Carbohydrate Research 343 (2008) 2771–2775

Contents lists available at ScienceDirect

Carbohydrate Research

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

The influence of cosolvents on hydrophilic and hydrophobic interactions. Calorimetric studies of parent and alkylated cyclomaltooligosaccharides in concentrated aqueous solutions of ethanol or urea

Giuseppina Castronuovo *, Marcella Niccoli

Department of Chemistry, University Federico II of Naples, Complesso Universitario Monte S. Angelo, Via Cintia I-80126 Naples, Italy

ARTICLE INFO

Article history:
Received 29 February 2008
Received in revised form 14 May 2008
Accepted 16 May 2008
Available online 24 May 2008

Keywords:
Cyclomaltooligosaccharides (cyclodextrins)
Enthalpic interaction coefficients
Excess properties
Microcalorimetry
Mixed solvents

ABSTRACT

Heats of dilution in water and in aqueous 7 mol kg^{-1} urea and 3 mol kg^{-1} ethanol of binary solutions containing cyclomaltohexaose, cyclomaltoheptaose, cyclomaltooctaose, 2-hydroxypropyl-cyclomaltohexaose (HPαCD), 2-hydroxypropyl-cyclomaltoheptaose (HPβCD), methyl-cyclomaltohexaose (MeαCD), methyl-cyclomaltoheptaose (MeβCD) and 2-hydroxypropyl-cyclomaltooctaose (HPγCD) have been determined at 298.15 K by flow microcalorimetry. The purpose of this study is to gain information about the influence of urea and ethanol, which have different effects on water structure, on hydrophilic and hydrophobic interactions. The pairwise interaction coefficients of the virial expansion of the excess enthalpies were evaluated and compared to those previously obtained for binary solutions of cyclomaltohexaose and cyclomaltoheptaose. The particular behaviour of cyclomaltooligosaccharides in water is put in evidence with respect to that shown by simple oligosaccharides. The values of the interaction coefficients greatly change in dependence of the solvent medium. They are negative in water for unsubstituted cyclomaltooligosaccharides, and positive for the alkyl-substituted ones, thus marking the major role of the hydrophobic interactions. In concentrated aqueous ethanol, coefficients are negative, while they are positive in concentrated aqueous urea. Urea solvates the hydroxyl group provoking the attenuation of hydrophilic and hydrophobic interactions. Instead, the presence of the cosolvent ethanol, which lowers the relative permittivity of the medium, enhances the strength of hydrophilic interactions.

1. Introduction

Cyclomaltooligosaccharides (cyclodextrins, CDs) are (1→4)-linked cyclic glucooligosaccharides. The most commonly studied of these substances are cyclomaltohexaose (αCD), cyclomaltoheptaose (βCD) and cyclomaltooctaose (γCD) with 6, 7 and 8 glucose units, respectively.[1,2] The primary and secondary hydroxyl groups of the glucose units make the exterior of the molecule hydrophilic, while the interior surface of the truncated cone structure (the cavity), normally considered as the site of the guest molecule, is largely hydrophobic. The hydrophobic cavities allow inclusion complexes to form with a large variety of organic and inorganic compounds in different solvents, including water.[2–7] Applications have been described of molecular modelling techniques to the study of the static and dynamical features of CDs, as well as their participation in the formation of inclusion complexes.[8]

In preceding papers from this laboratory, efforts were made to understand the factors determining the formation of the complexes between CDs and alkylated substances in solution.[9–12] The purpose of the present study is to obtain more information about the forces acting in the binary aqueous solutions of parent and chemically modified CDs. To that, a calorimetric study at 298 K is reported of the heats of dilution in water and in concentrated aqueous urea or ethanol of cyclomaltohexaose (αCD), cyclomaltoheptaose (βCD), cyclomaltooctaose (γCD), 2-hydroxypropyl-cyclomaltohexaose (HPαCD), 2-hydroxypropyl-cyclomaltoheptaose (HPβCD), methyl-cyclomaltohexaose (MeαCD), methyl-β-cyclomaltoheptaose (MeβCD) and 2-hydroxypropyl-cyclomaltooctaose (HPγCD). Urea and ethanol have different effects on water structure: the former is a well-known chaotropic agent, while the latter is a prevailingly hydrophobic structure maker. We shall analyze how the modifications induced in the structure of water by the two cosolvents modify the interactions between pairs of hydrated molecules. Through this kind of study, we hope it will be possible to relate the properties of the binary solutions with the inclusion requirements in the host–guest complexes.

The parameters that are most commonly used to characterize nonbonding interactions in solution are the pairwise coefficients of the virial expansion of the excess thermodynamic properties of a solution.[13–15] The physical meaning of these parameters is linked to the variations in thermodynamic properties when two

* Corresponding author. Tel.: +39 081 674239; fax: +39 081 674090.
E-mail address: giuseppina.castronuovo@unina.it (G. Castronuovo).

0008-6215/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2008.05.016

hydrated molecules are brought from an infinite separation, where solute–solvent interactions are dominant, to a finite separation where solute–solute, water-mediated interactions are operating. Then, they are very useful to get information about the mechanism through which two hydrated molecules interact in solution. The model of overlapping hydration spheres of hydrated molecules accounts qualitatively for the sign and magnitude of these coefficients. Just on the basis of the signs and values of the enthalpic pair interaction coefficients, it has been already inferred that parent CDs in aqueous solutions are very particular substances, since they behave very differently from other simple saccharides, rather resembling higher molecular weight polyhydric alcohols.[16,17] Cyclomaltooligosaccharides, especially their alkylated derivatives, are essential in pharmaceutical applications as they provide versatile carrier and delivery systems for drug molecules.[18–23] From that arises the importance of further investigating their binary solutions in different aqueous solvents. Indeed, the possibility to modify the forces acting in these solutions by changes in the structure of the solvent can be useful when designing new cyclomaltooligosaccharides derivatives with more suitable characteristics to include specific drugs.

2. Results

Heats of dilution were measured at 298 K in water, and in the presence of a constant amount of ethanol or urea. For the investigated systems, dilution is an exothermic or endothermic process and the derived enthalpic interaction coefficients are positive or negative, respectively.

In Table 1, the pairwise enthalpic interaction coefficients are reported for the binary aqueous solutions of parent and modified CDs. In the same Table, some literature data are also shown with the aim of presenting a complete thermodynamic framework of the behaviour of the cyclomaltooligosaccharides studied in this laboratory.[9,16,26–28]

In water, the coefficients for unsubstituted CDs are large and negative, the most negative characterizing the smallest cyclomaltooligosaccharide, αCD. On the contrary, methyl- and hydroxypropyl-substituted CDs are characterized by large and positive pairwise enthalpic interaction coefficients. Methyl derivatives are characterized by coefficients which are larger than those for the hydroxypropyl derivatives. It should be noted that the enthalpic coefficient reveals to be a useful parameter to distinguish among CDs of different degree of substitution. For instance, the coefficient for MeβCD (DS = 12) is 24,000 J kg mol^{-2}, while that for MeβCD (DS = 10–14) is 28,000 J kg mol^{-2}. The same occurs for HPβCD, DS = 3 and DS = 6.3, whose coefficients are 4812 and 15,498 J kg mol^{-2}, respectively.

In 3 mol kg^{-1} ethanol, coefficients for parent CDs become more negative than in water, while those for the alkylated derivatives change from positive to negative. The more positive the value of the coefficient in water, much more pronounced is the change in its value on passing to ethanol (as an example, see MeβCD, DS = 12).

In 7 mol kg^{-1} urea, all coefficients are positive. Parent CDs change from negative in water to positive in urea, cyclomaltooctaose presenting the largest difference. Instead, methyl and hydroxypropyl derivatives have only a small decrease in the positive values of their coefficients. In Table 1, three data refer to αCD, βCD and MeβCD in phosphate buffer: coefficients are positive, thus indicating a behaviour qualitatively similar to that in urea.

3. Discussion

The structure of the smallest cyclomaltooligosaccharide, αCD, in water can be assumed to resemble that of αCD hexahydrate in the crystalline state. Namely, the 'void' molecule of αCD has two water molecules entrapped in the cavity, hydrogen-bonded to each other and to two glucopyranose rings.[29,30] The pairwise enthalpic interaction coefficient for αCD is negative: on the basis of this sign, it can be classified as a prevailingly hydrophilic structure-breaking solute.[31] According to an interaction model that provides the presence of configurations between two hydrated molecules stabilized by the juxtaposition of groups having the same action on water structure, the negative sign is due to the juxtaposition of the external hydrated hydroxyl groups.[32] For βCD and γCD, coefficients are negative, too, but smaller than that for αCD, thus indicating that the larger macrocycles are structure breaker solutes less effective than αCD. Literature reports that αCD undergoes a tense → relaxed transition upon formation of a complex,[30] hydrogen-bonded water molecules being squeezed out of the cavity, relaxing to the bulk. To the contrary, βCD and γCD do not experience that conformational transition, and they would present relaxed conformations even in the absence of a guest, with the larger cavities more exposed to bulk solvent. The negative coefficients characterizing parent CDs in water make their behaviour to be different from that of other saccharides, which are characterized by positive coefficient. Cyclization is a factor that greatly influences interactions between molecules, since the rigidity of the cycles, compared to the flexibility of a linear chain, leads to face-to-face configurations stabilized by hydrophilic interactions which act through the juxtaposition of hydroxyl groups. As a fact, parent CDs mimic the behaviour of some higher members of polyols, whose enthalpic interaction

Table 1
Pairwise enthalpic interaction coefficients[a] for parent and modified cyclomaltooligosaccharides in water and in aqueous mixed solvents, at 298 K

Substance	Water	Ethanol 3 mol kg^{-1}	Na_2HPO_4 0.5 mol kg^{-1}	Urea 7 mol kg^{-1}
αCD	−3920 ± 60[b]	−7130 ± 50	1360 ± 60[c]	2760 ± 60[d]
MeαCD (DS 11)	18,200 ± 800	−5200 ± 300		12,530 ± 60
2HPαCD (DS 4.5)	9400 ± 200	−1400 ± 100		8500 ± 200
βCD	−2800 ± 200[e]	−10,300 ± 300[e]	4200 ± 100[f]	1200 ± 30[d]
MeβCD (DS 12)	24,100 ± 300	−6300 ± 200		15,000 ± 400
MeβCD (DS 10–14)	28,400 ± 500[f]		68,000 ± 1000[f]	
2HPβCD (DS 3)	4800 ± 100	−7100 ± 700		7200 ± 300
2HPβCD (DS 6.3)	15,500 ± 200	−1000 ± 200		10,700 ± 600
γCD	−3160 ± 90	−10,900 ± 300		5700 ± 400
2HPγCD (DS 4.5)	12,700 ± 200	−2700 ± 300		11,900 ± 200

[a] Units: J kg mol^{-2}. Errors reported are the 95% confidence limits.
[b] Ref. 16.
[c] Ref. 26.
[d] Ref. 27.
[e] Ref. 28.
[f] Ref. 9.

coefficients change sign depending on their stereochemistry.[17] For those substances, the hypothesis was that the separation of the hydrophilic and hydrophobic domains in the solute molecule determines sign and values of the pairwise enthalpic coefficients. Parent CDs should behave in the same way since primary and secondary hydroxyl groups make them hydrophilic molecules at the exterior, while the interior cavity is largely hydrophobic. The value of the pairwise enthalpic interaction coefficient should, then, be thought as composed of two contributions, one originating from the overlapping of the hydrated hydrophilic exterior and the other from the displacement of structured water present in the hydrophobic cavity. The former contribution is negative for the hydrophilic–hydrophilic interactions, while the latter is positive. For αCD, the cavity is smaller and the negative contribution due to the overlapping of hydrated external hydroxyls is prevailing. On the contrary, the cavities of βCD and γCD are larger, and, unlike αCD, they would participate to the interaction between two hydrated molecules. That positive contribution makes less negative the coefficients, notwithstanding the larger size of the macrocycles. The finding that interactions between two CD molecules are prevailingly hydrophilic is in agreement with the literature dealing with the cyclomaltooligosaccharide capability of self-assembling in aqueous solutions. In particular, it is found that αCD spontaneously forms films, at aqueous solutions/air interfaces, consisting of self-assembled nanotubes. The building blocks of the films are CD dimers that are bridged, on average, by 9–10 hydrogen bonds formed by the hydroxyl groups located at the edge of the truncated cone.[33]

Replacing hydroxyl groups with methyl or hydroxypropyl groups leads to a completely different thermodynamic behaviour. Coefficients are large and positive: hence, these solutes can be classified as prevailingly hydrophobic structure-makers. The large values of the coefficients originate from the relaxation of many structured water molecules from the cavity and from the hydrophobic hydration shell of the external methyl or hydroxypropyl groups when two hydrated molecules approach each other. The coefficients for the methyl substituted CDs are very large, while those for the corresponding hydroxypropyl derivatives are less positive notwithstanding the fact that the hydroxypropyl group has a longer alkyl chain. Probably, in front of increased hydrophobic interactions, there is a negative contribution from hydrophilic interactions between the terminal hydroxyl groups. Finally, it should be noted that coefficients increase on going from αCD to γCD, at a given number of glucopyranose rings and alkyl groups. That confirms the hypothesis of an increasing participation of the hydrophobic cavity to the pairwise interaction on passing from the smaller to the wider CDs.

Several factors can determine the influence of a cosolvent on the non-bonding intermolecular interactions acting in solution: among them, the changes in the dielectric constant of the medium, in the structure of bulk water and in the hydration of the CDs under examination. In concentrated urea, the coefficients for parent CDs change from negative to positive. That reproduces the behaviour shown by glycine, formamide and other hydrophilic solutes, whose pair enthalpic interaction coefficients become positive or less negative at increasing urea concentration.[34] According to studies on ternary aqueous solutions of hydroxylated substances, urea interacts mainly with hydrophilic domains, since hydrophilic–hydrophilic interactions are favoured for a negative contribution to the excess Gibbs energy.[35] Then, it seems reasonable to hypothesize that, in concentrated aqueous solutions of urea, urea solvates prevailingly the external hydroxyl groups of the CD so that the consequent steric hindrance attenuates hydrophilic interactions between them. This transition to a thermochemically unfavourable behaviour has been partially ascribed to the enhanced relative permittivity of the medium determined by urea, an effect reducing polar hydrophilic interactions. Both effects reduce the negative contribution to the enthalpic interaction coefficients, which become positive.

Alkylated cyclomaltooligosaccharides in concentrated urea are characterized by positive coefficients, too, but smaller with respect to those in water, with the only exception of 2HPβCD (DS = 3). For these substances, coefficients are determined by three contributions. One contribution, due to the interaction of urea with hydroxyl groups, attenuates hydrophilic interactions, and so does the second one due to the increased dielectric constant of the medium. The third one originates from hydrophobic interactions between the hydration shells of the alkyl groups. In preceding papers dealing with the influence of urea on hydrophobic solvation of alkanols, we found that the favourable interaction of urea with the functional group disturbs the hydrophobic hydration. Also in concentrated urea, hydrophobic interactions are attenuated so that, upon the interaction between two hydrated molecules, the number of water molecules relaxing to the bulk is smaller. In the present case, the first two effects should increase the values of the coefficients, while the third one should lead to a decrease. Indeed, the coefficients obtained are smaller than those in water (see Table 1), thus indicating that the prevailing effect is an overall decrease of hydrophobic interactions. On the contrary, 2HPβCD (DS = 3) is characterized by a coefficient larger than that in water, probably because the degree of substitution is too low: in that case the two contributions leading to the increase of the coefficient prevail on the attenuation of hydrophobic interactions. In phosphate buffer, coefficients are positive for the natural CDs, and increase towards more positive values for the alkylated ones, an indication that phosphate ions behave qualitatively as urea. The very large coefficient for methyl-cyclomaltoheptaose underlines that the interaction between two hydrated molecules is a process ruled mainly by hydrophobic interactions, hence an entropy-driven process.

Coefficients for parent CDs and their alkylated derivatives become negative or more negative in 3 mol kg^{-1} ethanol than in pure water. The addition of ethanol, less polar than water, lowers the relative permittivity of the aqueous medium potentiating hydrophilic interactions between the external hydroxyl groups of the CDs. On the other hand, ethanol, a well-known structure maker solute promotes the ice-like structure of liquid water. Its presence as cosolvent lowers the energetic level of the bulk: hence, water molecules released upon pairwise interaction, relax from the hydration shells to a more structured medium. As a consequence, with respect to water the coefficients for parent CDs should become more negative than in water, while those for alkylated CDs should diminish or even change sign. The data reported in Table 1 support this interaction mechanism. Hence, ethanol makes alkylated CDs, which are prevailingly hydrophobic structure maker solutes ($h_{xx} > 0$ in water), to be described by negative enthalpic coefficients, which usually characterize prevailingly hydrophilic structure breaker solutes ($h_{xx} < 0$ in water). The same occurs for 1-alkanols, 1,2-diols and α-ω-diols in highly concentrated aqueous ethanol (9 mol kg^{-1}).[27,36]

The behaviour shown by parent and substituted cyclomaltooligosaccharides seems to have a common basis. Whatever the solvent, in fact, the coefficients become less positive or more negative at increasing number of free hydroxyl groups. In concentrated aqueous ethanol, the coefficients vary between -1000 J kg mol^{-2} and $-11{,}000$ J kg mol^{-2}, following the change in the strength of hydrophilic interactions. In water or concentrated aqueous urea, instead, coefficients vary in a wider range of values, -4000 J kg mol^{-2}–28,000 J kg mol^{-2}. This occurs in the presence of several contributions, among them are the hydrophobic interactions between the hydrated alkyl chains of the alkylated derivatives and the attenuation of hydrophobic and/or hydrophilic

interactions due to the action of the structure-breaking urea. For the derivatives with a small number of free hydroxyl groups, coefficients in water are more positive than in urea, an indication that in the latter medium the attenuation of hydrophobic interactions is the prevailing effect.

To conclude, cyclomaltooligosaccharides and their alkylated derivatives are good systems for obtaining information on the influence of cosolvents on hydrophobic and hydrophilic interactions. Although aware that the hypothesis of conformational changes experienced by the macrocycles in the different solvent media cannot be rejected, here the large variability in the values of the pairwise enthalpic interaction coefficients has been explained in terms of a balance among various, sometimes contrasting effects. This kind of study is very useful in that it tries to analyze how the features of solutes and solvent media determine the interaction between two hydrated molecules.

4. Experimental

4.1. Materials

Cyclomaltooligosaccharides employed were purchased from Cyclolab, with the exception of αCD, βCD and methyl-cyclomaltoheptaose (substitution degree 10–14) which were Sigma products. Native cyclomaltooligosaccharides were of the highest commercially available purity (98–99.5% minimum). For methyl-cyclomaltohexaose and 2-hydroxypropyl-cyclomaltohexaose, the mean substitution degree is 11 and 4.5, respectively, as determined by NMR. For methyl-cyclomaltoheptaose, 2-hydroxypropyl-cyclomaltoheptaose and 2-hydroxypropyl-cyclomaltooctaose the mean substitution degree is 12, 6.3 or 3.0 and 4.5, respectively, as determined by the same technique. All cyclomaltooligosaccharides were dried on phosphorus pentoxide, under reduced pressure, for 2 h at 25 °C. Solutions were prepared by weight: water was twice distilled and filtered on a Millipore membrane.

4.2. Calorimetry

Measurements of the heats of dilution were carried out using a Thermal Activity Monitor (TAM) from Thermometric, equipped with a flow cell and a P2 peristaltic pump (from Watson Marlow) for the pumping of solutions into the cells of the calorimeter. The calorimeter operated in a room whose temperature was fixed at (25.0 ± 0.5) °C. Thermally pre-equilibrated solutions were used, and the temperature control in the calorimeter was granted by a thermostatted water bath whose temperature varies within 0.01 °C. That allows a temperature stability of $\pm 2 \times 10^{-4}$ degree for a long time (about 8 h). The values of the dilution enthalpies, ΔH_{dil}, were obtained from

$$\Delta H_{dil}(m_x^i \rightarrow m_x^f) = (dQ/dt)/P_w$$

where (dQ/dt) is the heat evolved or absorbed per unit time, P_w is the total mass flow-rate of water per unit time, and m_x^i and m_x^f are the initial and final molalities, respectively. ΔH_{dil} is given in J kg^{-1} of solvent in the final solution.

4.3. Treatment of the data

According to the treatment of solution properties originally proposed by McMillan–Mayer[13] and specifically applied to those of aqueous solutions of nonelectrolytes by Kauzmann[14] and other authors,[15] an excess thermodynamic property can be expressed as a function of molalities through a virial expansion of pair and higher order interaction coefficients, j, as follows:

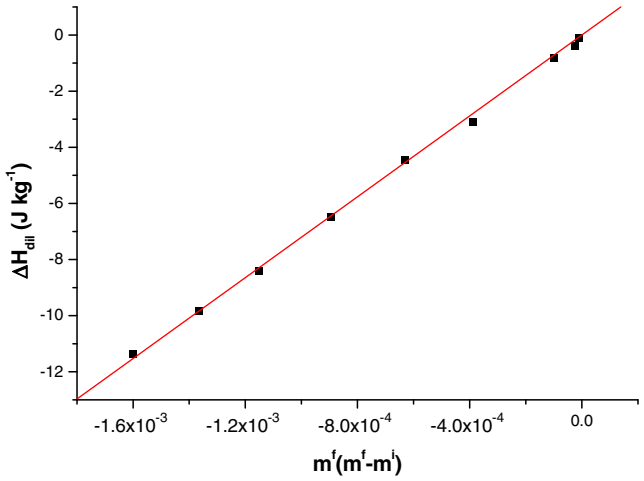

Figure 1. Dilution enthalpy versus $m^f(m^f - m^i)$ for 2-hydroxypropyl-cyclomaltoheptaose(DS = 3) in 7 mol kg^{-1} urea; m^f and m^i are the final and initial molalities, respectively, of the examined cyclomaltooligosaccharide.

$$J^E = \sum_{i=1} \sum_{k=1} j_{ik} m_i m_k + \text{higher terms} \quad (1)$$

For two-component solutions containing a solute x and the solvent, virial coefficients of the power series of the excess enthalpies, h, as a function of molalities can be easily derived from the enthalpies of dilution, ΔH_{dil}, as follows:

$$\Delta H_{dil}(m^i \rightarrow m^f) = h_{xx} m^f (m^f - m^i) + h_{xxx} m^f (m^{f2} - m^{i2}) + \text{higher terms} \quad (2)$$

where m_x^i and m_x^f are the molalities of the x solute before and after the dilution process, respectively. The h coefficients appearing in Eq. 2 represent the enthalpic contributions to the Gibbs free energy coefficients characterising the interaction between pairs, triplets, or higher order interactions. They implicitly account for all variations of solvent–solvent and solute–solvent interactions.[14,15] The values of the self coefficients for each solute are obtained by dilution of binary solutions in water or in a mixed solvent. To determine the coefficients, a least square procedure was used. Owing to the limited range of concentrations explored, only pairwise coefficients were found to be necessary for the best-fitting of experimental data. To show an example, in Figure 1 the dilution enthalpy is reported against the abscissa $m^f(m^f - m^i)$ for 2-hydroxypropyl-cyclomaltoheptaose in 7 mol kg^{-1} aqueous urea. The good linearity of the data for all the studied cyclomaltooligosaccharides, whatever the solvent, ensures that only pair interactions are operating in the concentration range explored. It is known that CDs are capable of self-aggregating in water to form columnar structures. However, different methods indicated that the fraction of the associates is very small (no more than 1%), or that the aggregation is limited to the formation of dimers.[24,25] From the present data we infer that, at the concentration employed (10^{-2}–10^{-3} mol kg^{-1} of solvent), only pair interactions determine the behaviour of the cyclomaltooligosaccharides in aqueous solutions.

References

1. Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344–362.
2. Szejtli, J. *Pure Appl. Chem.* **2004**, *76*, 1825–1845.
3. Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875–1917.
4. Rekharsky, M. V.; Inoue, Y. *J. Am. Chem. Soc.* **2000**, *122*, 4418–4435.
5. Clarke, R. J.; Coate, J. H.; Lincoln, S. F. *Adv. Carbohydr. Chem. Biochem.* **1988**, *46*, 205–211.
6. Connors, A. *Chem. Rev.* **1997**, *97*, 1325–1358.

7. Saenger, W. In *Inclusion Compounds*; Atwood, J. L., Davies, J. E. D., MacNicol, D., Eds.; Academic Press: London, 1984; Vol. 2, pp 231–259.
8. Lipkowitz, K. B. *Chem. Rev.* **1998**, *98*, 1829–1854.
9. Castronuovo, G.; Niccoli, M. *J. Inclusion Phenom. Macrocycl. Chem.* **2005**, *53*, 69–76.
10. Castronuovo, G.; Niccoli, M. *Bioor. Med. Chem.* **2006**, *14*, 3883–3887.
11. Castronuovo, G.; Niccoli, M. *J. Inclusion Phenom. Macrocycl. Chem.* **2007**, *58*, 289–294.
12. Castronuovo, G.; Niccoli, M.; Varriale, L. *Tetrahedron* **2007**, *63*, 7047–7052.
13. McMillan, W. G., Jr.; Mayer, E. *J. Chem. Phys.* **1945**, *13*, 276–305.
14. Kozak, J. J.; Knight, W. S.; Kauzmann, W. *J. Chem. Phys.* **1945**, *48*, 675–690.
15. Friedman, H. L.; Krishnan, V. *J. Solution Chem.* **1973**, *2*, 119–140.
16. Barone, G.; Castronuovo, G.; Del Vecchio, P.; Elia, V.; Muscetta, V. *J. Chem. Soc., Faraday Trans. 1* **1986**, *82*, 2089–2101.
17. Barone, G.; Cacace, P.; Castronuovo, G.; Elia, V. *Carbohydr. Res.* **1983**, *119*, 1–11.
18. Brewster, M. F.; Loftsson, T. *Adv. Drug Delivery Rev.* **2007**, *59*, 645–666.
19. Uekama, K.; Hirayama, F.; Irie, T. *Chem. Rev.* **1998**, *98*, 2045–2073.
20. Junquera, E.; Ruiz, D.; Aicart, E. *J. Colloid Interface Sci.* **1999**, *216*, 154–160.
21. Junquera, E.; Baonza, V. G.; Aicart, E. *Can. J. Chem.* **1999**, *77*, 348–355.
22. Junquera, E.; Aicart, E. *J. Phys. Chem. B* **1997**, *101*, 7163–7171.
23. Hedges, A. R. *Chem. Rev.* **1998**, *98*, 2035–2044.
24. Gonzales-Gaitano, G.; Rodriguez, P.; Isasi, J. R.; Fuentes, M.; Tardajos, G.; Sanchez, M. *J. Inclusion Phenom. Macrocycl. Chem.* **2002**, *44*, 101–105.
25. Topchieva, I. N.; Spiridonov, V. V.; Kalashnikov, Ph. A.; Kurganov, B. L. *Colloid J.* **2006**, *68*, 98–105.
26. Castronuovo, G.; Elia, V.; Iannone, A.; Niccoli, M.; Velleca, F. *Carbohydr. Res.* **2000**, *325*, 278–286.
27. Castronuovo, G.; Elia, V.; Niccoli, M.; Velleca, F. *J. Inclusion Phenom. Macrocycl. Chem.* **2002**, *44*, 229–232.
28. Castronuovo, G.; Elia, V.; Niccoli, M.; Velleca, F. *J. Inclusion Phenom. Macrocycl. Chem.* **2003**, *45*, 91–97.
29. Klar, B.; Hingerty, B.; Saenger, W. *Acta Crystallogr., Sect. B* **1980**, *36*, 1154–1165.
30. Saenger, W.; Noltemeyer, M.; Manor, P.; Hingerty, B.; Klar, B. *Bioorg. Chem.* **1976**, *5*, 187–195.
31. Barone, G.; Cacace, P.; Castronuovo, G.; Elia, V. *J. Chem. Soc., Faraday Trans. 1* **1981**, *77*, 1569–1577.
32. Castronuovo, G.; Elia, V.; Velleca, F. *Curr. Top. Solution Chem.* **1997**, *2*, 125–142.
33. Hernandez-Pascacio, J.; Garza, C.; Banquy, X.; Diaz-Vergara, N.; Amigo, A.; Ramos, S.; Castello, R.; Costas, M.; Pineiro, A. *J. Phys. Chem. B* **2007**, *111*, 12625–12630.
34. Andini, S.; Castronuovo, G.; Elia, V.; Pignone, A.; Velleca, F. *J. Solution Chem.* **1996**, *25*, 835–846.
35. Okamoto, B. Y.; Wood, R. H.; Thompson, P. T. *J. Chem. Soc., Faraday Trans. 1* **1978**, *74*, 1990–2007.
36. Castronuovo, G.; Elia, V.; Moniello, V.; Vellica, F.; Perez-Casas, S. *Phys. Chem. Chem. Phys.* **1999**, *1*, 1887–1892.