Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 64 (2006) 212–217
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Processes of dehydration and rehydration of raffinose pentahydrate investigated by thermal analysis and FT-IR/DSC microscopic system

Wen-Ting Cheng, Shan-Yang Lin *

Department of Medical Research and Education, Taipei Veterans General Hospital, Shih-pai, Taipei 11217, Taiwan ROC

Received 10 September 2005; received in revised form 2 November 2005; accepted 11 November 2005
Available online 20 December 2005

Abstract

This study deals with the investigation of dehydration or rehydration process of raffinose pentahydrate by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and Fourier transform infrared (FT-IR) microspectroscopy equipped with thermal analyzer. Raffinose pentahydrate was compressed on one KBr pellet (1 KBr method) or sealed within two KBr pellets (2 KBr method) for FT-IR determination. Thermogram results from DSC indicate that three endothermic peaks at 56, 73 and 85 °C were observed, likely corresponding to the loss of one, two, and 2 mol of water respectively, and corresponding to specific weight loss in the TGA curves. The total weight loss during TGA analysis was about 15.43% and was almost equal to the loss of 5 mol of water from raffinose pentahydrate. The thermal-dependent FT-IR spectra for raffinose pentahydrate revealed that the peak intensity at 1651 cm^{-1} was reduced gradually with temperature up to 50 °C, decreased significantly above 50 °C, and disappeared completely above 100 °C, due to the evaporation of water. The peak at 1651 cm^{-1} was shifted to 1639 cm^{-1} from 48 °C for 2 KBr sample and maintained its peak position even heating to 150 °C. The former peak was assigned to the scissoring vibration mode of the crystal water in raffinose pentahydrate, the latter peak was due to the free liquid water dehydrated from the raffinose pentahydrate but which was still sealed within two KBr pellets. The 62 °C-preheated raffinose sample could rehydrate to pentahydrate under 30 °C, 70%RH isothermal condition for 180 min via shifting the IR peak from 1645 to 1651 cm^{-1} with time. Once three water molecules were dehydrated from raffinose pentahydrate by preheating sample above 81 °C, its rehydration process seemed to be difficult. The extent of dehydration for raffinose pentahydrate might play a key role to influence the process of rehydration for the preheated raffinose samples.

Keywords: Raffinose pentahydrate; Dehydration; Rehydration; DSC; TGA; FT-IR/DSC

1. Introduction

In the development of biopharmaceutical products, sugars or saccharides are often added to formulations to improve the stability and increase the long-term storage of biomolecules (Imamura, Ogawa, Sakiyama, & Nakanishi, 2003; Roy & Gupta, 2004). The stabilization of biomolecules influenced by sugars, particularly in the dehydration process of freeze-drying, has been proposed by two major hypotheses, the water substitution hypothesis and the glass state theory (Crowe, Carpenter, & Crowe, 1998; Franks, 2003). It has been reported that the stabilizing effect of sugars varies with the type, amount and hydration state of the particular sugar (Imamura et al., 2003; Izutsu, Aoyagi, & Kojima, 2004; Liao, Brown, Quader, & Martin, 2002). Thus, it is important to better understand the physicochemical and thermodynamic properties of sugar used.

Raffinose is an oligosaccharide in which galactose is connected to the glucosyl group of sucrose via α-1, 6 linkage. Raffinose possesses a pentahydrate in its molecular structure that is 5 mol of water per 1 mol of raffinose. Three water molecules have been reported to locate in a tunnel of the raffinose structure, while two other water molecules are situated outside the tunnel (Jeffrey & Huang, 1990; Saleki-Gerhardt, Stowell, Byrn, & Zografi, 1995). The hydrogen bonding between water molecules and raffinose in the molecular structure is complex. Recently, it is of interest as a potential excipient in stabilizing biomolecules, since its specific stabilizing properties are reported to be superior to other sugars such as lactose, maltose and sucrose (Chatterjee, Shalaev, & Suryanarayanan, 2005; Davidson & Sun, 2001; Tsuzuki, Imamura, Yamamoto, Satoh, & Okazaki, 1997).

Raffinose has been used as a pharmaceutically acceptable carrier or stabilizer for pharmaceutics and biopharmaceutics, a cryoprotectant for biopharmaceutics, and a predominant

* Corresponding author. Tel.: +886 2 2875 7397; fax: +886 2 2873 7200.
E-mail address: sylin@vghtpe.gov.tw (S.-Y. Lin).

0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.11.024

component of preservation solution for biological materials or organs in clinical transplantation (Fischer et al., 2001; Hincha, Zuther, & Heyer, 2003; Patton, Foster, & Platz, 2004). Raffinose has been reported to be the most potentially strong lyoprotectant, since it may form the most fragile glass in the amorphous state (Chatterjee et al., 2005; Kajiwara, Franks, Echlin, & Greer, 1999). Although, the recrystallization of freeze-dried raffinose might cause the loss of activity of biomolecules (Chatterjee et al., 2005). Although several studies have focused on investigation of the solid-state properties of raffinose (Chatterjee et al., 2005; Davidson & Sun, 2001; Saleki-Gerhardt et al., 1995), little is known about the detailed process of dehydration or rehydration of raffinose pentahydrate.

A novel microscopic Fourier transform infrared spectroscope equipped with differential scanning calorimetry (FT-IR/DSC) has been used in our previous studies (Lin, Li, & Wei, 2004; Wang, Lin, Chen, & Chuang, 2001; Wang, Lin, Chen, & Cheng, 2004) to simultaneously determine the correlation between the thermal response and the structural change of drugs, polymers, and proteins. This one-step synchronous operation is fast, simple, sensitive, precise and reproducible. Recently, we have successfully used the thermogravimetric analysis (TGA), DSC, and FT-IR/DSC microspectroscopy to investigate the polymorphic transition of trehalose dihydrate in the dehydration, rehydration, and solidification processes (Lin & Chien, 2003). This technique has clearly shown (1) the dehydration process from solid-like water to liquid water in the trehalose dihydrate structure, (2) the rehydration process from anhydrous trehalose to the dihydrate form and (3) solidification of the liquid water to solid-like water in the dihydrate structure.

The purpose of this study is to explore the thermal-dependent characteristics of raffinose pentahydrate in the process of dehydration or rehydration by using these thermal and vibrational spectroscopic techniques.

2. Materials and methods

2.1. Materials

Raffinose ($C_{18}H_{32}O_{16}$ $5H_2O$, R-0514) was purchased from Sigma Chemical Co. (St Louis, MO, USA) and used without further purification. The KBr crystals for the pellets were obtained from Jasco Parts Center (Jasco Co., Tokyo, Japan).

2.2. Thermal analysis

Thermal analysis of raffinose was carried out via differential scanning calorimetery, DSC, (DSC-910, TA Instruments Inc., New Castle, DE, USA) at a heating rate of 3 °C/min with an open pan system in a stream of N_2 gas. Thermogravimetric analysis, TGA, was also performed (using a TGA-951, TA Instruments Inc., New Castle, DE, USA) at the same heating rate to measure the weight loss of the sample. Several raffinose samples were respectively preheated to 62, 81 or 125 °C by DSC for isothermal 5 min and cooled to 30 °C, and then determined again from 30 to 150 °C by DSC or TGA.

2.3. Thermal FT-IR microspectroscopic study

A trace powder of raffinose was smeared on one piece of KBr pellet and then directly compressed with an IR spectrophotometric hydraulic press (Riken Seiki Co., Tokyo, Japan) under 200 kg/cm^2 for 15 s. Pressure was quickly removed for the '1 KBr method'. In the '2 KBr method', another powder sample of raffinose was smeared and sealed onto two pieces of KBr pellets by compression with 200 kg/cm^2 for 15 s, followed by quick release of pressure.

Each compressed KBr disc was placed onto a micro hot stage (DSC microscopy cell, FP 84, Mettler, Greifensee, Switzerland). The DSC microscopy cell was placed in an FT-IR microscopic spectrometer (Micro FT-IR-200, Jasco, Japan) with a mercury cadmium telluride (MCT) detector. The system was operated in transmission mode. The temperature of the DSC microscopy cell was moni-tored with a central processor (FP 80HT, Mettler, Switzerland). The heating rate of the DSC assembly was maintained at 3 °C/min under ambient conditions. Each sample disc was equilibrated to the starting temperature (30 °C) and then heated from 30 to 150 °C. The thermal-responsive IR spectra were recorded while the sample disc was heated on the DSC microscope stage.

Isothermal FT-IR microscopic time-scan measurement was also carried out. Raffinose prepared by the 1 KBr method was respectively isothermal at 62, 81, 125 or 150 °C for 5 min and cooled to 30 °C, and then time-scanned at 30 °C for 180 min. During the course of study, the sample disc was first equilibrated at 30 °C and then time-scanned. Humidity was maintained at 70% RH. The time-scanned IR spectra were recorded.

3. Results and discussion

Raffinose is a trisaccharide which has a pentahydrate form in the crystal structure. This is the highest hydrate known of any oligosaccharides.

3.1. DSC and TGA studies on the dehydration of raffinose pentahydrate

The DSC thermograms and TGA curves of raffinose pentahydrate determined under an open pan system are shown in Fig. 1. It clearly indicates that three endothermic peaks at 56, 73 and 85 °C were observed from the DSC thermogram of raffinose pentahydrate (Fig. 1(a-1)). Several stages of weight loss in TGA curves were also seen. The total weight loss was about 15.43%, which was almost equal to the loss of 5 mol of water from raffinose pentahydrate (molecular weight: 594.5) (Fig. 1(a-2)). In order to explore the relationship between the stage of weight loss in each TGA curve and a corresponding endothermic peak in DSC thermogram, the raffinose sample was respectively preheated to 62, 81 or 125

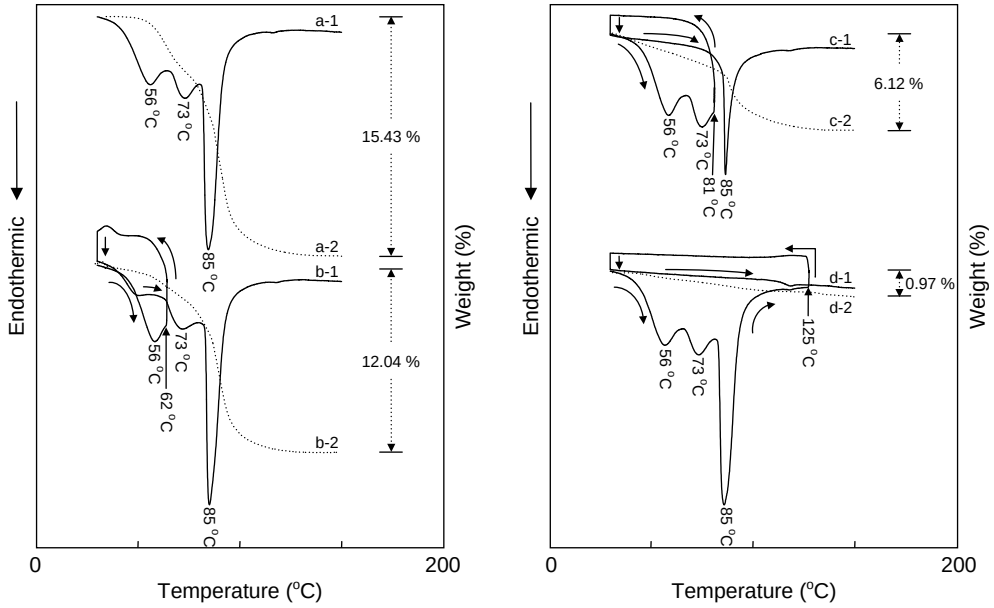


Fig. 1. DSC thermogram (solid line) and TGA curves (dash line) of raffinose pentahydrate. Key: (a-1) heating from 30 to 150 °C; (b-1) preheating to 62 °C for isothermal 5 min and cooled to 30 °C, then heating from 30 to 150 °C; (c-1) preheating to 81 °C for isothermal 5 min and cooled to 30 °C, then heating from 30 to 150 °C; (d-1) preheating to 125 °C for isothermal 5 min and cooled to 30 °C, then heating from 30 to 150 °C.

°C by DSC and cooled to 30 °C; and, measurements were then re-determined. Clearly, the endothermic peak at 56 °C disappeared from the second DSC thermogram, but two endothermic peaks at 72 and 85 °C still observed (Fig. 1(b-1)). Only 12.04% weight loss remained in the TGA curve, indicating an intact weight loss of 3.39% at this stage, which is approximately equal to the reduction of 1 mol of water (3.08%) from the sample (Fig. 1(b-2)). On the other hand, both

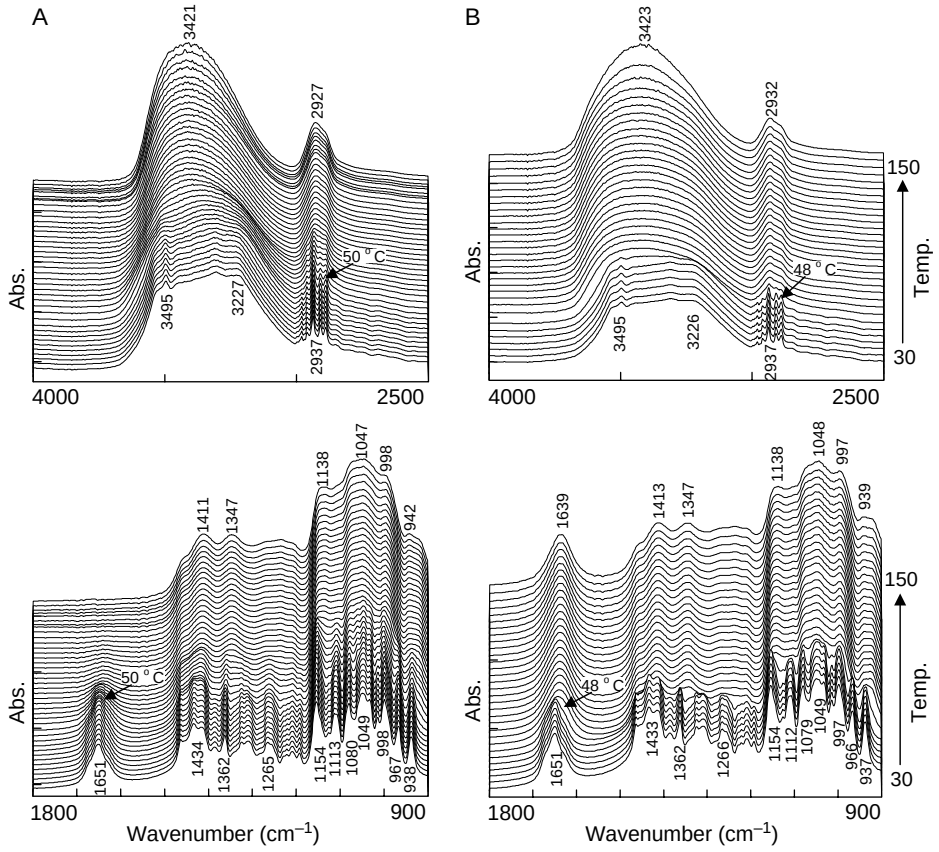
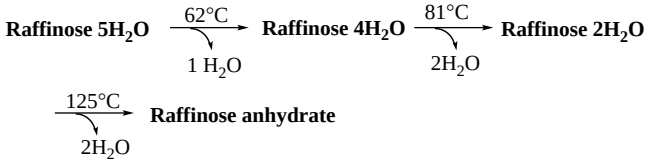


Fig. 2. Three-dimensional plots of thermal-dependent FT-IR spectra of raffinose pentahydrate prepared by 1 KBr (A) or 2 KBr (B) method within 4000–2500 cm^{-1} and 1800–900 cm^{-1}, as a function of temperature.

endothermic peaks at 56 and 73 °C disappeared from the second DSC thermogram, when the sample was preheated to 81 °C. Only one endothermic peak at 86 °C remained (Fig. 1(c-1)), with a weight loss of 6.12% (Fig. 1(c-2)). This means that 9.31% of intact water was lost from the 81 °C-preheated raffinose sample, which was almost equal to the weight loss of 3 mol of water (9.26%). When the raffinose sample was preheated to 125 °C, there was no endothermic peak remaining in the second DSC thermogram (Fig. 1(d-1)). The weight loss of 0.97% was obtained in the TGA curve (Fig. 1(d-2)), which might be due to the absorption of water from the atmosphere, when the 120 °C-preheated sample was cooled and transferred to TGA system. From the results of DSC thermograms and TGA curves, the process of dehydration for raffinose pentahydrate may be schemed as follows.



Jeffrey and Huang (1990) investigated the crystal structure of raffinose pentahydrate by single-crystal X-ray analysis, whereby three water molecules (waters 1, 2 and 4) per raffinose were located in a tunnel, but the other water molecules (waters 3 and 5) were situated outside the tunnel (Jeffrey & Huang, 1990; Saleki-Gerhardt et al., 1995). However, the hydrogen bonding between waters and raffinose seemed to be complex. According to their results, waters 2, 3 and 4 acted as acceptors/donors of hydrogen bonding. Waters 1 and 5 not only acted as hydrogen-bonding donors but also had longer and weaker hydrogen bonding than the other three water molecules. They proposed that waters 1 and 5 seemed most likely to be less tightly bound in the crystal structure, since only one hydrogen bonding directly interacted with raffinose. Although we do not know which water number was first dehydrated from the 62 °C-preheated sample in our DSC and TGA results, water 5 would seem to be the first candidate for dehydration due to its location and the previous report of relatively weak hydrogen bonding. When the raffinose sample was preheated to 81 °C, it is likely that three waters must be dehydrated, with two waters remaining in the sample. However, it is difficult to predict which water number will be removed from the sample.

3.2. FT-IR/DSC investigation of raffinose pentahydrate

Three-dimensional plots of thermal-dependent FT-IR spectra of raffinose pentahydrate prepared by 1 KBr or 2 KBr method are displayed in Fig. 2. The peaks near 4000–3000 cm^{-1} were assigned to the OH stretching vibration of water molecules in the pentahydrate of raffinose; the peaks within 3000–2800 cm^{-1} were due to the CH stretching of raffinose; the peak at 1651 cm^{-1} corresponded to the scissoring vibration mode of the crystal water in raffinose pentahydrate; the peaks within 1500–900 cm^{-1} belonged to the fingerprint region of CH deformation vibrations and CO stretching as well as OH bending modes of raffinose (Wolkers, Oldenhof, Alberda, & Hoekstra, 1998; Wolkers, Oliver, Tablin, & Crowe, 2004). Two peaks at 998 (997) and 966 (967) cm^{-1} represented the asymmetric and symmetric stretching vibrations of the α-(1→6) glycosidic bond (Kacurakova & Mathlouthi, 1996).

It is evident that the peak intensity at 1651 cm^{-1}, observed in the 1 KBr method, decreased gradually with temperature until approximately to approximately 50 °C, at which point it decreased sharply from 50 °C. This peak disappeared completely from 100 °C. The other peaks within the spectra fingerprint regions also changed from a sharp pattern to broader absorption peaks, which might be due to the formation of anhydrous raffinose (Wolkers et al., 2004). However, for the 2 KBr samples, the peak at 1651 cm^{-1} was shifted to 1639 cm^{-1} from 48 °C, but it maintained its peak position even when heating to 150 °C. The former peak was assigned to the scissoring vibration mode of the crystal water in raffinose pentahydrate; the latter peak was due to the free liquid water dehydrated from the raffinose pentahydrate (Akao, Okubo, Ikeda, Inoue, & Sakurai, 1998; Devlin, 1990). For the 1 KBr sample, the water might be evaporating from the KBr pellet with temperature, leading to the disappearance of the IR peak at 1651 cm^{-1}. However, in the 2 KBr pellets, the water dehydrated from the pentahydrate crystal might be sealed between the two pellets, resulting in an IR spectrum for free water appearing at 1639 cm^{-1}.

The comparison of DSC thermograms, TGA curves, and the changes in IR peak intensities of 1651 and 1639 cm^{-1} are revealed in Fig. 3. Obviously, the declining pattern of both TGA curves for raffinose powder and the peak intensity of 1651 cm^{-1} for 1 KBr sample exhibited the same tendency, which was also reflected to the changes in three endothermic peaks of DSC thermogram. On the other hand, the dramatic

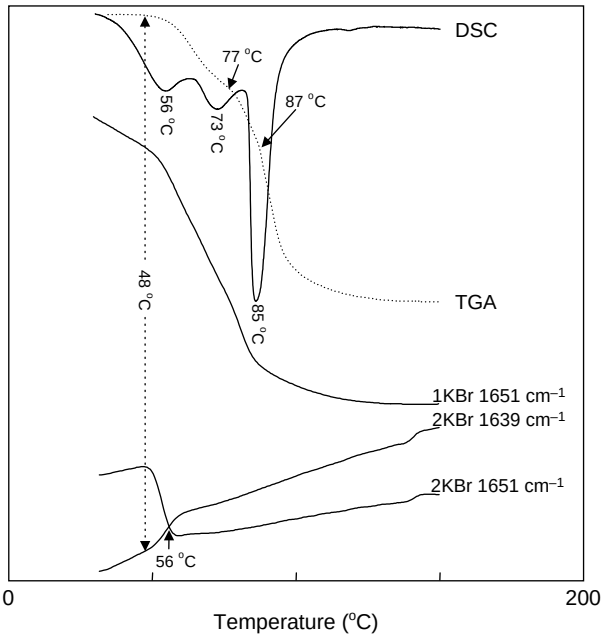


Fig. 3. The comparison of DSC themogram, TGA curve, and the changes in IR peak intensity of 1651 and 1639 cm^{-1} of raffinose pentahydrate

change in peak intensity of 1651 cm^{-1} for the 2 KBr sample was observed within 48–56 °C. At the same time, another IR peak at 1639 cm^{-1} simultaneously appeared. The thermal-dependent changes in IR peaks at 1651 (crystal water) and 1639 (free liquid water) cm^{-1} suggest that FT-IR/DSC microspectroscopy might be a potential technique to investigate the dehydration process of raffinose pentahydrate.

3.3. Isothermal rehydration process of preheated raffinose samples

It has been reported that the ability of cryoprotectant to form glasses may be a major factor in the long-term preservation of protein. This means that the stabilizer should not crystallize in the freeze-drying process and recrystallize during storage. Chatterjee et al. (2005) have recently found that anhydrous raffinose might crystallize during freeze-drying. Since raffinose pentahydrate appears to be losing weight at different states (Fig. 1), it is interesting for us to understand the rehydration process of raffinose sample from different hydrates.

Fig. 4 shows the changes in FT-IR spectra of raffinose pentahydarte prepared by the 1 KBr method and preheated isothermally at 62, 81, 125 or 150 °C for 5 min, then cooled to 30 °C, and isothermally re-studied at 30 °C, 70%RH for 180 min. It clearly indicates that, once the temperature at 30 °C was increased to 62, 81, or 125 °C, the original IR peak at 1651 cm^{-1} was shifted to 1642 cm^{-1}. This peak then disappeared via isothermal heating at 81 or 125 °C for 5 min. In contrast, the peak at 1642 cm^{-1} was maintained in the IR spectrum of the raffinose sample by isothermal heating at 62 °C for 5 min, implying that crystal water remained in the preheated sample. No IR peak appeared when sample was heated to 150 °C, indicating the changes from pentahydrate to anhydrate for raffinose sample.

Once all the isothermally preheated samples were cooled to 30 °C and then isothermally investigated at 30 °C, 70%RH for 180 min, two types of rehydration profiles can be obtained. The IR peak intensity at 1642 (1641) cm^{-1} for 81, 125 or 150 °C preheated sample was gradually increased with time, due to the absorption of water vapor from 30 °C, 70% RH condition. The appearance of this peak implies that it is difficult to rehydrate during 180 min, as compared with the complete pentahydrate formation by taking up water molecules from raffinose trihydrate (Saleki-Gerhardt et al., 1995). However, the cooled-sample which was then preheated isothermally at 62 °C showed the peak shifting from 1645 to 1651 cm^{-1} with time, which was similar to the peak at 1652 cm^{-1} for raffinose pentahydrate. According to the data of Fig. 1, raffinose pentahydrate isothermally preheated at 62 °C released only one dehydrated water molecule. However, after isothermally preheating at 81 °C, three dehydrated three water molecules are apparently released. Thus the appearance of IR peak at 1651 cm^{-1} for raffinose sample isothermally preheated at 30 °C, 70%RH might be due to the rehydration of raffinose tetrahydrate to pentahydrate. Once three water molecules have been dehydrated from the isothermally preheated raffinose

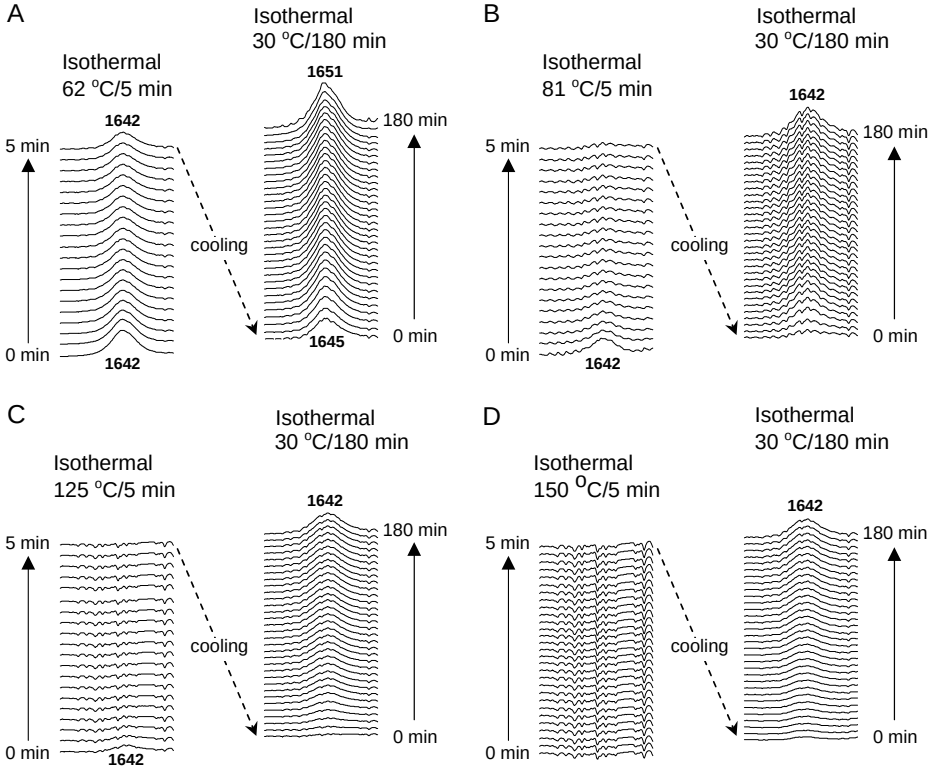


Fig. 4. The changes in FT-IR spectra of raffinose pentahydarte preheated to 62 (A) 81 (B) 125 (C) or 150 °C (D) for isothermal 5 min, then cooled to 30 °C, and isothermally again studied at 30 °C, 70% RH for 180 min.

sample, it seems hard to reconstruct the crystal structure of raffinose pentahydrate in such a short period, so it finally fails to perform the complete pentahydrate formation of raffinose.

4. Conclusions

Result of this study provides evidence that raffinose pentahydrate dehydrates to anhydrous raffinose in a step-wise sequence of weight loss through one, two and three molecules of water, respectively. However, the rehydration process of preheated raffinose sample is dependent on the preheating temperature. The extent of dehydration for raffinose pentahydrate might also influence the process of rehydration for the preheated raffinose sample.

References

Akao, K., Okubo, Y., Ikeda, T., Inoue, Y., & Sakurai, M. (1998). Infrared spectroscopic study on the structural property of a trehalose–water complex. *Chemistry Letters* , 759–760.

Chatterjee, K., Shalaev, E. Y., & Suryanarayanan, R. (2005). Raffinose crystallization during freeze-drying and its impact on recovery of protein activity. *Pharmaceutical Research*, *22*, 303–309.

Crowe, J. H., Carpenter, J. F., & Crowe, L. M. (1998). The role of vitrification in anhydrobiosis. *Annual Review of Physiology*, *60*, 73–103.

Davidson, P., & Sun, W. Q. (2001). Effect of sucrose/raffinose mass ratios on the stability of co-lyophilized protein during storage above the Tg. *Pharmaceutical Research*, *18*, 474–479.

Devlin, J. P. (1990). Vibrational modes of amorphous ice: Bending mode frequencies for isotopically decoupled H_2O and HOD at 90 K. *Journal of Molecular Structure*, *224*, 33–48.

Fischer, S., Hopkinson, D., Liu, M., MacLean, A. A., Edwards, V., Cutz, E., et al. (2001). Raffinose improves 24-hour lung preservation in low potassium dextran glucose solution: A histologic and ultrastructural analysis. *Annals of Thoracic Surgery*, *71*, 1140–1145.

Franks, F. (2003). Scientific and technological aspects of aqueous glasses. *Biophysical Chemistry*, *105*, 251–261.

Hincha, D. K., Zuther, E., & Heyer, A. G. (2003). The preservation of liposomes by raffinose family oligosaccharides during drying is mediated by effects on fusion and lipid phase transitions. *Biochimica et Biophysica Acta*, *1612*, 172–177.

Imamura, K., Ogawa, T., Sakiyama, T., & Nakanishi, K. (2003). Effects of types of sugar on the stabilization of protein in the dried state. *Journal of Pharmaceutical Sciences*, *92*, 266–274.

Izutsu, K., Aoyagi, N., & Kojima, S. (2004). Protection of protein secondary structure by saccharides of different molecular weights during freeze-drying. *Chemical and Pharmaceutical Bulletin (Tokyo)*, *52*, 199–203.

Jeffrey, G. A., & Huang, D. B. (1990). The hydrogen bonding in the crystal structure of raffinose pentahydrate. *Carbohydrate Research*, *206*, 173–182.

Kacurakova, M., & Mathlouthi, M. (1996). FTIR and laser-Raman spectra of oligosaccharides in water: Characterization of the glycosidic bond. *Carbohydrate Research*, *284*, 145–157.

Kajiwara, K., Franks, F., Echlin, P., & Greer, A. L. (1999). Structural and dynamic properties of crystalline and amorphous phases in raffinose–water mixtures. *Pharmaceutical Research*, *16*, 1441–1448.

Liao, Y. H., Brown, M. B., Quader, A., & Martin, G. P. (2002). Protective mechanism of stabilizing excipients against dehydration in the freeze-drying of proteins. *Pharmaceutical Research*, *19*, 1854–1861.

Lin, S. Y., & Chien, J. L. (2003). In vitro simulation of solid–solid dehydration, rehydration, and solidification of trehalose dihydrate using thermal and vibrational spectroscopic techniques. *Pharmaceutical Research*, *20*, 1926–1931.

Lin, S. Y., Li, M. J., & Wei, Y. S. (2004). Ethanol or/and captopril-induced precipitation and secondary conformational changes of human serum albumin. *Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy*, *60*, 3107–3111.

Patton, J. S., Foster, L., & Platz, R. M. (2004). Methods and compositions for pulmonary delivery of insulin. US Patent 6,685,967.

Roy, I., & Gupta, M. N. (2004). Freeze-drying of proteins: Some emerging concerns. *Biotechnology and Applied Biochemistry*, *39*, 165–177.

Saleki-Gerhardt, A., Stowell, J. G., Byrn, S. R., & Zografi, G. (1995). Hydration and dehydration of crystalline and amorphous forms of raffinose. *Journal of Pharmaceutical Sciences*, *84*, 318–823.

Tsuzuki, T., Imamura, K., Yamamoto, K., Satoh, T., & Okazaki, M. (1997). Thermal stabilization of freeze-dried enzymes by sugars. *Journal of Chemical Engineering of Japan*, *30*, 609–613.

Wang, S. L., Lin, S. Y., Chen, T. F., & Cheng, W. T. (2004). Eudragit E accelerated the diketopiperazine formation of enalapril maleate determined by thermal FTIR microspectroscopic technique. *Pharmaceutical Research*, *21*, 2127–2132.

Wang, S. L., Lin, S. Y., Chen, T. F., & Chuang, C. H. (2001). Solid-state trans-cis isomerization of captopril determined by thermal Fourier transform infrared (FT-IR) microspectroscopy. *Journal of Pharmaceutical Sciences*, *90*, 1034–1039.

Wolkers, W. F., Oldenhof, H., Alberda, M., & Hoekstra, F. A. (1998). A Fourier transform infrared microspectroscopy study of sugar glasses: Application to anhydrobiotic higher plant cells. *Biochimica et Biophysica Acta*, *1379*, 83–96.

Wolkers, W. F., Oliver, A. E., Tablin, F., & Crowe, J. H. (2004). A Fourier-transform infrared spectroscopy study of sugar glasses. *Carbohydrate Research*, *339*, 1077–1085.