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Complexation of Ephedrine with β-Cyclodextrin: An NMR Spectroscopy Study

THILIVHALI T. NDOU[1], SRINIVASAN MUKUNDAN, JR.[2], and ISIAH M. WARNER*
Department of Chemistry, Emory University, Atlanta, Georgia 30322, U.S.A.

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Abstract. This study focuses on the inclusion complex of ephedrine with β-CD. The association of β-CD and ephedrine has been examined using ^{1}H NMR and circular dichroism. The systematic shifts of the proton resonances of the phenyl moiety of ephedrine and that of the protons located inside the β-CD cavity, provide evidence of intracavity inclusion. Two-dimensional ROESY show preferential localization of ephedrine in close proximity with protons located inside the β-CD cavity. The systematic variation of circular dichroism spectra with increasing concentration of β-CD is used to estimate the apparent formation constant.

Key words: β-Cyclodextrin, ephedrine, nuclear magnetic resonance, circular dichroism.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides, the most widely used of which contain six to eight *D*-(+)-glucopyranose units (α, β, and γ, respectively). Each CD molecule is shaped like a torroidal, hollow cone with primary and secondary hydroxyl groups crowning the narrower and wider rims, respectively. The complexing properties of the CDs are largely attributable to their fairly hydrophobic cavity. This hydrophobicity is attributed to the glycosidic oxygens and the protons which line the interior of the CD cavity. The CDs are used for a wide range of applications including the stabilization of light sensitive compounds [1], enantiomeric separations [2], catalysis [3], and improving pharmaceutical formulations [4]. The bioavailability of drugs that are poorly soluble in water is markedly enhanced due to high solubility when in the complexed form. The inclusion complexes of CDs show a potential importance in the design of controlled chemical synthesis [5] and the CD cavity shows greater influence on photochemical reactions [6]. In addition, CDs have been successfully used to separate both structural and optical isomers [7]. The necessary criteria for the formation of an inclusion complex are that the guest must be hydrophobic and have the appropriate geometry.

* Author for correspondence; Current Address: Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, U.S.A.
* Current Address: Gillette Research Institute, Gaithersburg, MD 20879, U.S.A.
** Emory University School of Medicine, Atlanta, GA 30322, U.S.A.

Ephedrine (EPH) is a widely used adrenergic agent. For this reason, several researchers have shown much interest in the study of ephedrine. Many of the studies are aimed at determining the differences between the enantiomers of ephedrine in their pharmacological actions. Both gas liquid chromatography (GLC) and high performance liquid chromatography (HPLC) have been employed to study the chromatographic resolution of the enantiomers of ephedrine and its derivatives [8–11]. Mularz *et al.* [12] have attempted to characterize the resolution of enantiomers of ephedrine and its derivative, pseudoephedrine, by use of β-CD in the mobile phase.

Proton NMR spectroscopy has been utilized for the investigation of the stoichiometry of complexes [13], for the orientation of the host–guest system in solution [14–16], and for the molecular dynamics of the inclusion complex [17–19]. In such studies, nuclear cross-relaxation has been widely employed for the determination of molecular interaction by use of two dimensional (2D) nuclear Overhauser effect spectroscopy (NOESY) [20–22]. In addition, the experiments can be further extended by conducting them under spin-lock conditions, for the measurement of rotating-frame Overhauser effect spectroscopy (ROESY) [24–26]. The information obtained from such techniques has been shown to be valuable in determining whether complex formation occurs, as well as suggesting possible modes of interaction between two or more species in solution. For example, 2D ^{1}H NMR has been used to determine the complex structure and solution geometry of the CD complexes [17–19]. It is also well known that NOESY and ROESY can also yield valuable information about the spatial relationship of different spins (protons). For example, Schneider *et al.* [27] have recently demonstrated the use of ^{1}H NMR and 2D ^{1}H NMR spectroscopy to determine the mode of interaction between CD and guest molecules containing both phenyl and naphthyl units.

Knowledge of the formation constants for CD complexes is necessary in order to predict and better understand the interactions between CDs and guests in solution. The examination of these values can give a better understanding of the factors which affect complexation. A number of spectroscopic techniques have been used for investigation of cyclodextrin/guest inclusion complexes [28–30]. The spectral properties of the complexed guest molecule are often different from that of a guest free in solution. Consequently, spectral properties such as changes in fluorescence emission, UV/vis absorbance, ^{1}H NMR chemical shift and optical activity have been used to characterize cyclodextrin complexes. Optically active chiral molecules show marked changes in circular dichroism spectra upon formation of inclusion complexes with cyclodextrins [31]. Moreover, achiral molecules have also shown induced circular dichroism spectra as they are inserted into the optically active CD environment [32]. These circular dichroism spectral changes have also been used to determine the association constants for the complexation equilibria [33,34].

In this paper, the β-CD and ephedrine complex is investigated using ^{1}H NMR spectroscopy and circular dichroism spectroscopy. Results obtained from ROESY experiments are presented to show the preferential localization site of ephedrine

inside the β-CD cavity.

2. Experimental

2.1. INSTRUMENTATION

Proton NMR spectra were recorded on a General Electric GN-500 spectrometer operating at 500.1 MHz (^{1}H). Chemical shifts are in δ values using an internal reference of 3-(trimethylsilyl) propionic acid, sodium salt (TSP) in D_2O. Proton chemical shifts are accurate to within ±0.01 ppm. A ROESY spectrum with a 300 ms mixing time was acquired using 512 t_1 increments of 16 scans each with a relaxation delay of 2 s. Spectral windows of 6000 Hz and a 90° pulse width of 56 μs were employed for all measurements. All experiments were performed at 30 ±0.1 °C. The solvent resonance was suppressed by selective, continuous irradiation at all times except during t_1 and t_2 periods. The 2D data were transferred to a DEC MICROVAX II computer and processed with FTNMR software (Hare Research, Woodinville, WA).

Circular dichroism measurements were acquired with a Jasco J-600 spectropolarimeter using a 1.0 cm cylindrical quartz cell. The bandwidth and time constant were set at 1 nm and 4 s, respectively. The scan rate was 20 nm/min. A total of three scans were averaged for each run.

2.2. MATERIALS

(1R,2S)-(–)-Ephedrine (99%) were purchased from Aldrich (Milwaukee, WI). The β-cyclodextrin was obtained from American Maize Products (Hammond, IN). Deuterium oxide (99 atom % D, Aldrich) was the solvent used for the NMR studies. All reagents were used without further purification.

2.3. METHODS

2.3.0. *A. ^{1}H-NMR Measurement*

Influence of β-cyclodextrin. The concentration of ephedrine was fixed in all cases at 2.0×10^{-3} M, while the concentration of β-CD was varied from 1.0×10^{-3} to 8.0×10^{-3} M. To prepare ephedrine stock solution in D_2O, a weighted quantity was transferred to a dry vial and then D_2O was added to give a 1.0×10^{-2} M ephedrine solution. The stock solutions were adjusted to pH 10 (uncorrected) with NaOD. The solutions containing β-CD were prepared by placing a 100 μL aliquot of the 1.0×10^{-2} M ephedrine into an NMR tube and adding an appropriate amount of β-CD stock solution to make the desired concentration. All solutions were stored under nitrogen and left to equilibrate overnight before analysis. Blank solutions containing 1.0×10^{-2} and 2.0×10^{-3} M β-CD and ephedrine were prepared in D_2O and the pH adjusted to 10 using NaOD.

Two-dimensional ^1H NMR (ROESY). The concentrations of ephedrine and β-CD were 4.0×10^{-3} and 8.0×10^{-3} M, respectively. The sample mixture was first subjected to several lyophilization procedures to reduce the amount of water in the sample. The pH (uncorrected) of the solution was adjusted to 10 using NaOD.

2.3.0. *B. Circular Dichroism Measurement*

Influence of β-cyclodextrin concentation. A 4.0×10^{-4} M aqueous ephedrine stock solution was prepared by transferring a weighed quantity of ephedrine into a 100 mL flask and diluting to the mark with deionized water. To prepare an ephedrine solution in aqueous β-cyclodextrin, a weighed quantity of β-cyclodextrin was transferred into 10 mL flasks and the flasks were filled to the mark with a solution containing ephedrine. The ephedrine concentration was held constant at 4.0×10^{-4} M in all experiments. The concentration of β-CD were in the range 1.0×10^{-3} to 0.01 M. The solutions were buffered at pH 10 using $Na_2CO_3/NaHCO_3$. All solutions were allowed to equilibrate overnight prior to analysis. The experiments were conducted in triplicate and the spectra obtained were not smoothed.

3. Results and Discussion

3.1. Influence of Ephedrine on the Chemical Shifts of β-CD

The mode of interaction of ephedrine and β-CD has been inferred from ^1H NMR spectroscopy studies. These studies are specifically aimed at defining the mode of inclusion of ephedrine in the CD cavity. The resonance assignments of β-CD protons is well established [28–30], and our chemical shift data are consistent with those previously reported. Figure 1, part d, is the representative ^1H NMR spectrum of β-CD in D_2O, consisting of six kinds of protons. It is interesting to note a gradually increasing upfield shift of the signals between 3.76–3.70 ppm upon addition of increasing amounts of β-CD (Figure 1, parts a–c). The shifting signals are due to the H-5 proton resonance shifting upfield from the region where H-5 and H-6 overlap in the pure β-CD spectrum. The initial addition of β-CD is accompanied by an insignificant shift of the H-3 proton resonance, suggesting that ephedrine only leads to a strong shielding of the H-5 proton. This reflects a reduced interaction, in these instances, between H-3 protons and the aromatic protons of ephedrine, probably due to a partial penetration into the β-CD cavity. Thus, the shielding experienced by H-5 upon insertion of ephedrine inside the β-CD cavity, appears to lead to the amine and the hydroxyl group protruding into the bulk aqueous environment. This is in agreement with the ^1H NMR results of Mularz *et al.* [12] who reported a strong hydrogen bonding between ephedrine's ammonium and hydroxyl groups with the hydroxyl groups crowning the rims of the β-CD cavity. On the basis of molecular size, it is apparent that ephedrine can fit entirely into the β-CD cavity. Inspection of the CPK (Corey–Pauling–Koltun)

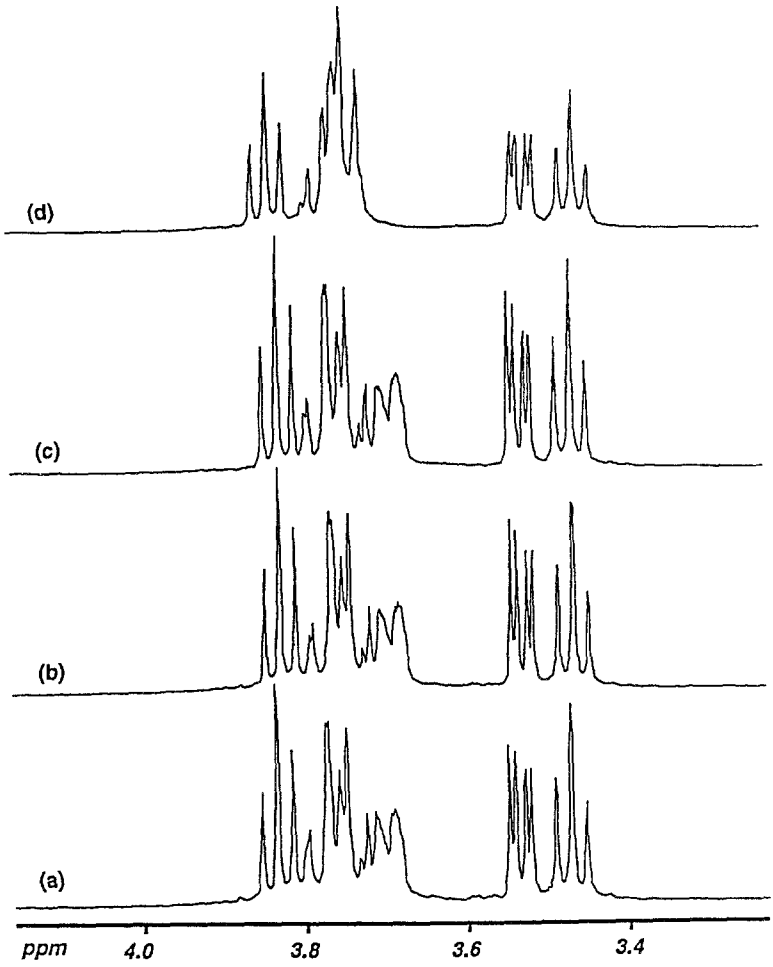

Fig. 1. The upfield region (3.2–4.2 ppm) of the ^{1}H NMR spectra of 2.0×10^{-3} M ephedrine and increasing concentrations of β-CD in D_2O. (a) 5.0×10^{-3} M, (b) 7.0×10^{-3} M, (c) 8.0×10^{-3} M β-CD and (d) β-CD alone.

space-filling models reveal that the phenyl moiety of ephedrine can actually fill the inside of the β-CD cavity.

It is interesting to note a systematic upfield variation of the chemical shift of the H-3, H-5 and H-6 proton resonances when the concentration of β-CD is further increased (Figure 1, part b and c). The H-3 and H-5 protons are located inside the CD cavity [26,35]. The shielding of H-3 provides the rationale for the assignment of the upfield shift of the H-5 proton resonances referred to above. The induced chemical shifts of H-3 and H-5 also reflect the proximity of these protons to the aromatic moiety of ephedrine. Thakkar and Dermarco [36] have rationalized the upfield shift of the H-3 and H-5 proton resonances in terms of the diamagnetic

anisotropy of the included phenyl ring moiety inside the CD cavity. Similar ^{1}H NMR induced chemical shifts of the protons inside the CD torus were reported upon inclusion complexation with aromatic substrates [37–40].

3.2. Influence of varying β-CD concentration on the chemical shifts of ephedrine

The influence of β-CD on the proton resonances of ephedrine was investigated to better understand the complexation behavior of β-CD/ephedrine. The aromatic region of the ^{1}H NMR spectra of ephedrine in D_2O is depicted in Figure 2. The resonance assignments of the protons of free ephedrine are observed as follows: δ 1.09 (doublet, 3H, terminal methyl); δ 2.23 (singlet, 3H, methylamine); δ 2.98 (multiplet, 1H, methine); δ 4.68 (doublet, 1H, benzylic) and δ 7.43 (multiplet, 5H, aromatic). In the absence of β-CD, the chemical shift of the ephedrine aromatic proton resonances consists of several peaks observed between 7.38–7.49 ppm. Further studies using 2D heteronuclear NMR methods are in progress to assign the specific aromatic proton resonances. The ephedrine proton shifts are sensitive to changes in the β-CD concentrations. When the concentration of β-CD is increased, the aromatic proton resonances are gradually shifted upfield with respect to their native chemical shifts (Figure 2, part a-b). This is in good agreement with the observations by Lehmann *et al.* [41] who reported a remarkable shielding of the protons of DL-1-phenylethanol and DL-phenylalanine upon inclusion inside the β-CD cavity. This is also consistent with the results of Inoue *et al.* [13] who reported a strong shielding of the aromatic proton resonances of L-phenylalanine when complexed within the CD cavity.

The extent of the line splitting varies significantly with individual resonances, suggesting variations in the degree of conformation of the individual protons of free ephedrine in solution. In contrast, in the presence of varying β-CD concentration, the conformational equivalency of the aromatic protons is reflected by the indistinguishable proton chemical shifts for the 5.0×10^{-3} M β-CD solution (Figure 2, part c). The aromatic proton resonances of ephedrine start to coalesce into a single, composite signal. The merging of the proton signals may indicate that the aromatic group of ephedrine is rotating within the β-CD cavity giving rise to the broad spectral feature due to time averaging of the individual signals. An alternate explanation could be that there is significant diamagnetic anisotropy within the cavity which causes the coalescence of all signals to a narrow chemical shift region. Perhaps, in the complexation process the ring protons interchange through a very rapid mechanism that happens to have a profound effect on their chemical shifts, the result being a single resonance observed upfield from the original signals observed in the pure ephedrine spectrum.

The loss of the multiplicity of the aromatic proton resonances and the magnitude and direction of the chemical shift variation more likely depend on the localization site of the ephedrine molecule as it interacts with increasing amounts of β-CD in so-

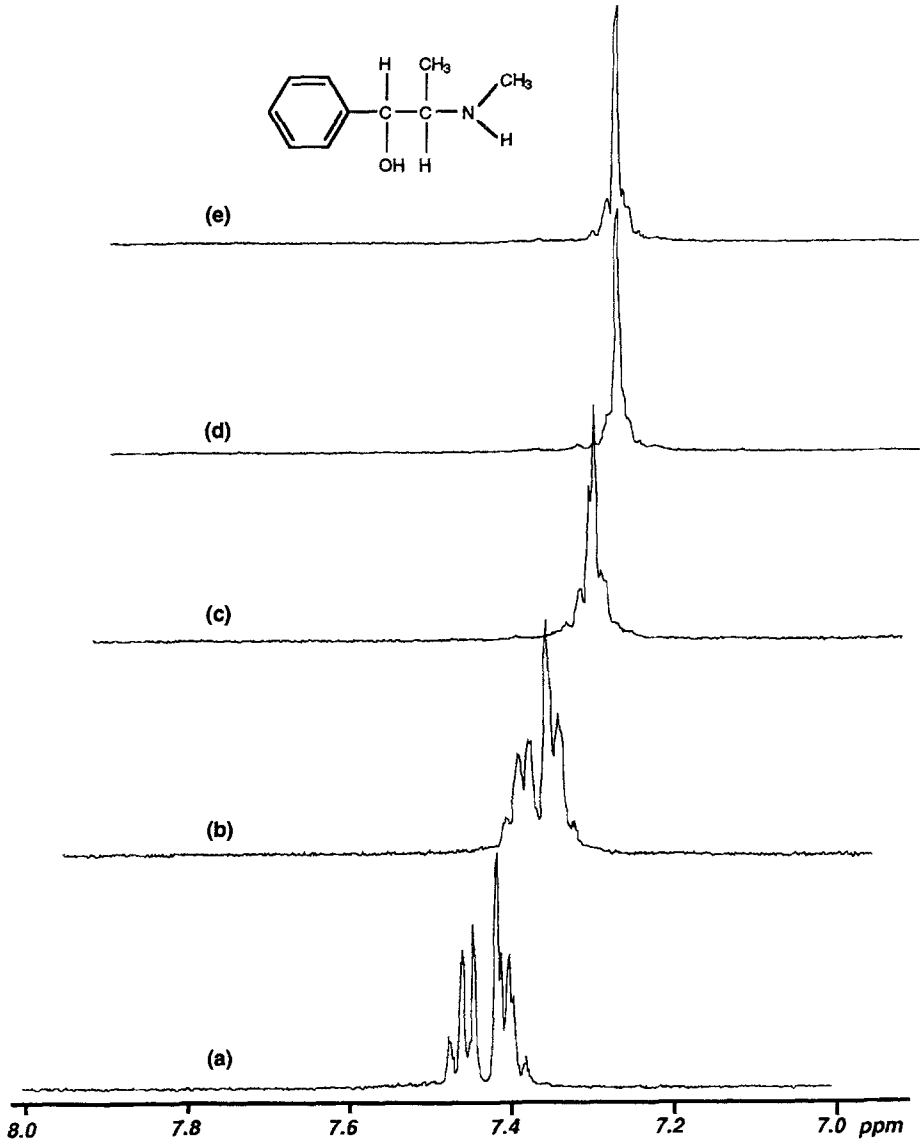

Fig. 2. The downfield region (8.0–7.0 ppm) of the ^{1}H NMR spectra of 2.0×10^{-3} M ephedrine and increasing concentrations of β-CD: (a) pure ephedrine, in the presence of (b) 1.0×10^{-3} M, (c) 5.0×10^{-3} M, (D) 7.0×10^{-3} M, and (e) 8.0×10^{-3} M β-CD respectively in D_2O.

lution. The signals arising from the aromatic protons show a systematic difference in chemical shifts with varying β-CD concentration. At 7.0×10^{-3} M β-CD the aromatic proton resonances show an upfield shifted singlet resonating at 7.27 ppm (Figure 2, part d). Upon further addition of β-CD (8.0×10^{-3} M β-CD) the shift

of the aromatic singlet remains constant at 7.27 ppm (Figure 2, part e). At this concentration, it could be inferred that most of the ephedrine molecules are effectively complexed with β-CD. Ephedrine evidently seeks out the inner hydrophobic environment, and is far removed from the bulk aqueous (D_2O) environment. The addition of β-CD to the ephedrine solution also gives a 0.21 ppm upfield shift of the terminal methyl signals. Therefore, the strong association between β-CD and ephedrine appears to take place through the phenyl ring and the terminal methyl group as indicated by changes in the NMR resonances of these groups. It is interesting to note that all the other protons outside the aromatic ring of ephedrine are of little diagnostic value since they have practically the same chemical shifts for each β-CD concentration. The chemical shifts are the same as that observed in the pure ephedrine spectrum.

In light of the given NMR proton assignments, it could be inferred that the phenyl moiety of ephedrine comes into close proximity to the H-3 and H-5 protons inside the CD cavity. Thus, the shielding experienced by the H-3 proton suggests that more of the ephedrine molecule is inserted deeper into the CD cavity. The above is further corroborated by the absence of changes in chemical shift of the protons outside the aromatic moiety of the ephedrine molecule. The systematic shifting of the ephedrine aromatic proton resonances in conjunction with the induced chemical shift changes of the CD H-3 and H-5 proton resonances, provide enough evidence to support the conclusion that ephedrine is complexed within the β-CD cavity. The H-1, H-2 and H-4 proton resonances of β-CD are unaffected, also suggesting that the association between ephedrine and β-CD does not occur on the outer surface of the β-CD torus.

3.3. Two-dimensional ROESY NMR

Previously, Mularz *et al.* [12] have demonstrated by use of ^{1}H NMR spectroscopy that ephedrine is included inside the cavity of β-CD. In that study, the depth of penetration and the conformation of ephedrine inside the CD cavity was not specified. However, the authors offered a discussion of the hydrogen bonding between the ephedrine ammonium and hydroxyl group with the β-CD hydroxyl groups, but which of the CD's hydroxyl groups (primary or secondary) were involved in the hydrogen bonding was also not specified. In addition, the geometry and molecular orientation of ephedrine inside the β-CD cavity could not be effectively determined by use of 1-D ^{1}H NMR spectroscopy alone. In this study, a 2D ROESY experiment was performed to determine the conformational behavior of ephedrine in the presence of β-CD. Wakamatsu *et al.* [42] have exploited proton NMR spectroscopy to elucidate molecular conformation. The NMR cross-relaxation data can provide qualitative information on the orientation of the guest molecules in solution [43]. Wüthrich [44] has reported on the use of a cross-relaxation process to determine the specific localization sites between two protons of different molecules interacting in space.

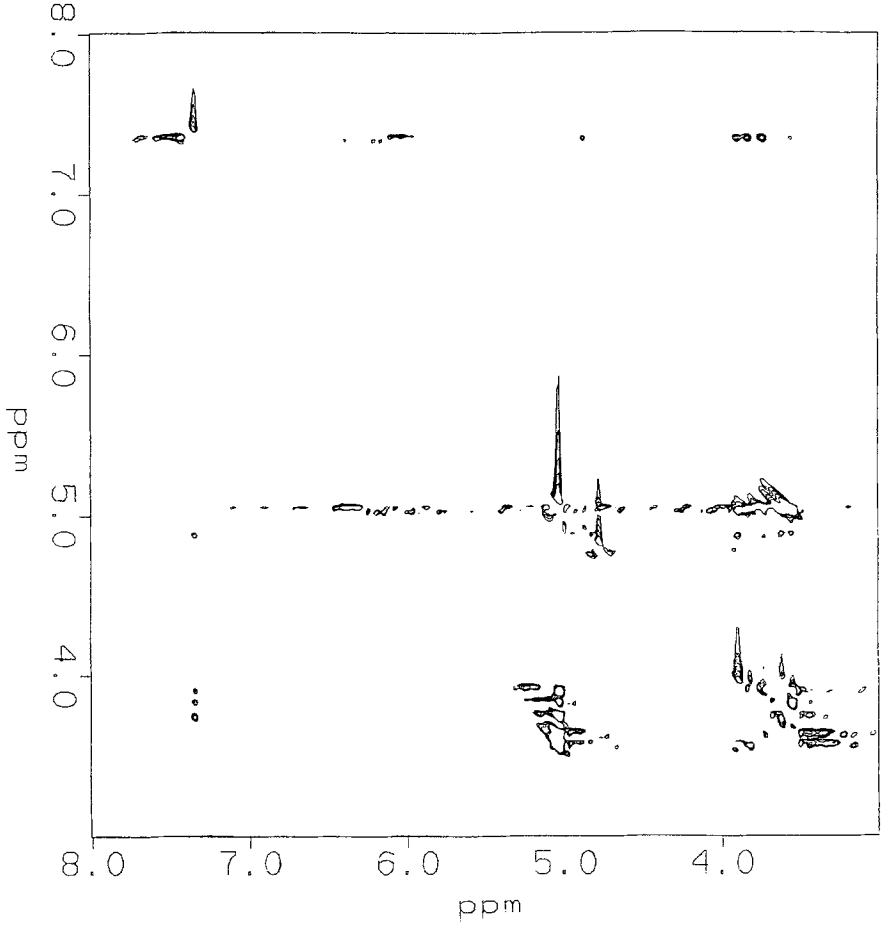

Fig. 3. ROESY spectrum of the β-CD/ephedrine complex in D_2O.

The 2D ROESY spectrum of ephedrine and β-CD in D_2O reveals the binding mode of ephedrine within the CD (Figure 3). The key features of the spectrum are the crosspeaks observed between the aromatic protons of ephedrine and the H-5 proton of β-CD (4.0–3.2 and 7.6–7.0 ppm). Moreover, weak crosspeaks are also observed between the β-CD H-3 and the ephedrine aromatic signals. These clearly suggest that the ephedrine aromatic protons are in close proximity to H-3 and H-5, and strongly indicate that ephedrine is preferentially inserted inside the β-CD cavity. In addition, a similar effect corresponding to the resonance for H-6 located on the rim of the primary hydroxyl side of β-CD is observed.

No crosspeaks have been observed between ephedrine protons and the H-1, H-2 and H-4 protons of β-CD (Figure 4). This leads to the conclusion that ephedrine does not associate on the exterior of β-CD. Thus, both the 1D ^{1}H NMR and 2D

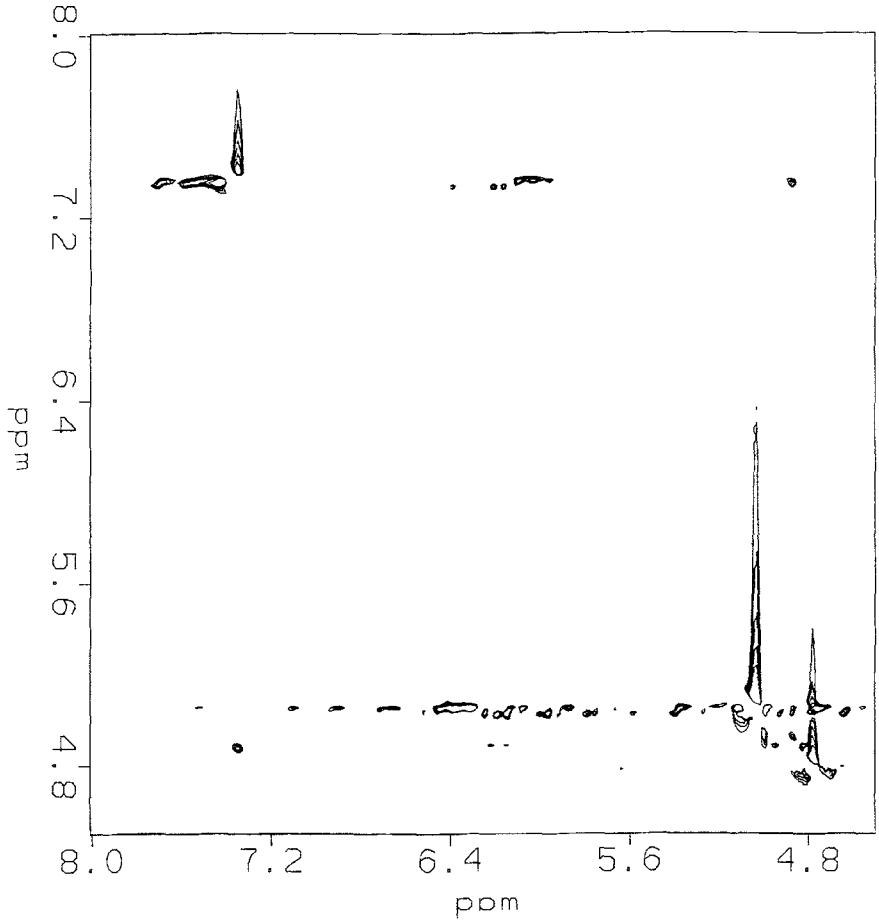

Fig. 4. ROESY spectrum of the β-CD/ephedrine complex in D_2O.

ROESY experiments suggest that ephedrine is inside the CD cavity. These data further suggest the polar alkyl chain to be in close proximity to the bulk aqueous environment (on the primary hydroxyl rim of the CD torus) and the aromatic moiety residing inside the CD cavity. Similar results of intracavity inclusion using 2D ^{1}H NMR spectroscopy have been reported elsewhere [43,45].

3.4. Influence of β-cyclodextrin on ephedrine circular dichroism

The circular dichroism spectrum of ephedrine consists of three positive bands at 253, 259 and 265 nm. The effect of the addition of increasing amounts of β-CD is shown in Figure 5. The molar ellipticity was measured at the maximum ephedrine peak of 265 nm. The molar ellipticity increases monotonically with increasing β-CD concentration, possibly due to the transfer of ephedrine from the aqueous

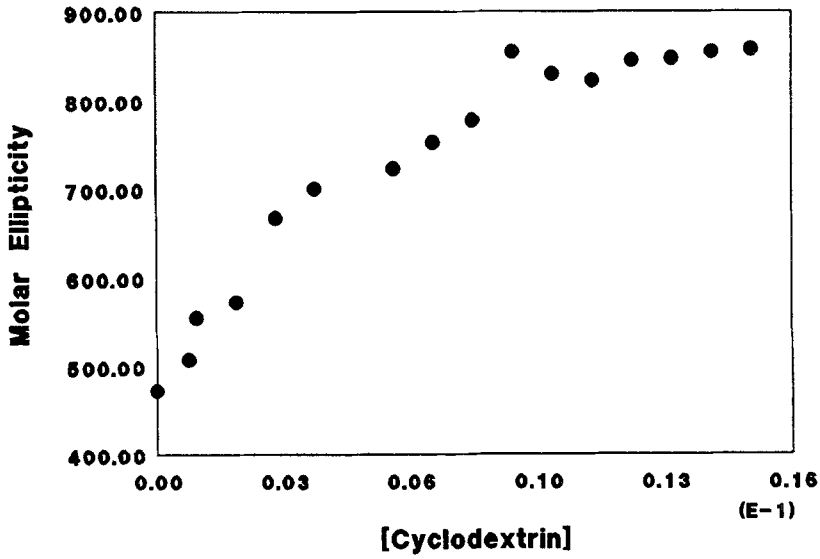

Fig. 5. The influence of varying β-CD concentration on the molar ellipticity of ephedrine.

environment to the hydrophobic β-CD cavity. According to Kirkwood–Tinoco oscillator theory [46] a positive enhancement in the 253, 259 and 265 nm bands of circular dichroism spectra of ephedrine observed upon addition of β-CD suggests an axial inclusion of ephedrine inside the β-CD cavity. In contrast, a perpendicular transition should result in a negative circular dichroism spectrum. This is consistent with the observations of Harata and Uedaira [47], Shimuzu *et al.* [48], Kodaka and Fukuya [49] and Kodaka [50] who reported similar orientations of guests within the CD cavity. When the β-CD concentration in a 4.0×10^{-4} M ephedrine solution is brought to 1.0×10^{-2} M, the curve starts to level off, indicating that the complexation process of ephedrine molecules within the β-CD cavity has reached equilibrium. It could be inferred that at the plateau region of the curve, ephedrine is located in a nonpolar environment and is far removed from the bulk aqueous environment. Furthermore, the observed increase in molar ellipticity suggests that the microenvironment of ephedrine complexed within the β-CD cavity is more conductive to absorption than the microenvironment of ephedrine in pure water.

Mularz *et al.* [12] have reported the existence of self-aggregates of ephedrine at higher concentrations. Such conclusions have been derived from ^{1}H NMR spectroscopy results. These results were based on the curvilinear plot obtained when $\Delta\delta$ (change in chemical shift) was plotted as a function of cyclodextrin concentration, suggesting more than one form of species or ephedrine aggregation in solution. To better characterize the interaction of ephedrine with β-CD, an understanding of the self-association of ephedrine should lead to a better interpretation of the data. The self association of ephedrine was investigated using UV/vis spectrophotometry.

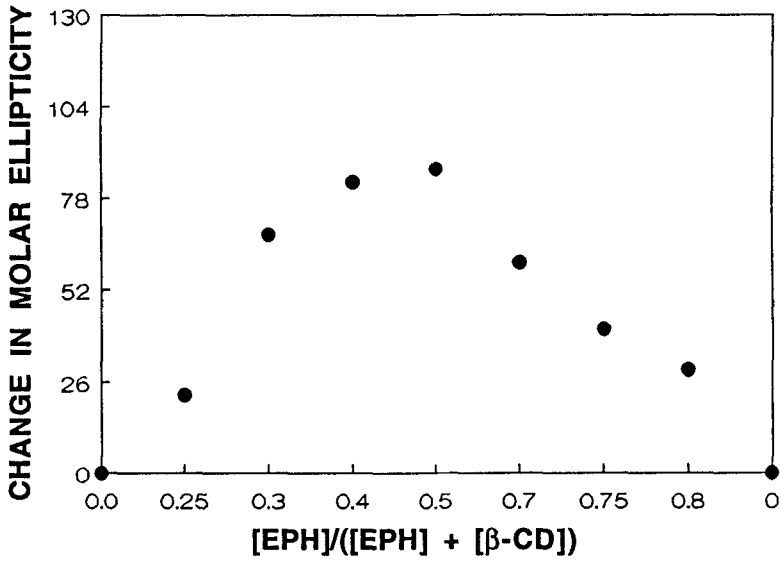

Fig. 6. Continuous variation plot monitored at $\lambda = 265$ nm.

Using cells of different pathlength (0.1, 0.2, 0.5 and 1.0 cm) the absorbance spectra of ephedrine at the concentration range tested did not show any concentration dependence. No significant spectral changes were observed, particularly with respect to the absorbance maxima of the different bands. In addition, the absorbance maxima remained relatively constant upon increasing concentration of ephedrine, indicating no change in the molar absorptivity of ephedrine. In this case, where there is a single component in solution, a constant molar absorptivity suggests the absence of self-aggregation of the drug itself. Similarly, the effects of temperature (25 to 60°C) on ^{1}H NMR of ephedrine (8.0×10^{-3} M) in D_2O did not show any evidence of self-aggregation of ephedrine. Therefore, the systematic increase in the circular dichroism spectrum of ephedrine with increasing β-CD concentration is not due to deaggregation of dimers/multimers and the subsequent incorporation of monomers into the β-CD cavity. This is clearly the result of an interaction of ephedrine monomers inside the β-CD cavity.

3.5. STOICHIOMETRIC RELATIONSHIP BETWEEN β-CYCLODEXTRIN AND EPHEDRINE

A knowledge of the formation constant for β-CD/ephedrine is necessary in order to predict and better understand the interactions between β-CD and ephedrine in solution. However, the results can be better interpreted with an understanding of the stoichiometry between β-CD and the ephedrine molecule. A continuous variation method was employed for circular dichroism spectral changes to evaluate the stoichiometry of the molecular complex. In this study, the total concentration

under the experimental conditions of [β-CD] + [EPH] was fixed at 2.0×10^{-3} M. The calculated molar ellipticity was corrected for the contribution from the molar ellipticity of ephedrine alone, and plotted as a function of the ephedrine molar fraction (Figure 6). The difference in the molar ellipticity goes through a maximum value at an ephedrine molar fraction of 0.5. The results provides a clear evidence of a 1 : 1 stoichiometry between β-CD and ephedrine. Similar circular dichroism stoichiometric results have been reported for the binary complexes formed between β-CD and benzoic acid [51], β-CD and benz[b]anthracene [52], β-CD and ferrocenecarboxylic acid [53] and 4′, 4″, 4‴-(21*H*, 23*H*-porphine-5,10,15,20-tetrayl)tetrakis[trimethylammonium]tetrachloride and profavin [54].

3.6. Formation constant of the β-cyclodextrin/ephedrine complex

Circular dichroism has been used to evaluate the apparent formation constant and to estimate the orientation of the guest inside the CD cavity [31,33,54–56]. The variation of the molar ellipticity of ephedrine at 265 nm is used to estimate the apparent formation constant between β-CD and ephedrine. The composition ratio of β-CD to ephedrine has been established as 1 : 1; therefore, the equilibrium constant, K, for a 1 : 1 association between β-CD and ephedrine (EPH) is given by

$$\beta\text{-CD} + \text{EPH} \rightleftharpoons \beta\text{-CD/EPH} \tag{1}$$

$$K = \frac{[\beta\text{-CD/EPH}]}{([\text{EPH}]_0 - [\beta\text{-CD/EPH}])([\beta\text{-CD}]_0 - [\beta\text{-CD/EPH}])} \tag{2}$$

where $[\beta\text{-CD}]_0$ and $[\text{EPH}]_0$ are the initial analytical concentrations of β-CD and ephedrine, respectively, and [β-CD/EPH] is the equilibrium concentration of the inclusion complex for a given β-CD concentration. Since the concentration of β-CD is large with respect to that of the complex, Equation 2 becomes

$$K = \frac{[\beta\text{-CD/EPH}]}{([\text{EPH}]_0 - [\beta\text{-CD/EPH}])[\beta\text{-CD}]_0} \tag{3}$$

It should be noted that, in this study, only ephedrine and the complex, β-CD/EPH, have optical activity, and as such the ellipticity of the solution is shown by the following expression.

$$\Theta = [\Theta_{\text{EPH}}]([\text{EPH}]_0 - [\beta\text{-CD/EPH}]) + [\Theta_{\text{B-CD/EPH}}][\beta\text{-CD/EPH}] \tag{4}$$

where $[\Theta_{\text{EPH}}]$ and $[\Theta_{\beta\text{-CD/EPH}}]$ are the molar ellipticity coefficients for the free and complexed ephedrine, respectively. Combining Equations 3 and 4 and then rearranging, we obtain

$$\frac{[\beta\text{-CD}]}{\Delta\Theta} = \frac{[\text{EPH}] + [\beta\text{-CD}] - [\beta\text{-CD/EPH}]}{\Delta\Theta_{\beta\text{-CD/EPH}}} + \frac{1}{\Delta\Theta_{\beta\text{-CD/EPH}}K} \tag{5}$$

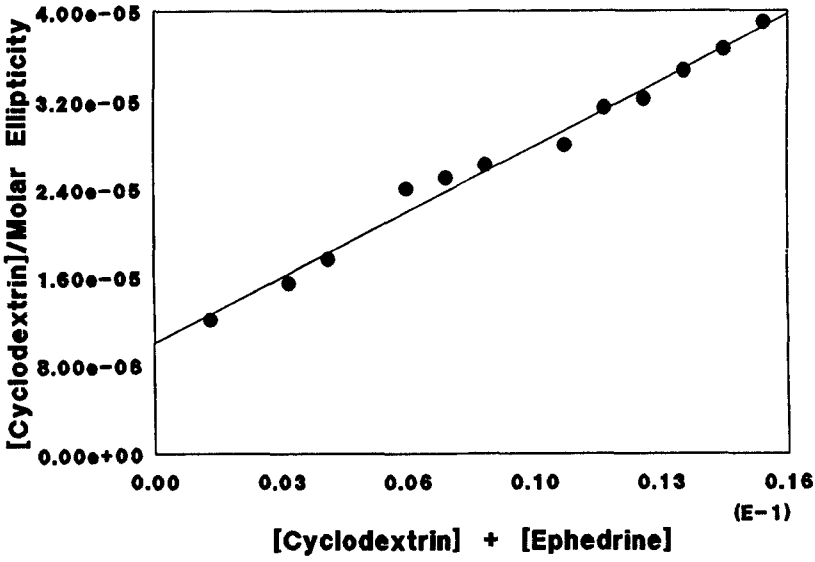

Fig. 7. The circular dichroism plot for the β-CD/ephedrine complex.

where

$$\frac{\Delta\Theta}{[\text{EPH}]} = \Theta - [\Theta_{\text{EPH}}] \tag{6}$$

$$\Delta\Theta_{\beta\text{-CD/EPH}} = [\Theta_{\beta\text{-CD/EPH}}] - [\Theta_{\text{EPH}}] \tag{7}$$

The apparent formation constant is first estimated approximately from a linear plot of $[\beta\text{-CD}]_0/\Delta\Theta$ vs $[\beta\text{-CD}]$ + [EPH] (Figure 7). Then, the best K is estimated from the plot of $[\beta\text{-CD}]/\Delta\Theta$ vs $[\beta\text{-CD}]$ + [EPH] – $[\beta$-CD/EPH] where $[\beta$-CD/EPH] is estimated by use of Equation 7. Finally, the best apparent formation constant, K, and the molar ellipticity of the complex, $[\Theta_{\beta\text{-CD/EPH}}]$ were simultaneously evaluated in a convergent iterative routine. The estimated binding strength was averaged for measurement at different wavelengths. The binding constant is estimated to be $177 \pm 10.0\ \text{M}^{-1}$ for the formation of a 1 : 1 β-CD/EPH complex.

While the apparent formation constant for the system under investigation here is relatively large, it should be noted that the formation constant will vary using HPLC and circular dichroism. Previous results for the formation of the β-CD/ephedrine complex by use of the HPLC method and assuming a 1 : 1 stoichiometry, provided a lower estimate of 27 and $30\ \text{M}^{-1}$ for (+)-ephedrine and (–)-ephedrine, respectively. The presence of acetonitrile and triethanolammonium acetate in the mobile phase to aid in a complete and efficient separation of diastereomers in the presence of β-CD, was thought to have contributed to the low values of the apparent formation constant. The low formation constant values cannot be solely attributed to the

presence of a second modifier in the mobile phase, but also to the fact that HPLC conditions are dynamic and the interactions may not be at equilibrium. In contrast, the estimated formation constant determined by use of circular dichroism was obtained with algorithms employing the initial concentration of β-CD. In addition, circular dichroism measurements were performed under the conditions that the solutions were allowed to equilibrate overnight before analysis; hence, the reactions should be complete.

4. Conclusion

The goal in this study was to determine the nature of the binding of ephedrine with β-CD. One dimensional NMR spectroscopy indicates an association between ephedrine and β-CD over the concentration range studied. Observed changes in the chemical shifts of the ephedrine resonances upon addition of β-CD suggest that the ephedrine phenyl moiety interacts with the β-CD cavity. This model was confirmed by 2D ROESY spectroscopy; crosspeaks were observed between the β-CD H-3, H-5, and H-6 signals and the signals of the ephedrine aromatic protons. The H-6 proton is located on the primary hydroxyl ring and the H-3 and H-5 protons are located within the β-CD cavity. No crosspeaks were observed between the β-CD H-1, H-2, and H-4 protons, located outside the β-CD cavity, and any ephedrine proton signals. The 1D ^{1}H NMR experiment indicated that at 7 mM β-CD, the complexation process has reached equilibrium, hence no more change in the NMR spectrum. Quantitative calculation of a binding constant, however, was precluded by the coalescence of all of the ephedrine aromatic resonances to a single chemical shift. This situation is being further studied using 2D heteronuclear experiments that are currently in progress. A binding constant of $177 \pm 10\,M^{-1}$ was determined using circular dichroism analysis. Further circular dichroism experiments that consider the interactions of the four optical isomers of ephedrine with β-CD are in progress in this laboratory.

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