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# The Inclusion of Diflunisal by γ-Cyclodextrin and Permethylated β-Cyclodextrin. A UV-Visible and $^{19}F$ Nuclear Magnetic Resonance Spectroscopic Study

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**Abstract.** The complexation of the diflunisal anion (DF) by γ-cyclodextrin (γCD) and permethylated β-cyclodextrin (βPCD) in aqueous solution at pH 7.00 at 298.2 K, has been studied by UV-visible and $^{19}F$ NMR spectroscopy. The formation of 1 : 1 and 1 : 2 γCD inclusion complexes proceeds through the two equilibria:

$$DF + \gamma CD \rightleftharpoons DF{\cdot}\gamma CD \qquad (K_1)$$

$$DF{\cdot}\gamma CD + \gamma CD \rightleftharpoons DF{\cdot}(\gamma CD)_2 \qquad (K_2)$$

characterised by $K_1 = (5.5 \pm 0.2) \times 10^4$ dm$^3$ mol$^{-1}$ and $K_2 = (2.3 \pm 0.2) \times 10^4$ dm$^3$ mol$^{-1}$ derived from UV-visible spectrophotometric data. The analogous βPCD complexes are characterised by $K_1 = (6.86 \pm 0.02) \times 10^4$ dm$^3$ mol$^{-1}$ and $K_2 = (8.75 \pm 2.7) \times 10^1$ dm$^3$ mol$^{-1}$. The variation of the $^{19}F$ chemical shift of DF on inclusion is consistent with the formation of 1 : 1 and 1 : 2 complexes also. Comparisons with related systems are made.

**Key words.** Cyclodextrin, diflunisal, equilibrium, spectrophotometry, nuclear magnetic resonance.

## 1. Introduction

The α-, β- and γ-cyclodextrins (αCD, βCD and γCD) are, respectively, six, seven and eight membered α-1,4-linked cyclic oligomers of D-glucopyranose of internal annular diameters of 5–6, 7–8 and 9–10 Å, which form inclusion complexes with a wide range of substrates in aqueous solution [1–9]. These complexes exhibit variations in their stoichiometries and stability constants as the size of the cyclodextrin and the nature of the substrate is varied. As a consequence of this, cyclodextrin inclusion complexes have been a vehicle for the study of inclusion phenomena in general, and have found practical applications which include their use as catalysts and microencapsulating agents for pharmacologically important compounds [2, 4, 7, 9].

Diflunisal [2-hydroxy-5-(2,4-difluorophenyl)benzoic acid] whose structure is shown below, is an anti-inflammatory drug [10]. The presence of the fluorine substituents of diflunisal facilitates the study of its inclusion by cyclodextrins using $^{19}F$ NMR spectroscopy [6, 7, 9] and UV-visible spectrophotometry as complementary methods to study the same phenomenon. Earlier studies of the inclusion of the diflunisal anion (DF) by αCD and βCD, show that in the first case a 1 : 1 complex, DF·αCD, only is formed, whereas in the second case both DF·βCD and DF·(βCD)$_2$ are formed [9].

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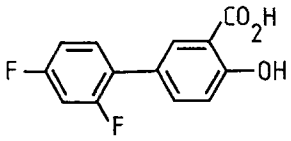


In this study, to elucidate further the factors of importance in inclusion complex formation, the effects on the stoichiometry and stability of DF inclusion complexes were investigated, by increasing the annular size of the cyclodextrin and replacing the cyclodextrin hydroxy groups by methoxy groups, using γCD and permethylated β-cyclodextrin (βPCD), respectively.

## 2. Experimental

Diflunisal (Merck, Sharp and Dohme) and β- and γ-cyclodextrin (Sigma) were stored as the anhydrous materials over $P_2O_5$ in a vacuum desiccator prior to use. Permethylated β-cyclodextrin (βPCD) was prepared as previously described [11] and excellent elemental analyses were obtained. Solutions of diflunisal and the cyclodextrins were made up in aqueous $KH_2PO_4/Na_2HPO_4$ buffer containing 10% $D_2O$, at pH 7.00 and 0.1 ionic strength. Diflunisal in the acid form ($pK_a$ *ca.* 3) is virtually insoluble in water, but its conjugate base is water soluble.

UV-visible spectra were run in silica cells on a Zeiss DMR 10 double-beam spectrophotometer equipped with a thermostatted (±0.1 K) cell block. All spectra were run in duplicate, and were recorded digitally at 1 nm intervals over the range 230–350 nm. $^{19}F$ NMR spectra were run on a Bruker CXP 300 NMR spectrometer at 282.35 MHz locked on the $D_2O$ deuterium frequency. An average of 5000 transients was collected for each spectrum into a 8192 point data base. The samples in 5 mm NMR tubes were thermostatted at 298.2 K. Chemical shifts were measured relative to a 2% solution of sodium trifluoroacetate in $D_2O$ sealed in a capillary. The use of this external reference was necessitated by the known ability of cyclodextrins to include trifluoroacetate. The errors introduced into the determination of the chemical shifts resulting from their measurement from this external reference have previously been shown to be negligible [6]. Both the UV-visible and $^{19}F$ spectroscopic data were analysed using a VAX 11-780 computer.

## 3. Results

The variation of the UV-visible spectrum of the diflunisal anion (DF) in the presence of γCD in the concentration range 0–$2.01 \times 10^{-2}$ mol $dm^{-3}$, in $KH_2PO_4/Na_2HPO_4$ buffer solution containing 10% $D_2O$, at 0.1 ionic strength, pH 7.00 and 298.2 K is shown in Figure 1. It is seen that at low [γCD] the molar absorbance of DF is increased, whereas at higher [γCD] the molar absorbance of DF decreases. (This spectral variation was obtained after correction for light scattering effects arising from γCD. The light scattering contribution to the observed absorbance was determined from spectrophotometric measurements of buffered solutions of γCD alone in the wavelength range 230–370 nm.) The spectral variation seen in Figure 1 is consistent with the formation of 1 : 1 and 1 : 2 inclusion complexes as shown in Equations (1) and (2). The variation of the molar absorbance in the range 240–255 nm was subjected to a non-linear least squares analysis [8] through Equation (3) which describes the variation of the observed molar absorbance ($A$) with concentration

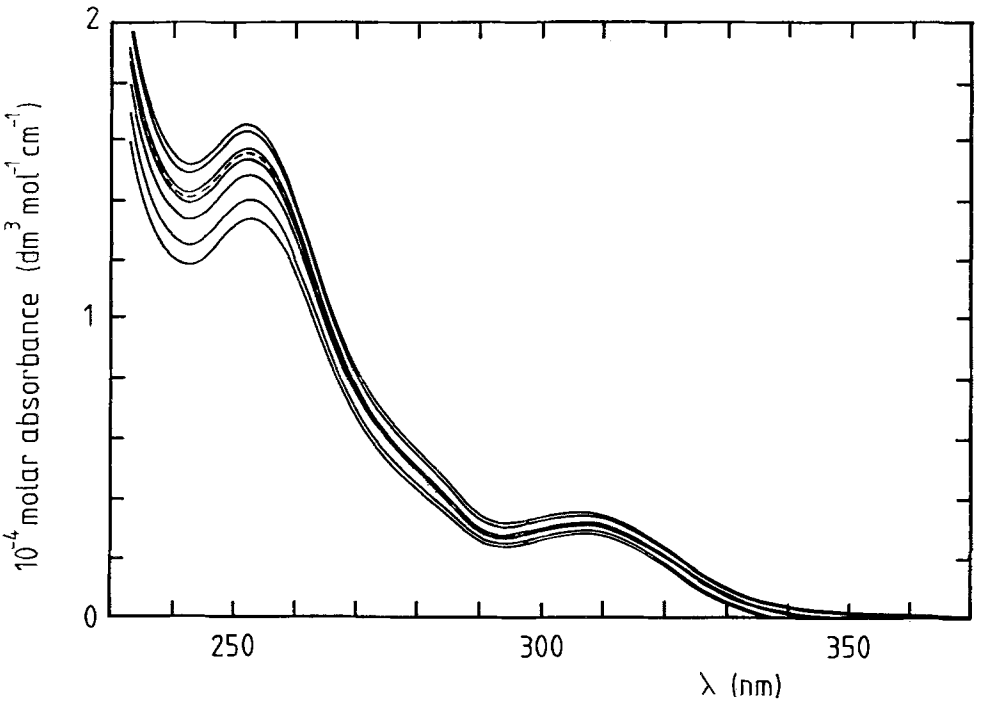


Fig. 1. The variation of the UV-visible spectrum of DF ($1.560 \times 10^{-5}$ mol $dm^{-3}$) in the presence of γCD in 10% $D_2O$ aqueous $KH_2PO_4/Na_2HPO_4$ buffer solution at 0.1 ionic strength, pH 7.00 and 298.2 K. The spectrum of DF alone is represented by the broken curve. The solid curves represent the spectra of solutions containing γCD. At 250 nm the molar absorbance decreases systematically as the total γCD concentration varies in the sequence: $2.00 \times 10^{-4}$, (9.89 and 1.94) $\times 10^{-5}$, 0, (1.91, 4.92 and 9.86) $\times 10^{-3}$, and $2.01 \times 10^{-2}$ mol $dm^{-3}$. These eight spectra illustrate the trend in spectral variation observed for the twenty solutions studied whose total γCD concentrations were encompassed by the range given above.

resulting from equilibria (1) and (2), and in which $\varepsilon_0$, $\varepsilon_1$ and $\varepsilon_2$ are the molar absorbances of DF, DF·γCD and DF·(γCD)$_2$, respectively. The derived $K_1$ and $K_2$ values are $(5.5 \pm 0.2) \times 10^4$ and $(2.3 \pm 0.2) \times 10^4$ $dm^3$ $mol^{-1}$. The variation of the UV-visible spectrum of DF in the presence of βPCD (Figure 2) differs from that observed for the γCD system, but is consistent with the formation of DF·βPCD and DF·(βPCD)$_2$ complexes in equilibria analogous to (1) and (2). The corresponding $K_1$ and $K_2$ values derived from the molar absorbance variation at 1 nm intervals in the range 240–259 nm are $(6.86 \pm 0.02) \times 10^4$ and $(8.75 \pm 2.7) \times 10^1$ $dm^3$ $mol^{-1}$, respectively.

$$\mathrm{DF} + \gamma\mathrm{CD} \overset{K_1}{\rightleftharpoons} \mathrm{DF}\cdot\gamma\mathrm{CD} \tag{1}$$

$$\mathrm{DF}\cdot\gamma\mathrm{CD} + \gamma\mathrm{CD} \overset{K_2}{\rightleftharpoons} \mathrm{DF}\cdot(\gamma\mathrm{CD})_2 \tag{2}$$

$$A = \varepsilon_0[\mathrm{DF}] + \varepsilon_1[\mathrm{DF}\cdot\gamma\mathrm{CD}] + \varepsilon_2[\mathrm{DF}\cdot(\gamma\mathrm{CD})_2] \tag{3}$$

From the dependence of the observed absorbances on concentration it is apparent that at least two inclusion complexes are formed under the conditions of the experiments. Of the several schemes examined, only that described by Equations (1) and (2) fitted the results satisfactorily.

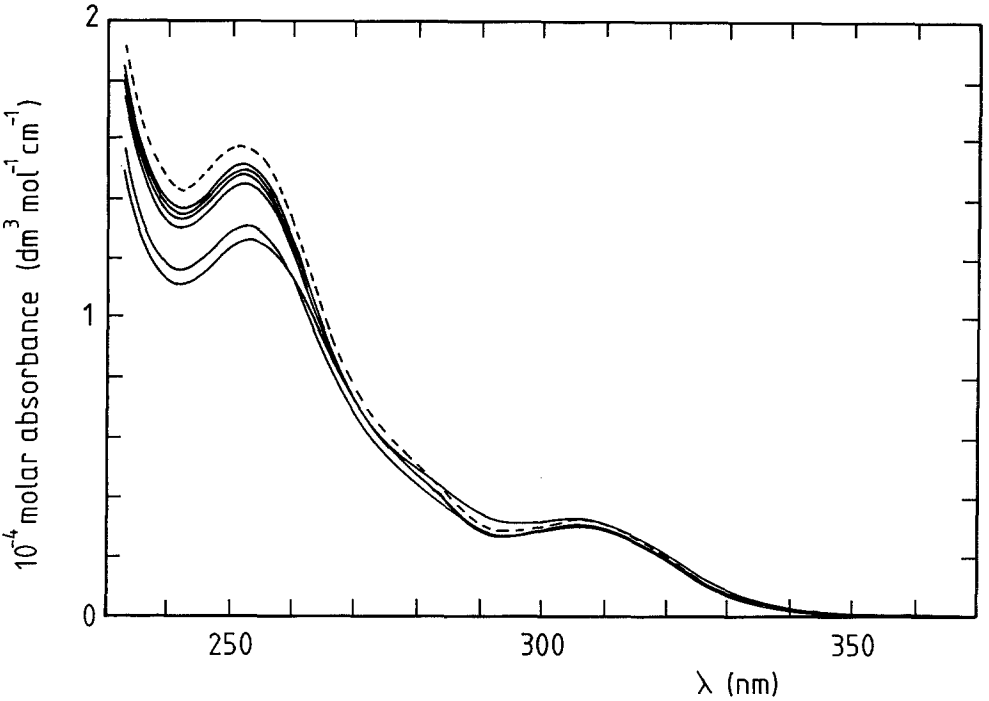


Fig. 2. The variation of the UV-visible spectrum of DF ($1.560 \times 10^{-5}$ mol dm$^{-3}$) in the presence of $\beta$PCD in 10% $D_2O$ aqueous $KH_2PO_4/Na_2HPO_4$ buffer solution at 0.1 ionic strength, pH 7.00 and 298.2 K. The spectrum of DF alone is represented by the broken curve. The solid curves represent the spectra of solutions containing $\beta$PCD. At 250 nm the molar absorbance decreases systematically as the total $\beta$PCD concentration varies in the sequence: 0, (1.92 and 7.49) $\times 10^{-5}$, (1.512 and 5.36) $\times 10^{-4}$, and (3.008 and 7.555) $\times 10^{-3}$ mol dm$^{-3}$. These seven spectra illustrate the trend in spectral variation observed for the eighteen solutions studied whose total $\beta$PCD concentrations were encompassed by the range given above.

The $^{19}F$ resonances of DF in a $5.00 \times 10^{-3}$ mol dm$^{-3}$ solution in 10% $D_2O$ at pH 7.00 at 298.2 K, appear at $-36.92$ ppm and $-39.40$ ppm, respectively, from an external reference of 2% sodium trifluoroacetate in $D_2O$. The resonance of the 2-F of the diflunisal anion is a multiplet (components at $-39.30$, $-39.33$, $-39.36$, $-39.39$ ppm of relative intensity 7.18, 14.8, 15.3, 6.87, respectively) which collapses to a doublet ($J_{F\text{-}F} = 7.22$ Hz) under broad-band $^1H$ decoupling. The resonance of the 4-F is also a multiplet (components at $-36.82$, $-36.85$, $-36.87$, $-36.90$, $-36.93$ ppm of relative intensity 7.13, 15.2, 20.0, 15.5, 8.50, respectively) which collapses to a doublet ($J_{F\text{-}F} = 7.20$ Hz) under broad band proton decoupling. In the DF/$\gamma$CD and DF/$\beta$PCD solutions studied some broadening of the hyperfine structure of these resonances was observed, but separate resonances for DF in the free state and its included states were not observed, consistent with exchange between these environments being in the fast exchange limit of the NMR timescale.

The biphasic variation of the DF $^{19}F$ chemical shifts (Figure 3) with total $\gamma$CD concentration (in the range 0–0.1090 mol dm$^{-3}$, similar to that employed in the UV-visible spectroscopic study) in $KH_2PO_4/Na_2HPO_4$ buffer containing 10% $D_2O$, at 0.1 ionic strength, pH 7.00 and 298.2 K, is also consistent with the two equilibria shown in Equations (1) and (2). Because the association processes are rapid on the NMR timescale, the observed $^{19}F$ chemical shift, $\delta$, is a time average of those for the various molecular species present

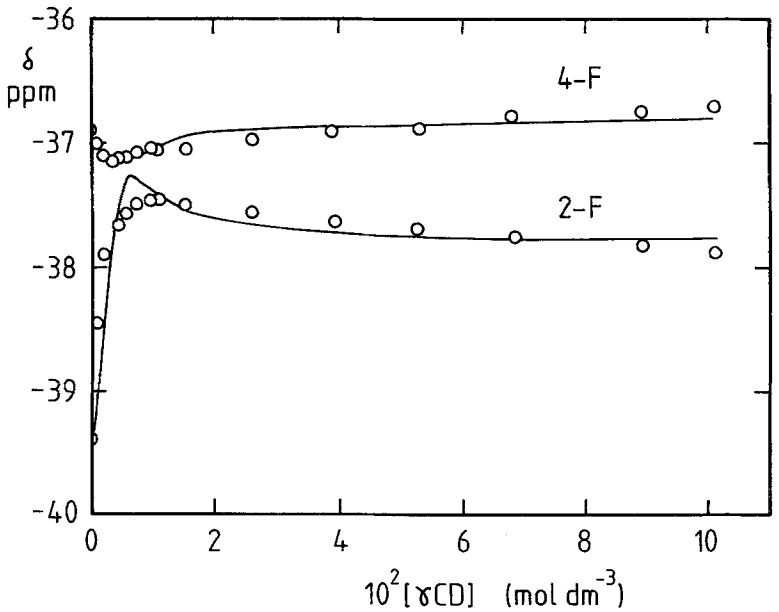


Fig. 3. Variation of the $^{19}F$ chemical shift of DF ($\delta$) ($5.00 \times 10^{-3}$ mol dm$^{-3}$) with total γCD concentration in 10% $D_2O$ aqueous $KH_2PO_4/Na_2HPO_4$ buffer solution at 0.1 ionic strength, pH 7.00 and 298.2 K. The upper and lower data sets refer to 4-F and 2-F respectively. The negative shifts signify upfield shifts from a 2% sodium trifluoroacetate solution in $D_2O$ external reference which is assigned to a shift of zero. The solid curves represent the best fits of these data to Equation (4) with $K_1$ and $K_2$ set equal to the values determined spectrophotometrically.

and may thus be calculated by Equation (4) in which $\delta_0$ is the shift of DF, $\delta_1$ is the shift of DF·γCD, and $\delta_2$ is the shift of DF·(γCD)$_2$ [9]. Because the $^{19}F$ NMR detection level for DF is several orders of magnitude below that of the UV-visible spectroscopic method in this case, a substantially higher total DF concentration ($5.00 \times 10^{-3}$ mol dm$^{-3}$) was employed. As a consequence of this and the high stabilities of DF·γCD and DF·(γCD)$_2$, the equilibrium DF and γCD concentrations are always very small compared to the DF·γCD and DF·(γCD)$_2$ concentrations, with the result that the $K_1$ and $K_2$ values [$(2 \pm 9) \times 10^5$ and $(0.5 \pm 2) \times 10^3$ dm$^3$ mol$^{-1}$, respectively] determined through a non-linear least squares fit of the $^{19}F$ shift data to Equation (4) are subject to large uncertainties. However,

$$\delta = \frac{\delta_0[\mathrm{DF}] + \delta_1[\mathrm{DF}\cdot\gamma\mathrm{CD}] + \delta_2[\mathrm{DF}\cdot(\gamma\mathrm{CD})_2]}{[\mathrm{DF}] + [\mathrm{DF}\cdot\gamma\mathrm{CD}] + [\mathrm{DF}\cdot(\gamma\mathrm{CD})_2]} \tag{4}$$

when $K_1$ and $K_2$ are set equal to the values obtained spectrophotometrically and the $^{19}F$ chemical shift data are again subjected to a non-linear least squares fit to Equation (4), the best fit curve is seen to approximate to the experimental $^{19}F$ chemical shift variation as is seen from Figure 3. (The $\delta_1$ and $\delta_2$ values derived through this fitting procedure appear in Table I.) Thus the UV-visible spectrophotometric data and the $^{19}F$ chemical shift data are both shown to be consistent with the establishment of equilibria (1) and (2).

The variation of the $^{19}F$ chemical shift with total βPCD concentration is also biphasic

Table I. Equilibrium constants and $^{19}F$ chemical shifts[a] for diflunisal-cyclodextrin systems in 10% $D_2O$ aqueous $KH_2PO_4/Na_2HPO_4$ buffer solution at 0.1 ionic strength, pH 7.00 and 298.2 K

| $K_1/10^3$/ dm$^3$ mol$^{-1}$ | $K_2/10^3$/ dm$^3$ mol$^{-1}$ | | $\delta_0$ ppm | $\delta_1$ ppm | $\delta_2$ ppm |
|---|---|---|---|---|---|
| α-cyclodextrin[b] | | | | | |
| 0.0170±0.0009[c] | – | (4-F) | −36.89±0.01 | −38.18±0.03 | – |
| | | (2-F) | −39.37±0.01 | −38.27±0.03 | – |
| β-cyclodextrin[b] | | | | | |
| 181±20[d] | 3.07±0.025[d] | (4-F) | −36.92±0.01 | −34.91±0.05 | −34.52±0.05 |
| | | (2-F) | −39.40±0.01 | −36.71±0.05 | −36.34±0.05 |
| γ-cyclodextrin[e] | | | | | |
| 55±2[d] | 23±2[d] | (4-F) | −36.91±0.01 | −37.23±0.09 | −36.80±0.07 |
| | | (2-F) | −39.38±0.01 | −37.11±0.09 | −37.81±0.07 |
| permethylated β-cyclodextrin[e] | | | | | |
| 68.6±0.2[d] | 0.0875±0.027[d] | (4-F) | −36.90±0.01 | −36.23±0.05 | −36.10±0.09 |
| | | (2-F) | −39.38±0.01 | −38.58±0.14 | −36.43±0.09 |

[a] A negative shift signifies an upfield shift from a 2% sodium trifluoracetate solution in $D_2O$ external reference, which is assigned a shift of zero. The $\delta_0$ values vary slightly with diflunisal concentration, and hence different values appear in the table for the αCD, βCD and γCD systems. The digital resolution was 0.007 ppm.
[b] Data from reference [9].
[c] Determined from $^{19}F$ chemical shift data.
[d] Determined from UV-visible spectrophotometric data.
[e] This work.

and is shown in Figure 4. A non-linear least squares fit of these data to Equation (5), [9]

$$\delta = \frac{\delta_0[\mathrm{DF}] + \delta_1[\mathrm{DF}\cdot\beta\mathrm{PCD}] + \delta_2[\mathrm{DF}\cdot(\beta\mathrm{PCD})_2]}{[\mathrm{DF}] + [\mathrm{DF}\cdot\beta\mathrm{PCD}] + [\mathrm{DF}\cdot(\beta\mathrm{PCD})_2]} \tag{5}$$

yields $K_1$ and $K_2 = (6.36 \pm 15.6) \times 10^4$ and $(8.52 \pm 14.7) \times 10^1$ dm$^3$ mol$^{-1}$, which are similar to those derived spectrophotometrically. The corresponding $\delta_1$ and $\delta_2$ values are shown in Table I and are also subject to substantial uncertainties as previously discussed. Overall, the UV-visible spectrophotometric data yield precise $K_1$ and $K_2$ values, and the $^{19}F$ NMR data semi-quantitatively support the existence of equilibria (1) and (2). A similar situation arose for the DF/βCD system [9]. In contrast $^{19}F$ shift data provided a reliable $K_1$ value (Table I) for the DF/αCD system as a consequence of the formation of a 1 : 1 complex only and its relatively small $K_1$, whereas the UV-visible spectral change was too small for quantitative determination of $K_1$ [9]. Thus in comparisons of the stabilities of the DF complexes the $^{19}F$ data is the more reliable for the αCD system, whereas the UV-visible spectrophotometric data is the more reliable for the βCD, γCD and βPCD systems.

## 4. Discussion

The greatest difference in stability of the 1 : 1 DF/cyclodextrin complexes formed (Table I) is between that formed by αCD and those formed by the three larger cyclodextrins, which indicates the existence of a critical size factor for the inclusion of DF. It is assumed that DF

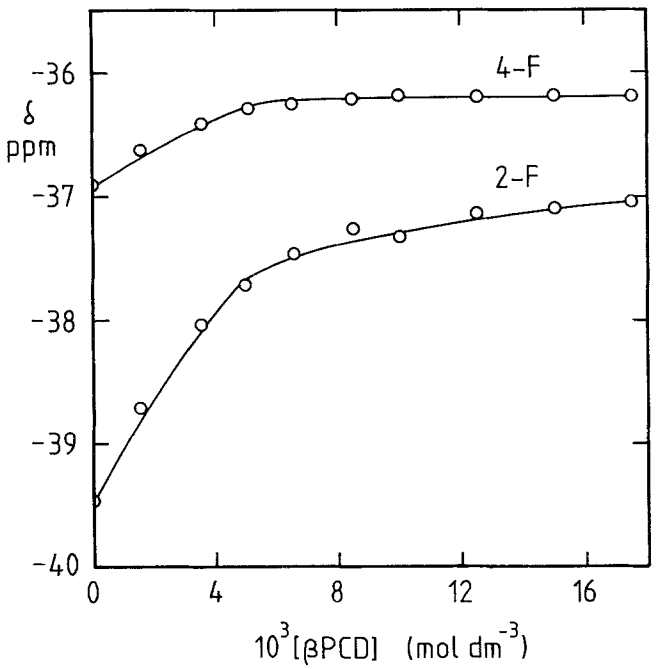


Fig. 4. Variation of the $^{19}F$ chemical shift of DF ($\delta$) ($4.81 \times 10^{-3}$ mol dm$^{-3}$) with total $\beta$PCD concentration in 10% $D_2O$ aqueous $KH_2PO_4/Na_2HPO_4$ buffer solution at 0.1 ionic strength, pH 7.00 and 298.2 K. The upper and lower data sets refer to 4-F and 2-F respectively. The negative shifts signify upfield shifts from a 2% sodium trifluoroacetate solution in $D_2O$ external reference which is assigned a shift of zero. The solid curves represent the best fits of these data to Equation (4).

enters the cyclodextrin annulus through the wider end delineated by the secondary hydroxy groups of αCD, βCD and γCD, and the secondary methoxy groups of βPCD, but it is not clear whether either or only one end of DF enters first. Thus $K_1$ and $K_2$ may be composite equilibrium constants as shown in Figure 5. Corey, Pauling, Koltun (CPK) models indicate that the annular diameter of αCD (5–6 Å) restricts the entrance of DF, and the greater annular diameter of βCD (7–8 Å) permits deeper penetration of DF, coincident with an increase in stability and $K_1$ by four orders of magnitude. The further increase in annular diameter of γCD to 9–10 Å produces a modest decrease in $K_1$, which suggests that βCD approaches the optimum size for the inclusion of DF in which the van der Waals interactions between DF and the interior of the annulus are maximised. Permethylation causes only a modest decrease in $K_1$ for DF·βPCD by comparison to $K_1$ for DF·βCD, but a substantial difference occurs in the stability of the 1 : 2 complexes where $K_2$ for DF·(βCD)$_2$ is 35 times greater than $K_2$ for DF·(βPCD)$_2$. A plausible explanation for the latter observation is that the methoxy groups of DF·(βPCD)$_2$ cause both steric hindrance to attainment of the optimum orientations for maximising van der Waals interactions between DF and βPCD, and eliminate hydrogen bonding interactions between the two adjacent βPCD; whereas in DF·(βCD)$_2$ the probability of hydrogen bonding occurring between the hydroxy groups delineating the annuli of the two adjacent βCD is substantial [12]. This explanation gains support from the observation that DF·(γCD)$_2$ (in which adjacent αCD can hydrogen bond to each other, and as a consequence of a larger annular diameter can probably adopt orientations favourable to complexation in a 1 : 2 stoichiometry more readily) is of moderately increased stability by comparison to that of DF·(βCD)$_2$, whereas

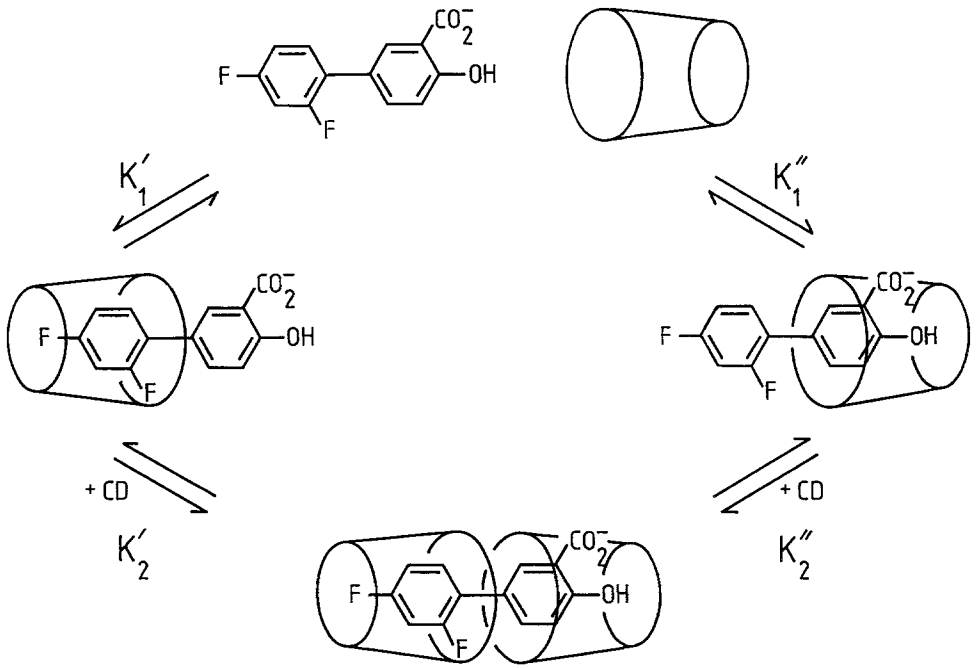


Fig. 5. A schematic representation of the inclusion of the diflunisal anion in which αCD, βCD, γCD and βPCD are represented as truncated cones. $K_1 = K_1' + K_1''$ and $K_2 = K_2' + K_2''$.

DF·$(\alpha CD)_2$ does not form due to the small annular diameter of αCD restricting the inclusion of DF and the close approach of two αCD. {In contrast several fluoro-cinnamates (FC) form FC·$(\alpha CD)_2$ complexes [6], which is probably a reflection of the smaller size of FC by comparison to DF.} Generally $K_1$ is greater than $K_2$ as anticipated on a statistical basis, but the variations in the relative magnitudes of $K_1$ and $K_2$ for a given system should also reflect the specific effects discussed above.

It has been suggested that upfield and downfield $^{19}F$ chemical shifts of fluorine substituents of included species in cyclodextrin complexes indicate a fluorine in a hydrophobic environment and adjacent to cyclodextrin hydroxy groups, respectively [13]. On this basis the observation that in DF·αCD the $\delta_1$ values of 2-F and 4-F are, respectively, downfield and upfield from their $\delta_0$ values for free DF (Table I) is consistent with 4-F being in a hydrophobic environment, whilst the 2-F is in a hydrophilic environment [9]. CPK models indicate that, when the difluoro end of DF is included in DF·αCD, the 4-F resides in the hydrophobic part of the αCD annulus while the 2-F interacts partly with solvent water and partly with the ring of secondary hydroxy groups at the annular entrance. In contrast the $\delta_1$ and $\delta_2$ values for both 2-F and 4-F in DF·βCD and DF·$(\beta CD)_2$ are downfield from their $\delta_0$ values, and the changes in chemical shift are greater than those observed for the αCD system (Table I), suggesting that both fluorines interact with βCD hydroxy groups. This could indicate that the larger annular diameter of βCD allows DF to position 4-F adjacent to the primary hydroxy groups at the narrower end of the annulus, and 2-F adjacent to the secondary hydroxy groups at the wider end of the annulus, and CPK models show this to be a structural possibility. The formation of DF·$(\beta CD)_2$ induces relatively small $^{19}F$ shift variations for 2-F and 4-F ($\delta_2$) suggesting that the second βCD largely includes the

carboxylate end of DF [9]. (These assignments and structural interpretations of the $^{19}F$ chemical shifts supersede those appearing in a preliminary account of a study of the αCD and βCD systems [14].) The observation in the present study that the variations of the $^{19}F$ chemical shifts of DF on inclusion by βPCD are qualitatively similar to those observed on inclusion by βCD suggests that the interpretation of the $^{19}F$ chemical shifts of DF included by αCD and βCD may not be interpretable predominantly in terms of hydrophilic and hydrophobic interactions, but instead may substantially reflect the proximity of fluorine substituents to the hydroxy oxygens, and the methoxy oxygens in the case of βPCD. Such a change in emphasis in the $^{19}F$ shift variations does not require any change in the probable structures of the inclusion complexes deduced from CPK models. The biphasic $^{19}F$ chemical shift variations exhibited by DF upon complexation by γCD seen in Figure 3 and Table I are quite different from those seen in the other three cyclodextrin systems, and emphasise the dominance of the nature of the cyclodextrin on both the $^{19}F$ shift variation and the structure of the inclusion complex. On balance it seems that the $^{19}F$ shift variations are very sensitive to the number of complexes formed, in a quantitative or semi-quantitative manner, but that the correlation of these shifts with specific interactions between the included species and the cyclodextrin is subject to uncertainty.

## Acknowledgements

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