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Journal of Chromatography A, 800 (1998) 327–332

JOURNAL OF CHROMATOGRAPHY A

^2H_2O effects on the inclusional complexation of β-cyclodextrin with sodium 2-naphthalenesulfonate in capillary electrophoresis and UV spectrophotometry

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Received 8 August 1997; received in revised form 14 October 1997; accepted 7 November 1997

Abstract

In capillary electrophoresis, deuterium isotope effects of ^2H_2O in the running buffer on the equilibrium constant (*K*) for the formation of an inclusion complex between β-cyclodextrin and sodium 2-naphthalenesulfonate (2NS) have been investigated. The *K* value for ^2H_2O is 27% greater than that for H_2O. A similar trend in the *K* value of 2NS has been obtained from the results employing a UV spectrophotometric method. © 1998 Elsevier Science B.V.

Keywords: Buffer composition; Deuterium isotope effects; Inclusion complexes; Sodium 2-naphthalenesulfonate; β-Cyclodextrins

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of six (α-CD), seven (β-CD) or eight (γ-CD) D-glucose residues. Because CDs are shaped like a truncated cone with a relatively hydrophobic cavity, they can accommodate a variety of organic compounds in their cavities to form inclusion complexes [1]. The formation of the inclusion complexes in aqueous solutions is affected by many factors such as pH. Phenol derivatives exist exclusively as neutral and anionic forms in acidic and alkaline solutions, respectively. The neutral and anionic forms show different binding abilities to form an inclusion complex with CDs [2,3]. Furthermore, CDs themselves exist as anions at pH values greater than 12.1 [4–6]. An equilibrium constant for the formation of an anionic CD–guest inclusion complex is different from that of a neutral CD–guest inclusion complex [7–9]. Solvents also affect the inclusional complexation between CDs and guests [10–13]. Although the inclusional complexation also occurs in organic solvents [14–16], it is generally depressed to a large degree compared to water. Since, in most cases, the inclusional complexation proceeds predominantly through the hydrophobic interaction between CD and a guest molecule, it is much more favorable for a guest to enter the CD cavity from a hydrophillic environment (water) compared to organic solvents which are less polar than water.

For *p*-nitrophenol, *p*-nitrophenolate, methyl orange, phenolphthalein and inorganic anions, Wang and Matsui have spectrophotometrically investigated the ^2H_2O effects on the equilibrium constants for the formation of inclusion complexes with α- and β-CDs [17]. They have revealed that the equilibrium con-

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0021-9673/98/$19.00
PII S0021-9673(97)01136-9

stants for these guests in ^2H_2O are 10–30% greater than those in H_2O. The observed increases in the equilibrium constant have been attributed mainly to a solvent $^2H/^1H$ isotope effect on hydrophobic and electrostatic interactions between the hosts and guests. The ^2H_2O effects on the equilibrium constant have similarly been examined for chlorogenic acid by Irwin et al. [18]. As in the cases of *p*-nitrophenol etc., about a 12% increase has been observed for an equilibrium constant in ^2H_2O relative to that in H_2O. In capillary electrophoresis, ^2H_2O buffers have been used to give enhanced resolution of a series of nucleotides and dansyl amino acids [19]. However, additives such as CDs have not been introduced in the ^2H_2O buffers.

In capillary electrophoresis using native or derivatized CDs, significant efforts have been directed to improvements in separation of various kinds of isomers including positional, structural, optical isomers, etc., for example, variations in pH values of running buffers, concentrations of CDs, organic modifiers such as alcohol, applied voltage, and so on [20–25]. In this respect, ^2H_2O may be used as a solvent modifier in improving the resolution in capillary electrophoresis. In this paper, we investigated the ^2H_2O effects on the equilibrium constant for the complexation between β-CD and sodium 2-naphthalenesulfonate by means of capillary electrophoresis as well as UV spectrophotometry.

2. Experimental

2.1. Chemicals

β-Cyclodextrin (β-CD), which was purchased from Nakalai Tesque, was recrystallized twice from water. Sodium 2-naphthalenesulfonate (2NS) from Tokyo Kasei Kogyo was recrystallized twice from ethanol. ^2H_2O (Uvasol; 99.8%) from Merck was used as received.

2.2. Samples

Sample solutions were prepared using boric acid–NaOH buffers (pH 10.0). The concentrations of 2NS for capillary electrophoresis and UV spectrophotometry were $5.0\cdot10^{-4}$ and $1.0\cdot10^{-4}$ mol dm^{-3}, respectively. In preparation of ^2H_2O solutions of 2NS, boric acid and NaOH, which were not deuterated, were used. p^{2}H values of ^2H_2O buffers were different from pH values of H_2O buffers; according to a relationship shown in a literature [26], a p^{2}H value corresponding to a pH of 10.0 was expected to be 10.4. As a pH value of running buffer is increased in capillary electrophoresis, the velocity of the electroosmotic flow is initially increased and reaches a plateau [27]. To perform experiments in the plateau region where the electroosmotic mobility does not scarcely vary with pH (p^{2}H), we selected H_2O solutions buffered at a pH of 10.0 as a running buffer.

2.3. Apparatus

Experiments in capillary electrophoresis were performed using a Hitachi U-2000 spectrophotometer and a Matsusada Precision Devices HCZE-30 PN as a detector and a high-voltage power supply, respectively. The detection wavelength of the spectrophotometer was 226 nm. The applied voltage was maintained at 15.0 kV throughout this work. A GL Science uncoated fused-silica capillary (70.0 cm× 0.05 mm I.D.) was used for capillary electrophoresis measurements. The effective length of the capillary was 30.0 cm (from an injection end to a detection window). Samples were hydrodynamically injected into the capillary tubing. Absorption spectra were recorded on a Shimadzu UV-260 spectrophotometer. All of the measurements were made at 25°C.

3. Results and discussion

3.1. Capillary electrophoresis of 2NS in H_2O buffers containing β-CD

Table 1 shows migration times of 2NS ($5.0\cdot10^{-4}$ mol dm^{-3}) in H_2O buffers (pH 10.0) containing several concentrations of β-CD. As the β-CD concentration is increased, the migration time of 2NS is shortened. Since 2NS has a negative charge, the formation of an inclusion complex of 2NS with β-CD increases the apparent molecular mass per charge for 2NS, leading to its slow electrophoretic

Table 1
Migration times of 2NS ($5.0\cdot10^{-4}$ mol dm^{-3}) in H_2O buffers (pH 10.0) and in ^2H_2O buffers (p^{2}H 10.4) at several β-CD concentrations

Concentration of β-CD/mol dm^{-3}	Migration time/s	
	H_2O	^2H_2O
0	452	641
$1.0\cdot10^{-3}$	380	509
$3.0\cdot10^{-3}$	322	443
$1.0\cdot10^{-2}$	291	402

flow-rate, which causes the shorter migration time in buffers containing β-CD.

When the electrophoretic mobilities for uncomplexed 2NS and a 1:1 inclusion complex of 2NS with β-CD are represented by μ_0 and μ_1, respectively, the observed mobility, μ_a, of 2NS in buffer is represented by:

$$\mu_a = (\mu_0 + \mu_1 K[\beta\text{-CD}]_0)/(1 + K[\beta\text{-CD}]_0) \quad (1)$$

where K and $[\beta\text{-CD}]_0$ are the equilibrium constant for the formation of the 1:1 β-CD–2NS inclusion complex and the initial concentration of β-CD, respectively. From Eq. (1), μ_1 is expressed as:

$$\mu_1 = \mu_a + (\mu_a - \mu_0)/(K[\beta\text{-CD}]_0) \quad (2)$$

When the migration times for a marker (neutral substance), a free guest (2NS), and the inclusion complex of β-CD with 2NS are represented by t_m, t_0, and t_1, respectively, and when the observed migration time for 2NS in buffer with β-CD is given by t_a, the following equations are derived:

$$\mu_0 = (1/t_m - 1/t_0)L/E \quad (3)$$

$$\mu_1 = (1/t_m - 1/t_1)L/E \quad (4)$$

$$\mu_a = (1/t_m - 1/t_a)L/E \quad (5)$$

Here, L and E are the length from an injection end to a detection window of a capillary and the electric field strength, respectively. Under the influence of the electric field, there is an electroosmotic flow (EOF) in a capillary. In Eqs. (3)–(5), the EOF is assumed to be identical to one another, although the slightly increased viscosity of buffer containing β-CD may slightly slow down the EOF; the t_m value in the presence of β-CD is assumed to be the same as that in the absence of β-CD. Throughout this work, the electric current, which is correlated with the magnitude of EOF [28], was little or not varied even in the presence of β-CD, suggesting that the above assumption is reasonable.

Substitutions of Eqs. (3)–(5) into μ_0, μ_1 and μ_a in Eq. (2), respectively, afford the following equation [29]:

$$1/t_a = 1/t_1 + (1/t_0 - 1/t_a)/(K[\beta\text{-CD}]_0) \quad (6)$$

Consequently, a K value can be evaluated from a slope of a plot of $1/t_a$ against $(1/t_0 - 1/t_a)/[\beta\text{-CD}]_0$. Fig. 1 exhibits the plot for 2NS, from which 450 mol^{-1} dm^3 is obtained as a K value. We also evaluated the K value according to the equation regarding mobilities [30]:

$$1/(\mu_a - \mu_0) = 1/(\mu_1 - \mu_0) + 1/((\mu_1 - \mu_0)K[\beta\text{-CD}]_0) \quad (7)$$

Eq. (7) is essentially equivalent to Eq. (6) because mobility is a function of time. From a plot based on Eq. (7), 380 mol^{-1} dm^3, which is about 15% smaller than the K value obtained from the plot based on Eq. (6), was evaluated as a K value.

From six separate experiments and analyses based on Eq. (6), a mean value of K is evaluated to be 450 ± 50 mol^{-1} dm^3. In a previous paper, we have estimated a K value of 2NS in phosphate H_2O buffers (pH 7.3) to be 480 ± 20 mol^{-1} dm^3 [29]. This

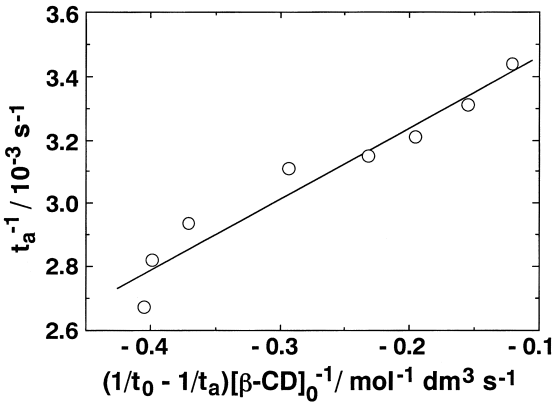

Fig. 1. Plot of $1/t_a$ against $(1/t_0 - 1/t_a)/[\beta\text{-CD}]_0$ for 2NS ($5.0\cdot10^{-4}$ mol dm^{-3}) in H_2O buffers (pH 10.0).

K value is nearly the same as the *K* value obtained in this study, suggesting that the buffer and pH exert little or no effects on the *K* value. As a mean *K* value from the analyses based on Eq. (7), 400±40 $mol^{-1}\,dm^3$ was estimated, which is about 11% less than the mean *K* value from the analyses based on Eq. (6).

A reported *K* value of 2NS, which has been based on a spectrophotometric method, is 340 $mol^{-1}\,dm^3$ [31]. An equilibrium constant for the self-association of the 1:1 β-CD–2NS inclusion complexes has also been evaluated to be 150 $mol^{-1}\,dm^3$ [31]. Even at the highest β-CD concentration of $1.0\cdot10^{-2}$ mol dm^{-3} in the present study, however, the concentration of the 2:2 β-CD–2NS inclusion complex is calculated to be less than 5% of the initial concentration ($5.0\cdot10^{-4}$ mol dm^{-3}) of 2NS by using the reported equilibrium constants, while the concentration of the 1:1 β-CD–2NS inclusion complex is calculated to be about 81% of the initial concentration of 2NS at the same concentration of β-CD. In this work, therefore, the existence of the 2:2 inclusion complex is negligible relative to the 1:1 inclusion complex. The *K* value (450±50 $mol^{-1}\,dm^3$) obtained on the basis of Eq. (6) is comparable to the reported *K* value (340 $mol^{-1}\,dm^3$), although the two evaluation methods are different from each other.

3.2. Capillary electrophoresis of 2NS in ^2H_2O buffers containing β-CD

In this study, p^2H values of ^2H_2O buffers were measured to be 10.4. The p^2H values are consistent with a reported relationship of $p^2H = pH + 0.4$ [26], since the pH values of the H_2O buffers were evaluated to be 10.0. As shown in Table 1, the migration time of 2NS in ^2H_2O buffer without β-CD is about 40% longer than that in H_2O buffer without β-CD. At 25°C, the viscosity (1.101 mPa s) of ^2H_2O is 1.23 times greater than that (0.893 mPa s) of H_2O. Consequently, the longer migration time for ^2H_2O is predominantly due to the increased viscosity of ^2H_2O compared to that of H_2O.

Fig. 2 illustrates a plot of $1/t_a$ against $(1/t_0-1/t_a)/[\beta\text{-CD}]_0$ for 2NS in ^2H_2O buffers. From four independent plots of $1/t_a$ against $(1/t_0-1/t_a)/[\beta\text{-CD}]_0$, a mean *K* value has been evaluated to be

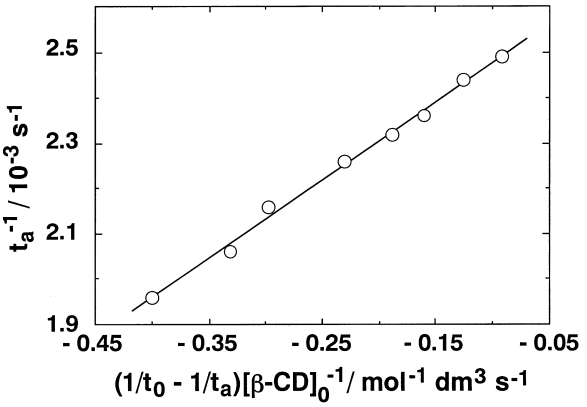

Fig. 2. Plot of $1/t_a$ against $(1/t_0-1/t_a)/[\beta\text{-CD}]_0$ for 2NS ($5.0\cdot10^{-4}$ mol dm^{-3}) in ^2H_2O buffers (p^2H 10.4).

570±20 $mol^{-1}\,dm^3$. This *K* value for ^2H_2O is 27% greater than that in H_2O. As stated previously, a similar trend that the *K* values in ^2H_2O are 10–30% greater than that in H_2O has been reported for *p*-nitrophenol etc. [17]. From double reciprocal plots based on Eq. (7), 530±30 $mol^{-1}\,dm^3$ was estimated as a mean *K* value, which is about 7% less than that obtained on the basis of Eq. (6). Consequently, a trend that the *K* value for ^2H_2O buffer is about 30% greater than that for H_2O buffer is the same in spite of the different analyses. It is known that hydrogen bonding of ^2H_2O is stronger than that of H_2O; as a medium, ^2H_2O possesses a highly ordered structure compared to H_2O. Consequently, environments surrounding β-CD and free 2NS molecules in ^2H_2O are varied from those in H_2O to some extent. This seems to enhance the inclusional complexation of β-CD and 2NS in ^2H_2O. In capillary electrophoresis, the ^2H_2O buffers cause the long migration time and the increased *K* value compared to the H_2O buffers. In some cases, therefore, the use of ^2H_2O may be useful in improving the resolution of substances.

3.3. Spectrophotometric investigation of an inclusion complex between β-CD and 2NS in H_2O buffers

Fig. 3 depicts absorption spectra of 2NS ($1.0\cdot10^{-4}$ mol dm^{-3}) in H_2O buffers containing several concentrations of β-CD. As the β-CD concentration is increased, the absorption peak at 274 nm is

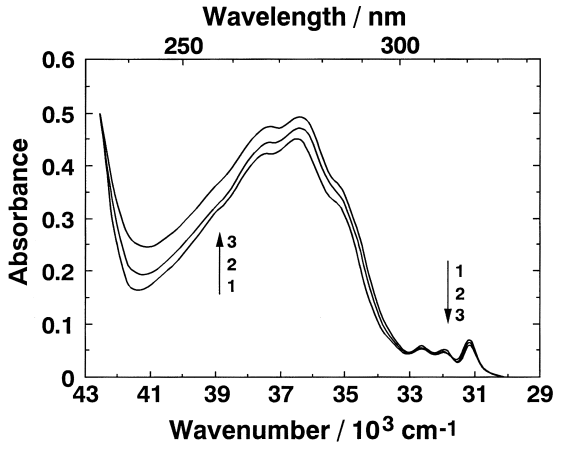

Fig. 3. Absorption spectra of 2NS ($1.0\cdot10^{-4}$ mol dm^{-3}) in H_2O buffers (pH 10.0) containing several concentrations of β-CD. Concentration of β-CD: (1) 0, (2) $2.0\cdot10^{-3}$, and (3) $5.0\cdot10^{-3}$ mol dm^{-3}.

slightly shifted to longer wavelengths, accompanied by an increase in absorption intensity of the 274-nm band. An isosbestic point is observed at 303 nm, indicating an equilibrium between free 2NS and the 1:1 β-CD–2NS inclusion complex. In such a case, a relationship holds between the absorbance (A) and the initial concentration of β-CD [32,33]:

$$1/(A - A_0) = 1/a + 1/(aK[\beta\text{-CD}]_0) \quad (8)$$

where A, A_0, and a are the absorbances of 2NS in the presence and absence of β-CD, and a constant including the 2NS concentration, respectively. Fig. 4

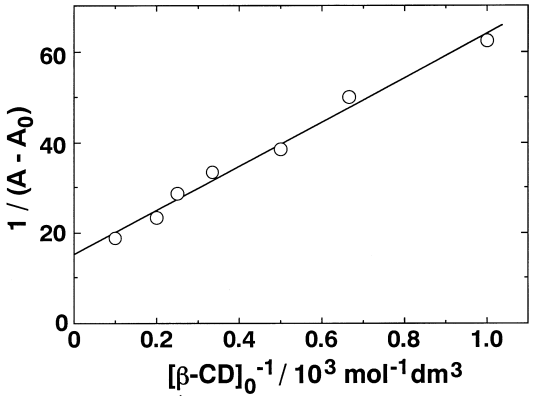

Fig. 4. Double reciprocal plot for 2NS ($1.0\cdot10^{-4}$ mol dm^{-3}) in H_2O buffers (pH 10.0) containing β-CD.

shows a double reciprocal plot of $1/(A-A_0)$ against $1/[\beta\text{-CD}]_0$ for 2NS ($1.0\cdot10^{-4}$ mol dm^{-3}) in H_2O buffers (pH 10.0). From this plot, a K value for 2NS is estimated to be 260 mol^{-1} dm^3. Averaging K values obtained from three independent experiments utilizing UV spectrophotometry, we obtained 240 ± 40 mol^{-1} dm^3 as the K value. This K value obtained from the spectrophotometric method is about half of that from the method employing capillary electrophoresis. In phosphate H_2O buffers (pH 7.3), 220 ± 30 mol^{-1} dm^3 has been evaluated as a K value, employing spectrophotometry [29]. This K value is nearly the same as that obtained in this study.

For phosphate H_2O buffers of pH 7.3, a similar result concerning the difference in the magnitudes of K values between the two different methods (capillary electrophoresis and spectrophotometry) has been observed [29]. As noted previously, the difference in the magnitude of K is not likely to be due to the buffer and pH. Although at present, it is unclear why the K values obtained from the two kinds of methods are different from each other, the difference in K may arise from some interactions between a capillary surface and 2NS (or inclusion complex). With respect to the method using spectrophotometry, the K value (240 ± 40 mol^{-1} dm^3) obtained in this work is comparable to the reported one (340 mol^{-1} dm^3).

3.4. Spectrophotometric investigation of an inclusion complex between B-CD and 2NS in ^2H_2O buffers

When the β-CD concentration was increased, a change in absorption spectrum of 2NS in ^2H_2O buffer (pH 10.4) was similar to that in H_2O (pH 10.0), indicating the formation of the 1:1 β-CD–2NS inclusion complex in ^2H_2O buffers. The K value of 2NS in ^2H_2O buffer (pH 10.4) was evaluated to be 350 ± 60 mol^{-1} dm^3, which was averaged from the data of three independent experiments based on Eq. (8). This K value for ^2H_2O is 46% greater than that (240 ± 40 mol^{-1} dm^3) for H_2O. The trend that the K value for ^2H_2O is greater than that for H_2O is parallel to that obtained from capillary electrophoresis. Using a near-infrared spectroscopic method, a K value for 2NS in ^2H_2O has been evaluated to be

205 $mol^{-1}\,dm^3$ [34], which is about 60% of the K value obtained from UV spectrophotometry.

4. Conclusion

In capillary electrophoresis, substitution of ^2H_2O for H_2O in mobile phase lengthens the migration times of 2NS. The longer migration times for ^2H_2O are most likely due to the increased viscosities of ^2H_2O buffers compared to H_2O buffers. For the β-CD–2NS system in capillary electrophoresis, the K value for ^2H_2O is 27% greater than that for H_2O. In UV spectrophotometry, the deuterium isotope effects of ^2H_2O on K are also observed for the β-CD–2NS system. In this case, a 46% greater K value for ^2H_2O has been obtained relative to the K value for H_2O.

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