Research Article


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Abstract

The stability constants for the inclusion complexes of β-cyclodextrin (β-CD) with various adamantane derivatives (ADA), namely the amantadinium (AM), rimantadinium (RIM), and memantinium (MEM) cations have been determined by UV-Vis spectrophotometry. All experiments have been performed at a pH of 1.7 and 25 °C on aqueous solutions adjusted to an ionic strength of 0.05 M (Na\textsuperscript{+}, H\textsuperscript{+})\text{ClO}_4. The competitive binding method has been used whereby methyl orange (MO) is first encapsulated by β-CD and is then substituted by ADA. It has been shown that the derivatives studied form host-guest type complexes. The calculated stability constants, reported as log K_1, were estimated to be 3.9 \pm 0.1, 5.1 \pm 0.2 and 3.3 \pm 0.1, for AM, RIM and MEM, respectively. The factors that govern the strength of binding ADA with β-CD have been discussed and an attempt was
made to rationalise the variation in the established stability constants for the ADA-β-CD complexes. General experimental conditions required for the determination of the stability constants of ADA with β-CD with the use of MO as an auxiliary agent were evaluated. The optimised experimental conditions are recommended. It has been concluded that MO, even though commonly used in this type of study, does not meet the optimal and recommended conditions.

Keywords β-cyclodextrin, amantadine, rimantadine, memantine, stability constants, UV-Vis spectrophotometry.

1. Introduction

Cyclodextrins (CD) are naturally occurring cyclic oligomers formed by the action of enzymes on starch. The three most common cyclodextrins are α-CD, β-CD and γ-CD. They have a truncated cone shape with a hydrophobic cavity and a hydrophilic exterior, thus enabling them to be suitable host molecules.1, 2 It is well-known that the cyclodextrins can host a variety of guests within their cavities,3, 4, 5 thereby altering the physical, chemical and biochemical properties of the guest molecule.6 Drugs are now frequently complexed with cyclodextrins, and as a result the solubility of the drugs is increased. Thus they are becoming important in the transportation of drugs in biological systems,7 and are of great interest in pharmaceutical and analytical chemistry.

The exterior of the CD structure has a high number of both primary and secondary hydroxyl groups, making it polar, whereas the interior is lined primarily with hydrocarbon units and glycosidic oxygens thereby making it relatively non-polar.2 The cavity diameter of β-CD is 7.8 Å.8 The stability of the inclusion complexes formed by cyclodextrins is dependent on the size and the hydrophobicity of the cavity, the chemical structure of the guest molecule, solvent, concentration of the cyclodextrin and temperature.9 Palepu et al have suggested that the size of the cavity is the most important factor influencing the strength of binding.10 The adamantyl group is one of the most suitable guest molecules that can be accommodated by β-CD due to its size.1, 10 The diameter of the adamantyl group is about 7 Å and fits well into the 7.8 Å cavity of β-CD.11
Cyclodextrins have been extensively studied by a number of techniques, such as NMR spectroscopy, capillary electrophoresis, spectrophotometric, chromatographic, and conductometric methods among others. Furthermore, they are increasingly becoming important in separation techniques in which they are used to separate enantiomers due to their stereoselective nature.

1-aminotricyclo[3.3.1.13,7]decane (amantadine), $\alpha$-Methyltricyclo[3.3.1.13,7]decane-1-methanamine (rimantadine) and 3,5-dimethyltricyclo[3.3.1.13,7]decan-1-amine (memantine) (seen in Figure 1 in their protonated forms) are compounds that exhibit anti-viral activity. Amantadine and rimantadine are used in the prophylaxis and treatment of the influenza A virus, and memantine exhibits anti-parkinsonian activity. It is for this reason that these compounds have been selected for this study. Hayden et al studied the toxicity effect of these substances and it has been shown that rimantadine is better tolerated by humans than its amantadine counterpart.

In this work we have studied the possibility of applying UV-Vis spectrophotometry with the aim to determine the stability constants for the inclusion complex formation of $\beta$-CD with the amantadinium (AM), rimantadinium (RIM) and memantinium (MEM) cations (see Figure 1). The competing equilibria (with methyl orange as an auxiliary agent) have also been modelled in search of analytical conditions that could provide the most significant input data for the optimisation procedures and refinement of the stability constants.

![Figure 1](image_url) Structures of the adamantane derivatives studied in this work.
2. Experimental

2.1. Materials and Instrumentation

β-CD (98%+) was purchased from Merck (Darmstadt, Germany). Methyl orange was obtained from LACHEMA (Brno, Czech Republic). The hydrochloride salts of the adamantane derivatives were used. Amantadine hydrochloride (99%+) was purchased from Sigma-Aldrich (Steinheim, Germany), rimantadine hydrochloride (99%) and memantine hydrochloride (99%+) from Lachema (Brno, Czech Republic). All ligands were used as received.

Aqueous solutions of β-CD, methyl orange and the adamantane derivatives were prepared using re-distilled water from a quartz still of Heraeus (Hanau, Germany). NaClO₄ and HClO₄ were used as the ionic strength and pH adjusters and were purchased from Lachema (Brno, Czech Republic). Where not specified, all reagents were of analytical grade and were used without any further purification.

UV-Vis absorbance spectra in the range 225-650 nm were recorded using a UV-2 Quartz UV-Vis Unicam spectrophotometer (Cambridge, UK). All measurements were carried out using standard 1 cm path-length quartz cells, 1 nm sampling wavelength and 120 nm min⁻¹ scan rate. The protonation constant of the methyl orange was determined via spectrophotometric titrations (I = 0.05 M NaClO₄, 25 °C). An appropriate volume of a stock solution of methyl orange was transferred to volumetric flasks. By addition of diluted perchloric acid or sodium hydroxide solutions, (both containing methyl orange, so as to maintain a constant concentration of MO at 2.00 × 10⁻⁵ M) the required pH was obtained. The pH of the analysed solutions was varied in the range between 1.6 and 6.3. The binding constant for β-CD–HMO was determined by measuring the effect of the increase in the concentration of β-CD (between 0 and 4.6 mM) on the spectrum of HMO ([HMO] = 1.00 × 10⁻⁵ M). Due to the problems associated with the solubility of β-CD in water (solubility of 1.85 g per 100 ml of water, or 16.3 mM, at 25 °C)²⁷ we have decided to work at the total concentration of β-CD in the region of 5 mM. The experiment was performed at I = 0.05 M (Na⁺,H⁺)ClO₄, at a constant pH of 1.7 and at 25 °C. A Radelkis OP-208/1 Precision Digital pH-meter (Budapest, Hungary) and a Radelkis pH sensitive combined glass electrode (calibrated with a use of standardised acid and base...
solutions) were used for the pH measurements. All experiments were conducted in duplicate.

2.2. Spectrophotometric Study

The binding constants for the association of β-CD and the adamantane derivatives (the abbreviation ADA will be used in reference to the derivatives collectively) were determined by observing the spectrophotometric effect of ADA on the β-CD–(HMO) system as both ADA and β-CD do not absorb in the wavelength region under investigation. The zwitterionic form of methyl orange (HMO), which is present in the acidic region was used as an indicator, thus enabling this technique to be used for the determination of the stability constants of interest. Methyl orange (MO) has often been used in the literature as the standard indicator to follow the host-guest complex formation. Addition of β-CD to the HMO solution results in a decrease in the absorbance as β-CD complexes HMO. This is because HMO is encapsulated by β-CD and as a result the concentration of uncomplexed HMO decreases. However, the addition of ADA to the solution containing the β-CD–HMO system, leads to an increase in absorbance, indicating the inclusion of ADA and the liberation of HMO. This method is described as the competitive binding method and is widely used.

2.2.1. Numerical Analysis

The reactions involved can be represented as

\[
\begin{align*}
H + MO & \overset{K_{H-MO}}{\rightleftharpoons} HMO \\
\beta-CD + HMO & \overset{K_{\beta-CD-HMO}}{\rightleftharpoons} \beta-CD-HMO \\
\beta-CD-HMO + ADA & \overset{K_{\beta-CD-ADA}}{\rightleftharpoons} \beta-CD-ADA + HMO
\end{align*}
\]

A study of complexes by any experimental technique involves two principle steps, namely modelling and optimisation of the stability constants for the complexes included in the model. It is often difficult to establish the number of species present in a solution, particularly when one experimental technique is employed and if some minor species
are formed. In a case such as in this study where an additional compound was employed (MO), it was important to identify all possible, major and minor, species. The existence of the latter ones could indicate, for instance, impurities in the reagents used.

The absorbance data were first evaluated with a factor analysis programme SIBYLA, used to predict the number of species present in solution. The programme uses the principles of factor analysis, which are concerned with the analysis of an absorbance matrix \( \mathbf{A} \) defined as \( N_\lambda \times N_p \). In this matrix, \( N_p \) stands for the number of spectra recorded (each spectrum recorded on a separate solution) and \( N_\lambda \) is the number of wavelengths in each spectrum. The readings in absorbance are taken at each \( N_\lambda \) wavelength. The number of absorbing components in the chosen spectral region is defined as \( N_c \). In matrix notation, the matrix \( \mathbf{A} \) can be written as

\[
\mathbf{A} = \mathbf{E} \mathbf{C}
\]

where, \( \mathbf{A} \), \( \mathbf{E} \) and \( \mathbf{C} \) represent the \( (N_\lambda \times N_p) \), \( (N_\lambda \times N_c) \) and \( (N_c \times N_p) \) matrices, respectively.

The rank of the matrix \( \mathbf{A} \) is given by

\[
\text{rank} (\mathbf{A}) = \min (\text{rank} (\mathbf{E}), \text{rank} (\mathbf{C})) = \min (N_\lambda, N_c, N_p)
\]

Therefore, provided that \( N_\lambda \) and \( N_p \) are equal to or greater than \( N_c \), then the rank \( (\mathbf{A}) \leq N_c \). As a result, the rank of the absorbance matrix \( \mathbf{A} \) gives an estimate of the number of species in the solution.

Furthermore, the spectrophotometric data were analysed by means of the programmes SQUAD and LETAGROP. The programmes refine values of the stability constant for the proposed equilibrium model, based on a non-linear least squares approach. The numerical data were evaluated by minimising the sum of squares of residuals, \( U \),

\[
U = \sum_{j=1}^{N_\lambda} \sum_{i=1}^{N_p} (A_{\text{exp}} - A_{\text{calc}})^2
\]

where \( A_{\text{exp}} \) and \( A_{\text{calc}} \) represent the experimental and calculated absorbance data, respectively, \( N_p \) and \( N_\lambda \) represent the number of solutions and number of wavelengths, respectively. Twenty wavelengths, surrounding the principal maximum wavelength,
were selected as the input to the programme. The standard deviation, \( \sigma(A) \), of the absorbance was also calculated.

This is defined by

\[
\sigma(A) = \left[ \frac{U}{N_p - n} \right]^{1/2}
\]

(7)

where \( n \) is the number of parameters estimated. The parameter \( \log_{10} K \) (logarithm of the stability constant) was optimised, when necessary, by both programmes and convergence was achieved when minimum values of \( U \) and \( \sigma(A) \) were obtained.

The species distribution and simulated diagrams for the systems under investigation have been constructed by employing the program MEDUSA.\(^{25} \) These relationships were used further in the analysis of the optimised experimental conditions.

3. Results and Discussion

3.1. Protonation Constant of Methyl Orange, \( K_{H-MO} \)

It was decided to establish the protonation constant of methyl orange, even though this value is known from the literature.\(^{26} \) There were several reasons for that. Among them, (i) one had to run spectra of the MO solutions anyway, to ensure that the reagents, as well as analytical solutions were of the required purity, (ii) the factor analysis was to be employed for the evaluation of the number of species formed between \( \beta \)-CD and ADA. Hence it was necessary to test this approach on a well-known system first, (iii) it was important to examine the suitability of the two programmes, SQUAD and LETAGROP, for the evaluation of spectroscopic data, and (iv) it was necessary to evaluate the analytical procedure employed in this work.

The spectra of the series of solutions obtained are given in Figure 2. It can be seen that there is an isosbestic point at 470 nm that represents one equilibrium in the solution. The absorbance maximum for the zwitterionic form of methyl orange occurs at 508 nm. The absorbance data were treated with the programme SIBYLA (as described above). It was found that the number of absorbing species in solution was equal to 2. One could conclude that the solution contained only the zwitterionic methyl orange
species (HMO) and the anionic species (MO) - no significant amount of impurities was found. The spectrophotometric data, between the wavelengths 400 - 600 nm were then evaluated with the programme SQUAD and LETAGROP. The results are summarised in Table 1.

![Image of absorbance spectra](image-url)

**Figure 2** Variation in absorbance spectra recorded with an increase in pH (from a to j) for the determination of the protonation constant of methyl orange. 

\[ [\text{MO}] = 2.00 \times 10^{-5} \text{ M}; \text{pH} = a: 1.67; b: 2.37; c: 2.67; d: 2.95; e: 3.26; f: 3.44; g: 3.81; h: 4.30; i: 5.23; j: 6.31. \]

**Table 1** Results for the calculation of the protonation constant of methyl orange by the programmes SQUAD and LETAGROP (I = 0.05M (Na⁺,H⁺)ClO₄, 25 °C).

<table>
<thead>
<tr>
<th></th>
<th>SQUAD</th>
<th>LETAGROP</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKₐ#</td>
<td>3.361 ± 0.001</td>
<td>3.361 ± 0.004</td>
</tr>
<tr>
<td>U</td>
<td>$8.45 \times 10^{-4}$</td>
<td>$8.19 \times 10^{-4}$</td>
</tr>
<tr>
<td>σ(A)</td>
<td>$1.80 \times 10^{-3}$</td>
<td>$1.64 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

# pKₐ = 3.39 (ref. [26])
The value of the protonation constant for methyl orange (log $K_{\text{H-MO}} = 3.361$) obtained in this study is in excellent agreement with that reported in the literature.\textsuperscript{26} Although the values for $U$ and $\sigma(A)$ differ slightly for both programmes used (they make use of different optimisation and minimisation procedures), the final estimate of the protonation constant was identical. Hence it can be seen that there is great confidence in the value obtained. Most importantly, however, it verified the analytical procedure used. The same procedure was used for evaluation of the stability constants for the complexes $\beta$-CD–HMO and $\beta$-CD–ADA.

3.2. Complexation of HMO by $\beta$-CD

Under the experimental conditions employed (pH = 1.7) the methyl orange exists as a zwitterion with a deprotonated sulphonic group and a protonated azo group (HMO). Only a negligible amount of MO with the deprotonated azo group is present. This is important, as the protonated form of methyl orange is the strongest absorbing species in the wavelength range of interest, and all the calculations performed in this work were based on the variation in the absorbance spectra of HMO.

The spectra recorded during the titration of a sample of HMO with $\beta$-CD are given in Figure 3. It can be seen that a rather small change in absorbance (about 0.3 absorbance units) was observed upon inclusion complex formation. Generally, the changes in absorbance, or any other signal measured, between the complexed and uncomplexed form should be greater to provide a larger variation in measured values used further by optimisation procedures. It appears that methyl orange, although it is widely used as the competitive complexant, does not seem to be the most suitable auxiliary ligand when the variation in the experimental signal is concerned. The latter statement can also be strengthened by the computed species distribution diagram for the $\beta$-CD–HMO system seen in Figure 4. The species distribution diagram was calculated with the use of the stability constant $K_{\beta$-CD-HMO} established in this work for the experimental conditions employed. It can be seen that only approximately 70% of the complexation is achieved when the concentration of 10 mM $\beta$-CD is reached. Much higher concentrations of $\beta$-CD would have to be used to achieve full complexation. This, however, could not be accomplished because of the solubility problem of $\beta$-CD at room
temperature. Under our experimental conditions, approximately 60% of the complexation was obtained. The UV-Vis data were evaluated by SIBYLA in a similar way as described for the spectra obtained on the methyl orange solutions. The factor analysis confirmed the presence of 2 species in solution, uncomplexed HMO and β-CD–HMO. Treatment of the spectral data in the region between 450 and 575 nm by the programme SQUAD resulted in log $K_{β-CD-HMO}$ equal to $2.48 \pm 0.03$. This value differs slightly when compared with values reported in the literature.\(^3, 4, 28, 29\) All these studies (in this work and in the literature) involved the HMO species. Shehatta et al\(^4\) have reported a stability constant which is one order of magnitude higher than the value determined in this work. However, they have studied the binding of HMO by β-CD in 0.1 M H$_2$SO$_4$ at an ionic strength of 0.5 M (Na$_2$SO$_4$), experimental conditions significantly different from those employed here.

![Absorbance spectra](image)

**Figure 3** Absorbance spectra for the complexation of β-CD with HMO as a function of the β-CD concentration. The total concentration of methyl orange was fixed at $1.00 \times 10^{-5}$ M. [β-CD] = a: 0; b: 0.196; c: 0.991; d: 1.94; e: 2.81; f: 3.81; g: 4.57 mM.

On the other hand, our log $K$ value of 2.48 fits well between the values 2.36 and 2.57 reported by Gelb\(^29\) and Matsui,\(^28\) respectively. As the binding of ADA by β-CD is
greatly influenced by the binding of HMO by β-CD, it was imperative that this stability constant be determined. The results obtained can be seen in Table 2. The stability constant $K_{\beta-CD-HMO}$ obtained in this work was used further to study the affinity of ADA to β-CD.

**Table 2**  Stability constants for the formation of the complex β-CD–HMO ($I = 0.05\text{M} (\text{Na}^+,\text{H}^+)\text{ClO}_4$, pH = 1.7, 25 °C).

<table>
<thead>
<tr>
<th>log $K_{\beta-CD-HMO}$</th>
<th>Statistical parameters</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.48 ± 0.03</td>
<td>$U = 7.37 \times 10^{-4}$</td>
<td>This work$^a$</td>
</tr>
<tr>
<td></td>
<td>$\sigma(A) = 1.70 \times 10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>2.36 ± 0.07</td>
<td></td>
<td>[29]</td>
</tr>
<tr>
<td>2.57</td>
<td></td>
<td>[28]</td>
</tr>
<tr>
<td>3.48</td>
<td></td>
<td>[4]</td>
</tr>
</tbody>
</table>

$^a$ as calculated by SQUAD

**Figure 4** Species distribution diagram of the methyl orange species computed at a fixed pH of 1.7 for the total concentration of methyl orange of $1.00 \times 10^{-5}$ M and the concentration range of β-CD from 0 to 10 mM.
3.3. Complexation of Adamantane Derivatives by β-CD

As the above discussion has demonstrated, the selection of appropriate experimental conditions plays a crucial role. It allows, when performed properly, to work at analytical conditions that provide the most important data for the refinement procedures. The protonation constant for amantadine found in the literature is 10.7. The other derivatives of ADA must also have rather high protonation constants. These are of no interest, however, because all experiments were to be performed at a low pH value at which all ADA would be fully protonated.

![Species distribution diagrams for the (β-CD–methyl orange) system computed for different values of the stability constant for the β-CD–ADA complex.](image)

**Figure 5** Species distribution diagrams for the (β-CD–methyl orange) system computed for different values of the stability constant for the β-CD–ADA complex. The total methyl orange and β-CD concentrations were fixed at $1.00 \times 10^{-5}$ and $5.00 \times 10^{-3}$ M, respectively, at pH of 1.7.

The computed species distribution diagrams for the β-CD–ADA systems are seen in Figure 5. Different values of the stability constant for the inclusion of the adamantane derivatives by β-CD were tested. It can be seen that for the different values of the stability constants, a significant change (from the point of view of refinement
procedures) occurs only in a limited concentration range of ADA. The variation in the solution composition seen in Figure 5 was used to predict the optimal experimental conditions. It is obvious that the maximum number of data points should be collected in the region (B) where the concentration of ADA varies between 4 and 6 mM. Collection of many data points out of this range (region (A) and (C)) would provide little or no information towards the calculation of the expected stability constants, although the greatest change in absorbance occurs in the region (A). This is because one cannot distinguish log $K$ values between 3.5 and 6.5 as all the curves seen in Figure 5 in the region (A) are fully overlapped up to [ADA] of about 3 mM. It is also seen that for log $K_{\beta$-CD-ADA} values that differ over two orders of magnitude (between 6.5 and 4.5), the concentration range in which meaningful and appropriate for refinement procedure data can be collected, is very small (in the vicinity of 5mM of ADA). This makes the study of these types of systems more complex and results, unavoidably, in an increase in the uncertainty of the calculated stability constants. For higher values of this stability constant, gathering of data points in the region (C) is of no use as complete complexation has already taken place. This further confirmed the importance of the simulated diagrams in predicting the necessary experimental conditions.

The simulated diagrams thus form an important part of this work as they point directly to the concentration region in which the experiment should be performed in order to establish conditions to reach reliable estimates of the stability constants under investigation. The initial solution composition was $[HMO] = 1.00 \times 10^{-5}$ M and $[\beta$-CD] = 5 mM. The change in absorbance of the $\beta$-CD–HMO solution was monitored as a function of increasing ADA concentration and selected spectra for titration with RIM are seen in Figure 6. Once again, the change in absorbance as complexation takes place can be seen to be rather small (about 0.09 absorbance units for AM and RIM, and 0.06 absorbance units for MEM was observed when studied in a broad concentration range of ADA, between 0 and 7.5 mM). Factor analysis proved the presence of the two species, uncomplexed HMO and $\beta$-CD–HMO. Selected data (in the concentration range of ADA between 4 and 6 mM) were analysed by the programme SQUAD in the wavelength region between 450 and 575 nm. Results obtained are seen in Table 3. The values for the stability constant for AM reported in the literature\textsuperscript{29, 31} compare very well
with the one that has been obtained in this work. The value obtained for the inclusion of RIM is higher than that in the literature,\textsuperscript{29} when the study was performed at significantly higher pH.

![Absorbance spectra](image)

**Figure 6** Variation in the absorbance spectra of HMO as a function of the RIM concentration at pH 1.7. The total methyl orange and β-CD concentrations were $1.00 \times 10^{-5}$ and $5.00 \times 10^{-3}$ M, respectively. [RIM] = a: 0; b: 1.00; c: 2.00; d: 2.50; e: 3.00; f: 3.50; g: 4.20; h: 7.50 mM.

It can be seen from Table 3 that RIM forms the strongest complex with β-CD, followed by AM and then by MEM. The reason for the lowest binding constant for the coordination of MEM could be due to the steric hindrance provided by the two methyl groups on the adamantyl group. While the adamantyl group, due to its non-polarity, enters the cavity, the ammonium group (memantine and amantadine) and the methyl-ammonium moiety (rimantadine) are situated closer to the periphery of the cavity. Thus the bulkiness of the methyl groups decreases a ‘good fit’ of the molecule in the cavity of β-CD. On the other hand, both RIM and AM do not possess any methyl groups in the 3- and 5-position of the adamantyl body. Furthermore, from the values that we have obtained it can be seen that RIM has a higher value for the stability constant than AM. Rüdiger et al,\textsuperscript{32} using NMR spectroscopy and computer-aided molecular modelling, have shown that in the case of adamantane-1-carboxylate, the entire moiety is
encapsulated by β-CD, with the adamantyl group ‘deep’ inside. According to Rüdiger, there is no room for water molecules inside the cavity, when adamantane-1-carboxylate is encapsulated. This is in a support of the above supposition of the steric hindrance caused by the two methyl groups in MEM. Moreover, molecular modelling calculations have shown that additional stabilisation is achieved by strong hydrogen bonding between the β-CD-2-OH groups (of the upper rim of the wide cavity end) and the COO⁻ group. In RIM the protonated ammonium group is one bond distance closer to the periphery than the ammonium cation in AM. This possibly allows for more significant stabilisation by hydrogen bonding between the β-CD-2-OH groups and the NH₃⁺ group in RIM than in AM.

Table 3  Results for the inclusion complex formation of β-CD and the adamantane derivatives from this work (I = 0.05M (Na⁺,H⁺)ClO₄, pH = 1.7, 25 °C), as computed by the programme SQUAD, and from the literature.

<table>
<thead>
<tr>
<th></th>
<th>log $K_{β-CD-ADA}$</th>
<th>statistical parameters</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>3.9 ± 0.1</td>
<td>$U = 8.32 \times 10^{-4}$</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\sigma(A) = 1.56 \times 10^{-3}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.93 ± 0.01</td>
<td></td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>3.95 ± 0.05</td>
<td></td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>3.92 ± 0.02</td>
<td></td>
<td>[33]</td>
</tr>
<tr>
<td>RIM</td>
<td>5.1 ± 0.2</td>
<td>$U = 8.82 \times 10^{-4}$</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\sigma(A) = 1.80 \times 10^{-3}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.48 ± 0.08</td>
<td></td>
<td>[28]</td>
</tr>
<tr>
<td>MEM</td>
<td>3.3 ± 0.1</td>
<td>$U = 3.26 \times 10^{-4}$</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\sigma(A) = 9.80 \times 10^{-4}$</td>
<td></td>
</tr>
</tbody>
</table>

a pH < 2.5; 25 °C; UV
b pH = 2.5; 25 °C; UV
c pH = 7.2; 25 °C; calorimetry
RIM has a chiral centre. In this case the stability constant determined is an average for both the D- and L- isomers. It is possible that the stability constants for the two isomers differ significantly. It is known that CDs are used effectively as chiral selectors as they are stereoselective agents.\textsuperscript{9, 33}

It is generally thought that the hydrophobicity of the molecule enhances the stability of the complex with CDs because of the favourable interaction with the hydrophobic interior of the CD.\textsuperscript{5} Tabushi et al\textsuperscript{35} investigated the binding of cyclohexane derivatives to $\beta$-CD and have shown that apolar guests are bound more strongly than polar guests. Based on the guest molecules they studied, they concluded that the hydrophobic interactions are the most important factor influencing the binding. Palepu et al\textsuperscript{10} have emphasised that although the ‘extreme snugness’ of ADA in the $\beta$-CD cavity is largely responsible for the strength of binding, the charge on the molecule, either positive or negative, decreases the strength of binding.

From our results, we have suggested that the distance between the polar exterior of the $\beta$-CD and the charged group on the encapsulated guest molecule greatly influences the strength of inclusion complex formation.

4. Conclusion
Simulated species distribution diagrams are important in the evaluation of the optimal experimental conditions thereby making the experiments practically viable. $\beta$-CD forms host-guest type complexes with the adamantane derivatives studied and when higher stability constants than those reported here would be expected, an auxiliary ligand with a larger stability constant with $\beta$-CD than HMO would be required to evaluate stability constants with reasonable accuracy. The variation in the stability constants found in this work for MEM, AM and RIM agrees with earlier findings\textsuperscript{32} (from molecular modelling and NMR spectroscopy). The size of the cavity, as well as the distance of the charged ammonium group (in MEM and AM) from the periphery of the cavity is related to the stability constants in this work. These are, in our opinion, the fundamental factors that govern the inclusion processes studied in this work. An extended study involving other adamantane derivatives would further clarify this point.
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References


