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Influence of ligand structure and water interactions on the physical properties of β-cyclodextrins complexes

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ABSTRACT

The water sorption and physical properties of freeze-dried β-cyclodextrin (BCD) and 2-hydroxypropyl-β-cyclodextrin (HBCD) were studied. The stability of the inclusion complexes of these cyclodextrins with different hydrophobic ingredients, such as myristic acid and α-terpineol, was investigated as a function of the storage time and water content of the systems. Besides increasing its solubility, BCD ring modification with hydroxypropyl groups conferred amorficity to the dehydrated matrices, and modified the sorption properties and their ability to form hydrates.

Both ligands decreased BCD and HBCD water adsorption, in comparison with the pure cyclodextrins. The water adsorption data and glass transition values obtained are consistent with the displacement of water molecules from the inner cavity of the CDs when the ligand is included. Encapsulation of non-polar ligands of linear hydrocarbon chain, like myristic acid, was initially incomplete, depending on the ligand/CD ratio, and increased with the time of storage and water content.

1. Introduction

Consumers' negative perception towards chemicals added to food, cosmetics and other household products has induced the companies to direct their attention towards compounds of natural origin (Adams, Demyttenaere, & Kimpe, 2003). α-Terpineol and myristic acid, constituents of essential oils, are good models of natural additives used both in food or pharmaceuticals. Myristic acid is a common saturated fatty acid, which has a long 13-carbon linear hydrocarbon chain terminated with a carboxylic acid moiety. In the food industry, myristic acid is approved for food uses by FDA, EC, FEMA, JECFA, and CoE (Burdock & Carabin, 2007) as a multipurpose food additive, as flavour adjuvant, defoaming agent, as cosmetic additive and coating of fresh citrus fruits (Becker et al., 2010). α-Terpineol is a naturally occurring cyclic monoterpene alcohol constituent of essential oils of many types of plants and flowers. Besides its usefulness for perfumes and cosmetics, it could be used as antifungal agent (Pitarokili, Couladis, Petsikos-Panayotarou, & Tzakou, 2002) and as ingredient in inhalants/decongestants pharmaceutical products (Tisserand & Balacs, 1995). Currently, research has been undertaken to show that α-terpineol, possess antitumour activity (Hassan, Gali-Muhtasib, Göransson, & Larsson, 2010). A limiting factor to the use of these hydrophobic natural food additives is their low stability, and/or their very low aqueous solubility (Fichan, Larroche, & Gros, 1999). The formation of inclusion complexes with cyclodextrins (CDs) may increase the solubility and stability by limiting the degradation or loss during processing and storage (Bhandari, D'Arcy, & Young, 2001; Karathanos, Mourtzinos, Yannakopoulou, & Andrikopoulos, 2007; Loftsson & Brewster, 1996). However, the application of CDs in the pharmaceutical and food ingredients development is limited by their own low aqueous solubility. Thus, numerous chemically modified CDs have been developed to counter the solubility limits by improving the hydrophilic/hydrophobic properties and the axial length of the cavity (Gould & Scott, 2005; Szejtli, 1998). 2-Hydroxypropyl-β-cyclodextrin (HBCD), a hydroxyalkyl derivative, is an alternative to α-, β-, and γ-CDs with enhanced water solubility (Gould & Scott, 2005). The modification of natural (parent) CDs generally converts them into amorphous, non-crystallisable derivatives, to provide high CD concentration in aqueous solutions (Szente & Szejtli, 1999). Moreover, the substitution pattern affects the stability of inclusion complexes too. It seems that both the substitution degree and the substitution pattern influence the stereo-specificity of HBCDs (Buvári-Barcza & Barcza, 1999). However, there are very few studies focusing on the effect of the substitution pattern of HBCDs on the formation and stability of inclusion complexes (Chao, Zhengyu, & Xuehong, 2008). On the other side, many studies and patents account for the use of CDs for drug and additives solubilisation, but there is a lack of information on the effect of relative humidity on the stability of inclusion complexes with CDs. The objective of this

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work was to analyse the water sorption and thermal properties of β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin, as affected by the relative humidity. The stability of the inclusion complexes of these cyclodextrins with the myristic acid and α-terpineol, two hydrophobic compounds of different structure was investigated in relation to the water content of the systems.

2. Materials and methods

2.1. Materials

β-Cyclodextrin (BCD) (containing 8 water molecules/molecule of BCD, Mr. 1135), and 2-hydroxypropyl-β-cyclodextrin (HBCD) (degree of substitution 0.6, Mr. 1380) were from Sigma Chemical Co. (St. Louis, MO, USA). The model systems selected in this work were a saturated carboxylic acid, myristic acid (Myr) from Sigma Chemical Co. (CAS No. 544-63-8) and a terpenic alcohol, α-terpineol (Terp), from Polymetron S.R.L, Germany. All other chemicals were of analytical grade and purchased from Mallinckrodt Chemical Works (St. Louis, MO, USA).

2.2. Preparation of the solid inclusion complexes

Inclusion complexes of BCD with α-terpineol or myristic acid as guests molecules (Terp/BCD or Myr/BCD respectively) were prepared by the coprecipitation method (Karathanos et al., 2007). Solutions of BCD (1.85 g/100 ml) were prepared and heated at 50 °C shaking until complete solubilisation of the CD (which took about 30 min). Terp or Myr were dispersed in the BCD aqueous solution in suitable proportions for Terp/BCD molar ratios 1:1 and 1:3 and for Myr/BCD molar ratios 1:1, 1:2, and 1:3. Ligands, such as terpenes (Choi, Soonttitantawat, Nuchuchua, & Min, 2009), and flavonoids (Tommasini et al., 2004) that are completely encapsulated in the cavity of the CDs, form complexes at molar ratio 1:1. Other ligands, such as fatty acids, are not completely encapsulated and the stable inclusion complex with CD would be obtained with 1:3 M ratios (Regiert, 2007). We thus chose different ratios to evaluate the degree of encapsulation. The systems were stirred at a constant rate for 3 h at 50 °C and for 3 h at room temperature. The obtained solutions were then stored overnight at 3 °C to promote the precipitation of the complexes. The suspensions were filtered (PTFE filters of 0.45 μm average pore diameter) and the filtrates frozen at −26 °C for 24 h and freeze-dried in a Heto Holten A/S freeze-dryer (operating at a condenser plate temperature of −111 °C, chamber pressure of 30 Pa, and shelf temperature of 25 °C). The secondary drying was also performed at 25 °C.

Inclusion complexes of HBCD with Terp and Myr were prepared by the freeze-drying method with molar ratios Terp/HBCD 1:1 and 1:3, and Myr/HBCD 1:1, 1:2, and 1:3. In this method, the aqueous solution of HBCD (10.0 g/100 ml) containing the ligand was stirred for 3 h at 50 °C, cooled to ambient temperature and then frozen at −26 °C for 24 h and freeze-dried.

Once dehydrated, the systems were equilibrated to different relative humidities (RH) and the water sorption isotherms, glass transition, and melting events in the samples were studied. Since the employed BCD was obtained as an octahydrate, it was dried in a vacuum oven (at 90 °C for 48 h) up to water content smaller than 3% dry basis (d.b.), before the experiments.

2.3. Determination of the water sorption isotherms

Sorption isotherms were determined by the standard isopiestic static-gravimetric method. After freeze-drying, samples of HBCD, BCD or their complexes with Terp or Myr were distributed into glass 5 ml vials (around 200 mg/vial) and placed into vacuum desiccators containing saturated salt solutions which provide different relative humidities (RH): LiCl, $KCOOCH_3{\cdot}5H_2O$, $MgCl_2{\cdot}6H_2O$, $K_2CO_3{\cdot}2H_2O$, $NaBr{\cdot}2H_2O$, NaCl, KCl, and K_2SO_4 for 11, 22, 33, 43, 57, 75, 84, and 97% RH at 25 °C ± 1 °C respectively (Greenspan, 1977).

The water content of the samples was determined as a function of storage time until reaching the equilibrium condition (mass differences lower than 0.0005 g). The longer time to reach this condition of constant mass was 2 weeks.

2.4. Determination of water content

The total water content of the samples was determined by difference in weight before and after drying in a vacuum oven (Fistreem OVA031, UK) at 96 °C ± 2 °C during 48 h. These drying conditions had been proved to be adequate to assess constant weight after drying and were selected from previous studies. The determinations were performed in triplicate and the average value was reported. The calculated confidence interval for a 95% certainty was between 3% and 4% of the absolute values. Results were express in % d.b.

2.5. Differential scanning calorimeter (DSC)

DSC was used to verify the formation of complexes in the solid state, the release of the guest molecule during storage and to determine the glass transition temperature (T_g) of the analysed systems (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009; Mourtzinos, Kalogeropoulos, Papadakis, Konstantinou, & Karatahanos, 2008).

A DSC, Mettler-Toledo equipment model 822 (Mettler Toledo AG, Switzerland), with a STARe Thermal Analysis System version 3.1 software (Mettler Toledo AG), was used for all the measurements. The instrument was calibrated with indium and zinc. All measurements were made at 10 °C/min, using hermetically sealed aluminium pans (Mettler, 40 μl capacity), and an empty pan was used as a reference. The confidence interval estimated for temperature values was 1 °C. An average value of three replicate samples was reported for each measurement.

The dynamic method was used to determine melting points (T_m) and heats of fusion (ΔH_m), expressed in J/g of the ligands (pure and in the complexes). The confidence interval estimated for the enthalpy values was 10 mJ. The temperature range of melting for Terp was between 34 °C and 42 °C, being the melting peak at 37 °C (Merck Index, 2006); T_m for Myr was between 48 °C and 56 °C with the melting peak at 52 °C (Food Chemical Codex, 1996). Each sample was heated at a rate of 10 °C/min from −20 °C up to 110 °C.

The percentage of free ligand (Myr or Terp), either that non-encapsulated during complex preparation or that released during storage was determined as the ratio of the fusion enthalpy of Terp or Myr in the system at time t, and the fusion enthalpy of the pure compound (180 J/g for Myr and 91 J/g for Terp, both measured in the same conditions of the samples). Then, the percentage of encapsulated ligand was expressed as indicated in the following equation (Mourtzinos et al., 2008):

$$\text{Encapsulated ligand } \% = 100^{*}\left(1-\frac{\Delta H_L}{\Delta H_0}\right) \quad (1)$$

where ΔH_L is the heat of melting of the ligand free in the systems and ΔH_0 is the heat of melting of the pure compound (Terp or Myr). Since the boiling point of Terp is 218 °C and of Myr 326 °C (Merck Index, 2006) the losses due to evaporation were considered negligible. The high melting enthalpy values of the employed ligands make the DSC method very sensitive.

The determinations were carried out in triplicate for each sample and the average value was reported. The calculated confidence