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A proton nuclear magnetic resonance study of the inclusion complex of naproxen with β-cyclodextrin

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Abstract

We report a ^{1}H-NMR study of the complex formed between naproxen (NAP) and β-cyclodextrin (β-CD) in aqueous medium. Our results confirm that inclusion occurs. Analysis of our data by the continuous variation method shows that the complex has 1:1 stoicheiometry. Using Scott's modification of the Benesi-Hildebrand method, we estimate the apparent stability constant of the complex at 298 K in alkaline medium to be 420 M^{-1}. Finally, with the aid of a molecular modelling program, we have determined the most probable structure of the complex. In general our results confirm those of previous studies, although our estimate of the apparent stability constant differs slightly from that reported on the basis of phase solubility results.

Key words: Naproxen; β-Cyclodextrin; Inclusion complex; ^{1}H-NMR; Molecular modeling

1. Introduction

^{1}H-NMR spectroscopy is one of the most useful techniques for investigating the stability, stoicheiometry and geometry of inclusion compounds, particularly when conflicting results have been obtained by other techniques (Djedaini et al., 1987; Djedaini and Perly, 1990). In the case of cyclodextrins, large variations of the chemical shifts due to protons located inside the cavity (H_3 and H_5), coupled with minimal variations of the shifts due to protons located outside the cavity (H_2 and H_4) in the presence of increasing amounts of the guest molecule, provide clear evidence of inclusion. Chemical shift differences can also be used to elucidate the structure of the inclusion compound.

Naproxen, (+)-6-methoxy-α-methyl-2-naphthaleneacetic acid, is a non-steroid anti-inflammatory drug, with pK_a 4.2, belonging to the arylalkanoic acid group. It has been reported that inclusion of drugs of this group in cyclodextrins leads to improved solubility and dissolution rates, and reduces secondary effects (Jones et al., 1984; Hirayama and Uekama, 1987). Phase solubility and UV spectroscopy studies (Otero-Espinar, 1990) have confirmed that interaction occurs be-

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tween naproxen and β-cyclodextrin in aqueous medium.

Here, we report a ^{1}H-NMR study of the complexation between naproxen and β-cyclodextrin in aqueous medium.

2. Experimental

2.1. Materials

Naproxen (NAP) was obtained from Sigma, Kleptose® β-cyclodextrin (β-CD; water content 12.7%) from Laisa (Roquette), and D_2O (deuterium content 99.8%) and NaOD (40% in D_2O, deuterium content 99.5%) from Fluka.

2.2. ^{1}H-NMR spectroscopy

NAP solutions (3 mM) were made up in D_2O following adjustment of pD to 9 with 40% NaOD. At pD values below 9, NAP is insufficiently soluble in D_2O to allow ^{1}H-NMR studies. β-CD solutions (3 or 15 mM) were made up in D_2O and concentrated to dryness three times to maximize substitution of hydrogen by deuterium. NAP and β-CD solutions were mixed as required by shaking in a water-free environment. ^{1}H-NMR spectra were obtained on a Bruker WM250 spectrophotometer at 250 MHz at 295 ± 0.5 K. The chemical shift at 4.8 ppm due to residual solvents (H_2O and HDO) was used as internal reference.

3. Results and discussion

3.1. ^{1}H-NMR spectra

The spectrum for NAP is shown in Fig. 1. For β-CD, assignment of peaks to protons was performed following Ueda and Nagai (1980). Spectra for NAP in the presence of different concentrations of β-CD, and for β-CD in the presence of different concentrations of NAP are shown in Fig. 2 and 3, respectively: in both cases there are clear differences with respect to the spectra for the individual components. Only the shifts for the H_3 and H_5 protons (located inside the cavity) and the H_6 proton (located on the cavity rim at the narrow end of the molecule) are appreciably shifted (Fig. 3), confirming that NAP only interacts with the inside of the cavity and thus that inclusion occurs. No new peaks are present in the spectra of the NAP-β-CD mixtures, indicating that complexation is a dynamic process, the included drug undergoing rapid exchange (relative to the NMR time scale) between the free and bound state, and thus that the exchange rate must exceed the reciprocal of the largest observed shift difference (in Hz) for any proton of

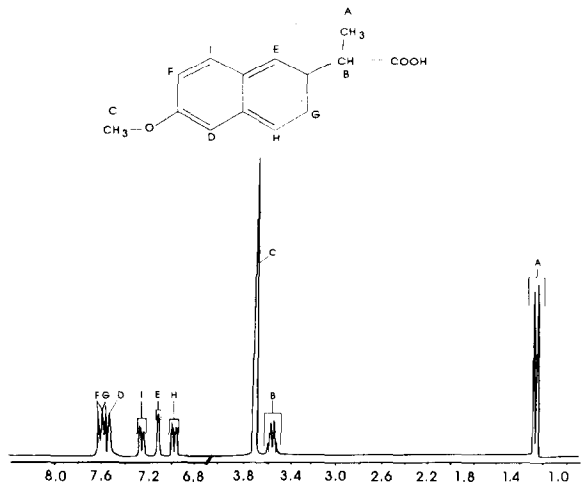

Fig. 1. ^{1}H-NMR spectrum for naproxen showing the assignment of peaks.

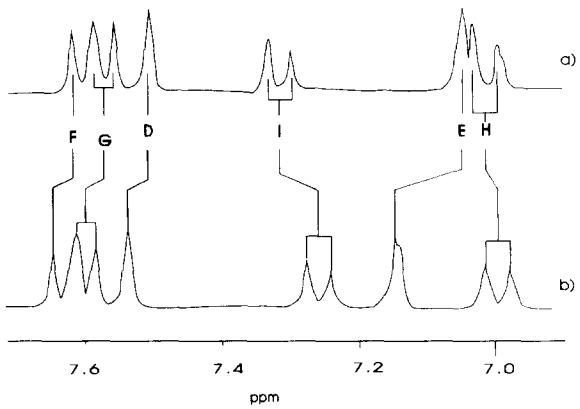

Fig. 2. ^{1}H-NMR spectrum for naproxen in the presence of different relative concentrations of β-cyclodextrin: (a) $r = 1$, (b) $r = 0.5$.

the guest molecule (Djedaini et al., 1987). Thus, complexation and release of the NAP molecule must occur at least 70 times per s.

3.2. Determination of stoicheiometry

The stoicheiometry of the complex was determined by the continuous variation method (Job, 1928). NMR spectra were obtained for a series of mixtures in which the total initial concentration of the two species was maintained constant ([NAP] + [β-CD] = 3 mM) but in which the ratio of initial concentrations (r = [NAP]/([NAP] + [β-CD]), or r = [β-CD]/([NAP] + [β-CD]) was varied between 0 and 1.

Under these conditions (Djedaini et al., 1990), if a physical parameter directly related to the concentration of complex can be measured and plotted as a function of r, its maximal value will be reached at $r = m/m + n$, where m and n are the naproxen and β-cyclodextrin proportions in the complex respectively.

When signals are rapidly averaged by the exchange betweeen free and bound states, the quantity $\Delta\delta_{obs} \cdot [NAP]_t$ or $\Delta\delta_{obs} \cdot [\beta\text{-CD}]_t$ (where $\Delta\delta_{obs}$ is the chemical shift difference between free NAP or β-CD and the observed value for a given ratio r; $[NAP]_t$ and $[\beta\text{-CD}]_t$ are the total concentrations of these compounds) will be proportional to complex concentration, thus they can be plotted against r.

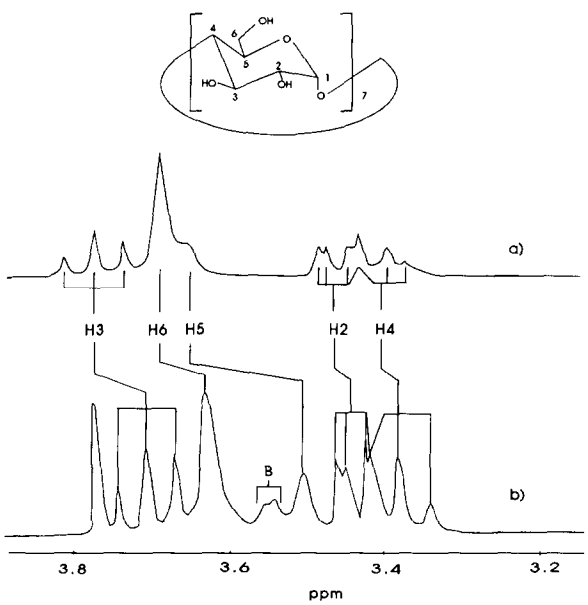

Fig. 3. ^{1}H-NMR spectrum for β-cyclodextrin in the presence of different relative concentrations of naproxen: (a) r = 1, (b) r = 0.5.

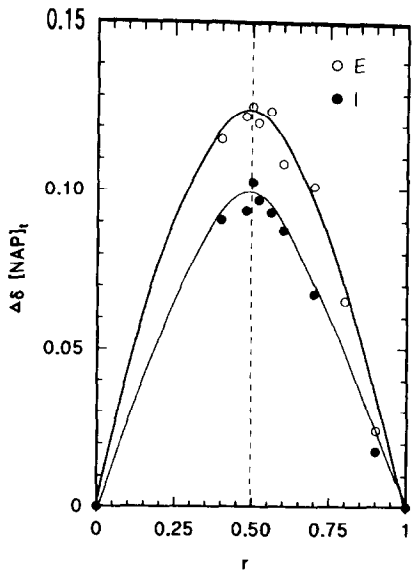

Fig. 4. Continuous variation plot for the most markedly affected (I and E) protons of naproxen in the presence of different relative concentrations of β-cyclodextrin.

The continuous variation plot of $\Delta\delta_{obs} \cdot [NAP]_t$ against r for the most markedly affected protons of NAP (Fig. 4) demonstrates that the complex has 1 : 1 stoicheiometry, since the maximum is at r = 0.5. The continuous variation plot for the most markedly affected protons of β-CD (Fig. 5) likewise peaks at r = 0.5. Note that this plot is more accurate than that for NAP, since in the case of β-CD each signal is stronger, being due to six identically positioned protons (one from each glucose monomer). Note also that the plots are symmetrical, indicating that only one type of complex is formed: competitive formation of other types of complex, or of dimers of the complex, would give rise to asymmetric curves (Djedaini and Perly, 1990; Djedaini et al., 1990).

3.3. Determination of the apparent stability constant

The apparent stability constant of the complex (at 295 K, pD 9) was determined by Scott's modi-

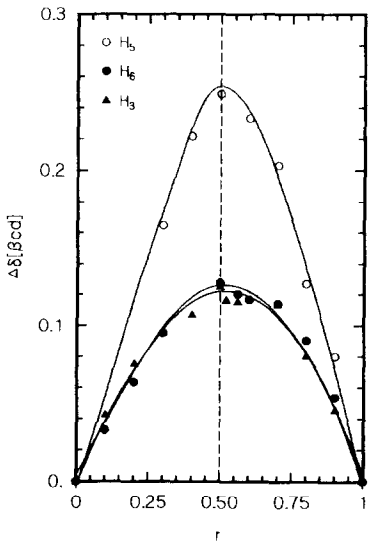

Fig. 5. Continuous variation plots for the H_5, H_6 and H_3 protons of β-cyclodextrin in the presence of different relative concentrations of naproxen.

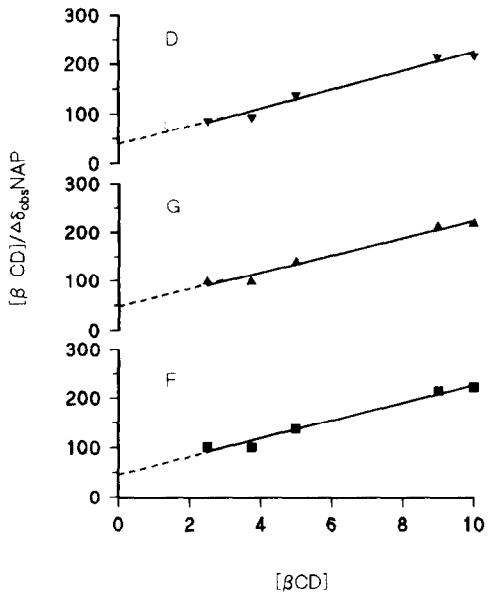

Fig. 6. Scott's plot for the D, F, and G protons of naproxen in the presence of different concentrations of β-cyclodextrin.

fication of the Benesi-Hildebrand method (Benesi and Hildebrand, 1949; Scott, 1956), which is applicable to NMR data for complexes with 1 : 1 stoicheiometry, in mixtures in which the complexing agent is present in large excess. Scott's equation is as follows:

$$[\beta\text{-CD}]_t/\Delta\delta_{obs} = ([\beta\text{-CD}]_t/\Delta\delta_c) + 1/K\Delta\delta_c$$

where $\Delta\delta_c$ is the chemical shift difference between a pure sample of the complex and the free component, and K denotes the apparent stability constant.

The slope of the plot of $[\beta\text{-CD}]/\Delta\delta_{obs}$ against $[\beta\text{-CD}]$ is thus equal to $1/\Delta\delta_c$ and the intercept with the vertical axis to $1/K\Delta\delta_c$, allowing estimation of K.

We determined shift differences in mixtures with a NAP concentration of 1 mM and β-CD concentrations ranging from 2.5 to 10 mM. For Scott's method, the most marked shift variations are generally used for the estimation of K; however, we found that the signals assigned to the NAP protons I and E (the most markedly affected) overlapped considerably. We therefore used the signals assigned to protons D, F and G, which were well-defined and reasonably strong. The corresponding plots (Fig. 6) allow estimation of K as 420 M^{-1} ($\sigma_{n-1} = 61.5$; Table 1). This value is appreciably different from that obtained in alkaline medium by phase solubility techniques (Otero-Espinar, 1990), which can be attributed to the errors arising from the use of concentrations, not activities, in phase solubility calculations.

The apparent stability constant allows estimation of the percentage of drug which will complex with the cyclodextrin at 298 K, in alkaline medium, and given equimolarity. With initial NAP and β-CD concentrations of 3 mM, 57% of the drug will be in complexed form. This is very low in comparison with the estimated value of 99% in acid medium (Otero-Espinar, 1990), which can be attributed to ionization of the drug at higher pH interfering with complex formation (since the β-CD cavity is hydrophobic).

Table 1
Apparent stability constants for the inclusion complex, estimated on the basis of data for protons F, G and D

Proton	K (M^{-1})	Correlation coefficient
F	385	0.98814
G	383	0.98710
D	491	0.98171

Table 2
Chemical shifts (ppm) for the protons of naproxen in the free state and in the pure complex

Proton	δ	δ_c	$\Delta\delta_c$
F	7.6335	7.6891	0.0556
G	7.6033	7.6599	0.0566
D	7.5410	7.5940	0.0530
E	7.2614	7.3778	0.1164
I	7.1493	6.9718	0.1775
H	6.9993	6.9604	0.0389
C	3.7237	3.7219	0.0018
A	1.2610	1.2319	0.0291

Scott's equation also allows estimation of $\Delta\delta_c$ values for the protons used in the calculation. For the remaining protons, $\Delta\delta_c$ values can be estimated indirectly on the basis of the data obtained by the continuous variation method, as follows:

$$\Delta\delta_c = \Delta\delta_{obs} \cdot [X]_t/[C]$$

where [X] is the total concentration of NAP or β-CD as appropriate, and [C] denotes the concentration of the complex. This value can be determined using the same equation using values of $\Delta\delta_c$ previously obtained.

The results of these calculations for NAP and β-CD are listed in Tables 2 and 3 respectively.

3.4. Determination of the structure of the complex

As can be seen from Table 3, the most marked chemical shift differences were recorded for the β-CD protons H_3 and H_5 (located inside the cavity) and H_6 (located on the cavity rim at the narrow end of the molecule), while the shift variations for the protons on the outside of the molecule were minimal: this is very strong evidence for the formation of an inclusion complex.

Table 3
Chemical shifts (ppm) for the protons of β-cyclodextrin in the free state and in the pure complex

Proton	δ	δ_c	$\Delta\delta_c$
H_1	4.8502	4.7896	0.0606
H_2	3.4275	3.3710	0.0474
H_3	3.7440	3.6032	0.1408
H_4	3.3642	3.3239	0.0403
H_5	3.6232	3.3421	0.2811
H_6	3.6589	3.5148	0.1441

All β-CD proton shifts were shielded. This indicates that the NAP molecule creates diamagnetic anisotropy effects in the interior of the cavity, due to the inclusion of groups which are very rich in π electrons (Djedaini et al., 1987; Zhang et al., 1990). The only large group with π electrons in the NAP molecule is the aromatic ring, suggesting that this group enters the cavity. This hypothesis is supported by the NAP data (Table 2): the maximum shift differences were for aromatic protons. These displacements are large, suggesting that the drug is included very deeply in the β-CD cavity.

The fact that NAP protons E, D, G and F were displaced upfield and I, H and C downfield is also of interest. An upfield displacement indicates that the proton is close to a host atom which is rich in π electrons, in this case that associated with oxygen atoms. A downfield displacement indicates weaker interactions (deshielding effect due to Van der Waals forces) with the hydrogen atoms (Zhang et al., 1990).

The NAP protons I and E show the most pronounced shift differences. The positive sign of the variation for proton E suggests that it locates close to the oxygen atoms in the β-CD cavity, while the negative sign of the displacement for proton I suggests that it locates at some distance from the oxygen molecules and within a hydrogen atom. Shift differences for the remaining aromatic protons were lower, but this does not indicate that they interact less strongly with the β-CD molecule (the β-CD data demonstrate that the entire aromatic ring is included within the cavity), but rather that they interact with both π electron-rich atoms and the hydrogen atoms of the β-CD molecule, the magnitude of the shift difference being dependent on the relative strength of the two types of interaction. The signal for proton H is deshielded due to Van der Waals interactions with the carbohydrate chain, but this effect is to some extent masked by the upfield displacement due to interactions with π electron-rich oxygen atoms. The displacement shifts for protons F, G and D, on the other hand, reflect the greater influence of interactions with oxygen

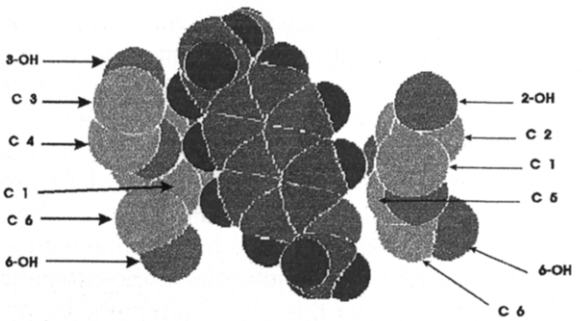

Fig. 7. Molecular model of the NAP-β-CD inclusion complex (transverse cross-sectional view), showing the position of the drug with respect to the hydroxyl groups at each end of the molecule, to an oxygen atom forming part of a glycosidic bond, and to carbon atoms.

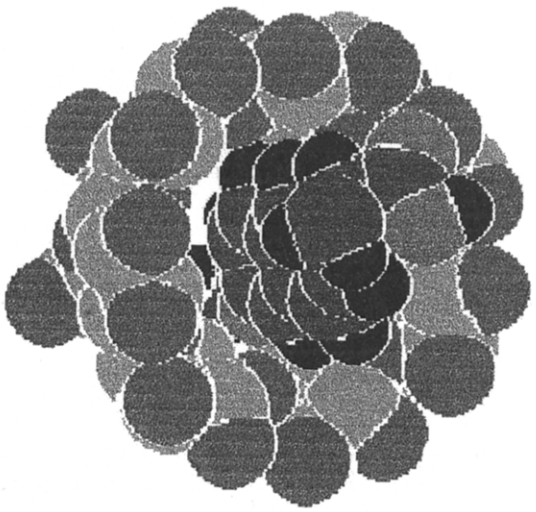

Fig. 8. Molecular model of the NAP-β-CD inclusion complex (frontal view).

atoms. The signal for proton C shows only a slight downfield shift, since its strong interaction with the H_6 protons of the NAP molecule is partly counteracted by interactions with freely rotating terminal hydroxyl groups. The signal for proton B is not detectable by one-dimensional ^{1}H-NMR, since it is overlapped by signals from the β-CD molecule. Finally, the slight downfield displacement of the signal for proton A cannot be due to Van der Waals interactions in the interior of the cavity, since our data show that it is the other end of the NAP molecule which enters the cavity. The displacement may be due to steric perturbation by the primary hydroxyl groups of the host molecule, or to a delocalization of charge into the aromatic ring of the drug molecule occurring as a result of inclusion.

The most probable structure of the inclusion complex in solution (Fig. 7–9) was determined using the ALCHEMY II molecular modelling program. The NAP model was built up from the naphthalene group using an energy minimization subroutine (maximum number of iterations 500, minimizer cut-off 0.1). The β-CD model was constructed using the subroutine CRYSTAL INPUT with X-ray diffraction data for β-CD (Harata et al., 1985). The molecular model was obtained using a manual docking in accordance with the NMR data. This model coincides closely with that obtained previously using the SIBYL molecular

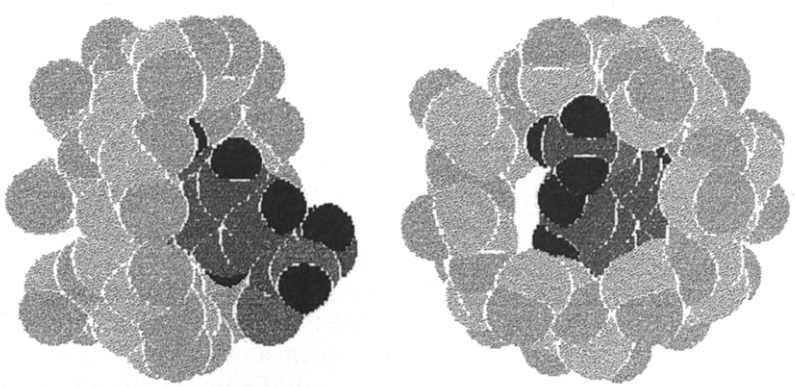

Fig. 9. Molecular model of the NAP-β-CD inclusion complex (lateral view).

modelling program (Otero-Espinar, 1990), and that reported by Bettinetti et al., (1991) on the basis of ^{13}C-NMR data.

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