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Ion-exclusion chromatography using mobile phases containing β-cyclodextrin

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ABSTRACT

Ion-exclusion chromatography of some aromatic carboxylic acids is performed using an Aminex HPX-87H organic acids column with a mobile phase comprising ethanol–water (20:80, v/v) containing camphorsulphonic acid and β-cyclodextrin. Retention volumes of the solutes increased with increasing mobile phase concentration of camphorsulfonic acid. On the other hand, some solutes (gallic, *o*-nitrobenzoic, acetylsalicylic and *m*-nitrobenzoic acids) showed a decrease in retention volume when the mobile phase concentration of β-cyclodextrin was increased. This behaviour was attributed to the formation of inclusion complexes in the mobile phase, leading to an equilibrium shift which decreases the amount of solute in the stationary phase and hence its retention. This provides an additional parameter for control of the solute retention. A theoretical equilibrium model is presented which considers the equilibrium reactions occurring in the chromatographic system. Retention data are then used to evaluate the equilibrium constants for these reactions. In this way, the inclusion constant for each solute may be determined.

INTRODUCTION

Ion-exclusion chromatography, first introduced by Wheaton and Baumann [1] in 1953, involves the use of strong anion- or cation-exchange resin for the separation of ionic solutes from weakly ionized or neutral solutes. In this mode of chromatography ionic solutes tend to be repelled (or excluded) from the resin, whilst neutral solutes are able to penetrate the resin and are hence retained. In addition to the charge on the solute, several other factors are known to play a part in the retention process; these include the size and especially the hydrophobicity of the solute [2].

The mechanism by which acidic solutes may be separated by ion-exclusion chromatography using a cation-exchange resin has been described previously [3–6]. We now introduce a further parameter which exerts an influence on retention in this form of chromatography, namely the concentration of an inclusion compound in the eluent. β-Cyclodextrin (CD) was selected as a model inclusion compound. The most characteristic property of cyclodextrins is their remarkable ability to form molecular inclusion compounds with some organic and inorganic compounds and also with ions. The stereoselective nature of CD inclusion has been exploited in high-performance liquid chromatography (HPLC) through the use of chemically bonded CD–silica stationary phases and by the application of CD as a mobile phase component. CD was first utilized as a mobile phase modifier by Uekama et al. [7] for ion-exchange chromatography and later applied [8–11] to reversed-phase HPLC. The main purpose of the present paper is to demonstrate that CD may also be utilized as an eluent component in ion-exclusion chromatography. A simple scheme describing the separation process occurring in this system is proposed and equations relating the distribution coefficient of a solute to the concentration of CD are derived. These equations are used to calculate in-

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clusion constants for some aromatic carboxylic acids.

THEORY

The ion-exclusion system under study consists of an eluent comprising an acid buffer (designated as HB) and CD, used in conjunction with a sulfonated cation-exchange resin. Aromatic carboxylic acids (designated as HR) are used as solutes. This system can be described after making some simplifying assumptions (some of which are similar to those used previously [3–6]: (i) An equilibrated column is used, so that the concentrations of the buffer and CD in the eluent remain constant during the experiment. (ii) The functional (sulphonic acid) groups on the resin are completely dissociated and their concentration is much higher than that of the solute. (iii) Only neutral molecules (that is undissociated solute, HR, and the inclusion complex, CD–HR) exist in the stationary phase. (iv) Both neutral and ionic species exist in the mobile phase. (v) The eluent buffer acid is completely dissociated and its concentration is much higher than that of the solute. (vi) CD exists only in the mobile phase [9,10] and its concentration is much higher than that of the solute. (vii) Eluent components (especially the buffer) do not form inclusion compounds with CD.

From assumption (v) we can write:

$$c_B = [B^-]_m \tag{1}$$

where c_B is the analytical buffer concentration and the subscript m refers to the mobile phase. Since the hydrogen ions originate only from the buffer dissociation, then:

$$c_B = [H^+]_m \tag{2}$$

From assumptions (vi) and (vii) we can write:

$$c_D = [CD]_m \tag{3}$$

where c_D is the analytical concentration of CD and $[CD]_m$ is the concentration of this species in the eluent. The chromatographic system can be described by the set of equilibria illustrated in Fig. 1. These equilibria incorporate the acid dissociation constant of the sample (K_a), the partition coefficients of the solute (K_1) and the inclusion complex (K_2), the formation constants for inclusion complexes of HR and R^- (K_3–K_7), and the acid dissociation constant of the inclusion complex (K_8). Expressions for these equilibrium constants are given below:

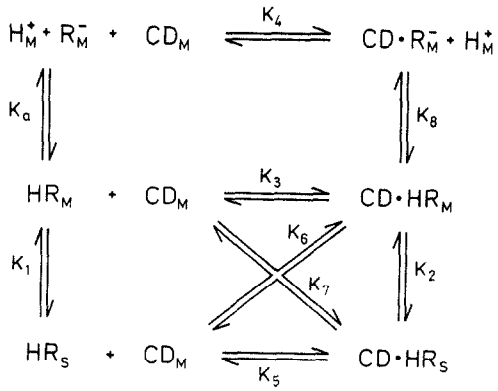

Fig. 1. Schematic representation of the equilibrium reactions existing in the chromatographic system under study.

$$K_a = \frac{[H^+]_m [R^-]_m}{[HR]_m} \tag{4}$$

$$K_1 = \frac{[HR]_s}{[HR]_m} \tag{5}$$

$$K_2 = \frac{[CD\text{–}HR]_s}{[CD\text{–}HR]_m} \tag{6}$$

$$K_3 = \frac{[CD\text{–}HR]_m}{[CD]_m [HR]_m} \tag{7}$$

$$K_4 = \frac{[CD\text{–}R^-]_m}{[CD]_m [R^-]_m} \tag{8}$$

$$K_5 = \frac{[CD\text{–}HR]_s}{[CD]_m [HR]_s} \tag{9}$$

$$K_6 = \frac{[CD\text{–}HR]_m}{[CD]_m [HR]_s} \tag{10}$$

$$K_7 = \frac{[CD\text{–}HR]_s}{[CD]_m [HR]_m} \tag{11}$$

$$K_8 = \frac{[CD\text{–}R^-]_m [H^+]_m}{[CD\text{–}HR]_m} \tag{12}$$

where the subscript s refers to the stationary phase. The overall distribution coefficient for the solute HR in the presence of CD is given by the concentration ratio of all forms of HR in the stationary phase to that in the mobile phase. That is:

$$K_9 = \frac{[HR]_s + [CD\text{–}HR]_s}{[HR]_m + [R^-]_m + [CD\text{–}HR]_m + [CD\text{–}R^-]_m} \quad (13)$$

K_9 can be calculated from the chromatographic retention data, as follows:

$$K_9 = \frac{V_R - V_m}{V_s} \quad (14)$$

where V_R, V_m and V_s are the retention, void and inner volumes, respectively. Under conditions where CD is absent from the mobile phase, the overall distribution coefficient becomes:

$$K_{10} = \frac{[HR]_s}{[HR]_m + [R^-]_m} \quad (15)$$

$$K_1 = \frac{K_{10}(c_B + K_a)}{c_B} \quad (16)$$

and from eqns 7–9, 13 and 16 it is possible to derive

$$K_9 = \frac{K_1 c_B + K_1 K_5 c_D c_B}{c_B + K_a + K_3 c_D c_B + K_4 K_a c_D} \quad (17)$$

Values of K_3, K_4 and K_5 can be estimated from values of K_9 obtained at different values of c_D and c_B. Thus, K_5 can be calculated by using two concentrations of CD, designated by the superscripts (1) and (2), using the equation:

$$K_5 = [K_1 c_B K_9^{(1)} K_9^{(2)} c_D^{(1)} c_D^{(2)} + K_9^{(1)} K_9^{(2)} c_D^{(1)} c_D^{(2)} (K_a + c_B)]/[K_1 c_B c_D^{(1)} c_D^{(2)} (K_9^{(2)} - K_9^{(1)})] \quad (18)$$

Similarly, K_3 and K_4 can be calculated using two concentrations of the eluent buffer [again designated by the superscripts (1) and (2)], using the equations:

$$K_3 = [K_1 K_9^{(1)} K_9^{(2)} c_B^{(1)} c_B^{(2)} (K_5 c_D + 1) + K_9^{(1)} K_9^{(2)} c_B^{(1)} c_B^{(2)}]/K_9^{(1)} K_9^{(2)} c_B^{(1)} c_B^{(2)} c_D \quad (19)$$

and

$$K_4 = (K_1 c_B^{(1)} + K_1 K_5 c_B^{(1)} c_D - K_9^{(1)} c_B^{(1)} - K_9^{(1)} K_a - K_3 K_9^{(1)} c_B^{(1)} c_D)/K_a K_9^{(1)} c_B^{(1)} \quad (20)$$

The remaining constants can then be calculated from the following equations:

$$K_7 = K_1 K_5 \quad (21)$$

$$K_6 = \frac{K_3}{K_1} \quad (22)$$

$$K_2 = \frac{K_7}{K_3} = \frac{K_5}{K_6} \quad (23)$$

$$K_8 = \frac{K_a K_4}{K_3} \quad (24)$$

EXPERIMENTAL

Instrumentation

Chromatographic studies were performed using a Millipore-Waters (Milford, MA, USA) Model 510 HPLC pump, a U6K universal injector and a Model 484 Tunable Absorbance Detector. Chromatograms were recorded using an ABB (Vienna, Austria) SE 120 recorder. The column used was a Bio-Rad (Richmond, CA, USA) Aminex HPX-87H organic acid analysis column (300 × 7.8 mm I.D.). The void and inner volumes of this column were determined by the method described in ref. 3 and were found to be 3.4 ml and 9.2 ml, respectively.

Reagents

CD was obtained from Chinoin (Budapest, Hungary) and (1*R*)-(−)-10-camphorsulphonic acid (98%) from Aldrich (Milwaukee, WI, USA). Other reagents were of analytical-reagent grade and were used without further purification. Water was triply distilled and was passed through a Millipore (Bedford, MA, USA) Milli-Q water purification apparatus. Eluents were filtered through a 0.45-μm membrane filter and were degassed in an ultrasonic bath prior to use.

RESULTS AND DISCUSSION

Chromatograms of solute acids (2-naphthol-6-sulphonic, sulphanilic, barbituric, gallic, *o*-nitrobenzoic, acetylsalicylic, salicylic and *m*-nitrobenzoic) with the mobile phases containing different concentrations of CD and camphorsulphonic acid (used here as a buffer) are presented in Figs. 2 and 3, respectively. The dependence of retention volume of the analysed acids on CD concentration is given in Fig. 4. These figures show that the addition of CD to the mobile phase reduces the retention volume of solutes forming inclusion complexes. The same effect has been observed in reversed-pase HPLC [8,10]. In contrast, an increase in the concen-

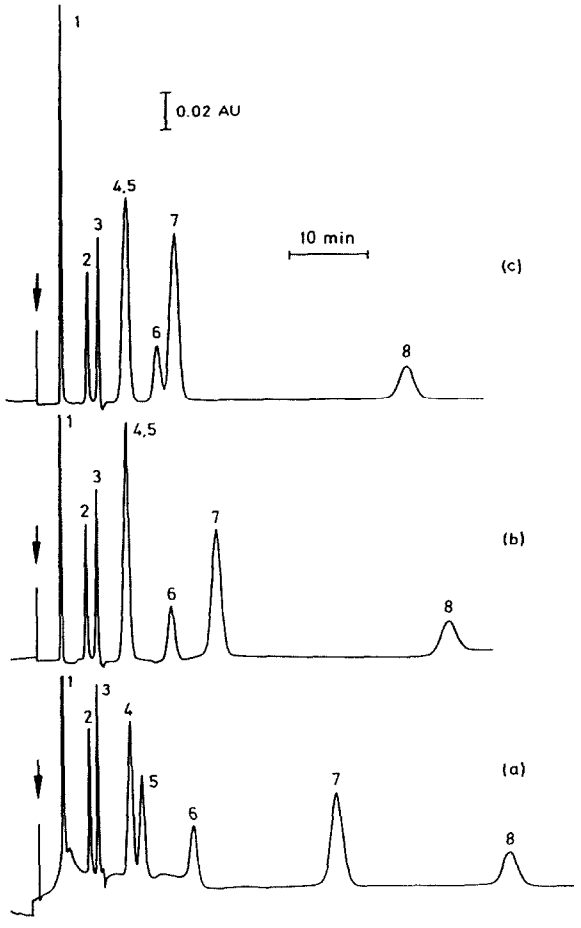

Fig. 2. Chromatograms of 2-naphthol-6-sulphonic (1), sulphanilic (2), barbituric (3), gallic (4), *o*-nitrobenzoic (5), acetylsalicylic (6), salicylic (7) and *m*-nitrobenzoic (8) acids at different CD concentrations in the mobile phase: (a) 0; (b) $5 \cdot 10^{-3}$ and (c) $1 \cdot 10^{-2}$ *M*. Conditions: Aminex HPX-87H organic acids column. Mobile phase: ethanol–water (20:80, v/v) containing 10^{-3} *M* camphorsulphonic acid and the indicated concentrations of CD, operated at a flow-rate of 0.5 ml/min.

tration of camphorsulphonic acid causes an increase in the retention volume. This results from the decreased dissociation of the solute acids and is in accordance with previous observations on retention in ion-exclusion chromatography [3,4]. Calculated values (from eqns. 16–24) of the thermodynamic constants are presented in Table I, with the exception of those for 2-naphthol-6-sulphonic acid, sulphanilic acid and barbituric acid since addition of CD to the mobile phase produced virtually no changes in retention for these species.

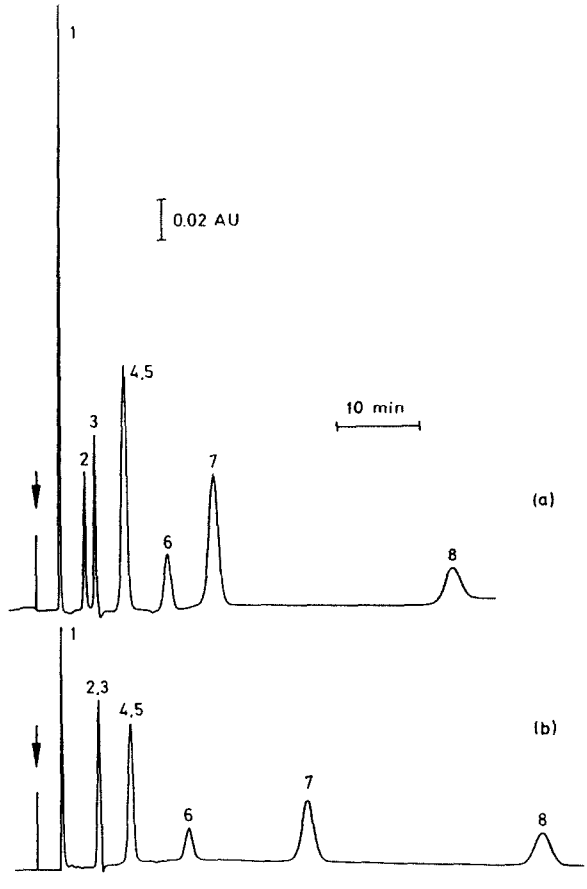

Fig. 3. Chromatograms of aromatic acids at different camphorsulphonic acid concentrations: (a) 10^{-3} *M*, (b) 10^{-2} *M*. Other conditions as for Fig. 2.

When a purely ion-exclusion mechanism operates, a dependence between the retention volume of a solute and its acid dissociation constant is observed. Retention volumes fall in the range between the column void volume and the sum of column void and inner volumes. The distribution coefficient falls in the range between zero (for strong acids, which are anionic and therefore are completely excluded) and one (for neutral solutes, which have unrestricted access to the occluded liquid in the stationary phase). In our experiments the mechanism for solute retention is rather more complex, as evidenced by the fact that the distribution coefficients (K_{10}) of aromatic carboxylic acids in the absence of CD are greater than 1. This is caused by hydrophobic interactions (K_1) between the solute and the resin and this effect is especially high on the polystyrene–divi-

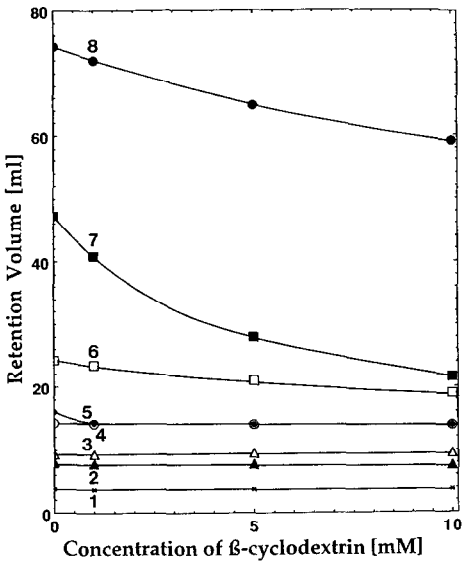

Fig. 4. Dependence of the retention volume of aromatic acid solutes on the concentration of CD in the mobile phase. Chromatographic conditions as for Fig. 2.

nylbenzene resin used as a result of π–π interactions. Of course, the distribution coefficient K_{10} is controlled not only by K_1 but also by the solute dissociation constant K_a.

The situation becomes even more complicated after the addition of CD to the mobile phase. For example the adsorption of the inclusion complex CD–HR (described by the constant K_2) is influenced by two dissociation constants (K_a and K_8). From a mathematical standpoint all unknowns can be obtained by solving eqns. 1–8, 13 and 15. Only the constants K_1–K_4 are necessary to describe the system, with the remaining constants being dependent. The constants K_3–K_7 each describe different mechanisms which might contribute to the retention of the solute. The values shown in Table I suggest that no single mechanism is predominant. However, some general trends may be elucidated.

It is known [8,9] that CD shows greatest interactions with aromatic compounds of the type investigated in this study. Some structural effect can also be observed by comparing the thermodynamic constants describing the formation of inclusion complexes (K_3–K_7 in Table I) for *o*- and *m*-nitrobenzoic acids. For each constant, the value obtained for *o*-nitrobenzoic acid is the higher, showing that the *ortho* isomer forms the stronger inclusion complexes. This behaviour probably results from steric effects and from the fact bonding with CD is more prevalent for the *ortho* isomer.

As can be seen from Fig. 1, solute complexation resulting in the formation of inclusion complexes existing in the mobile phase (K_3, K_4 and K_6) will result in decreased solute retention. Additionally, the inclusion complexes of the acids studied are characterised by smaller hydrophobic adsorption on the stationary phase, as evidenced by the relative magnitudes of K_1 and K_2 in Table I. These factors combine to suggest that decreased solute retention should be expected when inclusion complexes are formed, especially when the retention of the solute is high in the absence of CD. This behaviour is evident in Fig. 4. Comparison of K_3 and K_6 in Table I also suggests that the complexation of the adsorbed solute by CD is smaller than that for the solute in the mobile phase.

In conclusion we can state that it is possible to apply CD as a mobile phase modifier in ion-exclusion chromatography and that this modifier permits the attainment of selectivity effects which differ from those achieved by the conventional method of varying the pH of the mobile phase. Moreover, it is

TABLE I

VALUES OF THE THERMODYNAMIC CONSTANTS DEFINED BY EQNS. 5–15

Solute acid	K_a	K_1	K_2	K_3	K_4	K_5	K_6	K_7	K_8	K_{10}
Sulphanilic	$4.68 \cdot 10^{-4}$	0.73	3.0	177	250	200	241	730	$6.6 \cdot 10^{-4}$	0.5
Barbituric	$9.23 \cdot 10^{-5}$	0.7	0.8	159	54	159	229	187	$3.1 \cdot 10^{-5}$	0.6
Gallic	$3.89 \cdot 10^{-5}$	1.23	0.8	470	860	481	384	375	$1.1 \cdot 10^{-4}$	1.2
o-Nitrobenzoic	$6.76 \cdot 10^{-3}$	10.6	1.3	298	178	332	281	378	$4.0 \cdot 10^{-5}$	1.4
Acetylsalicylic	$2.69 \cdot 10^{-5}$	2.33	2.4	16	138	13	7.0	38	$2.3 \cdot 10^{-3}$	2.3
Salicylic	$1.00 \cdot 10^{-3}$	9.52	0.8	265	126	22	28	210	$4.8 \cdot 10^{-4}$	4.8
m-Nitrobenzoic	$3.2 \cdot 10^{-4}$	10.2	3.3	69	8.7	21	6.8	227	$4.1 \cdot 10^{-5}$	7.7

interesting to note that a few simple experiments enable us to calculate many thermodynamic constants. These constants are of fundamental importance in other chromatographic and electrochemical techniques which use CD. Whilst the precision of chromatographic methods for the calculation of such constants is not high, these methods are very rapid.

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