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# Investigation of the inclusion of the herbicide metobromuron in native cyclodextrins by powder X-ray diffraction and isothermal titration calorimetry

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ABSTRACT

We report the formation of inclusion complexes between the phenylurea herbicide metobromuron [3-(*p*-bromophenyl)-1-methoxy-1-methylurea] and β- and γ-cyclodextrin in the solid state. Formation of crystalline inclusion complexes by the kneading method was confirmed by powder X-ray diffraction and further structural characterization using the principles of isostructurality followed. In addition, $\Delta H^\circ$, $\Delta S^\circ$, $\Delta G^\circ$ and the association constants ($K$) at 298 K were determined for complex formation in solution using isothermal titration calorimetry. The magnitudes of $K$ for the formation of 1:1 complexes between metobromuron and α-, β- and γ-CD were estimated as 598, 310 and 114, respectively.



## 1. Introduction

Formulation and practical application of pesticides are often rendered difficult due to their adverse physicochemical properties such as poor solubility, chemical and thermal instability, mammalian toxicity, malodour, volatility, high soil mobility, persistence and poor wettability. Cyclodextrins (CDs) are relatively inexpensive, non-toxic, biodegradable and water soluble, having the added advantage of forming inclusion compounds with a large variety of molecules. Complexation between pesticides and cyclodextrins can result in products with superior performance (e.g., enhanced pesticide solubility and stability, reduction of volatility).[1] Metobromuron [3-(*p*-bromophenyl)-1-methoxy-1-methylurea] (Fig. 1) is a phenylurea herbicide that is used for the control of broadleaf weeds in cereal and vegetable crops, acting through the inhibition of photosynthesis.[2,3] The compound has a relatively low aqueous solubility ($3.3 \times 10^{-4}$ g/mL at 25 °C) and was selected as a representative phenylurea to test its affinity for CDs.

We recently reported that metobromuron (hereinafter MB) and other phenylurea herbicides form inclusion complexes with α- and β-CD, as evidenced by the differences in chemical shift of the $^1$H NMR signals of both the host and the guest.[4] Here we report a study of the complexation of MB with the native cyclodextrins (α-, β- and γ-CD) in the solid state and also the determination of the thermodynamics of association in solution as measured by isothermal titration calorimetry (ITC).

Powder X-ray diffraction (PXRD) and the principles of isostructurality were used to confirm and characterize the complexes in the solid state. The experimental PXRD patterns of putative complexes obtained by the kneading method were compared with an existing set of reference patterns for several isostructural series of the known CD inclusion complexes,[5] these reference patterns having been calculated using space group, unit cell and atomic coordinate data for complexes deposited in the Cambridge Structural Database.[6] A match between the PXRD pattern of the putative

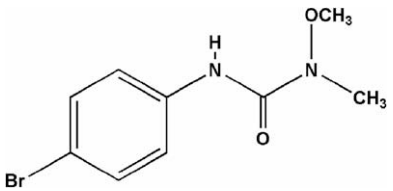


**Figure 1.** Chemical structure of the phenylurea herbicide metobromuron.

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complex and a reference pattern for a particular isostructural series immediately confirms complex formation and permits one to deduce the corresponding crystallographic space group as well as approximate unit cell dimensions.[5] One of many such previous applications of this technique is the characterization of the inclusion complex formed between the anaesthetic butamben and β-cyclodextrin.[7]

Recently ITC has become a popular method for characterizing the stoichiometry and thermodynamics of molecular interactions in solution. With improvements in the sensitivity of modern isothermal titration calorimeters, they are more frequently being employed in the determination of binding constants and the analysis of the thermodynamics of cyclodextrin–guest interactions.[8,9] The ITC technique measures the heat of reaction while analysis of the thermodynamic parameters along with other physicochemical properties of the interaction provides insight into how and why the molecules interact.[10,11] Titration microcalorimetry allows for the simultaneous determination of the equilibrium constant, enthalpy and stoichiometry from a single titration curve. This technique was therefore employed to investigate the interaction between MB and the native CDs in solution, with the objective of quantifying the thermodynamic parameters.

## 2. Experimental

### 2.1. Materials and solid complex preparation

Metobromuron was obtained from ChemService (Pennsylvania, USA); α-, β- and γ-cyclodextrin were obtained from Cyclolab (Budapest, Hungary). All compounds were used as received. The water content in the CD samples used for ITC studies was determined by TGA analysis. The solvent in each of the experiments was deionized distilled water (MilliQ).

Solid complex preparation was attempted by kneading equimolar amounts of cyclodextrin (host) with the herbicide (guest) over a period of 1 h while adding water dropwise. In a typical preparation 10–20 mg of guest was used.

### 2.2. Powder X-ray diffraction

PXRD patterns were recorded on a Huber Imaging Plate Guinier Camera 670 using nickel-filtered Cu K$\alpha_1$ radiation ($\lambda$ = 1.5405981 Å) produced at 40 kV and 20 mA by a Philips PW1120/00 generator fitted with a Huber long fine-focus tube PW2273/20 and a Huber Guinier Monochromator Series 611/15. Samples for PXRD were generally not amenable to sieving. For all samples the flat sample holder with model no. 0670.000.02 was used. The sample of paste-like consistency was applied to MYLAR® polyester film (Thin-Film Sample Supports, West Chester, Pennsylvania, USA) which was suspended in the flat sample holder. A $2\theta$ range of 4.0–100.0° was covered with a step size of 0.005° $2\theta$.

### 2.3. Isothermal titration calorimetry

Isothermal titration calorimetry was performed at 298 K using a VP-ITC Microcalorimeter (MicroCal, Inc., Northampton, MA). For each experiment, 28 aliquots of cyclodextrin solution of 10 μL each were titrated into the reaction cell (1.43 $cm^3$) containing the MB solution. The guest concentration in all experiments was 1.23 mM while the host concentrations were 27 mM for α-CD, 13 mM for β-CD and 28 mM for γ-CD. The concentration of the guest in the cell changed from 1.23 to 1.02 mM and that of the host from 0 to 4.42, 2.13 and 4.58 mM from the start to the end of the experiment in the case of α-, β- and γ-CD, respectively. To determine the actual concentration of CDs, the content of water in CD samples was determined by TGA analyses, the weighed amount therefore being corrected for the amount of water. The water content so determined was 9.1%, 14.2% and 6.6% w/w for α, β and γ-CD, respectively. All solutions were degassed before measurements commenced. The aliquots were added at 4-min intervals to allow the solution in the cell to equilibrate after each addition. Prior to each binding reaction experiment the heat of dilution was measured by titrating 28 aliquots (10 μL each) of the cyclodextrin solution into water in the reaction cell. The data obtained from the experiments were converted to binding isotherms. The isotherms for the heat of dilution were subtracted from the isotherms for the heat of binding before model-fitting. The first injection of 10 μL was discarded to eliminate diffusion effects of material from the syringe to the calorimetric cell. Each experiment was carried out in duplicate or triplicate to ensure reproducibility; the data from replicate experiments differed by less than 10%. Binding enthalpies ($\Delta H^\circ$), stoichiometry ($n$) and binding constant ($K$) were obtained by nonlinear fitting using MicroCal Origin software provided with the instrument. The software gave the relevant standard deviations based on the scatter of the data points in a single titration curve.

## 3. Results and discussion

### 3.1. PXRD and isostructurality

Attempts to form an inclusion complex between α-CD and MB in the solid state were unsuccessful, the PXRD pattern of the product of kneading having no correspondence with inclusion complex reference patterns. Instead, the experimental pattern is in very good agreement with that of polymorphic Form I of the host compound,[5] the major component by mass in the kneading experiment (Fig. 2a). We note that in separate experiments using the co-precipitation method (gradual addition of solid MB to a saturated aqueous solution of α-CD in 1:1 molar ratio at 60 °C under constant stirring), the same outcome resulted, that is, no solid inclusion complex formation.

The experimental PXRD trace of the product of kneading β-CD and MB closely resembles the reference traces for the *P*1 (triclinic) and *C*2 (monoclinic) channel-type dimeric structures for β-CD complexes (Fig. 2b). When comparing the experimental and reference patterns, the criterion for a match is close agreement between the respective angular positions of the peaks rather than between their relative intensities. This is because peak positions are determined by the lattice parameters and each corresponding lattice parameter of the members of an isostructural series generally spans a very narrow range (typically ~0.2–0.3 Å), whereas the chemically different guest molecules present in the various complexes comprising an isostructural series may lead to significant variations in the intensities of corresponding peaks. There is, in fact, very good agreement between the angular positions of the experimental and calculated peaks in Figure 2b and reasonable agreement between the peak intensities. Definitive complex formation is thus deduced, but an ambiguity does arise in the present instance: the two reference traces happen to correspond to isostructural series with coincidentally very similar lattice parameters (an explanation of this relationship is given below). Thus, definitive assignment of the complex between β-CD and MB (β-CD·MB) to the space group *P*1 or *C*2 is not possible from PXRD alone in this instance. Table 1 lists the possible space groups and the estimated unit cell dimensions, the latter being those of complexes belonging to the respective isostructural series.

The metric relationship between the unit cells in the space groups *C*2 and *P*1 for dimeric β-CD inclusion complexes is well known and was explored in a paper by Mentzafos et al.[12] When the metric transformation $a' = a + b$, $b' = b - a$, $c' = c$ is applied to the *P*1 unit cell, one obtains a unit cell with very similar dimensions to those of the *C*2

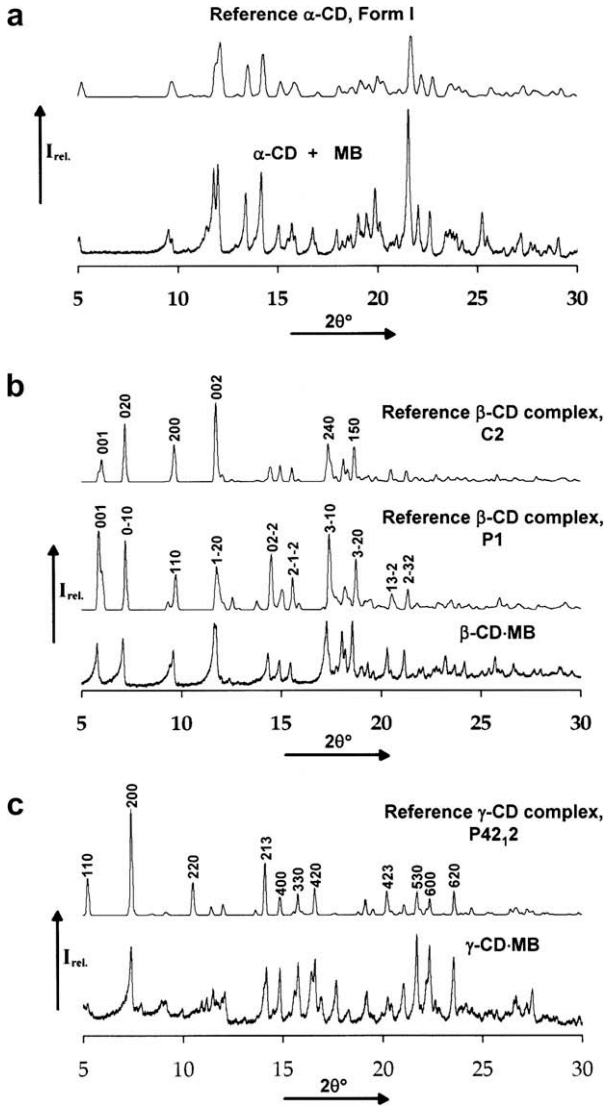



**Figure 2.** Experimental PXRD patterns for the solid products obtained by kneading MB with (a) α-CD, (b) β-CD and (c) γ-CD. Relevant reference PXRD patterns, with the major peaks indexed in (b) and (c), are included for comparison.

**Table 1**
Possible space groups and approximate unit cell parameters for the CD·MB complexes[5]

| Space group | $a$ (Å) | $b$ (Å) | $c$ (Å) | $\alpha$ (°) | $\beta$ (°) | $\gamma$ (°) | $V$ (Å$^3$) |
|---|---|---|---|---|---|---|---|
| $C2$ | 19.3 | 24.5 | 15.9 | 90 | 109 | 90 | 7109 |
| $P1$ | 15.6 | 15.6 | 15.9 | 102 | 102 | 104 | 3556 |
| $P42_12$ | 23.8 | 23.8 | 23.2 | 90 | 90 | 90 | 13,140 |

structure ($a$ = ~19, $b$ = ~24 and $c$ = ~16 Å, $\beta \sim 109°$). In addition, all β-CD dimers in both the $P1$ and $C2$ structures have the same orientation, as do their guest molecules. However, the space group $C2$ is characterized by a crystallographic diad at the interface of each dimer, whereas in the $P1$ structure, the dimeric complex possesses only a pseudo-twofold rotation axis at its interface. The consequence of the above-mentioned features is that the PXRD traces for the two structures are virtually indistinguishable. Single crystal X-ray intensity data, which reveal the crystal Laue groups, would be required for unequivocal space group assignment.

When comparing the PXRD trace of the putative γ-CD complex (γ-CD·MB) with that of the reference pattern, it is immediately apparent how well the peak angular positions agree (Fig. 2c). The assignment of γ-CD complexes is probably the easiest for the cyclodextrin complexes because most γ-CD complexes crystallize in the very rare tetragonal space group $P42_12$, which, having high symmetry produces relatively few peaks in the PXRD trace. The estimated unit cell parameters for the crystalline inclusion complex γ-CD·MB, based on isostructural analogy, are included in Table 1.

### 3.2. Isothermal titration calorimetry

Titration microcalorimetry allows for the simultaneous determination of the equilibrium constant, enthalpy and complex stoichiometry from a single titration curve using the least-squares nonlinear adjustment based on the Wiserman isotherm (Eq. 1).[13,14]

$$\left(\frac{dQ}{d[\mathrm{CD}]_T}\right)_P = \Delta H^\circ V_\circ \left[\frac{1}{2} + \frac{1 - X_R - r}{2\sqrt{(1 + X_R - r)^2 - 4X_R}}\right] \quad (1)$$

The above-mentioned equation relates the stepwise change in the heat of the system normalized with respect to the CD concentration added per injection, $(dQ/d\mathrm{CD})_P$, to the absolute value of the ligand to receptor ratio ($X_R = [\mathrm{MB}]_t/[\mathrm{CD}]_t$) at any point during the course of the titration. The parameters $\Delta H^\circ$, $V_o$ and $r$ are, respectively, the molar enthalpy of binding, the effective volume of the solution in the titration cell and a composition variable $1/[\mathrm{CD}]_t K_{eq}$.

By using Eqs. 1–3 it was possible to calculate the free energy and entropy parameters.

$$\Delta G^\circ = -RT \ln K \quad (2)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (3)$$

In Figure 3 plots of the power exchange against mole ratio MB/CD for a solution of MB titrated with solutions of α-, β- and γ-CD are shown. It can be seen that in all cases an exothermic reaction is observed, although in the case of γ-CD the amount of heat involved is much smaller than that in the other two reactions. This fact and the relatively low value of the equilibrium constant render the data for the interaction of MB with γ-CD less accurate than those for interactions involving the other two hosts. The reproducibility of the results in two experiments was in the order of 20%.

In order to determine the type of interaction that better fitted the data, the raw data were fitted to both a one-site model with $n$, $K$ and $H$ as adjustable parameters and to the sequential binding model. These two models correspond to the reactions indicated in Eqs. 4 and 5 and are designated in the software for the instrument as 'one single site model' and 'sequential binding model'.

$$\mathrm{MB} + n\mathrm{CD} \rightleftharpoons \mathrm{MB(CD)}_n \quad (4)$$

$$\mathrm{MB} + \mathrm{CD} \overset{K_1}{\rightleftharpoons} \mathrm{MB \cdot CD} \overset{K_2}{\rightleftharpoons} \mathrm{MB(CD)_2} \quad (5)$$

The data are summarized in Table 2. In the case of α-CD the best fit is obtained for the sequential binding mode ($\chi^2$ = 96), although a good fit is also obtained with the one set of sites model ($\chi^2$ = 512). The tendency for α-CD to associate in a higher stoichiometry than 1:1 is manifested in an experiment with higher total concentration (90 mM) of α-CD which gave the best fit for the one set of sites model using $n$ = 3. The latter result probably means that at this high CD concentration the MB associates with aggregated CD. It is now well established that CDs have a tendency to aggregate at high concentration in water solution.[15] In the case of β-CD the sequential binding procedure gave a good fit with $\chi^2$ = 8.4 but the best fit was obtained with the one set of sites model ($\chi^2$ = 3.87, and $n$ = 1). With γ-CD the best fit was obtained with the one set of sites model and $n$ = 1. Attempts to fit the data with other models failed, yielding results without physical meaning. For the 1:1 interaction of MB with CDs, two types of complexes can be envisaged (Scheme 1A and B).

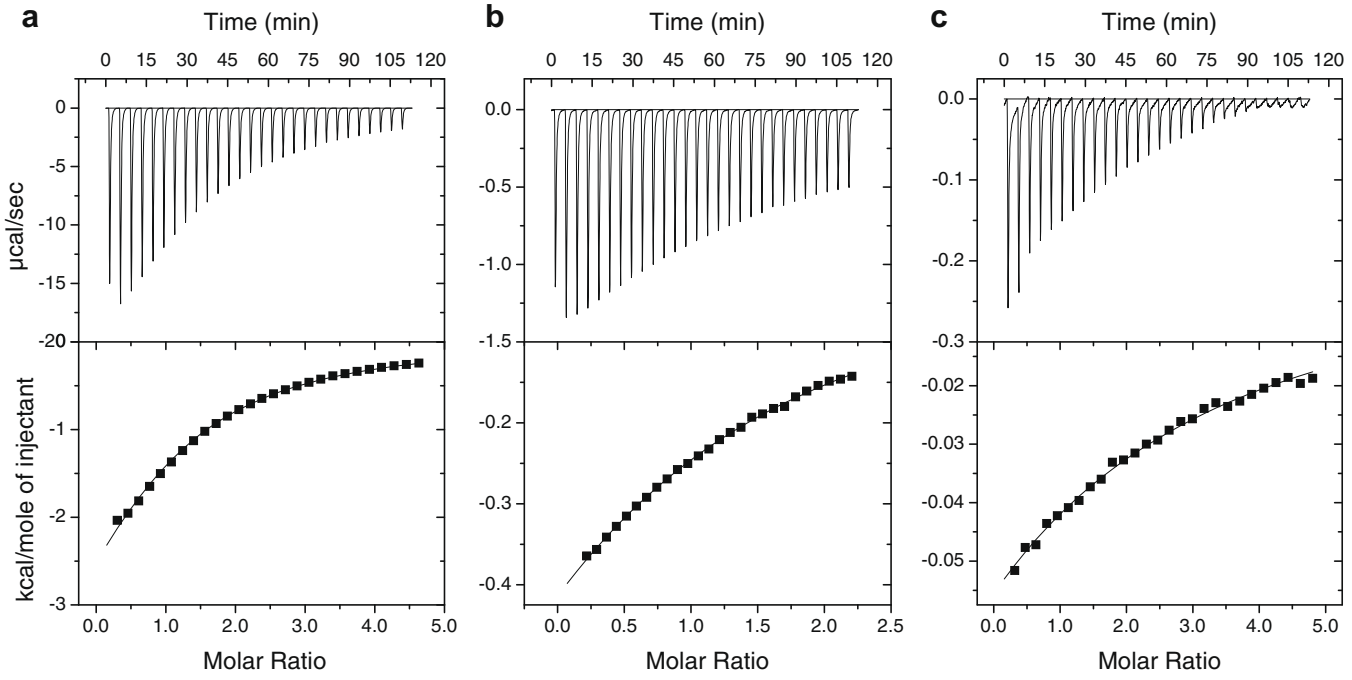


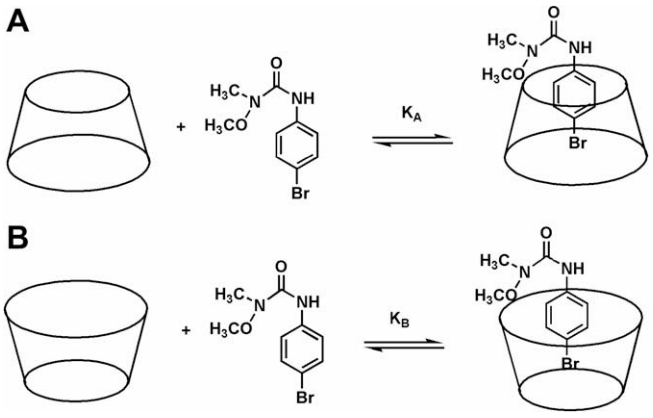

**Figure 3.** Calorimetric titration of MB with the native cyclodextrins: (a) α-CD, (b) β-CD and (c) γ-CD. Top: Raw data for 28 sequential injections (10 μL per injection) of cyclodextrin solution into the metobromuron solution. Bottom: Reaction heat isotherms obtained from the integration of the calorimetric curves. The line was drawn with the calculated parameters given in Table 2.

In our previous paper,[4] analysis of the induced chemical shifts for complexation of MB with α- and β-CD led to the conclusion that the main complex observed under the NMR conditions was one of type B for α-CD and of type A for β-CD. These results do not prove that complexes of other types may also be present as well, although in minor concentrations.[16] It is important to note that induced chemical shifts depend not only on the concentrations of the species but also on the rate of exchange, that is, forward and reverse rate constants for the formation of the complexes.[17] The data obtained by ITC indicate that there might be complexes of higher stoichiometry than 1:1 present and the *K* values determined are not comparable with those determined previously by NMR spectroscopy. Since the NMR experiments reported previously were carried out with a different sample of CDs we repeated the experiment using the same CD used for the ITC and the results obtained were similar to those reported previously.[4] Lack of agreement between association equilibrium constants of CDs with different guests, when determined by different techniques is not unusual[18] and it probably means that different techniques measure different types of complexes. In order to have a detectable signal in an ITC experiment it is required that the reaction should have a $\Delta H$ within the sensitivity of the instrument. If complexes such as those shown in Scheme 1 were to form, but one of them has a higher $\Delta H$ of formation, the equilibrium constant and heat of reaction measured would pertain to that reaction and it might be that the complex is not the same as that observed by the NMR technique. Furthermore, part of the discrepancies in the values reported previously with those reported here may be attributed to the different conditions employed; in the NMR experiment the temperature was 293 K and the highest CD concentration was 1 mM. In the present work the temperature was 298 K and the highest concentration was 4.42 and 2.1 mM for α and β-CD, respectively.

**Table 2**
Thermodynamic parameters $\Delta H°$, $T\Delta S°$, $\Delta G°$ and formation constants *K* for complexation between metobromuron and the native cyclodextrins at 298 K[a]

| CD | $\Delta H°$ (kcal/mol) | $\Delta S$ (cal/mol K) | $T\Delta S°$ (kcal/mol) | $\Delta G°$ (kcal/mol) | $K$ ($M^{-1}$) |
|---|---|---|---|---|---|
| α[e] | −(2.94 ± 0.03) | 5.74 | 1.71 | −4.65 | 2580 ± 80[b] |
| | −(2.74 ± 0.06) | 1.93 | 0.57 | −3.31 | 270 ± 4[c] |
| | −(5.69 ± 0.05) | −6.4 | 1.91 | −8.31 | 598 ± 13[d] |
| β[f] | −(0.649 ± 0.005) | 12 | 3.58 | −4.23 | 1230 ± 18[b] |
| | −(0.622 ± 0.017) | 8.41 | 2.51 | −3.13 | 196 ± 3[c] |
| | −1.47 ± 0.02 | 6.47 | 1.93 | −3.4 | 310 ± 5[d] |
| γ[g] | −0.442 ± 0.009 | 7.92 | 2.36 | −2.80 | 114 ± 3[d] |

[a] $[MB]_o$ = 1.23 mM.
[b] Sequential binding, $K_1$.
[c] Sequential binding, $K_2$
[d] One set of sites with $n = 1$. The calculation using *n*, *H* and *K* as adjustable parameters gave $n$ = 0.9–1.1.
[e] 27 mM solution in the titrating syringe.
[f] 13 mM solution in the titrating syringe.
[g] 28 mM solution in the titrating syringe.




**Scheme 1.** Formation of two possible 1:1 inclusion complexed between metobromuron and cyclodextrins.

To compare the thermodynamic parameters for the three CDs, we will consider the values corresponding to the 1:1 interaction. The three complexation reactions have negative $\Delta G^\circ$ values and are therefore spontaneous processes, implying that binding interactions are favoured.[19] In addition, the standard formation enthalpies ($\Delta H^\circ$) are negative in all cases indicating that the association is slightly exothermic in the order $\alpha > \beta > \gamma$. Considering the entropy changes for the 1:1 complexes we see that they are negative for α-CD and positive for β- and γ-CD. These trends appear to be general and are shown by many different guest molecules.[20]

The strength of interaction depends on the width of the CD cavity, the volumes of α-, β- and γ-CD being 174, 262 and 427 Å$^3$, respectively.[21] The entropy effect is due to the total result of the host–guest combination which includes a negative contribution to entropy due to the association of two molecules and the restriction of degrees of freedom, in particular those of the guest molecule, and a positive contribution due to the release of water from the cavity. The differences in the proportion by which the binding entropy and enthalpy contribute to the Gibbs energy reflect the differences in the interaction established between the various CDs and the MB molecule.

The general picture of hydrophobic hydration is that the introduction of a hydrocarbon into water gives rise to a structural rearrangement with increased order of the water close to the solute. The entropy increases when the water is released upon removal of the solute, while the enthalpy change is small (at room temperature). Assuming that the volume of a water molecule is ~30 Å$^3$, the number of water molecules that α-, β- and γ-CD can accommodate are 6, 9 and 14, respectively.[22] The negative entropy change for α-CD complex formation indicates that the situation for water molecules inside the cavity is different. It was proposed[18] that they are not able to develop a full hydrogen bonded network inside the cavity leading to an increased disorder, probably due to the high curvature of the inside of the cavity. When the water molecules are released, the hydrogen bonds reform, which leads to an increased order and release of heat. In this regard, it is interesting to note that Briggner and Wadso[23] observed a significantly lower heat capacity $C_p$ of water in α-CD hydrates than for water in the β-CD and γ-CD hydrates. The $C_p$ value of $H_2O$ molecules is 14.1 cal K$^{-1}$ mol$^{-1}$ in α-CD hydrate while it is 17.0 and 16.7 cal K$^{-1}$ mol$^{-1}$ for the β- and γ-CD hydrates, respectively. The two latter values are close to $C_p$ for liquid water (17.9 cal K$^{-1}$ mol$^{-1}$). The low average $C_p$ value for the water molecule bound to α-CD agrees with the suggestion that these water molecules are hydrogen-bonded to a lesser extent.

The reduction of entropy may be ascribed to the rigid architecture and new conformation assumed by species upon complexation, which confers greater molecular volume and smaller rotational and translational degrees of freedom when compared with free substances. Finally, it should be noted that one possible contribution to the entropy change is the conformational entropy of the urea substituent which decreases when the compound is included in the cavity.

One important effect that may contribute to the enthalpy change is the van der Waals interaction between the guest and the inside of the cavity. As noted earlier, $\Delta H^\circ$ is less negative for the inclusion compounds formed with β-CD; this might be caused by the smaller curvature inside the β-CD cavity, to which the water molecules can better adapt. All water molecules are expected to be expelled upon complexation of both α-CD and β-CD.

Heat capacity changes in binding reactions are attributed to water reorganization upon transference of the drug from bulk water to its binding site;[24,25] therefore, based on heat capacity measurements[26] it was shown that less reorganization of the water occurs with a drug binding to γ-CD than with one binding to β-CD, implying that the original bulk water structure is retained after drug binding in the larger γ-CD cavity.

The decrease in the magnitude of the binding enthalpies can be attributed to the retention of the water in the γ-CD·MB cavity allowing for a 'looseness' of the guest molecule fits in the γ-CD cavity. The increase in the entropic component may be attributed to this looseness in terms of a higher configurational entropic contribution in the γ-CD binding affinity as well as to changes in the solvent entropic contribution.

The formation of the complex α-CD·MB is accompanied by $\Delta S^\circ < 0$, possibly indicating conformational restrictions of the guest while the fact that $|\Delta H^\circ| > |T\Delta S^\circ|$ indicates an enthalpically driven inclusion process governed by van der Waals interactions.[12,19,27–29]

## 4. Conclusion

The use of PXRD alone to establish inclusion complex formation in the solid state and to extract structural information is well illustrated by the examination of samples obtained by kneading the herbicide metobromuron with each of the host compounds α-, β- and γ-CD. In the case of α-CD, no complex formation occurs and in the PXRD pattern of the material obtained by kneading, that of the host dominates. Complex formation between metobromuron and β-CD was definitely established, but unequivocal space group assignment was not possible in this case owing to a special lattice relationship between the two alternative complex crystal structures. Inclusion complex formation between the guest and γ-CD was proven and unequivocal determination of its space group and estimation of unit cell data were possible by PXRD alone.

In contrast to the solid-state results, all three native CDs were shown to form inclusion complexes with MB in solution. It is clear from the thermodynamic data that the size-fit relationship between host and guest makes an important contribution to the magnitude of the association constants reported here. The larger macrocyclic cavity dimensions of γ-CD allow greater conformational freedom of the guest resulting in larger entropy values. Furthermore, the data suggest that the driving forces of cyclodextrin inclusion complexation are mainly van der Waals and hydrophobic interactions.

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