Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **16:** 255–266, 1993.

Inclusion of the Herbicide Difenzoquat (1,2-Dimethyl-3,5-diphenyl-pyrazolium) by β-Cyclodextrin in Aqueous Solution

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(Received: 30 June 1993; in final form: 19 October 1993)

Abstract. The herbicide difenzoquat, 1,2-dimethyl-3,5-diphenyl-pyrazolium methylsulphate, forms a weak inclusion complex with βCD having 1 : 1 stoichiometry and a formation constant of $K = 70 \pm 12$ mol^{-1} L. The complexation changes the solution conductivity and substantially enhances the fluorescence intensity. The probable configuration of the inclusion complex is the insertion of the pyrazole ring into the cavity, leaving both phenyl substituents outside.

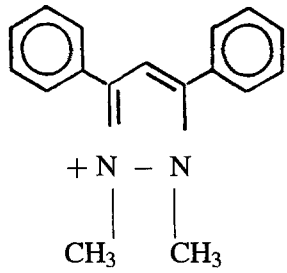
Key words: Cyclodextrin, difenzoquat, pyrazolium, fluorescence, conductivity, complex.

1. Introduction

Cyclodextrins (CDs) are oligosacharides which consist of six, seven, and eight glucopyranosyl units linked in 1–4 positions and are denoted as α-, β-, and γ-CD, respectively. The molecular shapes of CDs are truncated cones with internal diameters [1] in the range 0.42–0.88 nm in α-CD, 0.56–1.08 nm in β-CD, and 0.68–1.20 nm in γ-CD. Such cavities allow the selective accommodation of a variety of guest molecules or ions according to their size and polarity. The interaction of ionic surfactants, organic cations and zwiterions with cyclodextrins (CD) have been the subject of several studies [2–6]. The amphiphilic character of the host allows the formation of inclusion complexes, which results in a change of the conductivity, spectral characteristics and other parameters of the parent compounds. The stoichiometry of the resulting host–guest aggregates can be elucidated on the basis of conductivity data. Most of the surfactant–CD studies have been devoted to compounds characterized by a long alkyl chain and a polar head group, which enters the CD cavity with the alkyl chain. Funasaki [7] indicated a substantial controversy between reported formation constants, which in some cases span over three orders of magnitude and probably originate from neglect of other stoichiometric species

involved in the binding process, such as two chains in one cavity, one chain binding to two CD cavities, etc.

This study deals with a different type of compound, the herbicide difenzoquat (abbreviated here as DFQ) or 1,2-dimethyl-3,5-diphenyl-pyrazolium methylsulphate [8],



$CH_3SO_4^-$

which we recently proved to be highly surface active on a polarized interface [9]. In contrast to long-chain surfactants, the hydrophobic substituents in DFQ are the phenyl groups, which should yield a much simpler stoichiometry than the cases treated by Funasaki *et al.* [7]. In addition, DFQ yields intensive fluorescence spectra and has been suggested as a fluorescent probe by von Wandruszka [10]. The inclusion process, if any, can therefore be investigated by two independent techniques: conductivity measurements and fluorescence spectroscopy.

Inclusion of DFQ in the cyclodextrin cavity may also be interesting from the point of view of potential risk of a persistent contamination of starch-rich plants. Our attention to this subject was recently attracted by certain controversial reports on the stability and degradation [11,12] of DFQ, which is used under various trade names ('Finaven' and 'Avenge', American Cyanamide Co.). Furthermore, the CD treatment was suggested as a means by which the germination of grains could be retarded [13], allowing protection against the influence of herbicides administrated during the sowing. The assessment of the possible inclusion of herbicides in CD will be an important future research topic.

2. Experimental

2.1. Materials

Difenzoquat was purchased in a crystalline form from E. Ehrenstofe, Augsburg (Germany) and was used as received. Cyclodextrins were from Fluka AG, Buchs (Switzerland). The other reagents used as supporting electrolytes were of reagent grade. Water was distilled twice from an all-quartz still or was deionized by means of an Elgastat UHQ purifier yielding water of conductivity 15–18 MΩ/cm.

2.2. METHODS

Samples for UV spectroscopy were prepared by dilution of 5 mM stock solution of DFQ into a 5 mL volumetric flask adding the weighed amount of CD, when applicable, yielding a solution containing the required concentrations of DFQ and CD. Solutions for fluorometric measurement were prepared as follows: an aqueous 1 mM stock solution of difenzoquat was pipetted into a 1000 mL volumetric flask and diluted with deionized water to yield a concentration of 1.4×10^{-6} mol L^{-1}. Samples with the required concentration of CD were made up by transferring the corresponding amount of CD into a volumetric flask which was then filled with the abovementioned dilute solution of DFQ. Conductometric solutions were of concentration 1.53×10^{-3} mol L^{-1}, prepared in 50 mL flasks. Concentrations of cyclodextrins ranged from 2×10^{-5} mol L^{-1} to 1.25×10^{-2} mol L^{-1}. Absorption and fluorescence measurements were made at ambient temperature (22 ± 1°C), conductivity data were obtained in the water bath of an ultrathermostat at 20 ± 0.1°C.

2.3. APPARATUS

Absorption spectra were measured on a Varian UV-visible spectrophotometer model DMS300 using 1 cm quartz cuvettes. The scan rate was 20 nm/min, and the slit width 2 nm. Fluorescence emission spectra were recorded using a Perkin Elmer luminescence spectrometer model LS–5. The excitation wavelength was 260 nm or 226 nm, with excitation and emission bandwidth 5 nm. The formation constants were evaluated on the basis of the intensity of a band at 365 nm. ^{1}H-NMR spectra were measured on a Bruker spectrometer model AMX300 operating at 300 MHz. Chemical shifts were related to the signal of the sodium salt of 3-(trimethylsilyl)propionic acid in D_2O used as an external standard. Conductivity measurements were made using a Tesla conductivity bridge (Czechoslovakia) model BM590 operating at a frequency of 1 kHz. The conductivity cell used a pair of platinized platinum electrodes.

3. Results and Discussion

3.1. CONDUCTIVITY OF DFQ SOLUTION IN THE PRESENCE OF CD

The cationic character of DFQ offers the possibility of investigating the complexation reaction with CDs on the basis of conductivity measurements. The cation of DFQ has a positive charge located on one of the nitrogen heteroatoms; however, the ion has at the same time two phenyl substituents which introduce a partially hydrophobic character. In the concentration range used in this study (less than millimolar concentrations) there is no evidence of the formation of micelles and the conductivity depends linearly on the DFQ concentration in the investigated range from 1 μM to 10 mM. By measuring the conductivity of 1.53 mM DFQ solutions

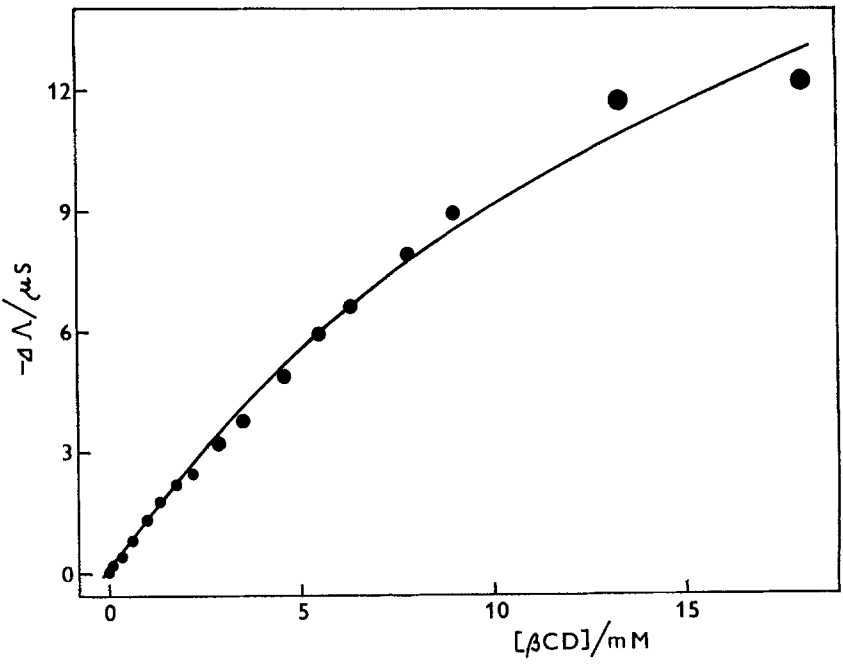


Fig. 1. The decrease of conductivity of an aqueous solution of 1.53×10^{-3} mol L^{-1} DFQ at 20°C as a function of the concentration of βCD. The full curve represents the best fit according to Equation (1) using fitted values $K = 56.9$ mol^{-1} L and $\Delta\lambda = 26.1$ μS.

in the presence of increasing concentration of CDs we observed a decrease of conductivity in the case of βCD, whereas the other two CDs do not influence the conductivity at all. Conductivity data were used for studies of inclusion phenomena of ionic surfactants and the formation constant of a complex with stoichiometry 1 : 1 can be evaluated on the basis of an equation proposed by Satake [2,3]. Other stoichiometries have to be treated according to the general rigorous theory for equilibrium concentrations given by Funasaki [7]. In the present case the Satake formalism proved to be adequate and conductivity data were analyzed according to the following equation [2,3]:

$$\Delta\Lambda = \frac{\Delta\lambda}{2K[\mathrm{DFQ}]}\left[K([\mathrm{DFQ}] + [\beta\mathrm{CD}]) + \right.$$

$$\left. +1 - \sqrt{[K([\mathrm{DFQ}] + [\beta\mathrm{CD}])]^2 - 4K^2[\mathrm{DFQ}]\,[\beta\mathrm{CD}]}\right] \qquad (1)$$

where $\Delta\Lambda$ is the difference between the conductivity of the DFQ solution in the absence of βCD and in the presence of a given βCD concentration and $\Delta\lambda$ is the difference of ionic conductivities of free and complexed DFQ. Since the conductivity still decreases at the highest available concentration of βCD, $\Delta\lambda$ must be

estimated along with K by two-parameter nonlinear regression. The experimental data for βCD inclusion and the best fit curve are shown in Figure 1: analogous measurements with the other two CDs are omitted because no change of conductivity was observed. The resulting estimate of $K = 57 \pm 8$ mol L^{-1} agrees reasonably with the estimation by spectral methods given below and indicates only weak complexation of DFQ.

3.2. Emission fluorescence in DFQ + CDs systems

The absorption spectrum of DFQ is characterized by a maximum at 255 nm and a shoulder at 235 nm, which is in agreement with the properties of substituted pyrazoles [14,15]. The position of the maximum does not depend on the concentration of DFQ. The absorbance slightly increases in the presence of increasing concentration of βCD without the appearance of an isosbestic point on spectra, and the ratio between the absorbance at the principal band (255 nm) and at the wavelength of the shoulder decreases progressively. Small changes of the absorbance do not ensure sufficient data precision and hence we evaluated the complex formation from fluorescence spectra, which were considerably more sensitive.

Difenzoquat is a strong fluorescence emitter with excitation maxima in aqueous media at 226 nm and 260 nm, and a maximum of emission at 373 nm. The fluorescence emission characteristics were described by von Wandruszka [10] and our experiments are in accordance with previous findings: the observed position of a band is not affected, either by the concentration of DFQ or by the wavelength of the excitation radiation. The ionic character of DFQ, together with the presence of hydrophobic phenyl substituents, led to the suggestion to use DFQ as a fluorescent probe. The fluorescence emission spectra of 1.4×10^{-6} mol L^{-1} DFQ solutions at excitation wavelengths of 260 nm and 226 nm are shown in Figures 2 and 3, respectively. While most inclusion complexes lead to a decrease of fluorescence, the present system is characterized by a higher intensity of the complexed form. The higher intensity of the fluorescence signal was observed in some systems [16] and a large increase of the fluorescence of dansyl chloride (5-dimethyl-amino-1-naphthalenesulphonyl chloride) was suggested as a method for the determination of CD [17]. The presence of an increased concentration of βCD increases the fluorescence emission and at the solubility limit of βCD the band is higher by a factor of 2.5. The emission obtained with 226 nm excitation is much less intense, resulting in a smaller signal-to-noise ratio. The band broadening in Figure 3 is therefore difficult to resolve for quantitative purposes: however, it is evident that the complexation of DFQ and βCD gives rise to a band with a maximum at about 330 nm. A single band of a complex can be observed in solutions where the ratio of CD to DFQ is in the range $10^6 : 1$. However, the concentration of such samples is difficult to specify due to strong adsorption and subsequent desorption of DFQ from the walls of glassware, etc. Hence we used data from Figure 2 for determination of the formation constants of the DFQ–βCD inclusion complex and for verification

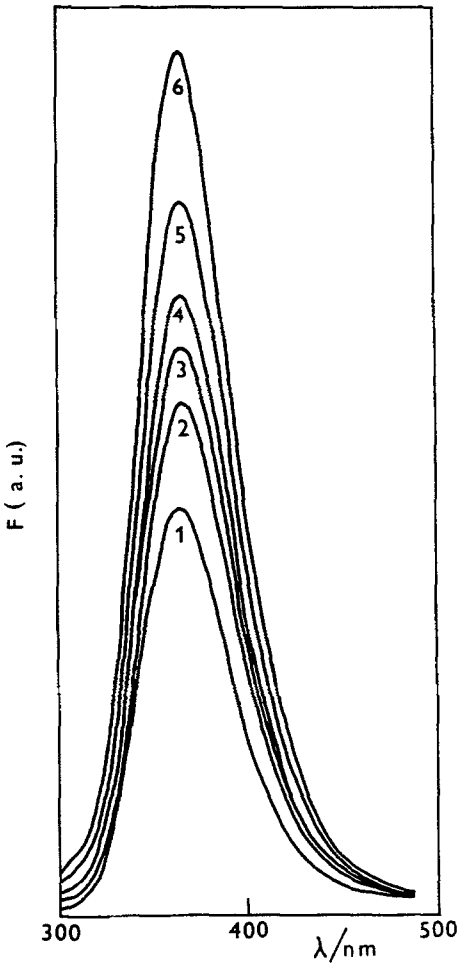


Fig. 2. Fluorescence emission spectra at an excitation wavelength of 260 nm of 1.4×10^{-6} mol L^{-1} DFQ in water at various concentrations of βCD: (1) 0, (2) 1.2×10^{-3} mol L^{-1}, (3) 2.0×10^{-3} mol L^{-1}, (4) 2.9×10^{-3} mol L^{-1}, (5) 4.7×10^{-3} mol L^{-1}, and (6) 8.5×10^{-3} mol L^{-1}.

of the stoichiometry. The other two cyclodextrins (αCD and γCD) influence the fluorescence intensities to a much smaller extent (for comparison of the effect of all three CDs see Figure 4).

The stoichiometry between CD and the guest in most cases is 1 : 1: however, this is not a general rule and other stoichiometric ratios, such as 1 : 2 or 2 : 1, have been reported. In view of the arguments given above we will assume the formation of a 1 : 1 complex and we verify this assumption by investigation of the appropriate linearized function of the fluorescence intensity vs. the CD concentration. In order to clarify the conditions and definitions we give below the

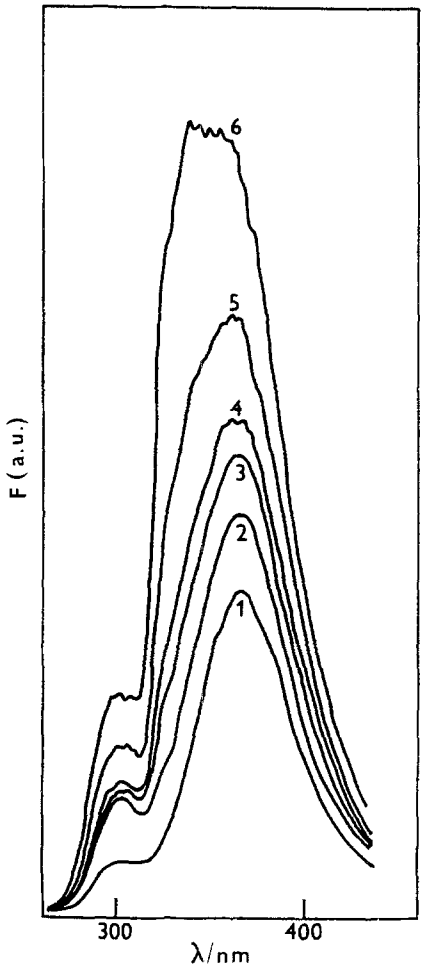


Fig. 3. Fluorescence emission spectra at an excitation wavelength of 226 nm of 1.4×10^{-6} mol L^{-1} DFQ in water at various concentrations of βCD: (1) 0, (2) 1.2×10^{-3} mol L^{-1}, (3) 2.0×10^{-3} mol L^{-1}, (4) 2.9×10^{-3} mol L^{-1}, (5) 4.7×10^{-3} mol L^{-1}, and (6) 8.5×10^{-3} mol L^{-1}.

fluorescence–concentration relationship, though similar equations can be found in the literature [18]. The equilibrium constant for an inclusion reaction involving a 1 : 1 complex

$$\mathrm{DFQ} + \mathrm{CD} = \mathrm{DFQ} \cdot \mathrm{CD} \tag{2}$$

is defined as

$$K = \frac{[\mathrm{DFQ} \cdot \mathrm{CD}]}{[\mathrm{DFQ}]\,[\mathrm{CD}]} \tag{3}$$

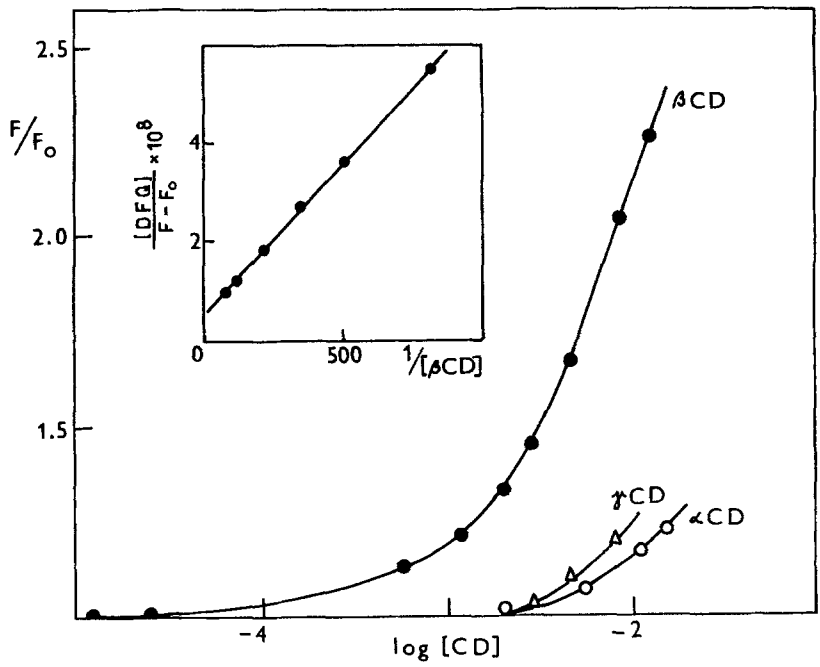


Fig. 4. The relative increase of the fluorescence emission intensity at 365 nm with excitation at 260 nm as a function of the concentration of αCD, βCD and γCD. The insert shows linearization of data according to Equation (6) yielding the formation constant for the βCD complex $K = 82$ mol^{-1} L.

where [DFQ], [CD] and [DFQ · CD] are the equilibrium concentration of the species involved. The experimental concentrations of host and guest are such that [CD] $\gg$ [DFQ · CD] and Equation (3) can be expressed as

$$K = \frac{[\mathrm{DFQ} \cdot \mathrm{CD}]}{([\mathrm{DFQ}]_0 - [\mathrm{DFQ} \cdot \mathrm{CD}])\,[\mathrm{CD}]_0} \tag{4}$$

where zero subscripts denote the analytical concentration of DFQ and CD. The quantum yield Q of the fluorescent complex is given by the general expression relating the fluorescence intensity of the complex F, concentration [DFQ·CD], and a constant G which characterizes the fluorophore and the instrumental parameters

$$Q = \frac{F}{G[\mathrm{DFQ} \cdot \mathrm{CD}]}\,. \tag{5}$$

Combination of Equations (4) and (5), after a simple rearrangement gives the expression which can be used for the estimation of K

$$\frac{[\mathrm{DFQ}]_0}{F} = \frac{1}{KGQ[\mathrm{CD}]_0} + \frac{1}{GQ}\,. \tag{6}$$

The fluorescence F of a complex which will be used in the evaluation of K is obtained by subtracting the fluorescence signal of DFQ in the absence of CD from the total fluorescence. It has been pointed out that Equation (6) may introduce errors [18]; however, in the present case there is no shift of fluorescence maxima and the signal of the inclusion complex is substantially enhanced over the fluorescence of free DFQ. The only deviation from linearity we observed was when the concentration of CD was comparable with the concentration of DFQ, and these data were excluded from the regression procedure. The linear least-squares fitting according to Equation (6) yields the value of the formation constant $K = 82 \pm 12\,\mathrm{mol}^{-1}$ L. A good fit of data at the same time confirms the assumption of a 1 : 1 stoichiometric ratio (in the case of a 2 : 1 complex one should observe a linear dependence on $1/[\mathrm{CD}]^2$ instead of $1/[\mathrm{CD}]$). The other two CDs enhance the fluorescence by less than 20%. In such a case the application of Equation (6) is not accurate (see above) and hence the corresponding values of K were not evaluated. We can only estimate that formation constants for αCD and γCD are at least one order of magnitude smaller.

3.3. Mode of interaction

The ionic character of DFQ is due to the presence of quaternary nitrogen and at the same time the compound exerts hydrophobicity due to two phenyl substituents. There are two possible modes of inclusion: (a) phenyl in; or (b) pyrazolium ring in. Even though the formation constant determined above indicates only a weak interaction, we attempted to distinguish the interaction mode on the basis of ^{1}H-NMR measurements of βCD solution in the absence and in the presence of DFQ (Figure 5). The NMR spectrum of βCD has been assigned by several authors [19–21] and consists of six types of signals (the numbering of H atoms, given in the literature is used here): the H-1 doublet (4.96 ppm), the H-3 triplet (3.86 ppm), an intensive unresolved peak of H-5 and H-6 (3.80–3.72 ppm), the two doublets of H-2 (centered at 3.55 and 3.52 ppm), and finally the H-4 triplet (3.47 ppm). The location of the H-3 and H-5 atoms inside the CD cavity enables one to detect the guest interaction with the interior of the CD torus and thus discriminate against the interactions with H-atoms on the external surface of CD. The change of the NMR spectrum of βCD in the presence of various guests is almost always very small and the system presented here behaves analogously. The presence of DFQ shifts the resonances of the intense band of H-5 and H-6 upfield; at the same time the bands become better resolved, thus suggesting the interaction with the guest by means of the interior of the CD cavity. Signals of the H-3 and H-6 hydrogens are more strongly shifted (an average of 22 Hz), whereas the other cyclodextrin signals change only by 14 Hz. The NMR spectrum of DFQ consists of a signal due to the phenyl substituents at 7.57 ppm, hydrogen in position 4 on the pyrazole ring at 6.90 ppm, and two methyl substituents at 4.00 ppm. Furthermore, there is a signal of the methyl group (3.65 ppm) from the methylsulphate anion which

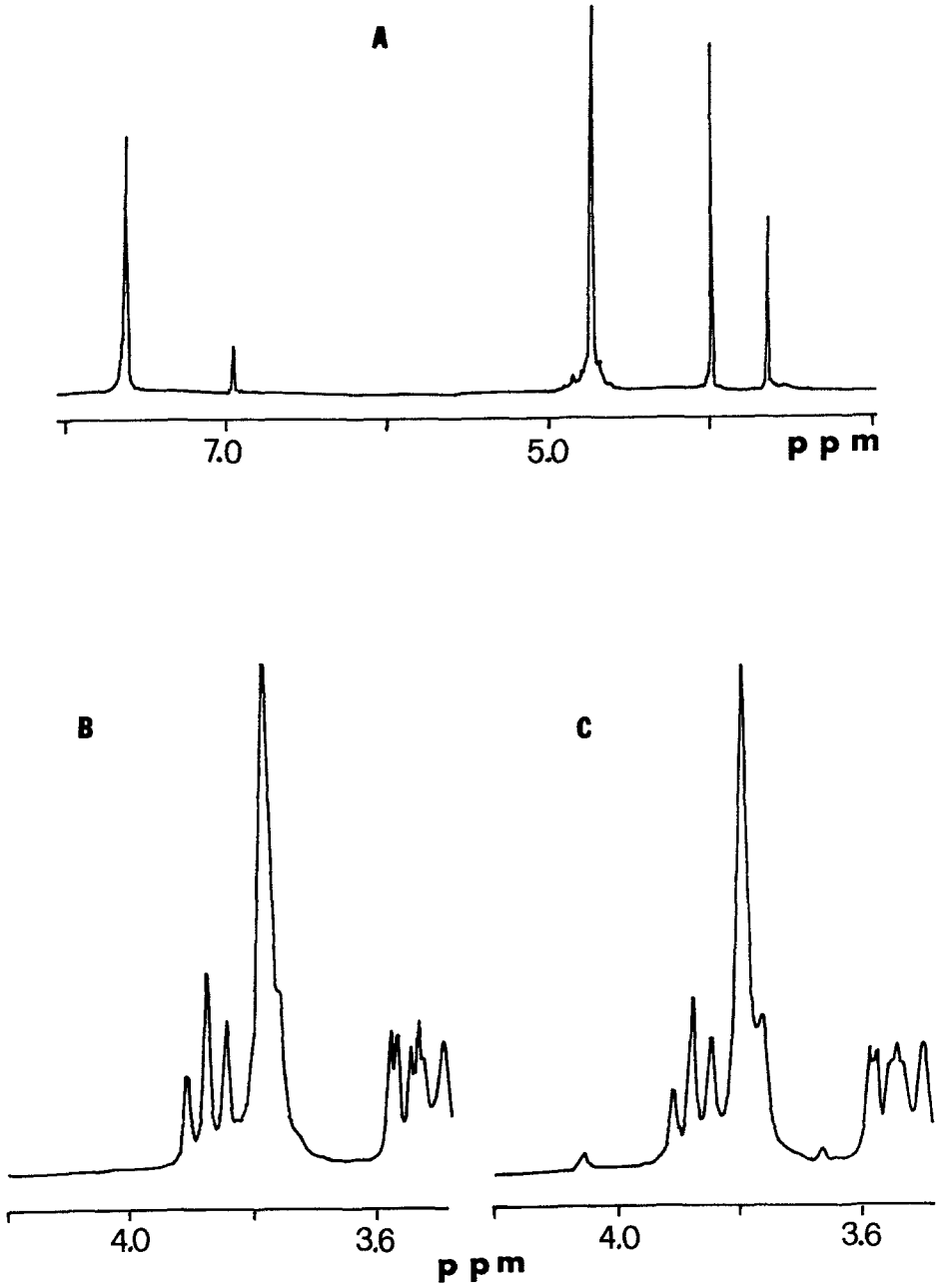


Fig. 5. The ^{1}H-NMR spectrum of (A) 5×10^{-4} mol L^{-1} DFQ in D_2O; (B) 10^{-2} mol L^{-1} βCD in D_2O and (C) mixture of 5×10^{-4} mol L^{-1} DFQ and 10^{-2} mol L^{-1} βCD. For the line assignment see text.

remains unaffected by the presence of βCD in solution. Two respective methyl peaks have integrated signals in the ratio 2 : 1 and the phenyl hydrogens to H4-pyrazole integrated signals are in the ratio 10 : 1, as expected. In the presence of βCD the signals of the phenyl hydrogens remain unaffected whereas the methyl groups on the hetero-ring are shifted by 30.8 Hz.

The small observed changes of NMR signals are comparable with data on the acridine complex [19] and in our view do not allow unambiguous conclusions to be drawn. However, data indicate a more probable configuration of the inclusion complex with the pyrazolium ring inside the CD cavity.

The value of the formation constant reported here ($K = 70 \pm 12$ mol^{-1} L) cannot be directly compared with chemically and structurally similar compounds because of the lack of adequate data. The methyl substituents exert a certain steric hindrance because the electrochemically demethylated product [21] yields a slightly higher formation constant. The unsubstituted pyrazolium chloride does not have a hydrophobic character, as can be inferred from the absence of surface activity (evidenced from comparison of electrochemical data of both cations) and at the same time does not form an inclusion complex, at least within the detection limits. This is in accordance with a general requirement that the guest has to be hydrophobic in order to form a stable inclusion complex.

4. Conclusion

Difenzoquat forms a weak complex with βCD having 1 : 1 stoichiometry. The complexation changes the solution conductivity and substantially enhances the fluorescence intensity. The most probable configuration of the inclusion complex is the insertion of the pyrazole ring in the cavity leaving both phenyl substituents outside.

Acknowledgements

One of the authors (M.P.C.) gratefully acknowledges the MPI financial support. The authors are indebted to the Grant Agency of the Academy of Sciences of the Czech Republic (grant No. 44062) and C.N.R. Rome for granting cooperation.

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