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Determination of the degree of substitution of chemically modified cyclodextrins by a microcalorimetric titration technique

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Abstract

The degree of substitution of chemically modified cyclodextrins (hydroxypropyl-β-cyclodextrin, hydroxyethyl-β-cyclodextrin and methyl-β-cyclodextrin) could be determined by a microcalorimetric titration technique. A linear relationship was obtained between the degree of substitution and the enthalpy of dilution. Secondly, a strong dependence of the heat effect on the concentration of cyclodextrin was demonstrated. The accuracy of measurements was proved by dilution experiments with propan-1-ol. The results are explained by comparison with enthalpy pair and triplet interaction coefficients of ethanol and propan-1-ol from the literature.

Key words: Microcalorimetry; Cyclodextrin; Degree of substitution; Microcalorimetric titration; Enthalpy of dilution; Partial molar enthalpy

1. Introduction

The degree of substitution (DS) is an important parameter for the characterization of chemically modified cyclodextrins. The degree of substitution mentioned here is defined as a measure of the extent to which the hydroxyl groups in each glucopyranose unit of the cyclodextrin have been substituted. It is important to distinguish between DS and molar substitution, which indicates how many moles of adduct have reacted with one mole of glucopyranose. As a result of this distinction the maximum value for DS is 3 (there are only three binding sites in one glucopyranose unit) whereas the degree of molar substitution theoretically could be unlimited, provided that the adduct itself has reactive sites. There is a need for rapid and reliable working techniques for the determination of DS, especially in the field of pharmaceutical applications with emphasis on quality control purposes and regulatory approval.

The routine method for characterizing the DS of cyclodextrins is ^{1}H-NMR spectroscopy carried out in deuterium oxide (Pitha et al., 1987). The DS is defined as the ratio of the signal of the anomeric proton of the native cyclodextrin at approx. 5 ppm to the signal which is due to the presence of methyl groups at approx. 1 ppm. The signal for 1 ppm only occurs for substituted cy-

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clodextrins, e.g., methylated or hydroxyalkylated derivatives.

Another approach for obtaining information about DS and the approximate distribution of substitution is characterization by fast atom bombardment mass spectrometry. This method was introduced by Pitha et al. (1986), who determined the DS of hydroxypropyl-β-cyclodextrin (HP-β-CD). The technique is highly sophisticated and therefore not suitable for routine analysis. As stated by Szente and Strattan (1991), neither of these methods alone can distinguish between adducts attached to each other (e.g., polymerization of propylene oxide in the case of HP-β-CD) and binding to the cyclodextrin. The authors propose to use the data of both techniques in combination in order to evaluate DS and to estimate the degree of adduct polymerization.

A third method is the determination of total hydroxypropyl content of HP-β-CD by removing these groups by acid hydrolysis in the presence of hydrogen iodide and subsequent quantitative analysis of isopropyl iodide by gas chromatography. This procedure is briefly described by Szente and Strattan (1991). However, all these methods are relatively time consuming and expensive. In this paper, a microcalorimetric titration method is presented for the determination of DS of cyclodextrins by performing simple dilution experiments.

The interaction of solute molecules becomes stronger with increasing concentration. An estimation performed by Moelands et al. (1992) indicates that there is not enough water in a 50% (w/v) solution of HP-β-CD (DS ≈ 1) to hydrate all ether and hydroxyl groups. They further claim that the high solubility of HP-β-CD is due to strong interactions between non-hydrated groups if one takes into account the large apparent enthalpy of dilution. This hypothesis is supported by the findings of Yoshida et al. (1988, 1989) who reported moisture sorption experiments with β-cyclodextrin, HP-β-CD and hydroxyethyl-β-cyclodextrin (HE-β-CD) which indicate that the derivatives are more hygroscopic than native cyclodextrin. It should be possible to confirm the hypothesis of interaction between non-hydrated groups by microcalorimetric titration experiments which determine the enthalpy of dilution. Different cyclodextrins with increasing DS were examined, namely, HP-β-CD, HE-β-CD and methyl-β-cyclodextrin (M-β-CD). Hence, with higher DS there should be greater enthalpies of dilution. This must be the case provided that all cyclodextrins are used at constant molal concentration. If this assumption proves to be valid it would be possible to estimate the DS of an unknown sample.

2. Materials and methods

2.1. Materials

The cyclodextrins used were obtained from different suppliers. They are listed in Table 1 according to their chemical modification. The procedures for measuring DS are not fully known, so reference was made to specifications of the manufacturers. The moisture content of the samples was determined with an infrared balance

Table 1
Cyclodextrins used throughout the study, their degree of substitution (DS) and the manufacturers

Cyclodextrin	DS	Manufacturer
Hydroxypropyl-β-cyclodextrin	0.45	Janssen Pharmaceutica (Beerse, Belgium)
Hydroxypropyl-β-cyclodextrin	0.55	Amaizo-Products Co. (Hammond, U.S.A.)
Hydroxypropyl-β-cyclodextrin	0.6	Wacker Chemie GmbH (Munich, Germany)
Hydroxypropyl-β-cyclodextrin	0.9	Wacker Chemie GmbH (Munich, Germany)
Hydroxyethyl-β-cyclodextrin	0.5	Chinoin (Budapest, Hungary)
Hydroxyethyl-β-cyclodextrin	0.6	Wacker Chemie GmbH (Munich, Germany)
Hydroxyethyl-β-cyclodextrin	1.0	Wacker Chemie GmbH (Munich, Germany)
Hydroxyethyl-β-cyclodextrin	1.6	Wacker Chemie GmbH (Munich, Germany)
Methyl-β-cyclodextrin	0.6	Wacker Chemie GmbH (Munich, Germany)
Methyl-β-cyclodextrin	1.0	Chinoin (Budapest, Hungary)
Methyl-β-cyclodextrin	2.0	Chinoin (Budapest, Hungary)

(Sartorius 7093 01, Göttingen, Germany). Propan-1-ol (p.a., Merck, Darmstadt) was dried with a 4 Å molecular sieve (Merck, Darmstadt) before use. All other substances were of analytical grade and purchased from Merck (Darmstadt, Germany). Double-distilled water was used throughout the study.

2.2. Methods

2.2.1. Apparatus

The calorimetric measurements were performed with the 2277 Thermal Activity Monitor (TAM) (Thermometric AB, Järfälla, Sweden), an isothermal heat-conduction microcalorimeter. This system has been described by Suurkuusk and Wadsö (1982). It was used in the titration mode equipped with a 1 ml stirred titration vessel. The cyclodextrin solutions were contained in a 1 ml gas-tight Hamilton syringe, attached to a computer-operated syringe drive (Hamilton Microlab M, Bonaduz, Switzerland). All experiments were carried out at 25°C.

2.2.2. Sample preparation and procedure

All cyclodextrins were dissolved in phosphate buffer (0.015 M) of pH 7.4. The titration vessel was charged with 0.9 ml of pure buffer and equilibrated. Aliquots of 20 μl of cyclodextrin solution were injected every 40 min. Two different experimental designs were used throughout the study. For the estimation of DS the concentration of cyclodextrin was fixed at 0.15 mol kg^{-1} for all samples. 10 injections were made. In a second approach the concentration dependence of enthalpy of dilution was to be investigated. Thus, only one cyclodextrin (HP-β-CD, DS 0.55) was used in the concentration range 0.025–0.35 mol kg^{-1}. Five injections were made in each series. The signals were collected as a heat flow (μW). By integrating the heat flow curve the cumulative heat Q (mJ) is obtained. As demonstrated by calibration experiments (see section 2.2.4) the calorimeter works accurately. Usually the coefficient of variation was below 3%. Even for heat flow curves of lower intensity, i.e., closer to the detection limit of the system the coefficient of variation for Q between different runs was lower than 5%. Samples for repeated experiments were freshly prepared on another day.

2.2.3. Calculations

The experiments could not be performed in ideal dilute solutions, therefore, some assumptions had to be made. In ideal mixtures there is no interaction between the molecules, and the enthalpy H of the system consists of the sum of enthalpies H_i of the single components,

$$H = \sum_{j=1}^{k} n_i H_i \tag{1}$$

where n_i is the number of moles.

In real mixtures the enthalpy H of the system can be described as the sum of partial molar enthalpies $\bar{H}_i$ of the different species:

$$H = \sum_{j=1}^{k} n_i \bar{H}_i \tag{2}$$

All partial molar quantities are not constant but depend on the concentrations of the components in the mixture (Wedler, 1982). One often used quantity is the partial molar enthalpy of solution at infinite dilution ($\bar{H}^{\infty}$), which provides a measure of the effect of one mole of solute in an infinite amount of solvent (here water). This enthalpy could be compared to the enthalpy of solution for a given substance. In this study the solid cyclodextrins were previously dissolved in water in order to perform the titration experiments. Therefore, with this procedure no enthalpies of solution were estimated but enthalpies of dilution ($\Delta_{\mathrm{dil}} H$) for a fixed concentration (here 0.15 mol kg^{-1}). Enthalpies of dilution of different cyclodextrins obtained by calorimetric titrations were transformed to partial molar enthalpies at infinite dilution ($\bar{H}^{\infty}_{\mathrm{CD}}$). Partial molar quantities are useful when interpreting solute-solvent interactions (Franks and Reid, 1973). The partial molar enthalpy is evaluated as:

$$\Delta \bar{H}_{\mathrm{CD}} = \left(\frac{\partial H}{\partial n_{\mathrm{CD}}} \right)_{T,p,n_{\mathrm{water}}} \tag{3}$$

where T is temperature, p denotes pressure and n_{CD} and n_{water} are the numbers of moles of cyclodextrin and water, respectively. From this

equation a linear relationship can be derived, resulting in the partial molar enthalpies of the two components in a given mixture (Wedler, 1982; Atkins, 1988):

$$\Delta_{dil}H_m = \Delta\overline{H}_{CD} - (1 - x_{CD})\frac{d_{dil}H_m}{dx_{CD}} \quad (4)$$

where $\Delta_{dil}H_m$ is the molar enthalpy of dilution ($\Delta_{dil}H$ divided by the total number of moles in the mixture). Although a 0.015 M phosphate buffer was used, for calculation the solvent was treated as pure water; $\Delta\overline{H}_{CD}$ represents the partial molar enthalpy of cyclodextrin; and x_{CD} is the mole fraction of cyclodextrin.

By plotting ($\Delta_{dil}H_m$) vs ($1 - x_{CD}$) and fitting Eq. 4 to the measured data the partial molar enthalpy of cyclodextrin for a given composition of the mixture could be estimated.

2.2.4. Calibration

The system was chemically calibrated by dilution of a 10% (w/w) aqueous propan-1-ol solution. The measured enthalpies of dilution were compared to literature data (Briggner and Wadsö, 1991). The correction term was found to be a negligible 1.0019 and was therefore not allowed for in calculation of the cumulative heats. An electrical calibration was avoided, due to the fact that a standardized dilution experiment resembles the experimental conditions much more. The fraction of heat flow not recorded can be large because of alternative pathways between vessel and heat sink (Briggner and Wadsö, 1991). Since the electrical heater is not positioned in the vessel large differences could occur.

Secondly, a comparison was performed to check the reliability of the calculated data (see section 2.2.3). 1 μl of pure propan-1-ol was injected in a volume of 3 ml of water. 10 injections were made. The final concentrations were judged to be low enough to take the experimental solution enthalpies as identical to the enthalpy of solution at infinite dilution ($\Delta_{sol}H^{\infty}_{propanol}$) (Hallén et al., 1986), which is confirmed by the linear relationship between concentration of propan-1-ol and the measured enthalpies. $\Delta_{sol}H^{\infty}_{propanol}$ was estimated to be -9.15 kJ mol^{-1} (standard deviation of 10 injections: ± 0.48 kJ mol^{-1}). The relatively high standard deviation is due to irregularities of the pump system; 1 μl is the smallest volume which can be dispensed. The same data were treated as described in section 2.2.3 and the partial molar enthalpy of propan-1-ol at infinite dilution ($\overline{H}^{\infty}_{propanol}$) was determined as -9.22 kJ mol^{-1} and is therefore in close agreement with the previous value. Compared to -10.16 kJ mol^{-1} (Hallén et al., 1986) these values are significantly lower but altogether in the same range. The difference could be due to small amounts of water in propan-1-ol, since no fractional distillation was performed before use. Another problem could be the inefficiency of mixing in the 3.5 ml vessel and a resulting loss in heat flow between vessel and heat sink (Briggner and Wadsö, 1991).

Nevertheless, the procedure demonstrated the reliability of the calculations. The method of estimating the partial molar enthalpies was preferred owing to the fact that in solutions of cyclodextrins even at low concentrations interactions between the molecules exist. This results in a deviation from linearity so that the assumption of infinite dilution is not valid. By extrapolation to the theoretical case of infinite dilution this effect is excluded (see Fig. 2). It is expected that different chemical modifications of cyclodextrins with extremely different heat effects (see Fig. 1) can be more easily compared.

3. Results

Two calorimetric records for HP-β-CD (DS 0.9) and HE-β-CD (DS 0.5) are depicted in Fig. 1. The curve for HE-β-CD is represented in bold-face. The record for HP-β-CD (DS 0.9) is a heat flow curve typical for all other experiments. As stated above, all samples for the comparison of different chemical modifications were of the same concentration. The increase of concentration in the vessel from 3.26×10^{-3} to 2.73×10^{-2} M caused a decrease in peak area.

The corresponding values for the heat flow curve of HP-β-CD and HE-β-CD are listed in Table 2. For HP-β-CD a decrease in peak area can be seen, as expected from Fig. 1. The peaks

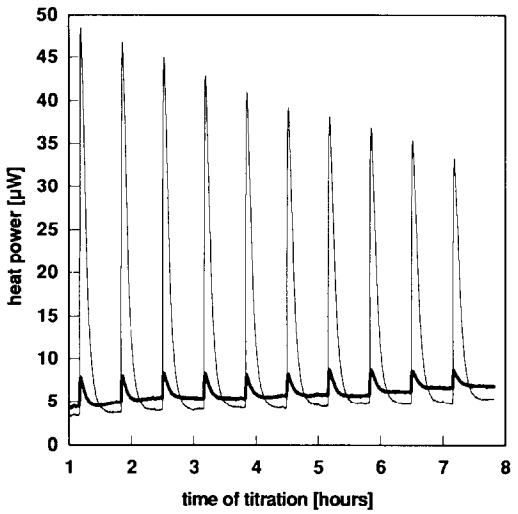

Fig. 1. Heat flow titration curves of hydroxypropyl-β-cyclodextrin (DS 0.9) and hydroxyethyl-β-cyclodextrin (DS 0.5) (bold-face line).

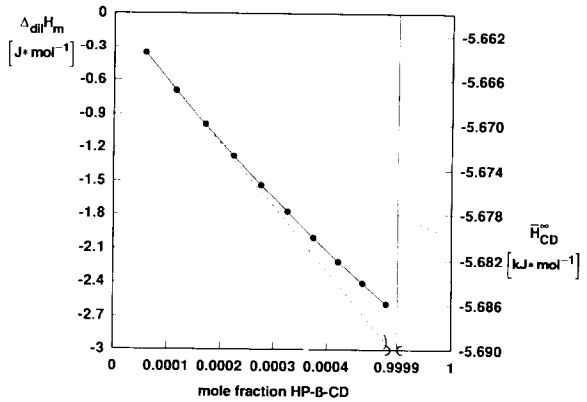

Fig. 2. Plot of the molar enthalpy of dilution vs mole fraction of hydroxypropyl-β-cyclodextrin (DS 0.9) (left ordinate) and extrapolation to the partial molar enthalpy at infinite dilution ($\bar{H}^{\infty}_{CD}$) (dashed line; intersection with the right ordinate).

and the corresponding values for HE-β-CD at a concentration of 0.15 mol kg^{-1} in Table 2 clearly show no trend, all being of the same magnitude. This cyclodextrin was the only one of all the samples tested without a decrease in the enthalpy of dilution during the course of experiment. It must be taken into consideration that this sample yielded the smallest effect of all cyclodextrins investigated, which was close to the resolution of the instrument. For this reason a second experiment was performed by doubling the concentration in the syringe (0.30 mol kg^{-1}). In this case the enthalpy of dilution also decreased with increasing concentrations in the vessel (see Table 2). From these results it can be concluded that HE-β-CD with DS 0.5 behaves in the same way as all other cyclodextrins and therefore the deviation at 0.15 mol kg^{-1} is only due to resolution problems of the measuring instrument.

Table 2
Integrated values of the heat flow curves of hydroxyethyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin with a degree of substitution of 0.5 and 0.9, respectively

Enthalpies of dilution (mJ)		
HE-β-CD (DS 0.5) (0.3 mol kg^{-1})	HE-β-CD (DS 0.5) (0.15 mol kg^{-1})	HP-β-CD (DS 0.9) (0.15 mol kg^{-1})
−2.91	−0.92	−18.41
−2.78	−0.96	−17.43
−2.73	−1.11	−16.66
−2.75	−1.04	−15.74
−2.55	−0.91	−15.30
−2.55	−0.80	−14.74
−2.30	−1.01	−14.11
−1.98	−1.29	−13.71
−1.88	−1.19	−13.29
−1.97	−0.88	−12.67

In Fig. 2 a plot of molar enthalpy of dilution ($\Delta_{dil}H_m$) vs mole fraction of HP-β-CD (DS 0.9) in the vessel is presented. Fig. 2 is split into two different parts: the left ordinate shows the measured enthalpies of dilution ($\Delta_{dil}H_m$) in J mol^{-1} and the right ordinate corresponds to extrapolated values of the partial molar enthalpies at infinite dilution ($\bar{H}^{\infty}_{CD}$) in kJ mol^{-1} (see Eq. 4). A linear regression was performed for the first three points and the fitted line was extrapolated to a mole fraction of cyclodextrin equal to unity. At the point of intersection with the right ordinate $\bar{H}^{\infty}_{CD}$ can be read (dashed line). In this way $\bar{H}^{\infty}_{CD}$ was determined for each of the different cyclodextrins. Fig. 2 demonstrates a decrease of the enthalpy of dilution with increasing concentration in the reaction vessel.

The partial molar enthalpies at infinite dilution ($\bar{H}^{\infty}_{CD}$) for a definite concentration in the syringe are depicted in Fig. 3. For all cyclodextrins investigated a linear relationship between

$\bar{H}^{\infty}_{CD}$ and DS was obtained. The exothermic $\bar{H}^{\infty}_{CD}$ increases with enlarged DS. The coefficients of correlation for HP-β-CD, HE-β-CD and M-β-CD are 0.9988, 0.9955 and 0.9979, respectively. For the two more hydrophilic derivatives HE-β-CD and HP-β-CD a large difference in magnitude is obtained with a shift to higher enthalpies for HP-β-CD. The more lipophilic derivative M-β-CD parallels HP-β-CD, but intersects with HE-β-CD at DS 0.6.

As mentioned above, all measurements were performed with a fixed concentration of 0.15 mol kg^{-1} in the syringe. In a further experiment the concentration dependence of the enthalpy of dilution was demonstrated. Fig. 4 outlines the results of the dilution experiments with HP-β-CD (DS 0.55) in the concentration range from 0.05 to 0.35 mol kg^{-1}. $\bar{H}^{\infty}_{HP\text{-}\beta\text{-}CD}$ is plotted vs the concentration of HP-β-CD dispensed by the syringe. The single points could be described by the following quadratic equation,

$$\bar{H}^{\infty}_{CD} = -0.50 - 7.48(c_{CD}) - 48.77(c_{CD})^2 \qquad (5)$$

where c_{CD} is the concentration of HP-β-CD in the syringe (mol kg^{-1}). This relationship is shown by the fitted line. If the respective function for the concentration dependence of any cyclodextrin is known, it is possible to estimate $\bar{H}^{\infty}_{CD}$ for all concentrations in that range.

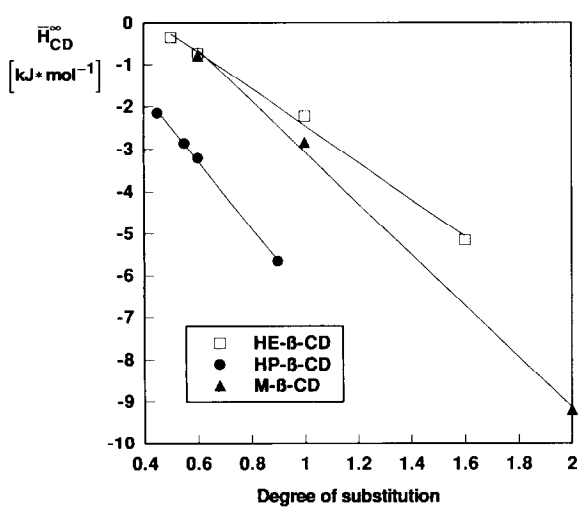

Fig. 3. Relationship between the partial molar enthalpy at infinite dilution ($\bar{H}^{\infty}_{CD}$) and the degree of substitution of the respective cyclodextrin derivative.

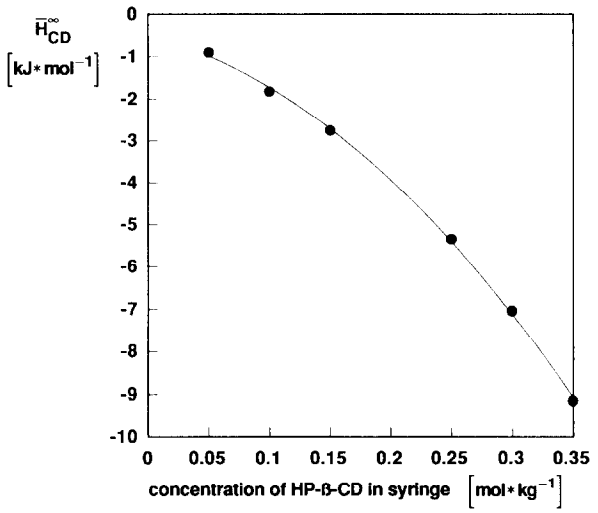

Fig. 4. Partial molar enthalpy at infinite dilution ($\bar{H}^{\infty}_{CD}$) of hydroxypropyl-β-cyclodextrin (DS 0.55) dependent on the concentration of the solution dispensed.

4. Discussion

For a correct interpretation of the data obtained some theoretical considerations on hydrophobic interactions (Moelands et al., 1992) should be taken into account. For this reason the results are discussed in terms of some thermodynamic annotations. The results for HE-β-CD and HP-β-CD could be interpreted by the findings of Franks et al. (1976) on hydrophobic interactions of ethanol and propan-1-ol in aqueous solution. They discussed the cooperativity of hydrophobic interaction by comparing the molecular pair and triplet interactions of ethanol and propan-1-ol according to the McMillan-Mayer solution theory (McMillan and Mayer, 1945). They calculated enthalpy coefficients which reflect the alkyl group interactions in aqueous solution. The enthalpy pair interaction coefficients for ethanol and propan-1-ol in aqueous solution at 25°C are 243 and 559 J kg mol^{-2}, respectively. The enthalpy triplet interaction coefficients are 65 and 158 J kg^2 mol^{-3} for ethanol and propan-1-ol, respec-

tively. These values were confirmed later by Hallén et al. (1986). Franks and co-workers stated that the extent of hydrophobic interaction increases with the number of carbon atoms in the alkyl chain. Hence, the pair and triplet interaction for propan-1-ol is stronger than for ethanol. They also emphasized the entropic origin of the hydrophobic interaction. Although attention must be paid to the temperature dependence of enthalpy interaction coefficients (Franks and Pedley, 1983), the interpretations of Franks and coworkers are consistent. The McMillan-Mayer theory cannot be applied without reservation to chemically modified cyclodextrins which are a combination of sugars and 'mixed' solutes with hydrophilic and lipophilic sections, since this theory may not be entirely suitable for hydrophilic sugars (Franks and Reid, 1973). However, some similarities between HP-β-CD/HE-β-CD and the mixed solutes ethanol and propan-1-ol are evident. Moelands et al. (1992) claimed that the high solubility of HP-β-CD is due to strong interactions between non-hydrated groups. This is consistent with our results. The more hydrophobic HP-β-CD results in a greater $\overline{H}^{\infty}_{CD}$ than HE-β-CD does, due to the stronger hydrophobic hydration of the hydroxypropyl group. The enthalpy of dilution is an exothermic effect for all cyclodextrins investigated. However, with increasing concentration in the reaction vessel the magnitude of the enthalpy of dilution decreases. If an interaction between alkyl groups is possible, hydrophobically bonded water must be displaced. The dehydration of hydrophobic parts is surely an endothermic and entropy-driven process as in the case of ethanol and propanol or the process of micellization of nonionic surfactants (Hüttenrauch, 1984). Therefore, this could be an explanation for the decrease of enthalpy of dilution during the titration experiment. M-β-CD does not fit closely in this model because of its more lipophilic nature. However, if experiments with ethyl-β-cyclodextrin are performed, probably quite similar differences will arise between ethyl-β-cyclodextrin and M-β-CD as compared to HP-β-CD and HE-β-CD.

It should be borne in mind that this method is based on comparisons between different samples of known DS. Nevertheless, even with the heterogeneous material from different suppliers used in this study the linear relationship between DS and $\overline{H}^{\infty}_{CD}$ is evident. By accurate determination of the DS using ^{1}H-NMR spectroscopy or fast atom bombardment mass spectrometry (or better, a combination of both) the preparation of well-defined standard samples is possible. This is especially important in the case of quality control purposes when these samples are gained from the production process. The recording of a calibration curve with these standard samples is only required once. Recalibration of the instrument could be performed by similar dilution experiments with propan-1-ol (Briggner and Wadsö, 1991).

From a thermodynamic point of view it is unsatisfactory to deal with concentration dependencies. If it is necessary to obtain thermodynamic data for pure cyclodextrins, enthalpies of dissolution ($\Delta_{sol}H$) should be measured. The dissolution of a solid solute could be recorded with a microcalorimetric solution technique (Hallén and Wadsö, 1989). However, it seems that titration calorimetry is more convenient and gives more precise results. The results clearly demonstrate that the degree of substitution of chemically modified cyclodextrins can be determined by simple dilution experiments. If the substituent is known the procedure presented is highly accurate and easily applicable.

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