

# Impact of water on molecular dynamics of amorphous $\alpha$ -, $\beta$ -, and $\gamma$ -cyclodextrins studied by dielectric spectroscopy

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Dielectric, calorimetric, and x-ray diffraction measurements were carried out on  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrins, which are cyclic saccharides built by, respectively, six, seven, and eight glucose units connected via glycosidic linkage. Differential scanning calorimetry measurements indicated that each carbohydrate has a melting temperature located much above the temperature at which thermal decomposition begins. Moreover, calorimetric data revealed that it is possible to completely dehydrate each cyclodextrin by annealing them above 413 K. Unfortunately, it is impossible to obtain amorphous forms of cyclodextrin by simple cooling of the melt. Thus, a solid state amorphization method has been applied. X-ray diffraction studies demonstrated that by ball milling at room temperature we are able to obtain completely amorphous cyclodextrins. Finally, dielectric measurements were carried out to probe molecular dynamics in the amorphous state of cyclodextrins. It was found that there is only one relaxation process in amorphous hydrated cyclodextrins, while in dried samples two secondary relaxations are present. Moreover, we have shown that water has an enormous effect on the dynamics of both relaxation modes, i.e., with increasing content of water, the activation energy of the slow mode decreases, while that evaluated for the fast mode increases. We were not able to follow the dynamics of the structural relaxation process, because glass transition temperatures of amorphous cyclodextrins were found to lie above thermal degradation points.

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## I. INTRODUCTION

Cyclodextrins (CDs) are cyclic saccharides built up by D-glucose units connected via  $\alpha$  (1,4) glycosidic linkage. There are three naturally occurring cyclodextrins which are composed of six ( $\alpha$ ), seven ( $\beta$ ), and eight ( $\gamma$ ) glucose molecules. Larger CDs, having a greater number of D-glucose units are also possible [1,2]. However, special chemical synthesis needs to be applied to obtain such carbohydrates. The main characteristic feature of examined macromolecules is their toroidal shape with a hydrophobic cavity surrounded by a hydrophilic exterior composed of primary and secondary hydroxyl groups [3]. CDs have a great ability to form inclusion complexes with different kinds of guest molecules [4–6]. What is more, the dimension of the cavity varies with the increasing number of D-glucose molecules. Thus, it is possible to choose cyclodextrin as having the best complexation abilities with the given labile specimen. The other interesting feature concerning cyclodextrins is their hydrophobic interior which enables the inclusion of hydrophobic drugs having the appropriate shape and size. Consequently, CDs can act as a drug carrier [7–9] improving water solubility, chemical stability, and bioavailability of sparingly water soluble pharmaceuticals [10,11]. Moreover, the cavity of a CD may serve as an environment for the chemical reactions [12,13]. These few examples of potential applications of cyclodextrins made them very attractive materials from a technological as well as a scientific point of view.

Cyclodextrins occur naturally as hydrates. For example, there are six molecules of water per one molecule of

$\alpha$ -cyclodextrins or 11–12 water molecules as it is in the case of  $\beta$ -homologue. Because of that reason cyclodextrins give a unique opportunity to study the dynamics of bulk and confined water. For that purpose, x-ray diffraction, neutron scattering, and nuclear magnetic resonance measurements were carried out to characterize the structure as well as the distribution of water molecules inside cyclodextrins [14–16]. Among all of the cyclodextrins  $\beta$ -isomer was investigated the most. It was found that, seven disordered water molecules were located in each  $\beta$ -CD cavity, and five more water molecules were located in the interstitial sites between the  $\beta$ -CD macrocycles. Most importantly, these seven molecules occupying the interior of  $\beta$ -CD are distributed over eight possible positions. Moreover, at room temperature most water molecules and  $\beta$ -CD's hydroxyl groups are orientationally disordered and alternately form hydrogen bonds with different neighbors. However, this disorder is very dynamic and is associated with rapid flips of O-H groups between discrete alternative orientations. The other studies carried out on  $\beta$ -cyclodextrin and its two methyl substituted forms indicated that rotational and translational motions of the water bonded with the external hydroxyl group and that occupying cavities are slower than the motion of bulk water [17]. Recently Shikata *et al.* published a paper on the dynamics of water in aqueous solutions of different cyclodextrins [18]. They found that at  $T = 298$  K there are three relaxation processes. The faster one of the largest amplitude and relaxation times around 8 ps was assigned to the rotational relaxation process of bulk water. The next relaxation process also having large dielectric strength with relaxation time 20–27 ps was attributed to the exchange process of

water molecules between the hydrated state of CDs and the bulk aqueous phase. The slowest mode was connected to the rotational motion of CD molecules in water.

Based on the literature data one can conclude that a lot of studies have been carried out on crystalline structure, water solutions, or chemical modifications of CDs. However, there are no reports on molecular dynamics of amorphous CDs. It is surely connected to the chemical degradation of CDs at melting temperatures. Thus, it is impossible to obtain amorphous cyclodextrins by the cooling of melt, which is the most standard method of obtaining glassy materials. However, as was shown in papers by Tabary *et al.* [19] and Li *et al.* [20] one can convert crystalline CDs to an amorphous form by milling in high-energy planetary mill, or by application of a freeze drying method. In literature there are papers reporting that the glass transition temperature of  $\beta$ -cyclodextrin lies within the range of temperature  $T_g = 313\text{--}523$  K [21–23]. Thus, it is clear that  $T_g$  for this cyclic carbohydrate is not defined. In fact in Ref. [19] authors found that the glass transition temperature of  $\beta$ -cyclodextrin must be located much above the thermal degradation of this material.

In this paper we present dielectric data collected for hydrated and anhydrous amorphous  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrins. Amorphous forms of these materials were obtained by milling of crystalline forms in high-energy planetary mill. Afterwards, x-ray diffraction measurements confirmed their complete amorphization. Dielectric measurements carried out for hydrated amorphous CDs revealed the presence of only one relaxation process having an activation energy of  $\sim 60$  kJ/mol. It was also found that the dielectric loss spectra of amorphous cyclodextrins strongly depend on the content of water in the examined samples. One can add that in anhydrous cyclodextrins two relaxation processes were observed: the slowest one of small amplitude having an activation barrier lying within 80–96 kJ/mol and the faster one of large amplitude and  $E_a = 42\text{--}48$  kJ/mol. It is worth mentioning that the activation barrier for both relaxation modes is influenced by the addition of water. In this work, we have also compared the dielectric data measured for sucrose and trehalose with that obtained for anhydrous cyclodextrins. Such a comparison enabled us to conclude that the dynamics of amorphous

CDs and disaccharides are almost the same. Our data also revealed that the dynamics of the amorphous phase of cyclic carbohydrates is very similar. Finally, based on the dielectric data, a prediction about the rigidity of examined CDs is given.

## II. EXPERIMENT

### A. Materials

$\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrins were purchased as hydrates from Sigma Aldrich. Declared purity of the outcome materials was higher than 99%. The chemical structures of the investigated cyclodextrins are presented in Fig. 1.

### B. Ball milling

Grinding was performed using a planetary ball mill (Retsch, Germany). Ball milling of  $\alpha$ -cyclodextrin,  $\beta$ -cyclodextrin, and  $\gamma$ -cyclodextrin was performed at ambient temperature. The mass of each sample was, respectively, 6.44, 8.94, and 5.20 g.

A zirconium jar was filled with the investigated material and six balls (20 in. diameter) made of the same material were added. The rotation speed of 410 rpm was set. Each milling cycle lasted 30 min and was followed by a 15-min break. Total milling time was 30 h.

### C. Dielectric spectroscopy

Isobaric dielectric measurements at ambient pressure were carried out using a Novo-Control GMBH Alpha dielectric spectrometer ( $10^{-2}\text{--}10^7$  Hz), and in the case of sucrose the high frequency setup HP 9531 [ $10^6\text{--}10^9$  Hz] was also used. The samples were placed between two stainless steel flat electrodes of the capacitor with a gap of 0.1 mm. The temperature was controlled by the Novo-Control Quattro system, with the use of a nitrogen-gas cryostat. Temperature stability of the samples was better than 0.1 K.

### D. Differential scanning calorimetry

Calorimetric measurements of the milled samples of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrins were performed with Mettler Toledo DSC apparatus equipped with a liquid nitrogen cooling accessory

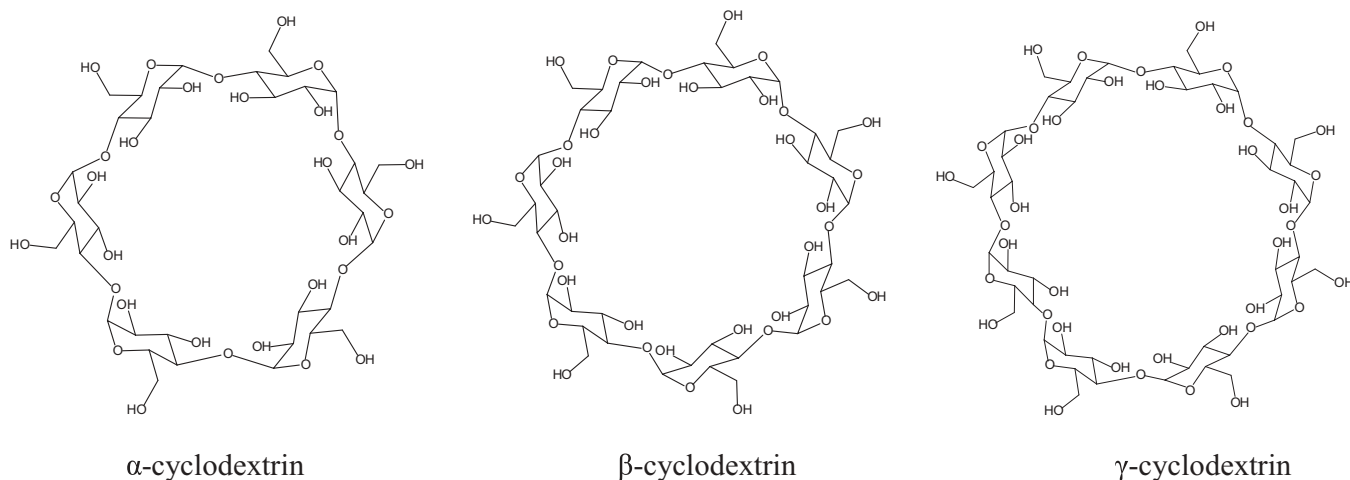


FIG. 1. Chemical structures of cyclodextrins.

and a HSS8 ceramic sensor (heat flux sensor with 120 thermocouples). Temperature and enthalpy calibrations were carried out by using indium and zinc standards. Aluminum crucibles (40  $\mu\text{L}$ ) with samples have been sealed with the top three punctures in order to enable water to volatilize during the sample heating. Samples were scanned at a rate of 10 K/min in the temperature range from 300 to 600 K.

### E. X ray

The x-ray diffraction experiment was performed at room temperature using the Rigaku-Denki D/MAX RAPID II-R diffractometer (Rigaku Corporation, Tokyo, Japan) with a rotating anode Ag  $K\alpha$  tube ( $\lambda = 0.5608 \text{ \AA}$ ), an incident beam (002) graphite monochromator, and an image plate in the Debye-Scherrer geometry as a detector. The pixel size was  $100 \times 100 \mu\text{m}$ . The sample was placed inside the glass capillaries (1.5 mm in diameter). The measurements were performed for the sample filled and empty capillaries and the intensity for the empty capillary was then subtracted. The beam width at the sample was 0.3 mm. The two-dimensional diffraction pattern was then converted into the one-dimensional intensity data using suitable software.

## III. RESULTS AND DISCUSSION

At first, x-ray diffraction measurements were carried out on  $\alpha$ -,  $\beta$ -, and  $\gamma$ - cyclodextrins received from a supplier. We found that two former cyclosaccharides were crystalline as received, while the latter one was amorphous (see Fig. 3). Since we were interested in studying the molecular dynamics of amorphous cyclodextrins we applied the most standard way to obtain glassy materials, i.e., cooling of the melt. However, during heating, the color of the white, crystalline powder changed into a brownish color. It was a clear sign that cyclodextrins undergo chemical degradation, commonly called caramelization. Hence, it is impossible to obtain amorphous cyclosaccharides by cooling of the melt. Therefore, another

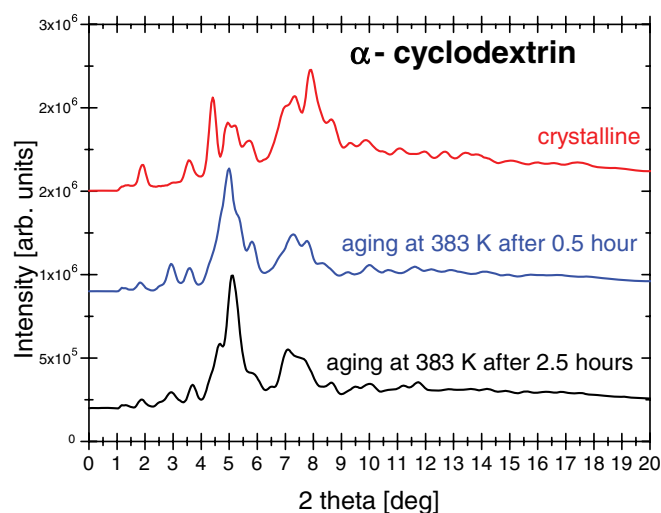


FIG. 2. (Color online) X-ray diffraction patterns obtained from the time dependent measurements carried out on  $\alpha$ -cyclodextrin at  $T = 383 \text{ K}$ .

method was applied. In literature it was suggested that it is possible to induce amorphization by dehydration of crystalline hydrates at temperatures much below melting temperature [24]. One can mention here that raffinose, which occurs naturally as the pentahydrate crystal, has no ability to form stable anhydrous crystals. It was shown that annealing at  $T = 333 \text{ K}$  leads to complete amorphization of this saccharide after 48 h [24]. It should be stressed that the glass transition temperature of raffinose was estimated to be equal to 382 K [25]. Thus, annealing well below  $T_m$  and  $T_g$  may induce complete amorphization of materials belonging to the same group as cyclodextrins. One can add that similar findings were also reported for trehalose [26,27] and carbamazepine [28]. Moreover, in literature it was shown that crystals of cyclodextrin collapse during storage at room temperature at relative humidity  $\text{RH} = 15\%$  [29]. Having these results in mind, annealing experiments on  $\alpha$ - cyclodextrin were carried out at  $T = 383$  and 423 K. In Fig. 2 x-ray diffraction patterns obtained during annealing at  $T = 383 \text{ K}$  are presented. It can be seen that during the first two hours of heating, the crystalline structure undergoes transformation. Further annealing does not bring any changes in the crystalline structure of  $\alpha$ -cyclodextrin. Similar results were obtained when experiment was repeated at 423 K (data not shown). Thus, based on the structural data obtained from x-ray measurements one can state that annealing of  $\alpha$ -cyclodextrin at high temperatures does not induce crystal-amorphous transformation. One should note that during heating of the crystalline sample, water evaporates causing reorganization of the crystalline structure of  $\alpha$ -cyclodextrin. Analogous effects were observed also in the case of  $\beta$ -cyclodextrin.

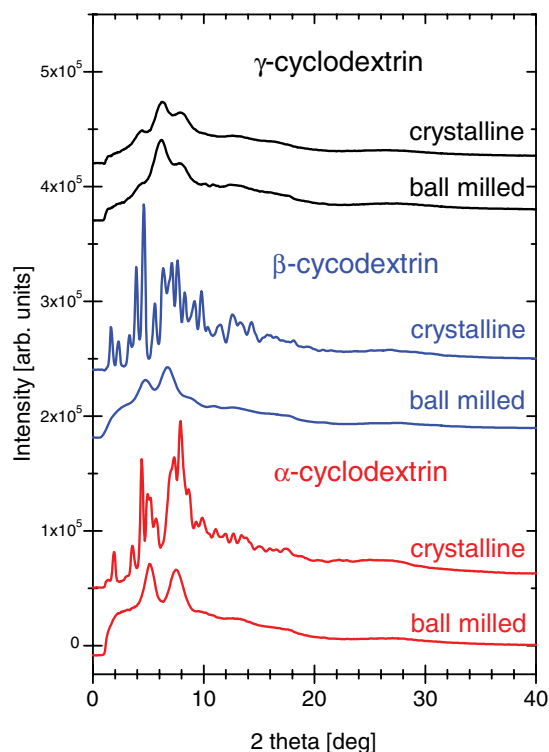


FIG. 3. (Color online) Diffraction patterns obtained for the crystalline and ball milled cyclodextrins.

Since any of the above described methods do induce amorphization of cyclodextrins, another technique, i.e., ball milling in high-energy planetary mill was applied. In the literature there are many reports indicating that this technique can be used to transform crystalline materials into an amorphous one [30,31]. Moreover, it was demonstrated that in the case of poorly soluble and thermally unstable substances ball milling seems to be the only way of obtaining materials in the amorphous form. However, to induce amorphization by mechanical milling some basic conditions must be satisfied. The most important one is that grinding must be performed below the glass transition temperature of the investigated material. It was shown that the efficiency of milling depends on the difference between the milling temperature and the position of the glass transition temperature  $T_g$ . When milling is carried out deeply below  $T_g$ , complete amorphization is attained much quicker and the presence of residual nuclei of the starting crystalline phase can be avoided. Consequently, thermally stable amorphous materials can be obtained. One can also add that as milling is carried out above the glass transition temperature, only crystal-crystal transformation between two polymorphic forms is possible [32]. Besides choosing the temperature at which milling is performed, the speed of rotation, time of milling, number of balls, and their diameter and amount of milled substance are very important parameters which need to be controlled and optimized to induce solid state amorphization via grinding. In the experimental part, the procedure of amorphization of cyclodextrin has been described in detail.

In Fig. 3 x-ray diffraction patterns obtained for initial materials as well as those milled in the high-energy planetary mill are presented. It can be seen that in the crystalline samples sharp Bragg's peaks are clearly visible, while in the milled ones only an amorphous halo is observed. Thus, we were sure that the applied procedure of milling enabled us to obtain completely amorphous cyclic carbohydrates. However, CDs were exposed to very extreme conditions such as mechanical stress, local heating, etc., so there was a possibility that they might undergo chemical degradation upon grinding. To verify this supposition additional NMR measurements were done (data not shown). We found that there was no trace of degradation of cyclodextrins. Hence, further measurements with the use of differential scanning calorimetry (DSC) as well as dielectric spectroscopy were carried out to describe the water evaporation and molecular dynamics of these materials.

At the beginning, calorimetric measurements were performed. The DSC thermograms for  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrins are presented in Fig. 4. As can be seen, in each heating run two endothermic peaks were detected: the first one (at lower temperatures) is associated with the presence of water, whereas the second one (at higher temperatures) is associated with thermal decomposition. The first peak is quite broad because water evaporation takes place over a wide range of temperatures. From the heat of evaporation of bulk water (2400 J/g at 373 K), we estimated the amount of moisture present in our samples as 9.7% for  $\alpha$ -cyclodextrin, 10.4% for  $\beta$ -cyclodextrin, and 9.8% for  $\gamma$ -cyclodextrin. One can add that the same amount of moisture was determined from the Karl Fischer titrimetry. In the past differential scanning calorimetry as well as thermogravimetry (TGA) were used to

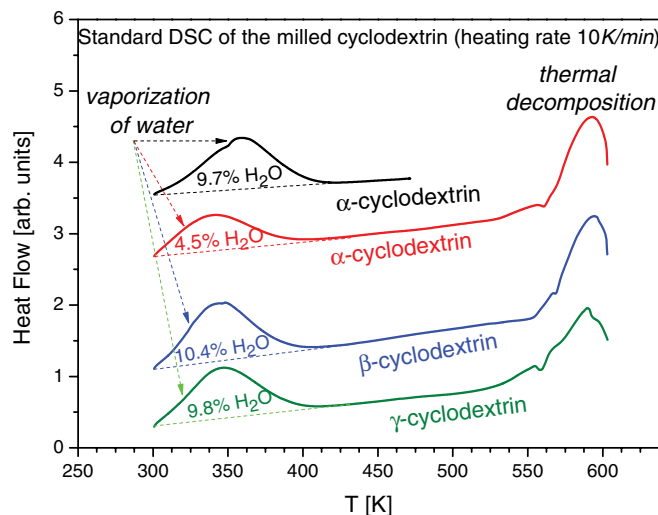


FIG. 4. (Color online) Thermograms obtained for the ball milled  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrins.

follow the kinetics of dehydration in cyclodextrin. Moreover, detailed studies with the use of a TGA method enabled us to characterize and describe the mechanism of evaporation of water from these materials [33], which proceeds in four steps. The first one is due to the release of surficial water, the next two correspond to the water present in the interstices, and finally the water trapped in the cavity. Unfortunately, our calorimetric data are not so precise and do not provide such detailed information about water evaporation from cyclodextrins. However, one can still estimate temperature regions at which water evaporates fastest and temperatures at which this process ends up. It is worth adding that in each case water evaporation is terminated at around  $T = 400$  K.

In the Introduction it was mentioned that the glass transition temperatures of cyclodextrins are imprecisely defined. Thus, it was necessary to estimate their  $T_g$ 's. However, in the range of studied temperatures we did not find any endothermic event which could be a signature of the glass transition. Thus, our data confirmed the finding reported by Tabary *et al.* [19] that  $T_g$ 's of cyclodextrins are located above temperatures at which thermal degradation begins. Finally, it should also be pointed out that DSC measurements demonstrated that amorphous cyclic carbohydrates obtained from the ball milling are very stable and do not crystallize even at very high temperatures.

Since we have determined water content in the investigated CDs from the DSC measurements and Karl-Fischer titrimetry, further dielectric measurements were carried out to describe their molecular mobility in the amorphous phase. In Fig. 5 dielectric loss spectra obtained for hydrated amorphous cyclodextrins are presented. As can be seen, in each case there is only one well-pronounced relaxation process shifting to lower frequencies with lowering temperature. It is interesting to point out that this relaxation process has huge amplitude which decreases with temperature. Moreover, it has asymmetric shape. Hence, one can suppose that this relaxation process has very similar dynamical properties to the  $\gamma$ -relaxation process in other saccharides. This issue will be investigated in a later part of this paper. In Fig. 5 we also present loss spectra measured for each cyclodextrin at the same temperature. It



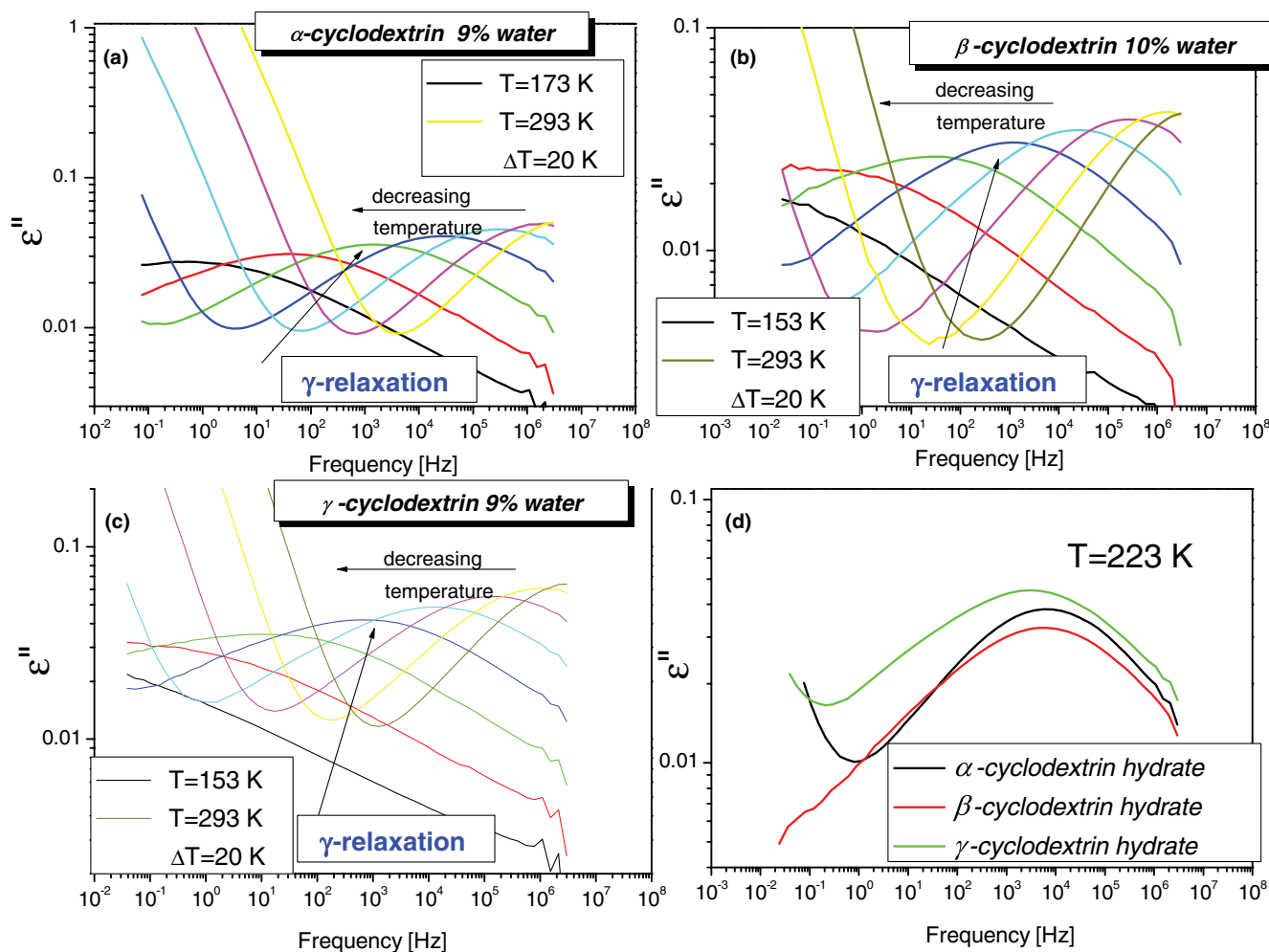


FIG. 5. (Color online) Dielectric loss spectra measured for hydrated ball milled  $\alpha$ - (a),  $\beta$ - (b), and  $\gamma$ - (c) cyclodextrins. In (d) the dielectric loss spectra of hydrated cyclodextrins measured at  $T = 223$  K are presented.

is visible that the maximum of the  $\gamma$  peak is located almost at the same frequency irrespectively of the sample. One can also note that the  $\gamma$  peaks of the investigated CD's differ in shape.

In the next step anhydrous samples were measured. It should be mentioned that complete dehydration of cyclodextrins was achieved by annealing them under vacuum at  $T = 413$  K for 2 h. Karl Fischer titrimetry confirmed that the moisture content was below 0.3% in each case. In Fig. 6 dielectric loss spectra obtained for anhydrous  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrins are presented. As illustrated, in anhydrous amorphous cyclodextrins there are two relaxation processes. They separate from each other with lowering temperature. It can also be seen that both relaxation processes differ significantly in amplitude. A very similar scenario was also observed in the case of disaccharides and polysaccharides [34]. Thus, it can be supposed that relaxation processes observed in the amorphous state of cyclodextrins are of the same origin as those found in chain saccharides.

In Fig. 6(d) dielectric loss spectra of  $\alpha$ -cyclodextrin having different moisture contents have been shown. It can be seen that the fast relaxation process ( $\gamma$ ) moves only slightly to higher frequencies with an increase in the content of water, thus, the relaxation time of the  $\gamma$  process is weakly dependent

on water concentration. A completely different situation is observed in the case of the slow relaxation mode ( $\beta$ ). This process also moves to higher frequencies, but a change in relaxation times is greater than two decades. One can mention that a similar scenario was found to occur in the glassy state of disaccharides [35], i.e., the position of the fast relaxation mode does not change with the addition of water, whereas the same relaxation time of the  $\beta$ -process changed more than a few decades.

Since we have found that there are a lot of similarities between the dynamics of the  $\beta$ - and  $\gamma$ -relaxations visible in disaccharides, polysaccharides, as well as cyclodextrins, we have decided to compare the loss spectra obtained for the latter carbohydrates with those measured for trehalose and sucrose [36] at the same temperature (see Fig. 7). As can be seen, the slow mode in disaccharides (sucrose and trehalose) is slower than the  $\beta$ -relaxation in cyclic carbohydrates. However, it should be stressed that differences in the relaxation times are not significant (less than one decade). Moreover, the maximum of the  $\gamma$ -relaxation peak is located in the same place, independently of the sample. Hence, basing on this simple comparison one can suppose that, in fact, the molecular origin of the  $\beta$ - and  $\gamma$ -relaxation processes in cyclic as well as chain

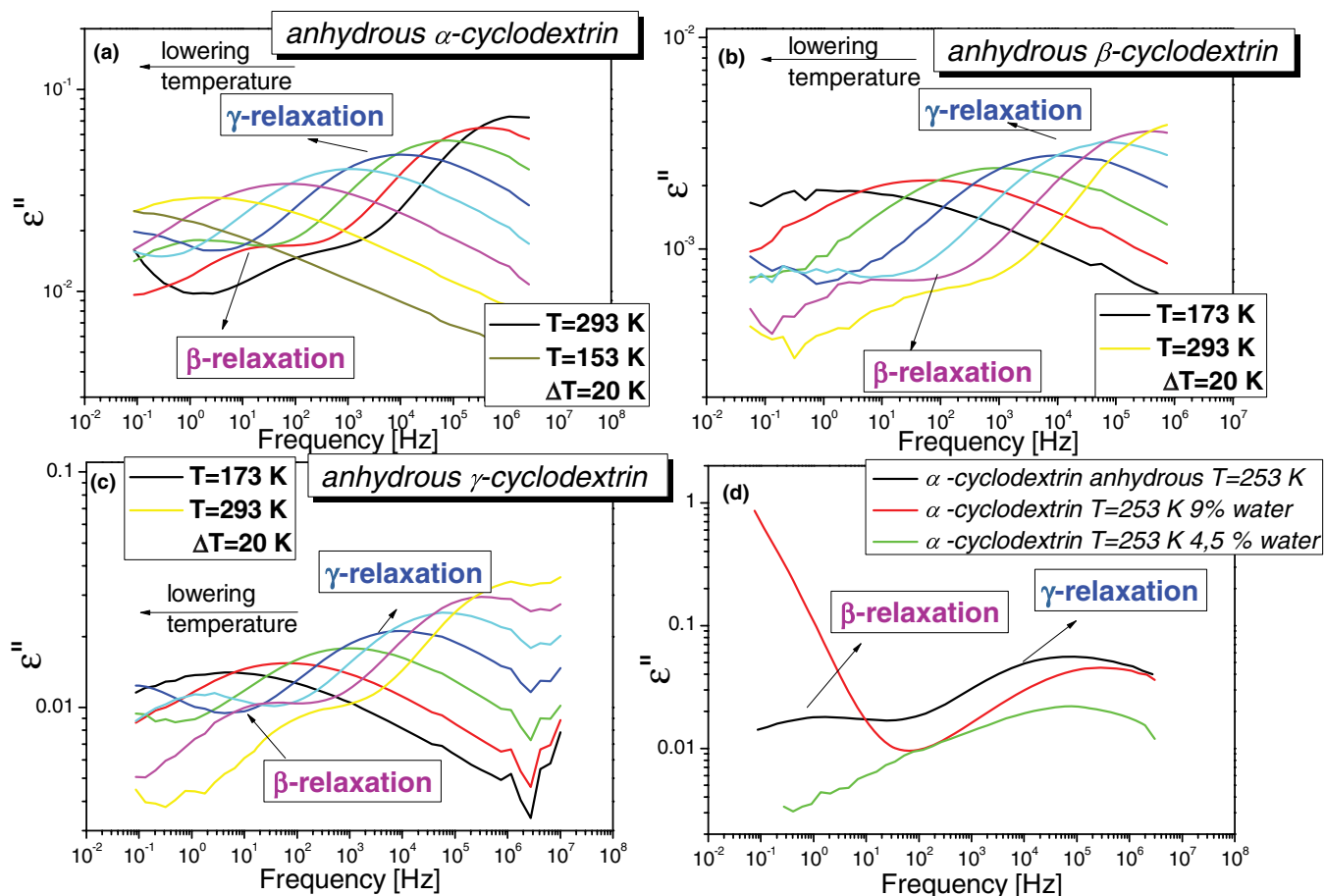


FIG. 6. (Color online) Dielectric loss spectra measured for the anhydrous ball milled  $\alpha$ - (a),  $\beta$ - (b), and  $\gamma$ - (c) cyclodextrins. In (d) the dielectric loss spectra of  $\alpha$ -cyclodextrin having different concentrations of water measured at  $T = 253$  K are presented.

saccharides is much the same. It is also worth to noting that the positions of the  $\beta$ -relaxation peaks in cyclodextrins are quite different, indicating that this mode is sensitive to the structure of the given cyclodextrin.

To get more detailed information about the dynamics of secondary relaxation, the dielectric loss spectra obtained for the anhydrous and hydrated cyclodextrins have been

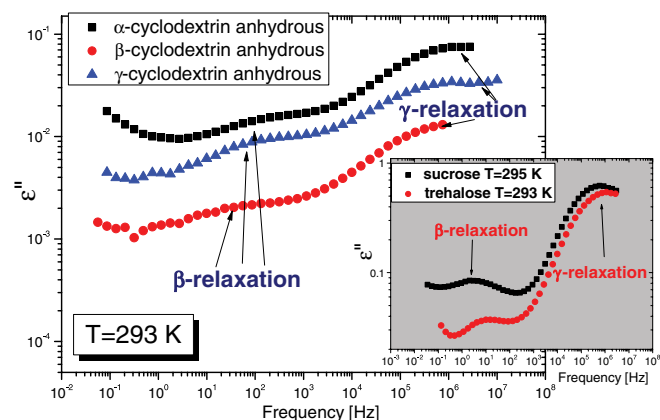


FIG. 7. (Color online) Dielectric loss spectra measured at  $T = 293$  K for  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrin. Inset presents the dielectric loss spectra obtained for sucrose and trehalose at indicated temperatures.

analyzed. The faster and slower modes were fitted to the Havriliak-Negami and Cole-Cole functions, respectively. Next a relaxation map has been constructed (see the left panel of Fig. 8). It should be added that temperature dependencies of the relaxation times of slow as well as faster relaxation processes were fitted to the Arrhenius equation

$$\tau_{\beta,\gamma} = \tau_0 \exp\left(\frac{E_a}{k_B T}\right). \quad (1)$$

All fitting parameters are collected in Table I.

#### A. Water influence on the dynamics of the fast relaxation ( $\gamma$ ) process in cyclodextrins

One can observe that the activation energy of the  $\gamma$ -relaxation process in hydrated cyclodextrins lies within the range 61–67 kJ/mol, while in anhydrous samples it is equal to 42–48 kJ/mol. Hence, one can conclude that water influences the activation energy of this mode very significantly. It should be added that a similar situation was also found in the case of mono- and chain saccharides. It was well presented in literature that the activation energy of a  $\gamma$ -relaxation process increases with the addition of water in such carbohydrates [37]. What is more,  $E_a$  of the  $\gamma$  process in hydrated saccharides was estimated to be equal to 62 kJ/mol [37]. Very similar values

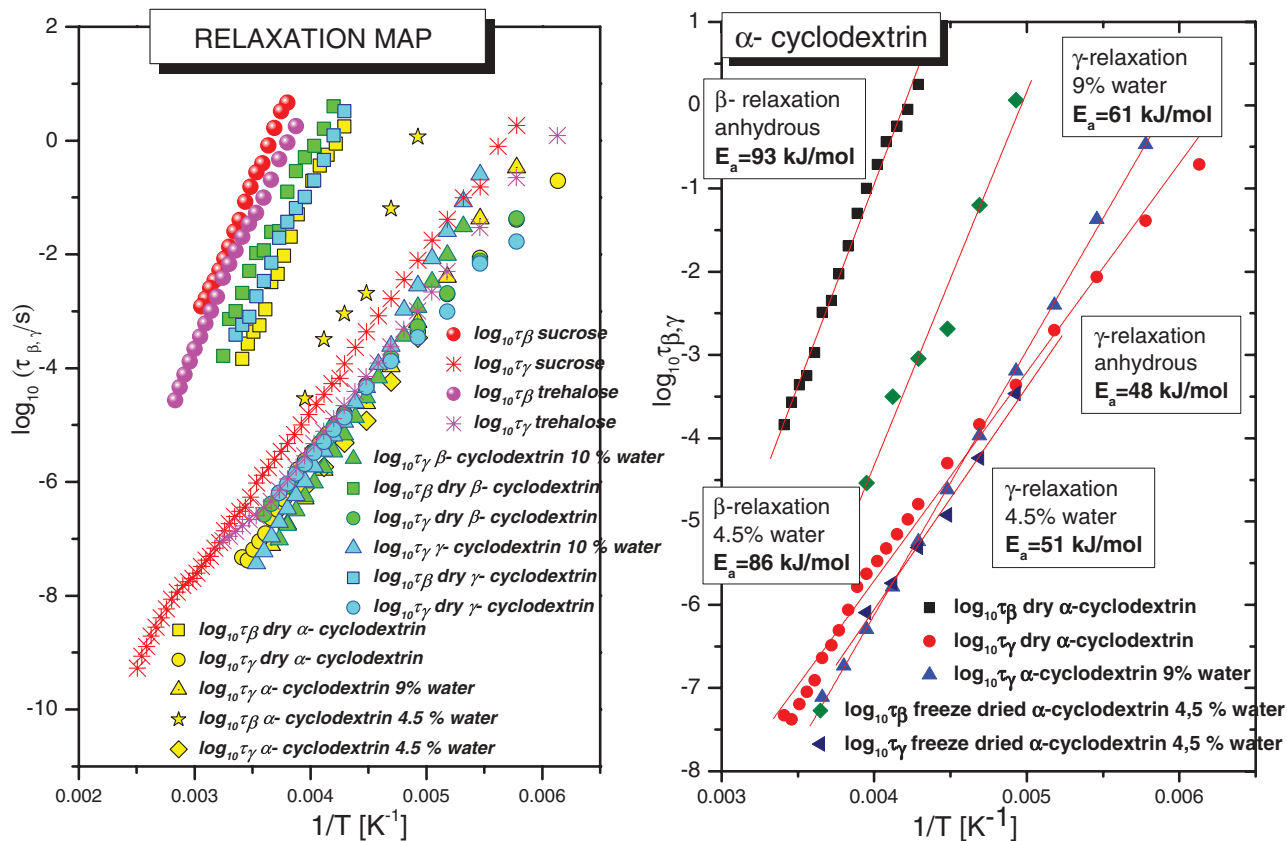


FIG. 8. (Color online) Left panel: Relaxation map of hydrated and anhydrous  $\alpha$ -,  $\beta$ -,  $\gamma$ -cyclodextrins. Relaxation times obtained for sucrose and trehalose were also included. Right panel: Relaxation times obtained for hydrated and anhydrous  $\alpha$ -cyclodextrins. Solid lines are fits to  $\beta$  and  $\gamma$  relaxations using Eq. (1).

of the activation barriers of the  $\gamma$  process indicate that the nature of this mode in cyclodextrins and chain saccharides is the same. Since we have identified motions of the exocyclic  $\text{CH}_2\text{OH}$  group to be responsible for the  $\gamma$ -relaxation process in saccharides [38], one can suppose that the same type of motions is also responsible for the fast relaxation mode of cyclodextrins. We can speculate herein on the mechanism of interaction between water and exocyclic hydroxymethylene groups in chain and cyclic saccharides. We suppose that water interacts with  $\text{CH}_2\text{OH}$  groups via hydrogen bonds, making it less flexible. Consequently, a greater activation barrier needs to be overcome to enable movements or rotation of this moiety.

However, to be sure of this concept additional molecular dynamics simulation should be performed.

### B. Water influence on the dynamics of the slow ( $\beta$ ) relaxation process in cyclodextrins

From the Arrhenius fits we have also estimated the activation energy for the slow ( $\beta$ ) relaxation process which is observed only in completely dried and partially hydrated cyclodextrins. For this relaxation process  $E_a$  varies from 93 to 80 kJ/mol, dependent on the sample. The greatest and the lowest activation barriers for this process were estimated for

TABLE I. Fitting parameters.

| Name   | Preexponential factor |                                   | Preexponential factor |                                    |
|--|-----------------------|-----------------------------------|-----------------------|------------------------------------|
|  | $\log_{10}\tau_0$ (s) | $E_a$ $\beta$ relaxation (kJ/mol) | $\log_{10}\tau_0$ (s) | $E_a$ $\gamma$ relaxation (kJ/mol) |
| $\alpha$ -cyclodextrin (dry)                 | -20.35                | 93                                | -15.72                | 48                                 |
| $\alpha$ -cyclodextrin (hydrated 9% water)   |                       |                                   | -18.84                | 61                                 |
| $\alpha$ -cyclodextrin (hydrated 4,5% water) | -22.24                | 86                                | -16.73                | 51                                 |
| $\beta$ -cyclodextrin (dry)                  | -17.49                | 83                                | -15.13                | 46                                 |
| $\beta$ -cyclodextrin (hydrated 10% water)   |                       |                                   | -19.87                | 66                                 |
| $\gamma$ -cyclodextrin (dry)                 | -17.57                | 80                                | -14.41                | 42                                 |
| $\gamma$ -cyclodextrin (hydrated 10% water)  |                       |                                   | -19.94                | 67                                 |
| Sucrose                                      | -18.76                | 96                                | -15                   | 52                                 |
| Trehalose                                    | -17.20                | 87                                | -16.06                | 51                                 |

the anhydrous  $\alpha$ - and  $\gamma$ -cyclodextrin, respectively. Moreover, we also noted that water decreases the activation barrier of the  $\beta$  process. Interestingly, the addition of water influences the activation barriers for the  $\beta$  and  $\gamma$  modes in completely opposite ways. This is well illustrated in the right panel of Fig. 8 constructed only for  $\alpha$ -cyclodextrin. One can see that the activation barrier for the slow and fast modes decreases and increases, respectively with the addition of water. Consequently, both relaxation modes tend to merge with the addition of water. Therefore, one can suppose that  $\beta$ - and  $\gamma$ -relaxation modes contribute to the observed pronounced relaxation process in hydrated samples.

In the context of what was reported above, one can mention the paper by Ermolina *et al.* [35], in which the effect of the addition of water on the dynamics of  $\beta$  relaxation in disaccharides was observed. As was found, water influences the dynamics of this process in the same way as a slow mode in cyclic carbohydrates. This can act as further evidence that the molecular motions underlying the  $\beta$  process in cyclodextrins and chain saccharides are of the same type.

Finally, we have also included in the relaxation map of cyclodextrins (left panel of Fig. 8) and Table I relaxation times as well as activation energies evaluated for the  $\beta$ -relaxation process detected in trehalose and sucrose, respectively. We found that the relaxation times and activation barriers estimated for the  $\beta$  process in disaccharides are very similar to the equivalent parameters determined for the slow mode in dry cyclodextrins. Hence, we found that the dynamics of the  $\beta$ -relaxation process seen in chain saccharides as well as in cyclodextrins is fairly the same.

At this point one can mention that the origin of the  $\beta$  process of disaccharides and polysaccharides was discussed in literature. Different authors proposed completely different explanations of this process [39–41]. However, our detailed dielectric studies combined with theoretical density functional theory computations enabled us to identify the motions responsible for the  $\beta$ -relaxation process [42–44] in chain saccharides. We found that this relaxation is connected to the motions of the glycosidic linkage and can be treated as a very useful source of information about the dynamics of the glassy state of disaccharides. Moreover, it was shown that based on the dynamical properties of  $\beta$  relaxation, one can calculate the structural relaxation time even deep below the glass transition temperature [45]. Hence, based on the above consideration we can certify that the slow relaxation process observed in the dried cyclodextrins is connected to the motions of the glycosidic linkage. Furthermore, based on our dielectric data we can predict the rigidity of cyclodextrins. Since we

have determined the activation barriers for the motion of the glycosidic bond to be the greatest in the case of  $\alpha$ -cyclodextrin, it can be regarded as the most rigid of all herein investigated cyclic carbohydrates. It is also worth noting that the mobility of glycosidic linkage increases with increasing the size of cyclodextrin.

#### IV. CONCLUSIONS

In this paper the molecular dynamics of three hydrated and anhydrous cyclodextrins ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) was investigated and fully described. As it turned out, there is only one relaxation process in hydrated and two modes in dried amorphous cyclic carbohydrates. Moreover, the effect of water on the dynamics of fast and slow relaxation modes was studied in detail. It was shown that the activation energies as well as the positions of peak maximums are affected by the addition of water. We found that the  $\beta$ - and  $\gamma$ -relaxation processes visible in the amorphous state of cyclodextrins have very similar dynamical properties as the relaxation modes detected in the glassy state of di- or polysaccharides. A comparison of dielectric spectra, activation energies, and relaxation times enabled us to conjecture that the same type of motions are responsible for the slow and the fast relaxation processes observed in cyclodextrins, and other chain carbohydrates. Based on the dielectric data collected for hydrated and anhydrous cyclodextrins we were able to make some predictions about the rigidity of cyclic saccharides. It seems that  $\alpha$ -cyclodextrin is the most rigid of all herein studied carbohydrates. What is more, we also found out that water makes the structure of a given cyclodextrin more flexible. We think that water weakens the internal hydrogen bonds formed between glucose units, making glycosidic linkage more flexible in each cyclodextrin. Consequently, this is the reason why we observe a decrease of activation energy of the  $\beta$  process with the addition of water. Finally, we have also shown that ball milling can be successfully applied to obtain completely amorphous cyclodextrins, avoiding their chemical decomposition.

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