ELSEVIER

Journal of Chromatography A, 722 (1996) 41–46

JOURNAL OF CHROMATOGRAPHY A

Binding forces contributing to reversed-phase liquid chromatographic retention on a β-cyclodextrin bonded phase

Tae Hwa Nah, Eun Hee Cho, Myung Duk Jang, Young Kyu Lee, Jung Hag Park*

Department of Chemistry, Yeungnam University, Kyongsan 712-749, South Korea

Abstract

Reversed-phase liquid chromatographic (RPLC) capacity factors for a number of organic solutes on a β-cyclodextrin (β-CD) bonded phase column in pure water mobile phase (k'_w) were compared with formation constants of inclusion complexes (K_f) between β-CD and the solutes based on the linear solvation energy relationship (LSER) in order to understand the types and relative strengths of various intermolecular forces between CD and the guest solute affecting the stability of inclusion complexes and hence retention in RPLC. A close fit of capacity factors (k'_w) with complexation constants indicated that inclusion complexation is the major driving force in retention on the β-CD-bonded phase in RPLC. Comparison of LSERs for K_f and k'_w showed that an increasing guest molecular size stabilizes the complex by virtue of increasing dispersive interactions between the hydrophobic interior of CD cavity and the guest and hence increases the RPLC retention, and that increasing guest dipolarity and hydrogen bond (HB) acceptor basicity lead to a decrease in the stability of the complex due to the stronger dipolar and HB interactions with water, and hence decrease the retention.

1. Introduction

Cyclodextrins (CD) are cyclic oligosaccharides traditionally formed by the action of *Bacillus macerans* amylose on starch. CDs contain six to twelve glucose units which are bonded through α-(1,4) linkages. Among them, the three smallest homologues are commercially available [1]. They have the shape of a hollow truncated cone, the interior of which forms a relatively hydrophobic cavity. The ability of CDs to form inclusion complexes with a variety of compounds has been extensively utilized in many industrial, pharmaceutical, agricultural and other applications [1,2]. CDs have also been extensively employed in separation science and their applications in liquid chromatography have recently been reviewed [3–5].

Although numerous papers have been published on binding forces affecting inclusion complexation of CD with the guest, the nature of the driving forces of complexation are not fully understood. However, it is widely accepted that the stabilities of CD inclusion complexes are governed by several forces, such as hydrogen bonding, hydrophobic interactions, dispersive interactions, dipolar interactions, release of distortional energy of and extrusion of high-energy water from CD upon inclusion of a guest and the molecular size and shape of the guest [1,2]. To date, only a few studies have been carried out to characterize the cavity of the CDs with regard to their polarity [6–8]. CDs have, depending on their size, 18–24 primary and secondary hydroxyl

* Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)00455-6

groups on their upper and lower rims, which may undergo hydrogen bonding (HB) interactions with incoming guest molecules. We have recently reported Kamlet–Taft solvatochromic HB donor acidity values (α) of (CDs) [9]. Despite the relevance of CD–guest interactions to several important areas of chemistry and biology, the types and quantitative estimation of relative importance of CD–guest interactions influencing the stabilities of CD inclusion complexes in aqueous media has not been studied in detail. Recently, we reported [10] the complexation constants (K_f) of β-CD with a number of organic solutes in water and examined them to obtain a better insight into the type and relative strength of CD–analyte interactions affecting the stability of the complexes based on linear solvation energy relationships [11,12].

Since the introduction of silica-bonded cyclodextrins as HPLC stationary phases, they have been widely used in separations of positional, geometrical and optical isomers as well as simple molecules in both normal- and reversed-phase LC [4,5]. The major contributor to the separation of these compounds is inclusion complexation and the stabilities of these complexes should affect the elution of the compounds. Factors governing the separation have been discussed by several groups [13–15]. It is known that for chiral recognition the analyte needs to fit into the CD cavity as closely as possible [13]. In addition to the size requirement, HB interactions of the analyte with hydroxyl groups of CD play a role [14] but are not essential to chiral recognition [15].

Arnold et al. [16] correlated energies of the molecular model (MM2)-optimized structures of inclusion complexes between substituted aromatic compounds and β-CD with RPLC retention times. A direct correlation was found between the inclusion complex stability and the retention times within a series of disubstituted aromatic compounds, indicating that inclusion complexation is the major driving force in the separation of these aromatics on a β-CD-bonded phase. However, the types and relative importance of CD–guest interactions influencing RPLC retention on a β-CD-bonded stationary phase have not been studied in detail.

In this work, we measured RPLC capacity factors (k') of a number of organic solutes on a β-CD-bonded silica stationary phase and compared them with the K_f values based on the linear solvation energy relationship (LSER) to understand the types and strengths of intermolecular interactions affecting retention in RPLC on β-CD-bonded stationary phases. It has been demonstrated that many disparate physicochemical, biochemical, toxicological and pharmacological properties of organic nonelectrolytes that depend on solute–solvent interactions and aqueous solubilities in a variety of media can be correlated, rationalized and predicted by the application of this methodology. Examples include octanol–water [17–19] and triolein–water partition coefficients [20], gas–blood partition coefficients [21], aqueous solubilities [22], inhibition of bioluminescence in *Photobacterium phosphoreum* (the Microtox test) [23], toxicities to the Golden Orfe fish [24] and binding to bovine serum albumin [20], biconcentration factors in fish [25] and retention behaviour of solutes in gas and liquid chromatography [26–32].

The LSER for a property (SP) of a solute that depends on solute–solvent interactions is given by

$$SP = SP_0 + mV_x/100 + s\Sigma\pi^* + b\Sigma\beta + a\Sigma\alpha \quad (1)$$

The $mV_x/100$ term measures a combination of the endoergic cavity formation process and exoergic dispersive interactions. V_x is the solute hard core volume calculated according to McGowan and co-workers [33,34]. The $s\Sigma\pi^*$ term measures exoergic dipole–dipole and dipole–induced dipole interactions. The $\Sigma\pi^*$ parameter measures the ability of a solute molecule to stabilize a neighbouring charge or dipole, and to induce a dipole in a neighbouring non-dipolar molecule [35]. Exoergic effects of HB interactions are measured by the $b\Sigma\beta$ and $a\Sigma\alpha$ terms. The $\Sigma\beta$ and $\Sigma\alpha$ parameters measure HB acceptor basicity and HB donor acidity, respectively [35]. SP_0 denotes the value of SP when all the interaction terms in the equation are zero. The coefficients m, s, b and a are obtained by multiple linear regression of SP vs. the solute parameters. The sign and magnitude of the

Table 1
Solute physical properties, inclusion complexation constants and capacity factors on the β-CD-bonded phase in water

Solute	$V_x/100$	$\Sigma\pi_2^*$	$\Sigma\beta_2$	$\Sigma\alpha_2$	K_f	k'_w
Acetaldehyde	0.406	0.67	0.45	0	0.23	0.30
Acetone	0.547	0.70	0.49	0.04	2.61	0.10
Acetonitrile	0.404	0.90	0.32	0.07	0.54	0.04
Tetrahydrofuran	0.622	0.52	0.48	0	29.60	0.39
Benzene	0.716	0.52	0.14	0	67.21	1.33
Toluene	0.857	0.52	0.14	0	124.47	2.23
Nitrobenzene	0.891	1.11	0.28	0	110.81	1.75
Benzaldehyde	0.873	1.00	0.39	0	59.70	1.22
Aniline	0.816	0.88	0.72	0.10	39.48	0.55
Benzyl alcohol	0.916	0.87	0.56	0.33	51.76	0.59
Methanol	0.308	0.44	0.47	0.43	0.11	
Ethanol	0.449	0.42	0.48	0.37	0.94	0.01
2-Propanol	0.590	0.36	0.56	0.33	4.23	0.04
1-Butanol	0.731	0.42	0.48	0.37	14.71	0.22
Cyclohexanol	0.904	0.54	0.57	0.32	572.67	2.55
Chloroform	0.617	0.49	0.02	0.15	27.01	0.79
Tetrachloromethane	0.739	0.38	0	0	160.04	7.21
Diethylamine	0.772	0.30	0.69	0	23.14	0.25
Dimethylsulfoxide	0.613	1.74	0.88	0	1.44	0.11
Dimethylformamide	0.647	1.31	0.74	0	2.67	0.21

Values of V_x were calculated according to McGowan and co-workers [33,34]. Values of $\Sigma\pi_2^*$, $\Sigma\beta_2$ and $\Sigma\alpha_2$ were obtained from Ref. [35]. Values of K_f were obtained from Ref. [10].

coefficients measure the direction and relative strength of different types of intermolecular interactions affecting *SP*.

Twenty compounds of widely varying chemical properties were chosen in order to ensure that all the possible types of intermolecular interactions are accounted for in the LSER. In the selection of the guest compounds the molecular sizes of the guests are also considered to ensure that they are all compatible with the size of the β-CD cavity. The properties of the selected organic compounds are listed in Table 1.

2. Experimental

Liquid chromatographic measurements were made on a β-CD-bonded silica column (Cyclobond I, Advanced Separations Technologies, Whippany, NY, USA) at 30 ± 0.1°C in methanol–water mobile phases. The capacity factors were averages of at least duplicate determinations. The void volume of the system was determined by a method suggested by Hinze et al. [36]. All measurements were made with an HPLC system from Tosoh (Tokyo, Japan) composed of a Model CCPD pump, a Model 8010 refractive index detector and a Model 8010 UV detector set to a wavelength of 254 nm. Retention times were taken at the peak maximum reported by a Hewlett-Packard Model 3396 Series II integrator. Samples were prepared in the mobile phase under study. Typically the column was flushed with 50 column volumes of mobile phase for each percentage change in composition from pure modifier to the analytical composition.

β-CD from Aldrich (Milwaukee, WI, USA) was purified by recrystallization from water and dried under vacuum at 80°C. All the organic compounds, of analytical-reagent grade, were obtained from various sources and were purified according to known procedures [37]. HPLC-

grade water and methanol (Fisher Scientific) were used throughout.

3. Results and discussion

In order to understand in detail which types of interactions between the solutes and β-CD affect the stability of β-CD–solute inclusion complex and RPLC retention on the β-CD-bonded stationary phase, we compared the LSERs for log K_f and RPLC capacity factors on a β-CD-bonded stationary phase in pure water mobile phase (k'_w), shown in Eqs. 2 and 3. The k'_w values (see Table 1) were obtained by linear extrapolation to 100% water of k' values measured in 10–30% methanol–water mobile phase.

$$\log K_f = -1.52(\pm 0.32) + 5.22(\pm 0.38)V_x/100 - 0.72(\pm 0.29)\Sigma\pi^* - 0.81(\pm 0.33)\Sigma\beta - 0.55(\pm 0.51)\Sigma\alpha$$
$$n = 20,\ r = 0.967,\ \text{S.D.} = 0.29 \qquad (2)$$

$$\log k'_w = -1.90(\pm 0.31) + 2.67(\pm 0.35)V_x/100 - 0.12(\pm 0.19)\Sigma\pi^* - 1.05(\pm 0.32)\Sigma\beta - 1.06(\pm 0.45)\Sigma\alpha$$
$$n = 19,\ R = 0.919,\ \text{S.D.} = 0.26 \qquad (3)$$

From the magnitude of each coefficient in Eqs. 2 and 3, we can see that the main term influencing the stabilities of the 1:1 complexes between CD and the solutes and RPLC retention is the solute size, which is followed by the hydrogen bonding and dipolar interaction terms.

The positive sign for the coefficient m indicates that increasing solute size (V_x) leads to increasing stability of the complexes in water and increasing RPLC retention. It is likely that the cavity formation process does not necessarily occur during the transfer of the solute from water to β-CD for the formation of the complex, since a cavity already exists in β-CD for the incoming solute whose size is compatible with the size of the cavity. The CD cavities are known to be hydrophobic [38] and therefore the energetically unfavourable polar–apolar interactions between the included water and the CD cavity are readily substituted without an appreciable expense of energy by the more favoured apolar–apolar interaction between the guest and the CD cavity [39]. Increasing stability of the complex with increasing solute size can be indicative of the fact that non-polar dispersive interactions between the solute and the CD cavity are an important factor affecting the stability. It is shown that the polarizability and hence dispersive interaction strength of a molecule increases with its size [25]. It follows that the coefficient m is a measure of non-polar (hydrophobic) dispersive interactions between the CD cavity and the solute. A much greater magnitude of the coefficient m than the other three coefficients then indicates that the formation of inclusion complexes of CDs and retention on the β-CD-bonded phase is dominated by non-polar (hydrophobic) dispersive interactions between the CD cavity and the solute.

The sign of the coefficient b is negative, indicating that increasing solute β leads to decreasing stabilities of the CD complexes and retention. Because water is a stronger HB donor acid ($\alpha = 1.17$ [40]) than β-CD ($\alpha = 0.14$–0.21 [9]), the hydrogen bonding between the solute and the hydroxyl groups on β-CD in water is highly improbable. Thus an increase in solute β should lead to increased hydrogen bonding with water, resulting in increased solubility of the solute in water and thus decreased stability of the inclusion complex between CD and the solute and decreased retention on the β-CD-bonded phase. The magnitude of the coefficient b (0.81 and 1.05) is much smaller than the coefficient m (5.22 and 2.67), indicating that the contributions of hydrogen bonding interactions to the formation of inclusion complexes and retention on the β-CD bonded phase are minor.

The sign of the coefficient a is negative, indicating that increasing solute α leads to decreasing stabilities of the CD complexes and retention on the β-CD-bonded phase. This also seems to indicate that water is a stronger HB base than β-CD. The HB basicity of β-CD has not yet been reported.

The sign of the coefficient s is also negative, indicating that increasing solute dipolarity leads

to a decrease in the stabilities of the CD complexes. Since water is more dipolar ($\pi^* = 109$ [40]) than β-CD ($\pi^* = 0.43$ [9]), an increase in solute π^* should lead to increased dipolar interactions with the more dipolar water, resulting in increased solubility of the solute in water and thus decreased stability of the complex between β-CD and the solute. This will cause retention of the solutes on the β-CD-bonded phase to decrease. The magnitude of the coefficient s (0.72 and 0.12) is again much smaller than that of the coefficient m, indicating that the contribution of dipolar interactions to the formation of inclusion complexes and retention is minor.

If inclusion complexation is the sole contributor to retention on the β-CD-bonded phase, it follows that inclusion complexation constants in water are well correlated with retention factors in a pure water mobile phase. The plot of log k'_w vs. log K_f is shown in Fig. 1. The straight line indicates the least-squares fit of the data. The reasonably close fit of k'_w with K_f is a strong indication that inclusion complexation is the major driving force in retention on the β-CD-bonded phase in RPLC. However, the scatter of the data points around the least-squares line indicates that non-inclusion interactions also affect retention. This is also seen from the fact that the magnitudes of the coefficients in the LSER equation for K_f (Eq. 2) are different from those in the LSER equation for k'_w (Eq. 3). This is probably due to additional non-inclusion interactions of solutes with interaction sites other than the bonded β-CD moieties on the silica support such as silanol groups.

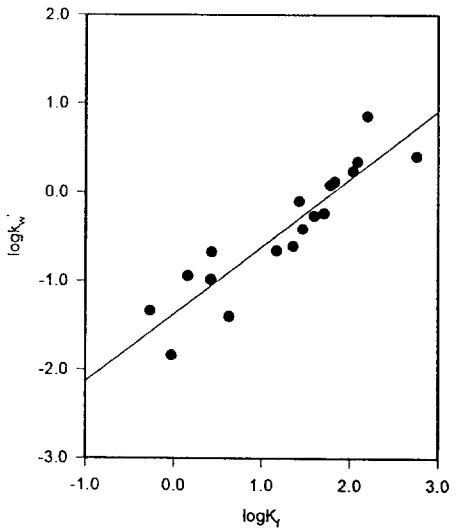

Fig. 1. Plot of log k'_w vs. log K_f.

References

[1] J. Szejtli, Cyclodextrin Technology, Kluwer, Dordrecht, 1988.
[2] W. Saenger, Angew. Chem., Int. Ed. Engl., 19 (1980) 344.
[3] W.L. Hinze, Sep. Purif, Methods, 10 (1981) 159.
[4] D.W. Armstrong and W. Demond, J. Chromatogr. Sci., 22 (1984) 411.
[5] D.W. Armstrong, A. Alak, K. Bui, W. Domond, T. Ward, T.E. Riehl and W.L. Hinze, J. Inclus. Phenom., 2 (1984) 533.
[6] K.W. Street, Jr., J. Liq. Chromatogr., 10 (1987) 655.
[7] A. Nag and K. Bhattacharyya, Chem. Phys. Lett., 151 (1988) 474.
[8] A. Nag, T. Kundu and K. Bhattacharyya, Chem. Phys. Lett., 157 (1989) 83.
[9] J.H. Park, M.D. Jang and M.J. Shin, J. Chromatogr., 595 (1992) 45.
[10] J.H. Park and T.H. Nah, J. Chem. Soc., Perkin Trans. 2, (1994) 1359.
[11] M.J. Kamlet and R.W. Taft, Acta Chem. Scand., Ser. B, 39 (1985) 611.
[12] R.W. Taft, J.L.M. Abboud, M.J. Kamlet and M.H. Abraham, J. Solution Chem., 14 (1985) 153.
[13] D.W. Armstrong, J. Liq. Chromatogr., 7 (1984) 353.
[14] D.W. Armstrong, T.J. Ward, R.E.D. Armstrong and T.E. Beesly, Science, 232 (1985) 1132.
[15] M. Yoshinaga and M. Tanaka, J. Chromatogr. A, 679 (1994) 359.
[16] E.N. Arnold, T.S. Lillie and T.E. Beesley, J. Liq. Chromatogr., 12 (1989) 337.
[17] R.W. Taft, M.H. Abraham, G.R. Famini, R.M. Doherty, J.L.M. Abbound and M.J. Kamlet, J. Pharm. Sci., 74 (1985) 807.
[18] M.J. Kamlet, R.M. Doherty, P.W. Carr, D. Mackay, M.H. Abraham and R.W. Taft, Environ. Sci. Technol., 22 (1988) 503.
[19] M.J. Kamlet, R.M. Doherty, M.H. Abraham, Y. Marcus and R.W. Taft, J. Phys. Chem., 92 (1988) 5244.
[20] J.H. Park and J.E. Lee, J. Korean Chem. Soc., 35 (1991) 438.
[21] M.J. Kamlet, D.J. Abraham, R.M. Doherty, R.W. Taft and M.H. Abraham, J. Pharm. Sci., 75 (1986) 350.
[22] M.J. Kamlet, R.M. Doherty, M.H. Abraham, P.W. Carr, R.F. Doherty and R.W. Taft, J. Phys. Chem., 91 (1987) 1996.

[23] M.J. Kamlet, R.M. Doherty, G.D. Veith, R.W. Taft and M.H. Abraham, Environ. Sci. Technol., 20 (1986) 690.
[24] M.J. Kamlet, R.M. Doherty, R.W. Taft, M.H. Abraham, G.D. Veith and D.J. Abraham, Environ. Sci. Technol., 21 (1987) 149.
[25] J.H. Park and H.J. Lee, Chemosphere, 26 (1993) 1905.
[26] J.H. Park, Y.K. Lee and J.B. Donnet, Chromatographia, 33 (1992) 154.
[27] J.H. Park and P.W. Carr, J. Chromatogr., 465 (1989) 123.
[28] P.C. Sadek, P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft and M.H. Abraham, Anal. Chem., 57 (1985) 2971.
[29] P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, W. Melander and Cs. Horvath, Anal. Chem., 58 (1986) 2674.
[30] J.H. Park, P.W. Carr, M.H. Abraham, R.W. Taft, R.M. Doherty and M.J. Kamlet, Chromatographia, 25 (1988) 373.
[31] J.H. Park, M.D. Jang and S.T. Kim, Bull. Korean Chem. Soc., 11 (1990) 297.
[32] J.H. Park, Bull. Korean Chem. Soc., 11 (1990) 568.
[33] J.C. McGowan, J. Chem. Technol. Biotechnol., 34A (1984) 38.
[34] M.H. Abraham and J.C. McGowan, Chromatographia, 23 (1987) 243.
[35] M.H. Abraham, J. Phys. Org. Chem., 6 (1993) 660.
[36] W.L. Hinze, T.E. Riehl, D.W. Armstrong, W. DeMond, A. Alak and T. Ward, Anal. Chem., 57 (1985) 237.
[37] J.A. Riddick, W.B. Bunger and T.K. Sakano, Organic Solvents, Wiley, New York, 4th ed., 1986.
[38] W. Schlenk and V.M. Sand, J. Am. Chem. Soc., 83 (1961) 2312.
[39] M.L. Grayeski and E. Woolf in L.J. Kricka (Editor), Analytical Applications of Bioluminescence and Chemiluminescence (Proceedings of 3rd International Symposium), Academic Press, London, 1984, p. 565.
[40] M.J. Kamlet, J.L.M. Abboud, M.H. Abraham and R.W. Taft, J. Org. Chem., 48 (1983) 2877.