Carbohydrate Research 345 (2010) 2085–2089

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

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Dissociation characteristic of the inclusion complex of cyclomaltohexaose (α-cyclodextrin) with 1-methylcyclopropene in response to stepwise rising relative humidity

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ARTICLE INFO

Article history:
Received 2 April 2010
Received in revised form 24 June 2010
Accepted 6 July 2010
Available online 4 August 2010

Keywords:
Cyclomaltohexaose
α-Cyclodextrin
1-Methylcyclopropene
Relative humidity
Dissociation

ABSTRACT

The dissociation of a crystalline complex of cyclomaltohexaose (α-cyclodextrin) and 1-methylcyclopropene has been studied in response to stepwise rising relative humidity at 50 °C using a dynamic vapor sorption instrument. The dissociation of the inclusion complex was monitored with a proton transfer reaction mass spectrometer. The increase in relative humidity generally triggered the complex dissociation. However, the dissociation was greatly retarded at 80% relative humidity, presumably owing to collapse of the crystalline structure. Abrupt dissociation was observed at 90% relative humidity which corresponded to complex dissolution. The changes in powder X-ray diffraction pattern of the inclusion complex during the storage period were also investigated.

Cyclomalto-oligosaccharides, more commonly known as cyclodextrins (CDs), are natural starch derivatives synthesized via degradation of starch catalyzed by cyclodextrin glycosyltransferase (CGTase). CDs make up a family of macrocyclic polymers that consist of α-D-glucose units linked by α-(1,4)-glycosidic bonds. Typical CDs comprise six to eight α-D-glucose monomers and are denoted as α-, β-, and γ-CD, respectively. Most of the industrial and pharmaceutical applications of CDs harness their ability to trap appropriately sized molecules within their relatively hydrophobic cavities resulting from the truncated molecular structure. There are a number of reviews on the application of CDs available covering different fields such as food,[1,2] agriculture,[2] cosmetics,[3] pharmaceutics,[4,5] artificial enzymes,[6] and water treatment.[7]

1-Methylcyclopropene (1-MCP) is an olefin compound consisting of a three-membered ring with a methyl group at the C-1 position. It can effectively inhibit ethylene perception in plants and thereby delay ethylene-driven responses, for instance fruit ripening and senescence. Recently, Schotsmans et al. published a review on articles that elucidate the physiological processes affected by 1-MCP and the mode of action of 1-MCP on the ethylene response pathway.[8] Food use registration of 1-MCP has been obtained in many countries for a wide variety of crops and produce.[9] 1-MCP has been continually attracting research interests because of its effective, non-toxic inhibition[10] of ethylene perception at extremely low concentrations over an extended period of time.[11] 1-MCP-related research could be categorized into two groups: one includes those that study the use of 1-MCP as a tool for investigation of the role of ethylene in plant physiology,[12–14] and the other covers the studies on 1-MCP-based technology for improvement of quality maintenance of agricultural products.[15–17]

1-MCP is a chemically unstable gaseous compound at ambient conditions that becomes an explosive hazard when compressed.[18] Inclusion complexation with α-CD greatly reduces explosiveness and stabilizes 1-MCP in the solid form which concomitantly eases its handling. In view of the fact that complex dissociation is an important aspect of an inclusion complex, we have kinetically characterized the solid-state thermal dissociation of the 1-MCP/α-CD complex.[19] Temperature and humidity are the two main extrinsic factors that influence the storage stability of an inclusion complex. The interaction of water with powdered materials is a major factor in formulation, processing, and product performance of solid dosage forms.[20] Rapid recrystallization of α- and γ-CD from aqueous solution has been reported by Hunt et al. to result in formation of the unstable columnar crystalline structure that transforms gradually into the relatively stable cage structure as a consequence of exposure to humidity.[21,22] During the preparation process of crystalline 1-MCP/α-CD complex, the complex crystallized seemingly upon its formation due to low aqueous solubility.[18] Water sorption may play an integral role in the crystalline

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0008-6215/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2010.07.006

structure and thus the stability of the complex. In the present study, we investigated the dissociation of crystalline 1-MCP/α-CD complex in response to stepwise rising relative humidity at a constant temperature of 50 °C.

The increase in relative humidity prompted the dissociation of 1-MCP/α-CD complex as shown in Figure 1. The inclusion ratio of 1-MCP/α-CD complex was determined by gas chromatography as described in our previous publication[18] to be 0.98 mol 1-MCP/mol α-CD, indicating that the inclusion complex initially contained approximately 5.2% of 1-MCP on weight basis. By integrating the data obtained from the experiments on dynamic complex dissociation (Fig. 1), an average of almost one fifth (≈17.6%) of the total amount of complexed 1-MCP was dissociated at the end of the experiments. Abrupt dissociation was observed particularly at 60% and 70% relative humidity (RH). This phenomenon may have resulted from the occurrence of capillary condensation in the pores or capillaries formed between adjacent complex crystals,[23] which was presumably reflected by the sharp rise in weight of the inclusion complex attributed to the rapid sorption of moisture at 60% and 70% RH (Fig. 2). Plausibly, the condensation at the surface of the complex crystals at 60% and 70% RH first led to surface dissolution of the inclusion complex that increased the mobility of the encapsulated 1-MCP, resulting in accelerated dissociation. A phenomenal retardation of the dissociation reaction was observed at 80% RH, which was indicative of the collapse behavior of the crystalline structure of the inclusion complex. Upon structural collapse, the diffusion distance of 1-MCP to the surface of the agglomerates increased thus retarding the apparent dissociation of the inclusion complex. Hence, the humidity level for initiation of structural collapse was determined to be 80% RH. Subsequently, a burst of dissociation was recorded at 90% RH, which was morphologically observed as dissolution of the complex crystals (Fig. 3g). Billings et al. showed that initiation of caking of bulk sucrose occurs at 80% RH, which agrees with the prediction by the Kelvin equation.[23] They also observed liquefaction at the surface of the sucrose above 94% RH. The Kelvin equation, describing the relationship between the size of capillary, the wetting angle, and surface tension,[23] can be used to calculate the capillary radius for any point on a general isotherm for adsorption on mesoporous materials (type IV isotherms).[24] According to capillary condensation hypothesis, all the capillaries of smaller radii than the Kelvin radius will be filled with

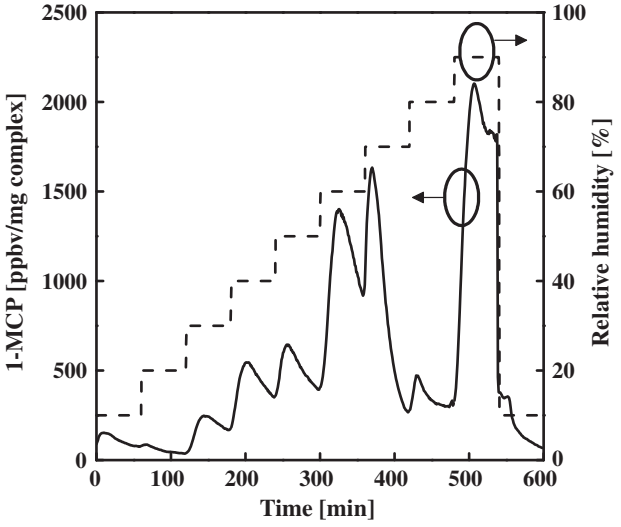

Figure 1. Typical real time dissociation profile of a 1-MCP/α-CD complex when subjected to stepwise increases in RH (10–90% RH, 10% increase in RH each hour, 50 °C). The solid line is the 1-MCP concentration in the effluent from the DVS and the dashed line represents the relative humidity in the DVS.

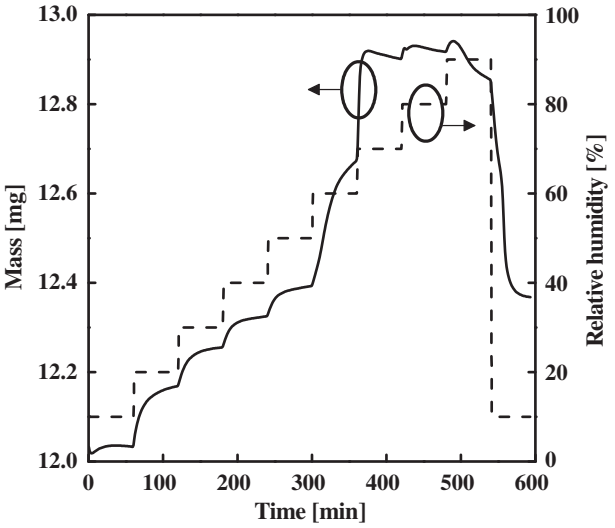

Figure 2. Typical real time weight change profile of 1-MCP/α-CD complex when subjected to stepwise increases in RH (10–90% RH, 10% increase in RH each hour, 50 °C). The solid line represents the weight change by the inclusion complex and the dashed line represents the relative humidity in the DVS.

water or solution of the tested material at the given pressure. Deriving from the Kelvin equation, the Kelvin radius, r_K is given by:

$$r_K = -\frac{2\sigma\cos\theta V_0}{(\ln\frac{P_v}{P_w})R(T+273.15)} \quad (1)$$

where V_0 [m^3/mol] and σ [N/m] are the molar volume and surface tension, respectively, of water or the saturated solution of the material, θ [−] the wetting angle, P_v [Pa] the water vapor pressure of the material, P_w [Pa] the saturated vapor pressure of pure water at absolute temperature, and R [J/K mol] the universal gas constant.

Sutter et al. related the decrease in the degradation rate of β-carotene encapsulated in freeze-dried mannitol/KH_2PO_4 powder to reduced oxygen diffusion from the surface to the inside of the powder due to the collapse of the amorphous matrix at 75% RH, 25 °C.[25] Soottitantawat et al.[26] and Dronen[27] also reported an increase in the release rate of encapsulated D-limonene with increasing water activity from several spray-dried matrices. The subsequent sharp decrease in the release rate was attributed to the glass transition and collapse of the matrices, after which the release rate increased with further increase in water activity.

In amorphous materials, water activity and glass transition temperature are the two main factors to be necessarily taken into account when dealing with the phenomena of bridging between particles and caking. It is noteworthy that the collapse phenomenon in amorphous materials is different from that of crystalline matrices, such as the 1-MCP/α-CD complex in this case. In crystalline materials, the adsorption of water at humidities below a critical RH will normally result in at the most 3–5 layers of water on the surface, which will not have the properties of bulk water and will generally not cause dissolution of the surface of the material.[28–30] However, certain crystalline materials become deliquescent in atmospheric water vapor above the critical RH.[30] From a bulk-phase thermodynamic perspective, the dissolution of a crystalline solid into its sorbed moisture would not be expected to occur below its critical RH. But capillary condensation of water vapor due to the Kelvin effect may always pose a threat to the stability of the crystalline material even at RH below the critical value. Kontny et al. proposed that relatively low levels of water vapor sorption on crystalline water-soluble solids at humidities below the critical RH could give rise to some form of 'surface dissolution' when there existed some subtle states of surface disorder brought

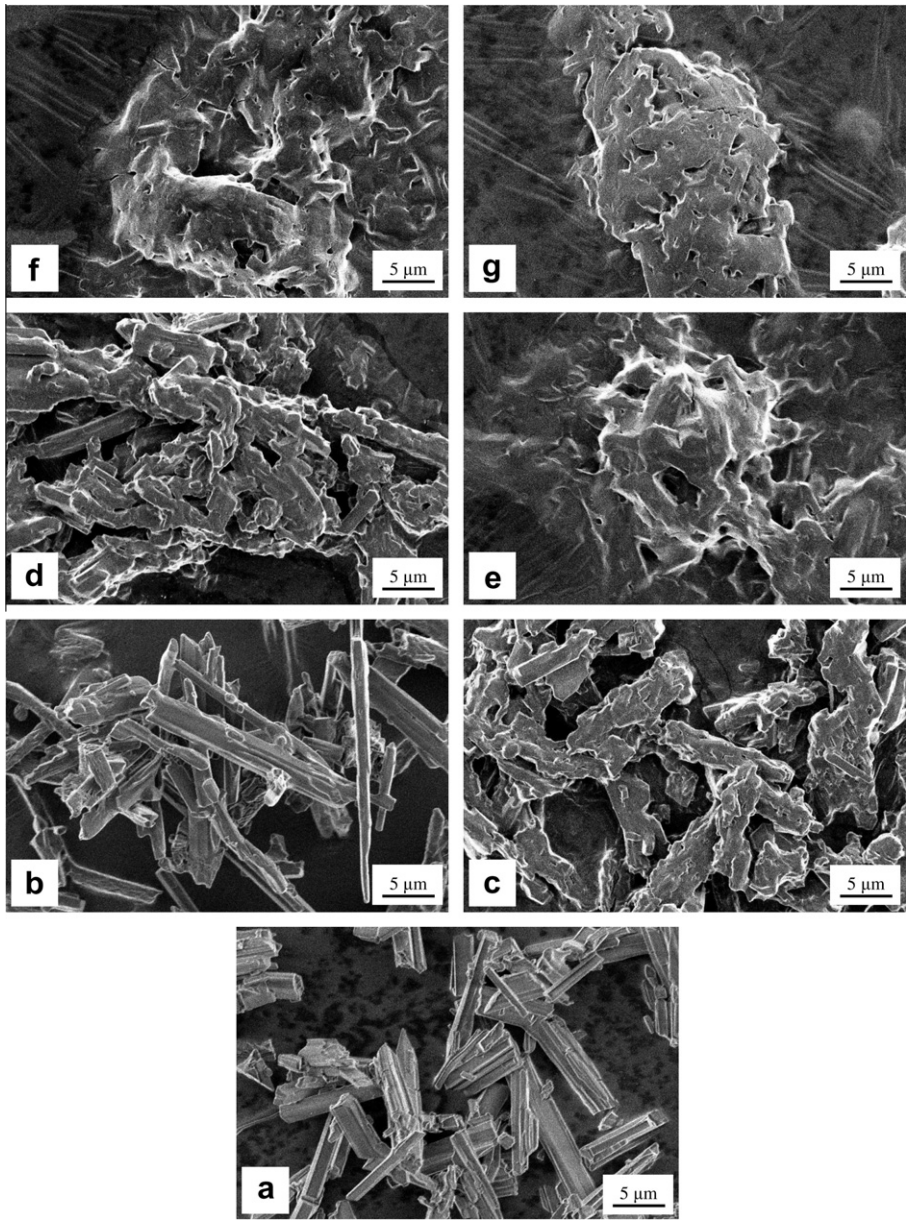

Figure 3. Scanning electron micrographs of 1-MCP/α-CD complexes at 3000-fold magnification before (a) and after exposure to stepwise increases in relative humidity from 40% (b) to 50% (c), 60% (d), 70% (e), 80% (f), and 90% RH (g).

about by various forms of mechanical disturbances.[29] Cleaver and Wong revealed a decrease in surface roughness of boric acid crystals with increasing RH caused by dissolution of submicron surface features and subsequent crystal growth on the flatter regions.[31] Mechanical pretreatments, for example grinding, may introduce some submicron features onto the surface of crystals which spur capillary condensation and thereafter surface restructuring. In the present study, the inclusion complex had not been subjected to any form of mechanical pretreatment and thus the surface dissolution of the inclusion complex at 60% RH might plausibly be attributed to capillary condensation that occurred in the pores formed between adjoining complex crystals.

As been mentioned earlier, an overall dissociation of 1-MCP/α-CD complex contributed to a final average weight loss of ca. 0.9%. Meanwhile, despite being negated by the weight loss caused by dissociation of 1-MCP, the inclusion complex recorded an apparent maximum weight increase of 7.8% on average, implying that moisture sorption was the factor dominating the weight change. The slight decrease in weight at 90% RH may be related to the significant loss of 1-MCP from the dissolved complex crystals in addition to the plateauing of moisture sorption.

There was a good agreement between the complex dissociation and the morphological changes of the crystalline inclusion complex. The scanning electron micrographs of the inclusion complexes (Fig. 3) showed that morphological changes took place with increasing storage relative humidity in the order of surface dissolution (60% RH), structural collapse (80% RH), and complex dissolution (90% RH).

The general observation from Figure 4 is that the increase in storage relative humidity effected crystallinity changes in the inclusion complex as reflected in the powder X-ray diffraction (PXRD) patterns. At higher humidities (80% and 90% RH), the reflections at high angles (2θ >13.5°) decreased in intensity and broadened, suggesting an appreciable loss of crystallinity. It is well-known that the channel-type packing structure of the inclusion complexes of long guest molecules with α-CD could be character-

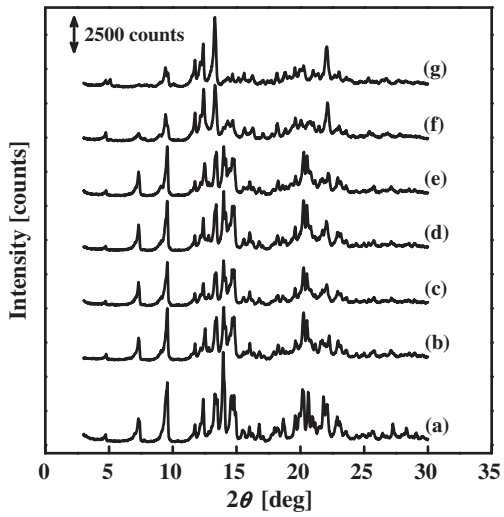

Figure 4. PXRD patterns of 1-MCP/α-CD complexes before (a) and after exposure to stepwise increases in relative humidity from 40% (b) to 50% (c), 60% (d), 70% (e), 80% (f), and 90% RH (g).

ized by a peak at 2θ of ca. 20° in the PXRD patterns.[32,33] During the encapsulation process, the 1-MCP/α-CD complex presumably crystallizes to form a channel-type packing structure which changes at humidities of 80% and 90% RH (Fig. 4), possibly due to increased molecular mobility and complex dissociation. Relative humidity of storage atmosphere was found to significantly affect the stability of the inclusion complex and therefore should be kept as low as possible and never exceed 60% RH within the range of storage temperature.

1. Experimental

1.1. General procedure

Pharmaceutical grade α-CD (Cavamax W6 Pharma) from Wacker Chemie AG (Munich, Germany) was purchased from Cyclochem Co., Ltd (Hyogo, Japan). The α-CD was dried in vacuo at 90 °C for 24 h. Unless otherwise indicated, all the chemicals used were of reagent grade. Distilled water was used throughout the experiments.

1.2. Preparation of inclusion complex

The guest compound, 1-MCP, was synthesized according to the procedure detailed in our previous publications[18,19] based on the method reported by Sisler and Serek[34] with some minor modifications. The complexation reaction between 1-MCP and α-CD was performed as described previously.[18,19] The wet precipitate of the inclusion complex recovered from the reaction was dried in vacuo at room temperature for 24 h.

1.3. Measurement of dynamic complex dissociation by DVS–PTR-MS

A dynamic vapor sorption or DVS instrument (DVS-1, Surface Measurement Systems, Ltd, Middlesex, UK) was utilized to control temperature and stepwise increase the relative humidity. Approximately 10–25 mg of sample was spread in a thin layer onto a flat bottomed, 16 mm diameter, quartz sample pan. The sample chamber was maintained at 50 °C and the flow rate of air over the sample was 31 mL/min with a response time of 3–4 min. The relative humidity of the sample chamber was programed to increase stepwise from 10–90% RH (10% RH increase at each step). Each humidity step was held for 60 min (nine steps totaling 9 h of sampling).

A proton transfer reaction mass spectrometer (PTR-MS) (Ionicon Analytik, Innsbruck, Austria) was utilized to monitor release of 1-MCP from the inclusion complex. Effluent from the DVS was introduced into the PTR via a heated (60 °C) copper transfer line. Three ion masses were measured during sampling: 21 ($H_3{}^{18}O^+$), 37 ($H_3O^+{\cdot}H_2O$) and 55 (1-MCP + H^+: 54 + 1 = 55). For 1-MCP (M_w = 54.1), ion 55 was found to be the most intense ion formed from 1-MCP during preliminary testing. Ions 21 and 37 were monitored as ionization indicators (reagent ions). Data points were taken every 60 s (one cycle), thus 60 cycles corresponded to a humidity step. DVS effluent was monitored during the 9-h run. The inclusion complex and pure α-CD as the blank were run in duplicate. The PTR-MS ion counts were converted into 1-MCP concentrations in parts per billion on volume basis [ppbv] and further corrected into parts per billion 1-MCP per mg of inclusion complex loaded into the DVS [ppbv/mg complex].

1.4. Powder X-ray diffraction (PXRD) analysis and scanning electron microscopy (SEM)

The cyclodextrin complex crystals were lightly packed into a standard glass sample holder. Subsequently, the top surface of the sample was smoothed using a glass microscope slide. Twelve loaded sample holders were prepared and placed in a bench-top temperature/humidity chamber (LHU-113, ESPEC Corp., Osaka, Japan) at 50 °C with a stepwise rise in relative humidity identical to that of the DVS, but commencing from 40% RH. Two sample holders were withdrawn from the chamber for duplicate PXRD analysis after every humidity step (60 min after changing RH). The PXRD patterns of the inclusion complexes were recorded on a RINT-TTR III X-ray diffractometer (Rigaku, Tokyo, Japan) with Cu Kα radiation (λ = 0.154 nm) generated at 300 mA and 50 kV. The samples were scanned at a scanning rate of 4°/min from 3° to 30° (2θ) by a sampling width of 0.02°. The divergence slit was set at 1.00 mm and the receiving slit was set open.

For SEM, the complex crystals were sprinkled onto a double-sided adhesive carbon tape (Nisshin EM Corporation, Tokyo, Japan) placed on a SEM stub. A total of twelve stubs were prepared. Similarly, the stubs were placed in the chamber and two stubs were removed after every humidity step for microscopic observation of the morphological changes resulting from an increase in relative humidity. A JEOL JSM-6060 scanning electron microscope (JEOL Ltd, Tokyo, Japan) was used for this purpose. The inclusion complexes on the stubs were coated with a thin sputtered Pt–Pd layer using a magnetron sputter coater (Model MSP-1S, Vacuum Device Inc., Tokyo, Japan). The coated samples were analyzed at an operating voltage of 2.0 kV and a spot sized of 30.

Acknowledgments

One of the authors, T.L.N., acknowledges the Japan Society for the Promotion of Science for the grant for JSPS Postdoctoral Fellowship for Foreign Researchers (No. P09113). This study is financially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (21·09113).

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