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'Novel trends in cyclodextrins encapsulation. Applications in food science'

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Abstract

Cyclodextrins (CDs) are cyclic oligosaccharides composed of linked glucopyranose subunits. The main property of CDs is that their hydrophobic inner cavity forms inclusion complexes with a wide range of guest molecules, while the hydrophilic exterior enhances CD solubility in water. Because of their molecular inclusion capability, the properties of the materials with which they complex can be significantly modified. Particularly, solubility and stability of bioactive compounds to be used as nutraceuticals, could be improved by encapsulation in CDs. The available thermodynamic data are consistent with an exothermic and spontaneous inclusion processes. Phase solubility studies in liquid systems along with studies of physical properties of solids complex, help to elucidate complex stoichiometry and guest-CD interactions.

The use of CD-complexes for improving molecules solubility and stability, for control release and as adjuvant in extraction processes, represents a promising innovative strategy in the food industry for the development of new ingredients and products.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides that can be produced by enzymatic modification of starch [1]. The most common of them are composed from 6 to 8 glucopyranose units, known as *α*, *β* and *γ* CD, respectively, being β-cyclodextrin (BCD) the most studied and widely employed.

CDs are of crystalline and non-hygroscopic nature, with non-reducing character. They are chemically and physically stable molecules [2,3] which structure resembles a toroid conical cylinder, with a hydrophobic interior and a hydrophilic exterior [4,5]. They are not absorbed in the upper gastrointestinal tract, but are completely metabolized by the colon microflora. *α, β* and *γ* CDs are 'generally regarded as safe' (GRAS) (Food and Drug Administration, US) for use as additives in food products, but their approval status depends on the country [2,4–7•].

The main property of CDs is that their hydrophobic inner cavity forms inclusion complexes with a wide range of guest molecules while the hydrophilic exterior enhances CD solubility in water [3,4,8–10]. Figure 1 shows the spatial representation of the BCD molecule and the estimated theoretical properties (molecular volume, octanol-water partition coefficient (logP), polar topological area (TPSA), total polar molecular surface (PSA)) obtained by the program *Molinspiration*. The equilibrium established in the process of inclusion is shown schematically in Figure 1b.

2. Interactions with water

2.1. Inclusion mechanism. Complex formation.

Although the central cavities of CDs are hydrophobic, half of the water molecules of the dodecahydrated BCD crystal are placed on specific sites in the CD cavity, and the rest distributed between the surface and the interstices of CD molecule [11]. This conformation is energetically unfavorable due to polar-non polar interactions and,

therefore, the system energy is reduced when those water molecules are replaced by guest molecules less polar than water [3,8,12,13]. Thus, the main driving force for the complex formation is the release of the water molecules from the inner CD cavity (considered to be in a high enthalpy state) [8,14]. A non covalent inclusion complex is formed by the process called "molecular encapsulation", in which CD ring strain is released and Van der Waals forces and hydrogen bond interactions take place [12,15–17]. The interactions with water determine not only the ability to form the complexes, but also their stability [16,18].

2.2. Phase solubility studies

The formation and dissociation constants of the ligand-CD complexes depend on the chemical structure and shape of guest and on the environment physicochemical properties. The stability of the inclusion complex is associated to the complex stability constant (K_s), which can be obtained from the phase solubility diagrams according to the method developed by Higuchi and Connors [20]. Phase solubility studies performed at different temperatures, and applying the integrated form of the van´t Hoff equation (for K_s vs temperature), allowed to calculate the enthalpy (ΔH) and entropy changes (ΔS) of the inclusion process [12,15,21–23•]. Although the inclusion of different ligands in BCD showed that the process is exothermic and spontaneous (negative ΔH and ΔG values, respectively, in Table 1), small changes in the structure of the ligand were evidenced in changes in the values of ΔH and ΔS. In particular, the inclusion of β-sitosterol was thermodynamically favored with regard to cholesterol, even though both sterols have very similar structures (Table 1).

2.3. Water sorption isotherms

Considering the high influence of water on complex formation and stability, the analysis of water sorption behavior of solid ligand-CD systems becomes of fundamental importance to define the appropriate storage conditions of dry formulations [16]. There are different methods to obtain combined solid systems of cyclodextrins and ligands [24]. One of the most commonly used has been the coprecipitation from a saturated CD solution in equilibrium with the ligand followed by freeze-drying [25–27]. Figure 2 shows the water sorption isotherm of BCD and for the solid complexes α-terpineol-BCD (TER-BCD) and cholesterol-BCD (CHOL-BCD). As increasing RH, the water content of BCD increases, but a plateau is reached at 52% RH, at the water content of 15% (d.b.), (approximately 11 mol of water per mol of BCD). This is consistent with the crystalline nature of hydrated BCD, which is maintained until at least 97% RH [15,28]. The TER-BCD and CHOL-BCD complexes (Figure 2) adsorbed less water between 11% and 97% RH (attributed to the displacement of water molecules from the inner cavity) and they did not show a plateau, which indicates that no hydrates were formed. Their isotherms followed a sigmoid-type behavior with a marked increase of water content above 85% RH. This behavior was also observed for BCD complexes with myristic acid [16], cholesterol, β-sitosterol, cinnamaldehyde and thymol [29,30]. The stability of the dried complexes is governed by the shape of the water sorption isotherms: the release of thymol and cinnamaldehyde from BCD complexes was detectable from 85% RH, coincidentally with the abrupt increase of water sorption observed in the isotherm [31].

2.4. Thermal transitions and stability.

The thermal events observed by differential scanning calorimetry (DSC) in pure freeze-dried CDs are water desorption (in the range 60-140 °C, only when determinations are performed in open or punctured capsules), hydrate interconversion or structural rearrangements at 175-180 °C and melting accompanied by decomposition at temperatures higher than 250°C [32••].

The glass transition temperature of BCD is hard to determine due to its crystalline nature. It is to be noted that once the complexes are formed, they are of amorphous nature and a clear glass transition can be detected [15,16], in agreement with the shape of the water sorption isotherm. Hence, the inclusion/encapsulation of the ligand in the CD leads to supramolecular changes in the matrix.

The complex formation can be assessed recording the molecular changes taking place during the guest inclusion by different methods (ultra violet-visible, nuclear magnetic resonance spectroscopy or circular dichroism, differential scanning calorimetry [2]). Differential scanning calorimetry (DSC) is one of the most widely used methods for evaluating the formation of solid state complexes [33,34]. The total or partial disappearance of the endothermic melting transitions corresponding to the guest molecule is a strong evidence of complete or partial inclusion in the CD [35–37]. Therefore, the relationship between the area of the melting peak obtained in the thermogram and the melting enthalpy value of the pure ligand is considered a good approximation to quantify the degree of ligand encapsulation [16,35]. Figure 3 shows the proposed structures for BCD complexes with different ligands (obtained with the Hyperchem 7.5 and VMD program) [29]. The degree of encapsulation determined by DSC varies with the ligand-CD molar ratio and with the structure and polarity of the ligand.

3. **Applications.**

3.1. Enhancement of aqueous solubilization of non polar components

3.1.1. Cyclodextrins as green extracting agents.

The recovering of bioactive compounds from wastes and by-products from agroindustrial production may help to add value by the production of natural additives

for food and cosmetic products. However, the use of organic solvents to extract them from vegetable resources may result in environmental pollution and personnel health risks. Thus, there is a need for replacing organic extraction solvents by green extracting agents with adequate extraction yields.

The use of CDs solutions for extraction processes is currently being studied to promote the aqueous extraction of both hydrophilic and hydrophobic compounds in a single stage, with the advantage of being a safe and non-polluting method [38••–40]. Recently, the BCD-assisted extraction processes of polyphenols from grape pomace, apple pomace and vine shoot cultivars have been optimized [41–44]. Cui et al. [45] reported that aqueous BCD offered a better yield to recover epigallocatechin from tea leaves than that obtained using an aqueous 50% ethanol solution. Compared to organic solvents, BCD-assisted extraction is more economic, safe and green.

3.1.2. Phytosterols encapsulation

Phytosterols (especially β-sitosterol) have technological application as antioxidants and are also effective in diminishing plasmatic cholesterol [46–49]. Phytosterols encapsulation in CDs can improve their low water solubility and control its release and overcome the limitations for its use as food or nutraceutical ingredient [29,47].

3.1.3. The case of propolis, a multicomponent system.

Propolis possesses many beneficial biological activities: antimicrobial, antioxidant, anti-inflammatory, antitumour, hepatoprotective, local anaesthetic [50–53]. Waxes, essential oils and phenolic compounds (pinocembrin, galangin, quercetine and chrysin) are mainly responsible of propolis properties [53,54]. For medical, dietetic and cosmetic uses of propolis, it is available as a 70% ethanolic extract [53], although aqueous solutions of propolis have similar or higher pharmacological activity [55-58].

Encapsulation in CDs is regarded as a solution to the limitations of propolis applications caused by its low water solubility and by its strong and unpleasant odour [57], enhancing also its bioavailability [29,59-61].

3.2. Stabilization of bioactive or functional compounds

An important step in the development of natural food additives or ingredients is the design of formulation procedures for their stabilization, solubilization and delivery. The use of CDs as nanoencapsulating amphiphilic molecules for such purposes introduces a new concept in the cosmetics, drug and food industries[6,62-64].

The stability of the inclusion complexes of the CDs with different hydrophobic components from essential oils was investigated as a function of the storage time and water content of the systems [16]. Rosemary components which have demonstrated beneficial biological activities, were successfully encapsulated in cyclodextrins (CDs), providing a convenient delivery and stabilizing vehicle [63].

The recent applications of cyclodextrins include enclosing or capture of unwanted components, such as trans-fats, allergens, mycotoxins, acrylamides, bitter compounds, such as vitamins, as well as the design of smart active packaging of foods [6].

Strategies to prepare host-guest inclusion complexes of β-cyclodextrin with vitamins of different hidrophyilicity, from nicotinic acid to the more hydrophilic ascorbic acid, have been explored to stabilize them in aqueous medium, and avoid unwanted tastes [65].

3.3. CD complexation for volatiles protection and elimination of unpleasant flavors

One of the most frequent applications of CDs is as nano-encapsulating carriers for improving physical and chemical flavor stability. This strategy also transforms flavors from liquid into more practical crystalline presentations. Besides, they are employed for

the modification or elimination of bitter and disgusting odours of foods and beverages or vegetable extracts [5,66]. These characteristics are also exploited in the formulation of pharmaceutical dosages [6,67].

3.4. Cholesterol sequestrant

CDs may be employed to separate cholesterol from food products like milk or eggs [8,68] studied the thermodynamics and factors affecting the optimum encapsulation conditions and stability of cholesterol complexes in β-cyclodextrin. These data could be essential to employ cyclodextrins for removing cholesterol or to incorporate functional ingredients (such as sitosterol) in the development of innovative food products.

3.5. CD derivatives and their uses

Various cyclodextrin derivatives have been prepared to overcome the limitations of natural cyclodextrins derived from their low solubility, both in water and organic solvents, and to improve their inclusion capacity [4].

The most common BCD derivatives in pharmaceutical applications include hydroxypropyl-β-cyclodextrin (HBCD), methyl- β-cyclo-dextrin (MBCD) and sulfobutylether-β-cyclodextrin (SBEBCD) [4].

The substitution of OH by hydroxypropyl groups in the external part of BCD increased the surface polarity of the molecule, without a great change of the nonpolar internal cavity. As a consequence, the interaction of the CD with water changes and this is manifested both in the water solubility and in the shape of sorption isotherm of solid systems. HBCD isotherm follows a sigmoid-type behavior (without the plateau observed in Figure 2 for BCD), which indicates that no crystalline hydrates are formed.

A sharp increase of water content at RH higher than 85% derived in structural collapse, which did not occur in BCD, of crystalline nature [30].

The introduction of other hydrophilic groups or of hydrophobic moieties (e.g. methyl-groups) also results in the increase of CDs water solubility, due to breakage of H-bonds and reduction of crystal lattice energy [69]. Conjugates of BCD units to highly hydrophilic polymers (polyethylene glycol, dextran) showed better solubilizing properties for poorly water soluble drugs and selective cholesterol extraction in comparison to conventional BCD derivatives [70], interesting for the development of hypercholesterolemia treatment [71].

Depending on their cavity size and the substituents, CDs are able to both remove and reload cholesterol, while other CDs are specific for phospholipids and fatty acids. These molecular recognitions of CDs have been employed for the selective manipulation of ordered lipid structures in living cells and model membranes [6••].

CDs derivatives were employed as active components (of low toxicity and biocompatible) in biomolecule stabilizing media [4,8,14,72,73].

The interactions of CDs with aromatic amino acids located at the proteins surface has been proposed to improve protein stability. Two approaches have been analyzed for the use of CDs derivatives to improve enzyme stability: a) the covalent modification of enzymes with CDs moieties or b) the covalent modification of polymers by including CD moieties, inducing advantageous interactions at the surface of the enzyme [74,75].

4. Concluding remarks

Although many types of encapsulation are available, the encapsulation in CDs has the unique characteristic of being a molecular encapsulation where a dynamic equilibrium between the CD and the ligand is established. CDs have the ability to form inclusion complexes with many guest molecules by taking up the whole molecule, or some part of it, into the cavity. This partial or total encapsulation will affect the physicochemical

properties of the guest molecules. Solubility and stability of bioactive compounds to be used as nutraceuticals, could be improved by encapsulation in CDs. The available data are coincident with an exothermic and spontaneus inclusion processes of hydrophobic or partially hydrophilic compounds. Phase solubility studies are helpful to elucidate complex stoichiometry and guest-CD interactions. Water adsorption behavior are consistent with the displacement of water molecules from the inner cavity of the CDs when the ligand molecule is included, and glass transition temperatures of the complexes have fundamental importance to define the appropriate storage conditions of dehydrated systems.

Theoretical calculations employing available software help to predict potential consequences of CD-ligand-water interactions, saving time and experimental resources.

The versatility of cyclodextrins and modified cyclodextrins is demonstrated in their many industrial applications, such as green extracting agents, solubility enhancer, stabilizer of bioactive or functional food ingredients, among others. Its unique features make CDs become an interesting option for the development of new biotechnological, pharmaceutical or food products.

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This article evaluates the use of α, β, and γ cyclodextrins as bioactive food supplements and nutraceuticals. The mechanisms behind these effects are reviewed together with their applications as solubilizers and stabilizers of dietary compounds such as unsaturated fatty acids, phytosterols, vitamins, flavonoids, carotenoids among others. Cyclodextrins versatility is based on their unique structure that enables them to form inclusion complexes with molecules of low hydrophilicity and proper geometrical size.

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Cyclodextrins allow a nanoencapsulation of different compounds at molecular level. Food ingredients formulated with cyclodextrins are stabilized against oxidation and heat. Food industry employs natural cyclodextrins with different goals. Whilst α-cyclodextrin acts as a prebiotic, β-cyclodextrin and γ- cyclodextrin are used in very diverse applications due to their ability to form inclusion compounds or complexes. Actually cyclodextrins are being used in the food and nutraceutical markets to solve stability, taste and odour problems of special ingredients.

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The inclusion complexation of guest molecules by cyclodextrins in aqueous solutions results in a substantial rearrangement and removal of the water molecules originally solvated to both the cyclodextrin and guest molecules, and this process also induces the release of water molecules from the cyclodextrin cavity into the bulk water. The binding is given mainly by van der Waals and hydrophobic interactions, although hydrogen bonding and steric effects are also important. The thermodynamic magnitudes obtained for complexes formation in cyclodextrins are a consequence of the weighted contributions of all these interactions.

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Despite the numerous and diverse successful applications of cyclodextrins, the mechanism of complexation and the relationship between structure and selectivity are still only partly solved and remain open for discussion. Thermodynamic studies could supply valuable information facilitating an understanding of the physico- chemical basis of the complexation processes.

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Complexation in cyclodextrin allows improving the extraction efficiency of natural compounds from plant matrices enhancing their solubility, stability and/or bioavailability. Extraction of phenolic compounds from plants with aqueous cyclodextrin solutions has been demonstrated as an efficient and green extraction process. In brief, cyclodextrins are an environmentally friendly additive for the rapid and effective extraction of both hydrophilic and hydrofobic compounds.

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Figure captions

Figure 1. a) Spatial molecular geometry and interatomic distances of the BCD cavity obtained with Hyperchem *7.5* program. The Molinspiration program was used for calculations of molecular volume, logP, polar topological area (TPSA) and its three-dimensional representation or polar molecular surface (PSA). In the PSA the polar and non polar areas are represented in red and grey respectively. b) Dynamic equilibrium established in the process of ligand-CD inclusion.

Figure 2. Comparison of BCD water adsorption isotherm with that of the complex terpineol-BCD (a) and cholesterol-BCD (b), in molar ratios 1:1 and 1:3. The isotherms of the complexes were adjusted with the GAB model.

Figure 3. Chemical structure of BCD complexes with α-terpineol (TER-BCD), myristic acid (MYR-CD), cholesterol (CHOL-BCD) and β-sitosterol (SITO-BCD). Molecular modeling with professional Hyperchem (version 7.5) and VMD.

Table 1. Comparison of the thermodynamic parameters obtained for the encapsulation of α-terpineol in BCD in present work with those obtained by other authors for different terpenes (thymol, geraniol), flavonoids (hesperidin, naringin) and sterols (cholesterol, β-sitosterol).

Ligands	ΔH, (kJ.mol^{-1})	ΔS, (J mol^{-1}K^{-1})	$\Delta G_{25,}$ (kJ.mol^{-1})
α-terpineol	-124	-355	-18
Geraniol[a]	-40	-60	-22
Thymol[a]	-47	-82	-22
Hesperidin[b]	-35.5	-71.3	-14.3
Naringin[b]	-50	-120	-14
Cholesterol	-43	-65	-23
β-sitosterol	-54	-121	-18

[a]Mourtzinos et al., 2008;[b]Tommasini et al., 2004

Highlights

- Cyclodextrins can form non covalent inclusion complexes by molecular encapsulation
- Thermodynamic parameters account for a spontaneous and exothermic inclusion process
- Isotherms shape confirm the displacement of cyclodextrin water molecules by the ligand
- Cyclodextrins allow enhancing solubility and stability of a wide range of compounds
- They are widely used in many pharmaceutical and food industrial products

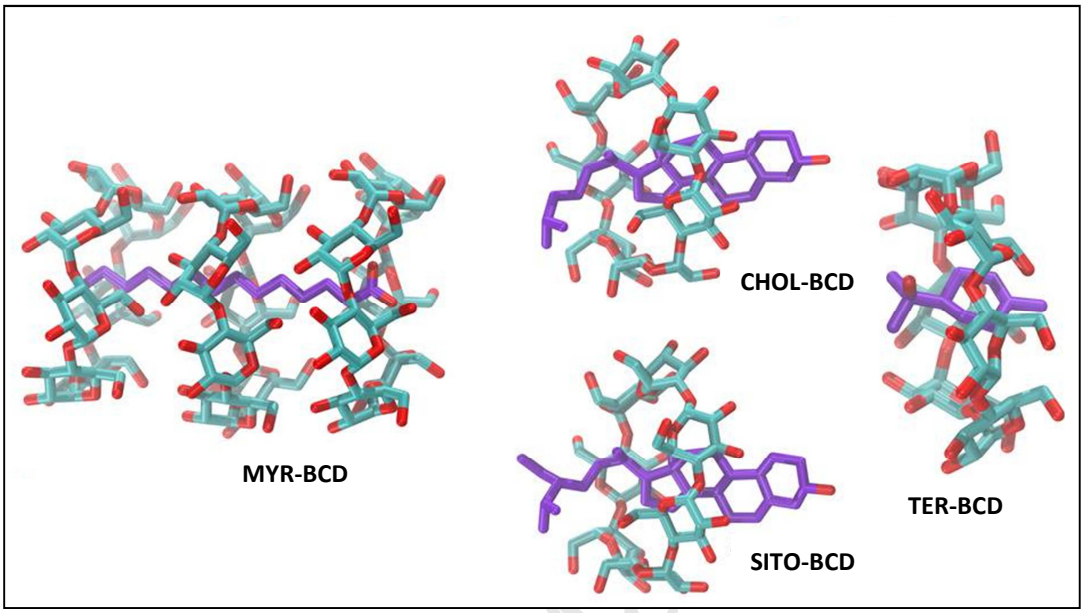


Figure 3

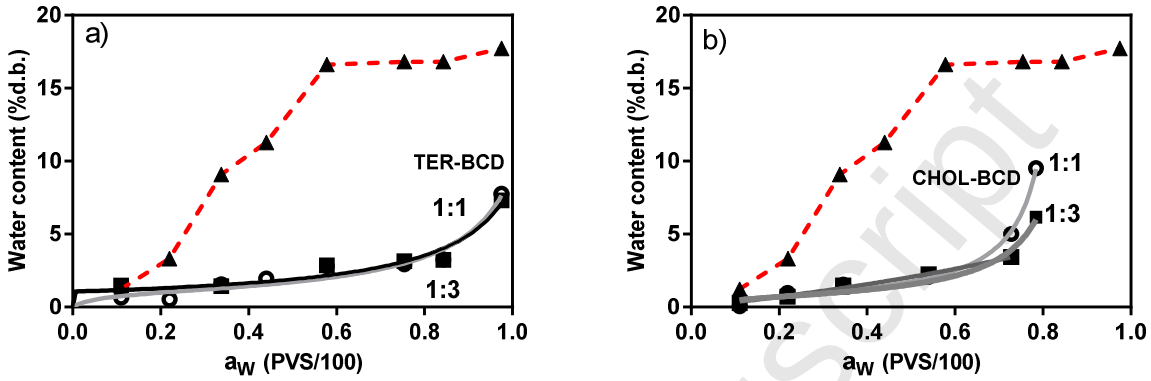


Figure 2

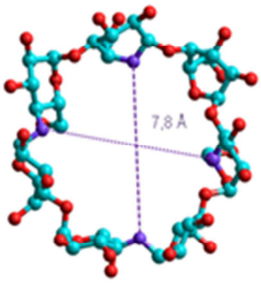
a)

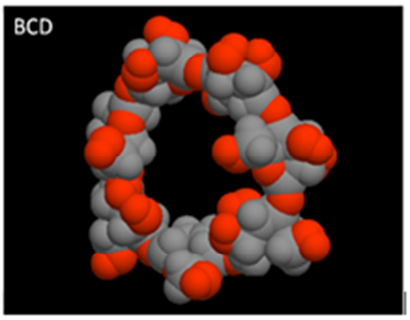
Chemical structure with interatomic distances

PSA: Surface polar area
Red: polar Grey: non polar

BCD

- **Volume= 926 A^3**
- **log P= -6,33**
- **TPSA= 554 A^2**





b)