents and scientific papers. Cyclodextrins can, in fact, solubilise and stabilise active compounds, increase their bioavailability, decrease their undesirable side effects, mask unpleasant flavours or odours or transform gases or liquids into solid powders, inter alia. Such fascinating properties explain their presence in many patented products intended for the agro-food industry as well as in cosmetology and pharmacy.

In a number of patents dealing with emulsions (Duchêne et al., 1996, 1999; Vaution et al., 1987), cyclodextrins are used, but, most often, without any detail on their role, or they are presented as stabilising agents but still without any information on their mechanism of action.

The present paper synthesises the literature on interactions between cyclodextrins and lipids, as well as their role in emulsion formulation, and gives an example of the work we have carried out on the role of cyclodextrins in the formulation of multiple emulsions.

2. Interactions cyclodextrins/lipids

Triglycerides being the main constituents of vegetable oils, this review on interactions between cyclodextrins and lipids deals first with cyclodextrins/fatty acids interactions and secondly with cyclodextrins/glycerides interactions.

2.1. Cyclodextrins/fatty acids

The cyclodextrin hydrophobic cavity can include, at least in part, fatty acid chains. The exact nature of the cyclodextrin (α-, β- or γ-cyclodextrin, or a derivative), and that of the fatty acid (chain length, double

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bonds), both have a significant influence on the inclusion characteristics.

Depending on the fatty acid chain length (C_4–C_{18}), one cyclodextrin (or more) can interact and include the carboxylic chain. For either short ($\leq C_8$) or long ($\geq C_{12}$) chain fatty acids, the highest affinity is obtained with α-cyclodextrin, which has the narrowest cavity (Gelb and Schwartz, 1989; Lopez-Nicolas et al., 1995). This can be easily explained by the fact that a shorter distance between atoms of host and guest molecules results in stronger interactions. For intermediate chain fatty acids ($\approx C_{10}$), part of the chain is outside the β-cyclodextrin cavity so these can better interact with α-cyclodextrin than with α-cyclodextrin at the same 1:1 molecular ratio (Szente et al., 1993). Of course, if the amount of α-cyclodextrin is not sufficient to reach the 2:1 molecular ratio with long chain fatty acids, then the interaction strength decreases, due to that part of fatty acid chain not included within the α-cyclodextrin. In such a case β-cyclodextrin can lead to better or, at least, similar interactions.

These results are confirmed by the study of the water solubility of fatty acids from C_6 to C_{12} in the presence of α- or β-cyclodextrin (Schlenk and Sand, 1961). The increase in solubility is higher in the presence of α-cyclodextrin from C_6 to C_9 fatty acids and then enhanced in the presence of β-cyclodextrin for C_{10} and C_{11} fatty acids, when it is almost the same with both cyclodextrins for C_{12} fatty acids. It can be supposed that C_{10} or C_{11} fatty acids are slightly twisted inside the larger β-cyclodextrin cavity, leading to better molecular interactions than with α-cyclodextrin. Starting with C_{12}, part of the chain is outside the cavity, in both α- and β-cyclodextrins.

When considering substituted cyclodextrins, it is important to take into account the effect of the substitution on the cyclodextrin cavity on accessibility by the guest molecule, and the possible direct interaction between the substituent and the guest molecule. For example, methylation in position 2 of β-cyclodextrin is favourable to a stronger interaction between the cyclodextrin and linoleic acid (Lopez-Nicolas et al., 1995). On the other hand, the randomised methylation of β-cyclodextrin (RAMEB) in positions 2, 3 and 6 leads to a lower interaction evidenced by a lower water solubility (Szente et al., 1993).

Inclusion of fatty acids in cyclodextrin results in an increase in their water solubility. However, when comparing C_6 to C_{12} fatty acids in the presence of either α- or β-cyclodextrin, Schlenk and Sand (1961) demonstrated that the increase is proportionally higher for long chain fatty acids, but that their water solubility remains very low compared with that of short chain fatty acids. This could be the consequence of the partial inclusion of the alkyl chain. This is confirmed by the work of Slotte and Illman (1996) who studied C_{14}–C_{16} fatty acid desorption from a monolayer deposited on a β-cyclodextrin aqueous solution. The desorption rate decreases linearly with increase in fatty acid chain length, demonstrating that due to the partial inclusion formed the complex solubility is low.

The presence of double bonds has been investigated by different authors (Szente et al., 1993; Jyothirmayi et al., 1991), but this did not lead to a clear conclusion despite the fact that doubles bonds result in less linear, more compact structure of fatty acids.

On the other hand the influence of the fatty acid chain length has been evidenced by Schlenk and Sand (1961), Gelb and Schwartz (1989) and Shimada et al. (1992). The number of cyclodextrins capable of interacting with fatty acids increases with an increase in the hydrocarbon chain length. However, the exact number of cyclodextrins involved in that process is somewhat unclear (Table 1).

Depending on the number of cyclodextrins involved in the complex formed, it will be characterised by one or more affinity constant(s) (Lopez-Nicolas et al., 1997)

$$CD_{free} + FA \rightleftarrows CD/FA_{complex} \quad (K_1)$$

$$CD/FA_{complex} + CD_{free} \rightleftarrows CD_2/FA_{complex} \quad (K_2)$$

The formation of inclusion compounds occurs through two types of interaction: (1) creation of hydrogen bonds between the carboxyl of the fatty acid chain and the hydroxyls in position 6 on the cyclodextrin; and (2) creation of hydrophobic inter-

Table 1
Stoichiometry of inclusion compounds α- or β-cyclodextrin/C_{18} or C_{12} fatty acid

References	C_{18}		C_{12}	
	α-CD	β-CD	α-CD	β-CD
Schlenk and Sand (1961)	3.1	2.7	2.0	2.0
Gelb and Schwartz (1989)	2.6	2.6	1.7	1.7
Shimada et al. (1992)	2.4	2.4	1.8	1.7

actions between the fatty acid hydrocarbon chain and the cyclodextrin cavity.

2.2. Cyclodextrins/glycerides

Vegetable oils are comprised of triglycerides together with di- and monoglycerides as well as free fatty acids. Cyclodextrins can form inclusion compounds as we have discussed with the free fatty acids or the fatty acid chains of the glycerides. According to Szente et al. (1993) the complex stability depends on the acylation degree and decreases in the order: free fatty acid > monoglyceride > diglyceride > triglyceride. Only fatty acids and monoglycerides lead to water-soluble complexes, complexes with triglycerides being insoluble. Kolossvary and Kolossvary (1996) studying, by computer assisted molecular modelling, the complexation in water medium of oleic acid and triolein by β-cyclodextrin, demonstrated that only the complex obtained with oleic acid is stable in water when that obtained with triolein is unstable. This result is confirmed by Laurent et al. (1994) who studied the desorption of monolayers of oleic acid, mono-, di- and triolein deposited at the surface of water to which β-cyclodextrin had been added. The presence of cyclodextrin leads to an increase in oleic acid or monoolein desorption rate, due to the formation of water-soluble inclusion complexes. Neither di- nor triolein are desorbed from their monolayers in the presence of β-cyclodextrin. On the other hand, there was a decrease in the triolein monolayer elasticity in the presence of β-cyclodextrin, when there is no such modification of the diolein monolayer. This demonstrates that no complex can be obtained between β-cyclodextrin and diolein, but a non-desorbed insoluble complex is obtained with triolein, the insolubility being due to the fact that only one fatty acid chain is included in the cyclodextrin. This insolubility of cyclodextrin/triglyceride inclusion complexes is a prerequisite for their potential role at the water/oil interface of emulsions.

3. Cyclodextrins and emulsions

Despite the number of patents in which cyclodextrins are claimed to have a stabilising effect in emulsions, rather few scientific papers present a thorough account to this subject.

In 1991, Shimada et al. investigated the emulsifying power of α-, β- and γ-cyclodextrins in the preparation of a 1:1 soybean oil/water emulsion. They found that the best emulsifying effect was obtained with β-cyclodextrin, for which only 0.25% was necessary to stabilise the emulsion, when 0.50 and 2% levels are necessary for α- and γ-cyclodextrin, respectively. Similar results were obtained by Tanaka et al. (1989), who demonstrated that, at low cyclodextrin concentrations, β-cyclodextrin had better emulsifying properties for sunflower oil, than α-cyclodextrin. But, different results were obtained by other authors Regiert et al. (1996) and Wimmer et al. (2000) working with sunflower oil found that the best results were obtained with γ-cyclodextrin. According to Riegert, inclusion of triglycerides would be easier in the wider cavity of γ-cyclodextrin rather than in that of either α- or β-cyclodextrin. Anyhow, this explanation, which was not proved, does not seem to be realistic. The differences obtained in these studies could be attributed, at least for part, to the different experimental conditions used, among which the cyclodextrin concentration.

A very interesting paper is that by Shimada et al. (1992). In this paper, the authors studied the influence of α- and β-cyclodextrin on soybean oil/water interfacial tension. These cyclodextrins, which do not modify

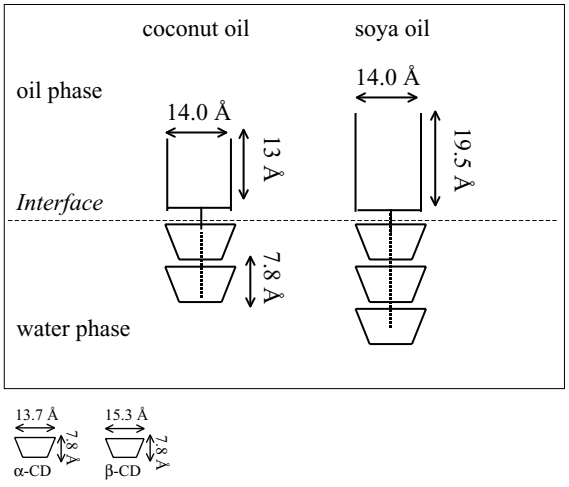

Fig. 1. Schematic representation adapted from Shimada et al. (1992) for proposed structure of CD/triglyceride inclusion complexes at vegetable oil/water interface. The sizes of fatty acid residues in soybean oil and coconut oil were those of C_{18} and C_{12}, respectively.

the surface tension water, decrease the oil/water interfacial tension proportionally to their concentration. Using the Gibbs' equation they calculated the surface excess and stacking of cyclodextrins at the interface. A partial inclusion complex is formed at the interface, the cyclodextrin molecules interacting with one fatty acid chain of the triglycerides. This super-molecule constitutes a surface active agent, the cyclodextrins containing the fatty acid chain being oriented toward the aqueous phase, when the two free fatty acid chains are oriented toward the oily phase. Such a mechanism explains the stabilising role of cyclodextrins in emulsion formulation. Furthermore, according to the fatty acid chain length, one, two or three cyclodextrins can interact. Fatty acid chains in C_{12} are the minimal length requested to obtain the surface active role of cyclodextrins, shorter chains leading to completely water-soluble complexes (Fig. 1). Furthermore, the interfacial film rigidity should play a role on the stability of the emulsions obtained (Laurent and Graille, 1994).

4. Cyclodextrins in the formulation of multiple emulsions

Multiple emulsions, which can contain active ingredients dissolved in each phase, represent very attractive drug delivery systems for the future (Grossiord and Seiller, 1998). They also could constitute a controlled release system for drugs incorporated in the internal phase(s). They could be used for either oral (o/w/o) or dermal administration (o/w/o or w/o/w). The use of cyclodextrin in their formulation is especially interesting because it allows the decrease in the high amount of classical surfactant necessary normally to stabilise the internal simple emulsion (primary emulsion) and the final multiple emulsion.

The work reported here, deals with the preparation of an o/w/o emulsion having soybean oil as oily phase (Yu, 2002). This work necessitated the preparation of a primary o/w emulsion, the assessment of its stability in the presence of model active ingredients, and finally the preparation of the multiple emulsion.

4.1. Preparation of blank o/w emulsions

We investigated the feasibility of o/w emulsions (60/40, w/w) soybean oil/water, using natural α-, β- or γ-cyclodextrins as stabilising agents. Soybean oil was chosen as a model oil, in order to be able to compare our results to those of Shimada et al. (1991, 1992).

Emulsions were prepared by adding the oily phase to the aqueous phase comprising a 0.1 M solution of α-, β- or γ-cyclodextrin (Yu et al., 2001). Stability studies were carried out at days 0 and 20 by assessing macroscopic and microscopic feature, centrifugation stability and rheological behaviour. It appeared that only α- and β-cyclodextrins were capable of leading to stable emulsions.

These results confirmed that only the α- and β-cyclodextrin allow optimum molecule organisation and interaction of the soybean triglycerides with the cyclodextrin cavities, the γ-cyclodextrin cavity is probably too wide. The results are in agreement with those of Shimada et al. (1991), but did not confirm those of Regiert et al. (1996), who worked with different oils and experimental conditions.

4.2. Preparation of o/w emulsions with model active ingredient

The stabilisation of blank emulsions was probably the consequence of the in situ formation of a surface agent resulting form the inclusion of part of the fatty acid chains of soybean triglycerides in the α- or β-cyclodextrin cavities. We investigated the possible competitive effect of model active ingredients which may displace the hydrocarbon chains from the complexes (Yu et al., 2001).

The model active ingredients were benzophenone and camphor whose affinity constants, calculated from Higuchi diagrams, are 30, 1900 and 280 M^{-1} for benzophenone and 80, 430 and 370 M^{-1} for camphor with α-, β- and γ-cyclodextrin, respectively. For the preparation of emulsions, the model active ingredients in the oily phase (47 mM) were added to the aqueous phase containing the cyclodextrin.

Benzophenone decreases significantly the stability of the emulsions prepared with β- or γ-cyclodextrin. In the case of β-cyclodextrin this can be easily explained by the high affinity constant between the two products. In the case of γ-cyclodextrin, the affinity constant is lower, and the decrease in stability is explained by the fact the emulsion has already a poor stability. The addition of a lipophilic active ingredient

in the oily phase in this case easily displaces the hydrocarbon chain, and destroys the weak surface active agent formed. On the other hand, the low affinity of benzophenone for α-cyclodextrin explains that it has no effect on emulsion stability, which is maintained.

In the case of camphor, destabilisation occurs for all the emulsions whatever the nature of the cyclodextrin. This can be easily explained for β- and γ-cyclodextrins for which camphor has a significant affinity, but it is more surprising for α-cyclodextrin for which camphor has only a low affinity. Nevertheless, higher affinity constants are mentioned in the literature by authors using chromatographical methods such as Bielejewska et al. (1999) who found an affinity constant of $147\,M^{-1}$ M. Furthermore, we demonstrated by circular dichroism, as well as by ^{1}H NMR, that there was a true inclusion of camphor in α-cyclodextrin (Yu et al., 2003). It seems that this interaction is sufficient to destabilise the emulsion.

4.3. Preparation of o/w/o multiple emulsions

As most of non-ionic surfactants are known for their ability to be included (at least partly) in cyclodextrins, we chose rather to stabilise the multiple emulsion by use of a thickening agent in the external oily phase (Yu et al., 1999, 2001). However, the cyclodextrin added in the aqueous intermediate phase can stabilise the second interface primary emulsion (o/w)/external oily phase (o).

The thickening agent, 10% of candelilla wax, was added in the oily external phase. Multiple emulsions were prepared according to the two-step method (Jaeger-Lezer et al., 1997): the primary emulsion previously described is first prepared, the outer phase is prepared separately by dissolving candelilla wax in soybean oil at 80 °C, and is added to primary phase after cooling to a semi-solid waxen phase.

Blank emulsions (Yu et al., 1999) are not very homogeneous with respect to globule size. In the case of γ-cyclodextrin, which leads to unsatisfactory simple emulsions, large internal globules (4–12 μm) are observed, characteristic of potentially unstable emulsions. Stability to centrifugation, when compared with that of primary simple emulsions, is only slightly decreased for α- and β-cyclodextrins (2 and 6% oily supernatant, respectively) but dramatically decreased for γ-cyclodextrin (60% oily supernatant).

When benzophenone is added to the emulsions (Yu et al., 1999), it increases the instability of emulsions prepared with either β- or γ-cyclodextrin. In the case of γ-cyclodextrin, the coalescence of internal globules can be observed, when, with β-cyclodextrin some crystals appear in the emulsion. On the other hand, when benzophenone is added to the emulsion prepared with α-cyclodextrin, its stability is maintained. Addition of camphor (Yu et al., 2003) confirms its effect on simple emulsions: all the multiple emulsions are destabilised.

4.4. Discussion

The work we carried out on the feasibility of multiple o/w/o emulsions is particularly interesting for several reasons.

First, during the preparation of blank primary emulsions, it confirms that cyclodextrins can be successfully employed if their cavity is adapted to the triglyceride fatty acid chains of the oily phase. In the case of soybean oil, only α- and β-cyclodextrins are suitable for the preparation of o/w emulsions, and γ-cyclodextrin cavity is probably too wide to lead an optimal interaction with the fatty acid chains.

Secondly, active ingredients can be added to emulsions stabilised by cyclodextrins, but they must not interact significantly with the cavity of the cyclodextrin employed, otherwise they displace the fatty acid chain and destabilise the emulsion. From this standpoint, we can imagine that high molecular weight active ingredients will not destabilise emulsions prepared with α-cyclodextrin.

Finally, for the first time, o/w/o multiple emulsions were prepared without any preformed surface active agent in the presence of cyclodextrins as the stabilising agent of the primary emulsion, but as in the case of simple emulsions, the addition of an active ingredient is possible only if its affinity for the cyclodextrin employed is low.

5. Conclusions

Because of their ability to include inside their cavity guest molecules of different types, and to confer on them new physico-chemical properties, cyclodextrins have been too often considered as all-purpose

excipients, without a clear idea of their true role in the formulation, or of their mechanism of action. In the past, this was most probably the case for many patents in which cyclodextrins were added in the preparation of emulsions.

This review of the literature, completed by the work we carried out on multiple emulsions, explains clearly how cyclodextrins can form in situ surface active agents by including a fatty acid chain of the glycerides of the oily phase. From this standpoint, small cyclodextrins, α or β, seem preferable to γ-cyclodextrin with its larger cavity. The conditions of emulsion stability in the presence of active ingredient, or any additive, are explained and are dependant on the possible competition between the additive and the fatty acid to enter the cyclodextrin cavity.

Last but not least, it was shown that multiple emulsions can be prepared in the absence of classical surface agents, by using an appropriate cyclodextrin. A good understanding of the inclusion mechanism will help to decide what kind of active ingredient can be formulated in such emulsions.

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