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# Substrate Binding to Cyclodextrins in Aqueous Solution: A Multicomponent Self-Diffusion Study

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**Abstract.** It is demonstrated that substrate binding to α- and β-cyclodextrins (CD) in solution can conveniently and directly be monitored from multicomponent self-diffusion data on these solutions, using the Fourier Transform NMR pulsed-gradient spin-echo technique. Included are aromatics and a series of alcohols ranging from methanol to octanol. Experimentally it was found that *n*-alcohols associate more strongly with α-CD than with β-CD. As the bulkiness of the alcohol increased, binding to β-CD was enhanced while the reverse effect was observed in the case of α-CD. For both cyclodextrins it was found that *n*-alcohol complexation in the homologous series was attributable to an increment in standard free energy of complexation of ~ − 3.0 kJ/mol for each $-CH_2-$ group, suggesting that the binding mechanism is of a hydrophobic nature.

**Key words:** Cyclodextrin, Self-Diffusion, Nuclear Magnetic Resonance, Inclusion–Complex, Hydrophobic Interaction, Alcohols.

## 1. Introduction

Cyclodextrins are cyclic oligosaccharides consisting of six or more D(+)-glucopyranose units having the shape of a hollow truncated cone, the interior of which forms a hydrophobic cavity. The remarkable ability of cyclodextrins to form inclusion complexes with a variety of substrate molecules have given them widespread application in chromatography [1,2] and catalysis [3,4,5,6]. Of special interest is the use of cyclodextrins as enzyme active-site models through their ability to selectively bind and catalyse certain chemical reactions of guest molecules [7,8,9].

A prerequisite for substrate binding to cyclodextrins is that the size of the substrate molecule enables it to fit into the cavity of the cyclodextrin. The forces responsible for complex formation have been ascribed to Van der Waals interactions, hydrophobic interactions and hydrogen bonding. Of these, hydrogen bonding seems to be of minor importance since it has been observed that modified cyclodextrins, incapable of hydrogen bonding, do not exhibit drastically different binding characteristics [10]. On the other hand, inclusion phenomena involving cyclodextrins occur almost exclusively in aqueous solutions, pointing to the significance of hydrophobic interactions [1]. However, as to the exact nature of the binding forces responsible for complex formation, controversy still exists.

In previous association studies involving cyclodextrins indirect experimental methods have been employed [1,11–15]. Apart from making the validity of the results dependent on a number of assumptions this has also restricted the experimental conditions under which the measurements could be performed and limited the range of substrates accessible for study.

The pulsed-gradient spin-echo NMR method as described by Stilbs [16,17,18] is capable of measuring individual self-diffusion coefficients in multicomponent systems and has previously been employed to study solubilization in micelles [18] and counter ion binding in

polyelectrolyte solutions [19]. It was therefore considered of interest to apply this technique for the study of association in cyclodextrin systems. Self-diffusion coefficients are well defined quantities which directly reflect the association behaviour of the interacting species. The binding of a variety of substrate molecules with α- and β-cyclodextrin was investigated. Included is a homologous series of alcohols ranging from methanol to octanol.

## 2. Experimental

### 2.1. NMR MEASUREMENTS

The measurements were performed on a JEOL FX–100 Fourier Transform NMR spectrometer operating at 99.6 MHz using internal $D_2O$ lock. All diffusion coefficients we measured at 25.0 ± 0.2 °C using techniques outlined earlier [16–18]. An example of the spectra obtained from a typical experiment is shown in Figure 1.

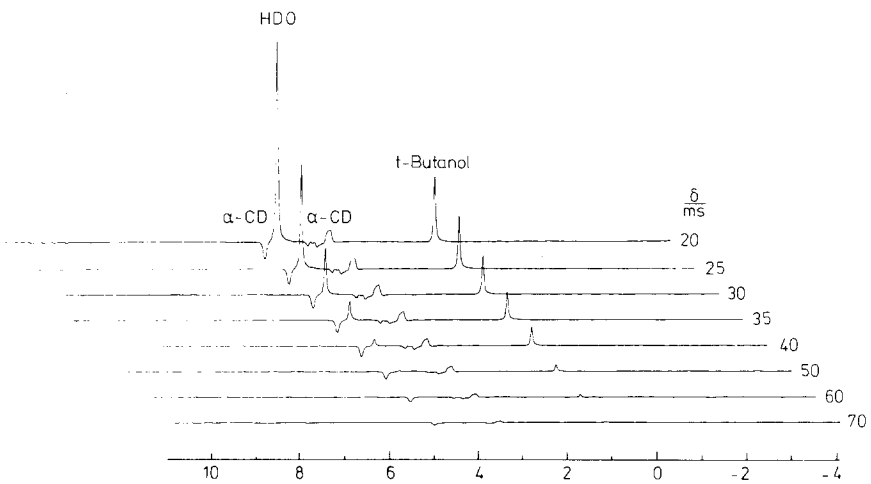


Fig. 1. A typical sequence of spectra by the Pulsed-Gradient Spin-Echo method. Shifts are given in ppm, assigning the HDO-signal a shift of 4.7 ppm. The δ-values represent the durations of the applied magnetic field gradient pulses. Signal amplitudes decrease as a result of diffusion with increasing δ-values. See references [16] and [18] with regard to the experimental procedures. Note the negative amplitude of the α-CD-signal at 5.0 ppm which is due to so-called J-modulation effects. Diffusion coefficients in this experiment were evaluated to be $(0.27 \pm 0.01) \times 10^{-9}\ m^2\ s^{-1}$ and $(0.68 \pm 0.01) \times 10^{-9}\ m^2\ s^{-1}$ for α-CD and *t*-Butanol, respectively.

### 2.2. SAMPLE PREPARATION

Stock solutions of cyclodextrin (Sigma Chemical Company) in $D_2O$ (Norsk Hydro, Rjukan, Norway) were prepared by weighing. The highest concentration of α-CD used was ~20 mM and that of β-CD ~10 mM. The substrate was then added to a portion of this stock solution. The concentration of substrate in the mixture was typically of the order of 10–20 mM. With the higher alcohols lower concentrations had to be employed in order to avoid precipitation of the cyclodextrin.

2.3. CALCULATIONS

Since there is a fast exchange on the NMR time scale between bound and free substrate molecules, an apparent self-diffusion coefficient is obtained, given by:

$$D_s^{obs} = p \cdot D_s^{bound} + (1 - p) \cdot D_s^{free} \tag{1}$$

which can be rearranged to yield:

$$p = \frac{D_s^{free} - D_s^{obs}}{D_s^{free} - D_s^{bound}}, \tag{2}$$

where $p$ is the fraction of complexed substrate molecules, $D_s^{bound}$ is the self-diffusion coefficient of the complex and $D_s^{free}$ that of the free substrate. It was found in all cases that the diffusion coefficient of cyclodextrin was unaffected upon complexation and equal to $0.27 \times 10^{-9}$ m$^2$ s$^{-1}$ (average value of a large number of measurements) for both $\alpha$- and $\beta$-cyclodextrin. This value of $D_s^{bound}$ was therefore used in all calculations. $D_s^{free}$ was measured separately in the absence of cyclodextrin in dilute (< 20 mM) aqueous ($D_2O$) solution. It can safely be assumed that $D_s^{free}$ is the same in the cyclodextrin solution, since any obstruction effect [20] due to the presence of cyclodextrin is negligible at the low concentrations employed. This is substantiated by the fact that $D_s^{free}$ and $D_s^{obs}$ are the same within experimental error in the cases where no binding occurs (see Table I). Furthermore, any small error in $D_s^{free}$ will partly cancel out in the calculation of $p$ as seen from Equation 2.

It is generally assumed that [1,3,11,21]cyclodextrins form 1 : 1 inclusion complexes with the kind of substrates and concentrations used in the present investigation, conforming to an equilibrium of the type:

$$S + CD \rightleftarrows S \cdot CD$$

with an association constant, $K_c$, defined by

$$K_c = \frac{C_{S \cdot CD}}{C_S \cdot C_{CD}} \tag{3}$$

Inserting Equation 2 into 3 yields:

$$K_c = \frac{p}{(1 - p)(C_{CD,\,tot} - C_{S,\,tot} \cdot p)}, \tag{4}$$

where $K_c$ can be calculated from a single substrate concentration. $C_{CD,\,tot}$ and $C_{S,\,tot}$ denote the total concentrations of cyclodextrin and substrate, respectively.
An alternative formulation of Equation 4 is:

$$\frac{p}{1 - p} = K_c \cdot C_{CD,\,tot} - K_c \cdot p \cdot C_{S,\,tot}, \tag{5}$$

where $K_c$ can be obtained from a plot of

$\frac{p}{(1 - p)}$ versus $p \cdot C_{S,\,tot}$, keeping $C_{CD,\,tot}$ constant.

Since the purpose of this investigation was to explore the applicability of this technique in connection with cyclodextrin complexation and not to make precision measurements of

TABLE I. Diffusion and cyclodextrin association data for some organic substrates[a]

| | | α-CD | | | β-CD | | |
|---|---|---|---|---|---|---|---|
| | $D_s^{free}$ | $D_s^{obs}$ | $p$ | $K_c/M^{-1}$ | $D_s^{obs}$ | $p$ | $K_c/M^{-1}$ |
| *n*-alcohols | | | | | | | |
| Methanol | 1.312 ± 0.007 | 1.295 ± 0.007 | 0.016 ± 0. 006 | 0.9 ± 0.4 | 1.34 ± 0.05 | no binding | – |
| Ethanol | 1.014 ± 0.003 | 0.96 ± 0.01 | 0.07 ± 0.02 | 4 ± 1 | 1.02 ± 0.01 | no binding | – |
| *n*-Propanol | 0.850 ± 0.005 | 0.71 ± 0.02 | 0.24 ± 0.03 | 20 ± 4 | 0.80 ± 0.02 | 0.09 ± 0.03 | $13^{+7}_{-6}$ |
| *n*-Butanol | 0.764 ± 0.004 | 0.62 ± 0.02 | 0.29 ± 0.04 | $46^{\pm 20}_{-10}$ | 0.70 ± 0.01 | 0.13 ± 0.02 | 19 ± 4 |
| *n*-Pentanol | 0.736 ± 0.009 | 0.51 ± 0.01 | $0.48^{+0.03}_{-0.02}$ | $210^{+50}_{-30}$ | 0.60 ± 0.01 | 0.29 ± 0.02 | 140 ± 40 |
| *n*-Hexanol | 0.69 ± 0.04 | 0.44 ± 0.01 | $0.60^{+0.03}_{-002}$ | 940 ± 300 | 0.42 ± 0.02 | 0.64 ± 0.05 | $340^{+100}_{-80}$ |
| *n*-Heptanol | 0.65 ± 0.04 | 0.33 ± 0.01 | $0.84^{+0.03}_{-0.02}$ | $2700^{+1300}_{-500}$ | 0.48 ± 0.02 | 0.45 ± 0.05 | $700^{+500}_{-250}$ |
| *n*-Octanol | 0.65 ± 0.04 | 0.33 ± 0.01 | $0.84^{+0.03}_{-0.02}$ | $7800^{+7000}_{-2200}$ | 0.38 ± 0.01 | 0.71 ± 0.03 | $2100^{+700}_{-500}$ |
| *iso*-alcohols | | | | | | | |
| *iso*-Propanol | 0.878 ± 0.005 | 0.808 ± 0.005 | 0.12 ± 0.01 | 8 ± 1 | 0.824 ± 0.002 | 0.089 ± 0.003 | 14 ± 1 |
| *iso*-Butanol | 0.788 ± 0.002 | 0.653 ± 0.003 | 0.261 ± 0.007 | $30^{+1}_{-2}$ | 0.680 ± 0.002 | 0.21 ± 0.01 | $43^{+4}_{-3}$ |
| *iso*-Pentanol | 0.734 ± 0.003 | 0.573 ± 0.003 | 0.35 ± 0.01 | $82^{+5}_{-6}$ | 0.559 ± 0.007 | 0.38 ± 0.01 | 147 ± 10 |
| *neo*-Pentanol | 0.728 ± 0.007 | 0.72 ± 0.01 | no binding | – | – | – | 410 ± 30[b] |
| *t*-alcohols | | | | | | | |
| *t*-Butanol | 0.722 ± 0.004 | 0.683 ± 0.009 | 0.09 ± 0.02 | 5 ± 1 | 0.67 ± 0.01 | 0.12 ± 0.02 | 17 ± 5 |
| *t*-Pentanol | 0.692 ± 0.002 | 0.575 ± 0.001 | 0.278 ± 0.002 | $34^{+0.4}_{-1}$ | 0.524 ± 0.003 | 0.398 ± 0.007 | 128 ± 6 |

TABLE I. (continued)

| | | α-CD | | | β-CD | | |
|---|---|---|---|---|---|---|---|
| | $D_s^{free}$ | $D_s^{obs}$ | $p$ | $K_c/M^{-1}$ | $D_s^{obs}$ | $p$ | $K_c/M^{-1}$ |
| secondary alcohols | | | | | | | |
| 2-Butanol | 0.772 ± 0.002 | 0.684 ± 0.007 | 0.190 ± 0.002 | 22 ± 0.5 | 0.70 ± 0.01 | 0.14 ± 0.02 | 22 ± 5 |
| 2-Pentanol | 0.712 ± 0.002 | 0.558 ± 0.003 | 0.35 ± 0.01 | $105^{+10}_{-8}$ | 0.61 ± 0.01 | 0.23 ± 0.02 | 59 ± 10 |
| 3-Pentanol | 0.707 ± 0.005 | 0.568 ± 0.001 | 0.32 ± 0.02 | 71 ± 2 | 0.624 ± 0.002 | 0.190 ± 0.005 | $54^{+2}_{-5}$ |
| acetins | | | | | | | |
| mono-Acetin | 0.645 ± 0.005 | 0.594 ± 0.007 | $0.14^{+0.02}_{-0.03}$ | 9 ± 2 | 0.62 ± 0.01 | 0.07 ± 0.02 | 16 ± 7 |
| di-Acetin | 0.617 ± 0.003 | 0.553 ± 0.004 | $0.18^{+0.02}_{-0.01}$ | $13^{+1}_{-2}$ | 0.596 ± 0.007 | 0.06 ± 0.02 | $15^{+7}_{-4}$ |
| tri-Acetin | 0.571 ± 0.001 | 0.519 ± 0.006 | 0.17 ± 0.02 | 12 ± 2 | 0.554 ± 0.002 | 0.056 ± 0.006 | 14 ± 2 |
| various substrates | | | | | | | |
| Methyl-cyclohexanol | 0.65 ± 0.02 | 0.61 ± 0.05 | no binding | – | 0.40 ± 0.01 | 0.66 ± 0.03 | $120^{+50}_{-30}$ |
| Benzyl-alcohol | 0.804 ± 0.008 | 0.67 ± 0.01 | 0.25 ± 0.02 | $22^{+3}_{-2}$ | – | – | 215 ± 10[b] |
| *N*-Acetyl-glucosamine | 0.498 ± 0.004 | 0.473 ± 0.007 | 0.11 ± 0.03 | 7 ± 2 | – | – | no binding[b] |

[a] $D$ is expressed in units of $10^{-9}$ $m^2 \times s^{-1}$ and error limits are 80% confidence intervals from a Monte-Carlo simulated error analysis described in reference [24].
[b] Evaluated according to Equation 5. Error limits are 80% confidence intervals.

binding constants, $K_c$-values were generally computed from a single substrate concentration, with a few exceptions (see Table I). If $K_c$ is evaluated from Equation 5, by measuring the individual self-diffusion coefficients for a number of different substrate concentrations, the precision in the $K_c$-values can be significantly improved.

With regard to the general applicability of the technique, it should be pointed out that it is best suited for conditions where $p \sim 0.5$. For $p$ close to 0 or 1 relatively small deviations from $D_s^{free}$ or $D_s^{bound}$ result, and the determination of $K_c$ consequently becomes quite uncertain. As compared with binding to, for example, micelles or polyelectrolytes [18,19], the technique is not quite as favourable owing to the relatively high $D_s^{bound}$ for these cyclodextrins.

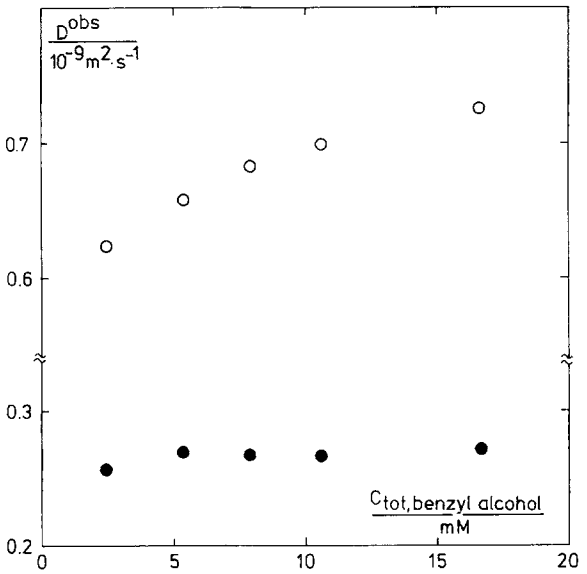


Fig. 2. The observed self-diffusion coefficient, $D^{obs}$, versus concentration of benzyl alcohol. (○) benzyl alcohol and (●) β-cyclodextrin. The concentration of cyclodextrin was 5.65 mM.

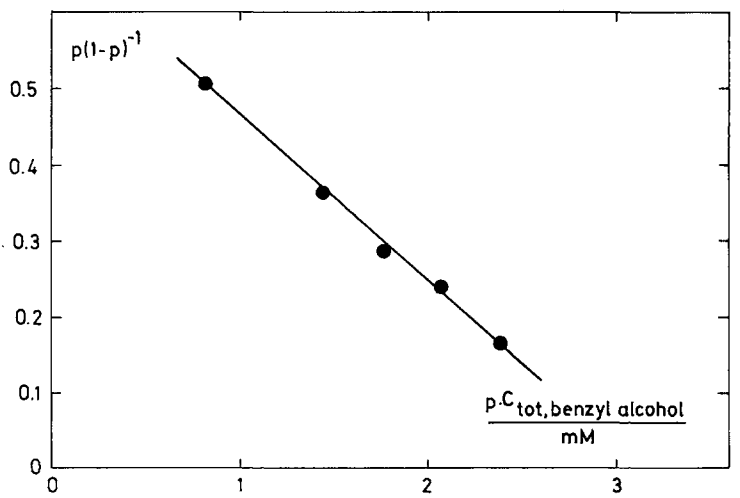


Fig. 3. Data from Figure 2 plotted according to Equation 5.

## 3. Results

Self-diffusion coefficients and association data are reported in Table I. Error limits for $p$ and $K_c$ in this table are indicative only in that they are based solely on error propagation from $D_s^{obs}$. In most cases binding constants were evaluated from a single measurement according to Equation 4.

For some of the substrates indicated in Table I the concentration dependence of $D_s^{obs}$ was determined and plotted according to Equation 5 in order to test the assumption that only 1 : 1 complexes are actually formed. A good fit to Equation 5 was observed in all cases. Representative plots for benzyl alcohol are shown in Figures 2 and 3.

The change in standard free energy, $-\Delta G^0$ (as calculated from $RT\ln K_c$) for transfer from the aqueous to the cyclodextrin phase for a homologous series of $n$-alcohols is depicted in Figure 4.

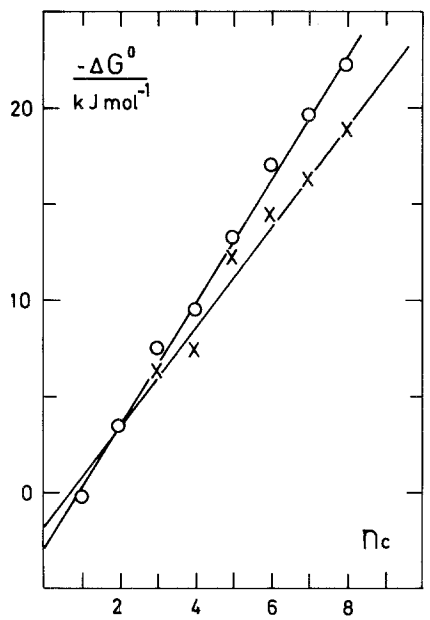


Fig. 4. The change in standard free energy, $-\Delta G^0$ ($= RT\ln K_c$) versus the number of carbon atoms, $n_c$, for the inclusion of a homologous series of $n$-alcohols in (○)α-cyclodextrin, $-\Delta G^0 = (3.2 \pm 0.1) \times n_c - (3.0 \pm 0.6)$ kJmol$^{-1}$ and (x)β-cyclodextrin, $-\Delta G = (2.6 \pm 0.3)\, n_c - (1.9 \pm 1.9)$ kJmol$^{-1}$. Error limits are 80% confidence intervals.

## 4. Discussion

The straight chain alcohols exhibit stronger binding to α-CD than to β-CD, indicating a closer fit into the smaller α-CD cavity, whereas the penetrant interacts less favourably with the larger β-CD cavity. This is substantiated by the findings of Matsui and Mochida [11], according to which both $\Delta H$ and $\Delta S$ were negative for binding of $n$-alcohols to α-CD whereas binding to β-CD was accompanied by positive values of $\Delta H$ and $\Delta S$. They concluded that associations

to α-CD is dominated by van der Waals interactions, while hydrophobic interactions are of primary importance in the complexation of *n*-alcohols with β-CD.

We find an increment in free energy per $-CH_2$-group for transfer of *n*-alcohols from the aqueous phase to the cyclodextrin cavity of $-3.2 \pm 0.1$ kJmol$^{-1}$ for α-CD and $-2.6 \pm 0.3$ kJmol$^{-1}$ in the case of β-CD. It is not surprising to note that the corresponding change in free energy for solubilization of *n*-alcohols is sodium dodecyl sulphate micelles, $\Delta G^0 = -2.6 \pm 0.3$ kJmol$^{-1}$ [18] is nearly the same, since in both cases a partitioning between an aqueous and a hydrophobic phase is involved. A similar value, $\Delta G^0 = -3.4$ kJmol$^{-1}$, for the increment in $\Delta G^0$ per $-CH_2$-group has been reported for the transfer of alcohols and carboxylic acids from water to hydrocarbon solvent [22,23]. It may thus be concluded that changes in $\Delta G^0$ of this order of magnitude are indicative of hydrophobic interactions.

The effect of increasing bulkiness on complex formation can be studied from a comparison of the $K_c$-values for the three primary pentanols, *n*-pentanol, *iso*-pentanol and *neo*-pentanol. It is seen in Table I that for α-CD, $K_c$ decreases with increasing bulkiness of pentanol while an opposite trend is observed in the case of β-CD. This is to be expected, since bulkier penetrants may be more efficiently accommodated by the larger β-CD cavity. The above arguments also apply to the isomers of the primary butanols and propanols investigated.

The data for the isomers of pentanol and butanol reveal that a primary position of the hydroxyl group favours complex formation with both α- and β-cyclodextrin as compared to secondary and tertiary alcohols with the same carbon skeleton as their primary counterpart. This probably reflects the difference in hydrophobicity between these alcohols. Using the partition coefficient as a measure of hydrophobicity this is in agreement with the findings of Matsui and Mochida [11] in their study of the partitioning of alcohols in a diethyl ether / water system.

In order to further stress the general applicability of this technique a number of additional substrates were also included in the present study (see Table I). The binding constants reported show satisfactory agreement with those of other investigators [1,11].

## 5. Conclusions

We conclude that measurements of self-diffusion coefficients using the FT NMR-PGSE method offers a convenient and reliable way of studying complexation in cyclodextrin systems. Assuming a simple two-site model between free and bound substrate molecules, binding constants can readily be obtained.

The technique is, furthermore, applicable to a wide range of substrate molecules contrary to other NMR methods used, i.e. measurement of chemical shifts, which is limited to substrates capable of inducing shift changes of considerable magnitude upon complexation, to make the evaluation of binding constants feasible.

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