Insights into the Chemistry of Adsorption of Water Molecules by Cyclodextrins as Studied by Near Infrared Spectroscopy

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ARTICLE INFO	ABSTRACT
	The nature of OH groups in α, β and γ cyclodextrins and their behavior towards adsorption of water molecules have been studied by using near infrared spectroscopy and derivative techniques. Pure dry cyclodextrins were exposed to surrounding air with known relative humidity and their near infrared spectra were measured by using a Perkin Elmer Spectrum one NTS FT-NIR spectrometer equipped with a transflectance accessory and a DTGS detector. The spectral evolution during the water molecular adsorptions was analysed using derivative techniques to understand and locate the water molecular adsorption sites in the cyclodextrin molecules.The results show that the β-cyclodextrin behaves differently compared to the other two cyclodextrins. The β-cyclodextrin adsorbs and acquires water through the C6-OH and C2, C3-OH groups and diffusion processes from the start. The other two cyclodextrins use their primary and secondary OH groups in adsorbing water molecules in the initial stages and through a slow diffusion process at latter stages.

INTRODUCTION

Starch is a polymer of α–glucose joined by 1,4-glycosidic bonds. The polymer strands take the shape of helix and there are mostly seven glucose units in one turn in the helical structure. Cyclodextrins are cyclic oligosaccharides formed during the enzymatic degradation of starch. The action of cyclodextrin glycosyl transferases (CGTases) on starch leads to the break down of starch into oligosaccharides containing six, seven and eight glucose units. The nature of helical structure leads to the formation of segments containing seven glucose units in abundance. The formation of oligosaccharides containing six and eight glucose units is less common. These oligosaccharides form cyclic structures by the action of CGTases (Dass *et al.* 2000). The cyclodextrins containing six, seven and eight glucose units are called α, β og γ-cyclo dextrin respectively (Fig. 1). There is also evidence for the natural existence of δ, ζ, ξ and η-cyclodextrins containing 9, 10, 11 and 12 glucose units respectively (Hirose *et al.*, 2001,).

The three cyclicdextrins α, β and γ-cyclodextrins have similar molecular shapes. The arrangement of glucose units in circle give cyclodextrins torus shaped molecular structure (Martin Del Valle, 2004). The upper aperture of the torus shaped cyclodextrin has a larger circumference than the base aperture.

Furthermore, the size of the cavity increases with increasing number of glucose units. Details regarding the molecular parameters can be found in http://www1.lsbu.ac.uk/water/cyclodextrin. html (accessed 27th of May 2015). The hydroxyl groups on C2 and C3 of the glucose units are located at the top of the torus and the hydroxyl group on C6 is located at the base of the torus. The hydroxyl group on C6 is free to rotate and can point inwards the base aperture. The central cavity of the cyclodextrins contains the carbon skeleton and the ethereal oxygen of the glucose units. Therefore, the cavity of the torus is apolar and the outer edges of the torus are hydrophilic. The hydroxyl groups on C2 and C3 in cyclodextrins can form hydrogen bonds and are less available for interaction with other molecules. Furthermore, the three hydroxyl groups on C6, C2 and C3 have different reactivities and this influences the chemistry that can be performed with cyclodextrin (Lindhorst, 2007) molecules.

Lilke most of the organic molecules containing OH groups, the cyclodextrins also have affinity for polar molecules. The cyclodextrins adsorb water molecules by forming hydrogen bonds with OH groups in the molecules. They can also acquire free water molecules in the cavity of the molecules. The amount of water that is adsorbed and how the adsorption processes vary between the molecules

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dependig on their structure and how the OH groups are affected in different structures.

There have been a large number of publications dealing with the cyclodextrins and their applications. Josef Szejtli (2004) has published and article that deals with the past, present and future of cyclodextrins. He has reviewed the important aspects of cyclodextrin research. Nitalikar *et al.* (2012) have published another review article on cyclodextrins. The articles that are dealing with the adsorption characteristics of cyclodextrins towards water molecules are rare. A few of the articles have looked at the interaction of water molecules with cyclodextrins using mid infrared spectroscopy (Egyed, 1990 and 1994; Gavira *et al.*, 2003). All these used curve fitting techniques to resolve the Infrared absorption bands arising due to the OH stretching vibrations and attempted band assignments for the vibrations. They all faced with the problem of very broad bands in the OH stretching regions of the mid-infrared spectrum and relied on the curve fitting methods used.
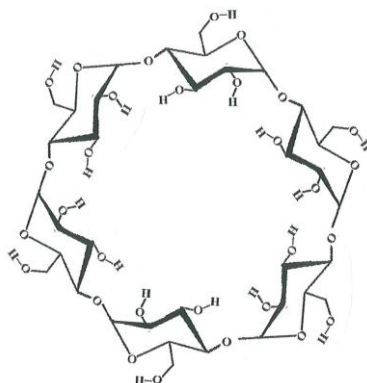

Fig. 1: Molecular structure of α-cyclodextrin.

Christy in a series articles has looked at the water molecular adsorption on different inorganic (Christy, 2010, 2011a and 2011b) and organic compounds (Christy, 2013a and 2013b). The use of near infrared spectroscopy and derivative techniques has helped to understand the nature of surface hydroxyl groups and their adsorption characteristics towards water molecules. The absorption due to the combination frequencies of stretching and bending OH vibrations fall in a convenient region in near infrared and analysis of the peak arising in this region would give information regarding the differences in water molecules adsorbed onto the surface. These differences would give information regarding the types of polar groups on the surface. Since cyclodextrins are compounds made up of glucose units and contain hydroxyl groups, the methodology used in the applications above could reveal information regarding the nature of the hydroxyl groups in cyclodextrins and how they adsorb water molecules from the surrounding air.

The aim in this article is to analyse the three cyclodextrins by using near infrared spectroscopy and gravimetry to reveal the nature of the hydroxyl groups in the molecules. Furthermore, the information from the analyses was used to understand and compare the water molecular adsorption characteristics of the three cyclodextrins.

Experimental:
Samples and procedures:
All the three α, β and γ- cyclodextrins were purchased from Sigma-Aldrich.

The evolution of the adsorption of water molecules on cyclodextrins were followed separately by NIR spectroscopy and gravimetry. All samples were first dried at 120 °C under vacuum using a ceramic heater (BA Electric Bunsen from Electrothermal, Staffordshire, U.K.). The samples were cooled to room temperature and the adsorption evolution of water molecules was followed. The adsorption experiments and infrared measurements were carried out simultaneously in the same room, which had a room temperature of 25.5 °C. The relative humidity during the water adsorption experiments and near-infrared measurements of cyclodextrins were controlled by a glycerol/water system (Forney and Brandl, 1992). The amount of glycerol was adjusted to produce the relative humidity needed for the experiment. The construction of a chamber to provide air with varying relative humidity during the near infrared measurements under the adsorption of water molecules is shown in Fig.2.

The gravimetric determination of water sorption was carried out on each dry cyclodextrin sample by placing the NIR sample cup containing 0.1 g of dry sample on a Mettler electronic balance that is provided with an environment of air with relative humidity of 25%. The balance was connected to a computer through an RS-232 port for data collection. A home written software communicated with the Mettler balance and recorded every increase in mass of the adsorbing cyclodextrin by 0.0002g. The data collected at the computer were imported into an Excel spread sheet and presented in the form of

graphs for comparison and discussion. The   50%.
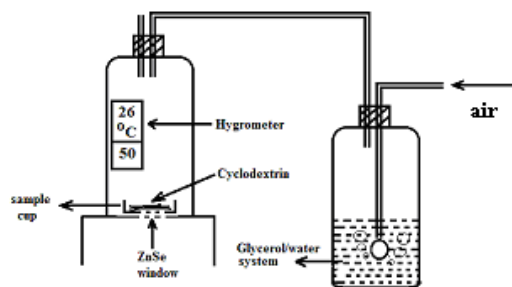experiments were repeated at a relative humidity of

Fig. 2: A figure showing the set up for humidity control during the experiments.

The near-infrared measurements were made using a Perkin- Elmer Spectrum One NTS FT-NIR spectrometer (Perkin- Elmer Ltd., Cambridge, U.K.) equipped with a transflectance accessory and deuterated triglycine sulfate detector. The measurements were made on the sample placed in another NIR sample cup. Around 0,1g of the cyclodextrin sample was quickly placed in the NIR sample cup and moved on to the ZnSe crystal of the transflectance accessory that is covered by a chamber providing air with the required relative humidity. The spectral evolution was recorded frequently in the range of $10000-4000$ cm^{-1}. A total of 30 scans were obtained at a resolution of 16 cm $^{-1}$. All NIR spectra were transformed to log(1/R) format and second-derivative profiles of the spectral data were

calculated by an algorithm developed by Savitzky and Golay with a 19-point derivative width. The fourth-derivative profiles of the spectra were obtained in the same manner from the second-derivative profiles.

RESULTS AND DISCUSSION

The near infrared spectra of cyclodextrins are shown in Figure 3. Their fourth derivative profiles in the region 7200-6900 cm^{-1} are shown in Fig. 4. A table showing the near infrared band assignments for the carbohydrate polymers is given in Christy, 2013a. The first overtones of the C2`-OH in the α, β and γ-cyclodextrins absorb at 7060, 7034 and 7055 cm^{-1} respectively.
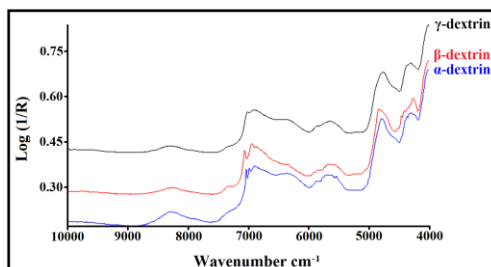

Fig. 3: The near infrared spectra of the α, β and γ cyclodextrins in the 10000-4000 cm^{-1} region.
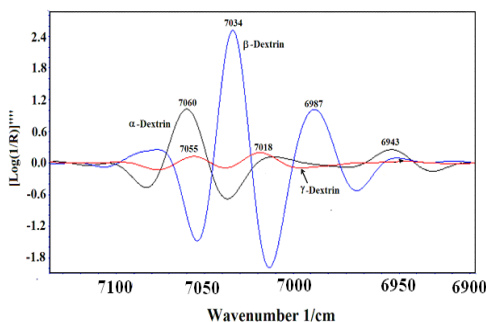

Fig. 4: Fourth derivative near infrared spectra of α, β and γ cyclodextrins in the 7200-6900 cm^{-1} region.

The appearance of relatively sharp peaks in the 7200-6900 cm^{-1} region in the Near infrared spectra of the α, β and γ cyclodextrins show that the intermolecular interactions taking place between the OH groups in these molecules are relatively less in order than in the carbohydrate polymers like amylose, amylopectin and cellulose. The NIR spectra of the water free dry cyclodextrins show clearly the variations in the first overtone of the free OH stretching vibrations. The α and β cyclodextrins exhibit clear absorptions from the free OH stretchings compared to γ-cyclodextrins. The β-cyclodextrin has an intense absorption for the first C2`-OH overtone (see Fig.5) compared to the first C2`-OH overtones of the α and γ-cyclodextrins. Furthermore, the fourth derivative profiles of the three cyclodextrins in the region 7200-6900 cm^{-1} show that the β-cyclodextrin has a free OH group that absorbs at a lower frequency compared to the OH groups in the other analogues. This indicates that the C2`-OH bond in β-cyclodextrin is weaker and more polarized than the C2`-OH groups in α and γ analogues. The possible inter molecular hydrogen bondings between C`2-OH and C3-OH pairs in the cyclodextrin molecules are shown in Fig. 5. The first overtones of the C6-OH in the α, β and γ-cyclodextrins absorb at 7018, 6987 and 6947 cm^{-1} respectively. The absorption position of the first overtone of the C2, C3-OH groups in cyclodextrins indicate the formation of strong hydrogen bond between the OH groups at carbon no. 2´ and 3 of β-cyclodextrin molecules compared to the hydroxyl groups in α and γ cyclodextrins. The strong hydrogen bonding between these hydroxyl groups gives β-cyclodextrin a rigid structure. This also explains why the β-cyclodextrin has limited water solubility compared to the other two analogues.
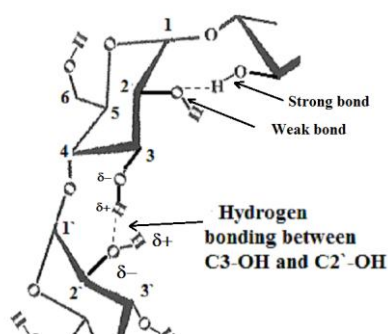

Fig. 5: A figure showing hydrogen bonding between C2'-OH and C3-Ohin cyclodextrins.
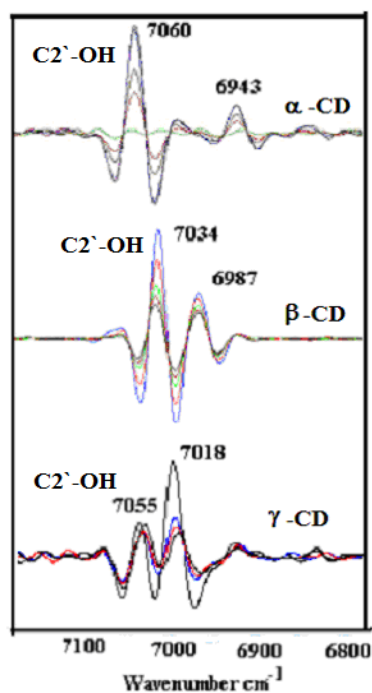

Fig. 6: Fourth derivative spectra in the 7100-6800 cm^{-1}. (α-CD: 0, 10, 24, 30 and 52 minutes; β-Cd: 8, 22, 33 and 54 min; γ-CD: 5, 13, 17 and 27 minutes).

The α-cyclodextrin has the weakest hydrogen bonding and the OH stretching of C2(C3)-OH absorbs around 7060 cm⁻¹. The molecule is strained and only four pairs of the C`2-OH and C3-OH pairs in the α-cyclodextrin engage in hydrogen bonding (Szejtli, 2004). The hydrogen bonds formed between these pairs are weaker than the hydrogen bonds between the pairs in β-cyclodextrin. The γ-dextrin has stronger hydrogen bonding than α-cyclodextrin and the OH stretching of C2`-OH absorbs around 7055 cm⁻¹. The molecule is non-coplanar and more flexible in structure (Szejtli, 2004) and the OH groups can be easily approached by water molecules. Therefore, γ-dextrin is more soluble than the other two analogues.

Fourth derivative spectra of the three cyclodextrins acquired during the adsorption evolution of water are shown in the region 7200-6800 cm⁻¹ (Fig. 6) and for β–cyclodextr in the region 5300-5100 cm⁻¹ (Fig. 7).

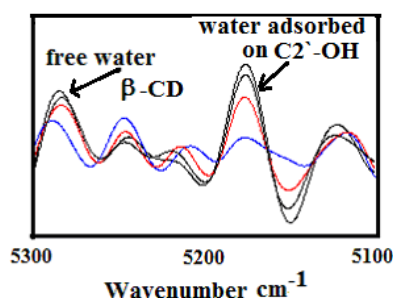**Fig. 7:** Fourth derivative spectra of β–cyclodextrin in the 5300-5100 cm⁻¹(β-Cd: 8, 22, 33 and 54 minutes)

The spectra in the OH overtone region show that the peaks decrease in intensities when dry samples are exposed to surrounding air. The exposure of the samples to the surrounding air allows the OH groups in the samples to adsorb water molecules by forming hydrogen bonds. The rates of depletion of the intensity of absorption of the C2`-OH groups of α, β and γ cyclodextrins at relative humidities 25% and 50% are shown in Fig. 8 and Fig. 9 respectively. The plots show an obvious strange behaviour of β-cyclodextrin in adsorbing water molecules at both relative humidities. The absorption intensity of the first overtone of the β-cyclodextrin C2`-OH groups is depleted at a faster rate compared to the first overtone absorption intensities of the other two cyclodextrins at both RHs. All the three cyclodextrins C2`-OH adsorb water at almost ten times faster rates at 50 % relative humidity than at 25% relative humidity. Furthermore, the spectra shown (Fig. 7) in the OH combination frequency region (5300-5100 cm⁻¹) that are due to the water molecules acquired by the cyclodextrins show another interesting behaviour regarding the acqusition of water molecules.
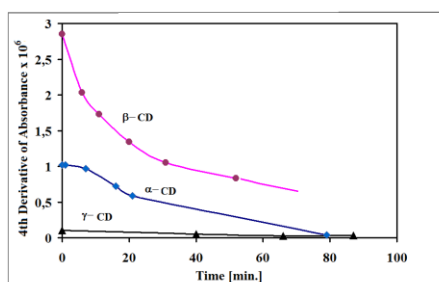

Fig. 8: Rates of depletion of intensities of absorptions of the first overtones of the OH groups attached to C2` in cyclodextrins at 25% RH.

The absorption arising at 5282 cm⁻¹ is due to free water molecules and the absorption arising around 5150 cm⁻¹ is due to the water molecules adsorbed on to C2`-OH groups by hydrogen bond formation. It is easy to see that the β-cyclodextrin acquires water molecules through two different processes namely by adsorption and by acquiring free water molecules in the cavity. These two processes take place from the start when the dry β-cyclodextrin is exposed to surrounding air. The spectra acquired for α-cyclodextrin show that the water is mainly acquired by the adsorption process during the initial stage of exposure to air. However, a similar plot obtained in the 5300-5100 cm⁻¹ region with the shelf α-cyclodextrin shows an absorption at 5284 cm⁻¹. This indicates the acquisition of some free water molecules by α-cyclodextrin. Similar processes are also observed for γ-cyclodextrins. There is no doubt that the behaviour of β-cyclodextrin in the acquisition of water molecules is unique and this can

only be attributed to the differences in the crystal structure. However, all the three cyclodextrins end up in acquiring water molecules in the cavities by diffusion (Braesicke *et al.*, 2000) and by adsorption process on to the primary and secondary OH groups.
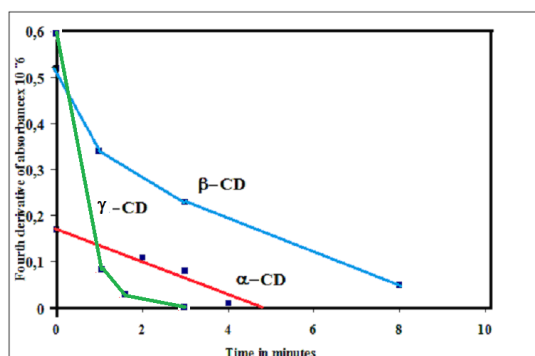

Fig. 9: Rates of depletion of intensities of absorptions of the first overtones of the OH groups attached to C2` in cyclodextrins at 50% RH.
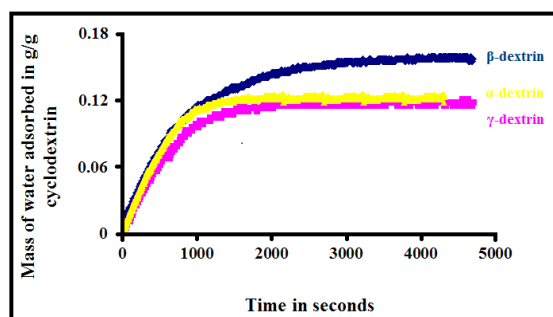

Fig. 10: Total acquisition of water by α, β and γ- cyclodextrins over a period of 80 minutes.

The acquisition of water by the three cyclodextrins at 50% relative humidity are shown in Fig. 10. The figure shows the total water acquired by the three cyclodextrins over a period of 80 minutes. The mass of water is the combination of water acquired by adsorption and diffusion processes.

Conclusion:

The effectivity of the second deerivative techniques applied to near infrared spectroscopic data is again demostrated in this article. The method was able to resolve the first overtones of the OH groups attached to carbon atoms in the glucose units of the cyclodextrins. The absorption position of the peak for first overtone of the OH stretching shows that the β-yclodextrin has the strongest intra molecular hydrogen bonding between OH groups attached to neighbouring glucose units.

Furthermore, the adsorption behaviour of the OH groups in cyclodextrins towards water molecules has been successfully investigated. The results clearly show the abnormal behaviour of the β-yclodextrin in adsorbing water molecules compred to the other two analogues. The dry β-cyclodextrin adsorbs water at a faster rate compared to α and γ-cyclodextrin and hold more water at the end.

The acquisition of water by cyclodextrins takes place through adsorption and diffusion processes. These processes are very effective in β-cyclodextrin compared to α and γ-cyclodextrins.

REFERENCES

Braesicke, K., T. Steiner, W. Saenger and E.W. Knapp, 2000. Diffusion of water molecules in crystalline beta-cyclodextrin hydrates, J. Mol. Graph. Model, 18(2): 143-52-167-168.

Christy, A.A., 2010 New insights into the surface functionalities and adsorption evolution of water molecules on silica gel surface: A study by second derivative Near Infrared Spectroscopy, Vibrational Spectroscopy, 54: 42-49.

Christy, A.A., 2011a. Near infrared spectroscopic characterisation of surface hydroxyl groups on hydrothermally treated silica gel, International Journal of Chemical and Environmental Engineering, 2 (1): 27-32

Christy, A.A., 2011b. Effect of Hydrothermal Treatment on Adsorption Properties of Silica Gel, Ind. Eng. Chem. Res., 50(9): 5543-554

Christy, A.A., 2013a. Chemistry of Desiccant Properties of Carbohydrate Polymers as Studied by

Near-Infrared Spectroscopy, Ind. Eng. Chem. Res., 52(12): 4510–4516

Christy, A.A., 2013b. Water Adsorption Characteristics of Acyclic Oligosaccharides of α-Gluose Studied by Near Infrared Spectroscopy. TOSpecJ, 7: 1-8

Dass, C.R. and W. Jessup, 2000. Apolipoproteins. A-I. Cyclodextrins and liposomes as potential drugs for the reversal of atherosclerosis. J. Parm. Parmacol, 52: 731-61.

Egyed, O., 1990. Spectroscopic studies on β-cyclodextrin, Vib. Spectrosc. 1: 225-227

Egyed, O. and V. Weiszfeiler, 1994. Structure determination of copper (II)-β-cyclodextrin complex by Fourier transform infraredspectroscopy, Vib. Spectrosc, 7: 73-77.

Forney, C.F. and D.G. Brandl, 1992. Control of Humidity in Small Controlledenvironment Chambers using Glycerol-Water Solutions. HortTechnology, 2(l): 52-54.

Gaviraa, J.M., A. Hernanza and I. Bratub, 2003. Dehydration of β-cyclodextrin An IR n(OH) band profile analysis, Vibrational Spectroscopy, 32: 137–146.

Hirose, T. and Y. Yamamoto, 2001. Hinokitol containing cyclo-olefin polymer composition sand their molding with excellent antimicrobial and gas barrier properties, Japanese Patent JP, 55480.

Lindhorst, T.K., 2007. Essentials of Carbohydrate Chemistry and Biochemistry, Willey-VCH Verrlag GmbH & Co. KGaA.

Martin Del Valle, E.M., 2004. Cyclodextrin and their uses:a review, Process Biochemistry 39 :1033-1046.

Nitalikar, M.M., D.M. Sakarkar and P.V. Jain, 2012. The Cyclodextrins: A review, Journal of Current Pharmaceutical Research, 10 (1): 1-6.

Szejtli, J., 2004. Past, present, and future of cyclodextrin research*, Pure Appl. Chem., 76:1825-1845.

http://www1.lsbu.ac.uk/water/cyclodextrin.html, accessed 27th of May 2015.