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Solvatochromic hydrogen bond donor acidity of cyclodextrins and reversed-phase liquid chromatographic retention of small molecules on a β-cyclodextrin-bonded silica stationary phase

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ABSTRACT

Values of the Kamlet–Taft hydrogen bonding (HB) donor acidity (α) and dipolarity/polarizability (π*) parameters for α-, β- and γ-cyclodextrins (CDs) are reported. They possess HB donor acidities in the range 0.21–0.12 and dipolarity/polarizability parameters of about 0.43. Both values are smaller than those for free aliphatic alcohol analogues. The reversed-phase liquid chromatographic retention behaviour of some small molecules on the β-CD bonded silica was compared with that on an octadecylsilylsilica based on the linear solvation energy relationship. It was found that the factors affecting retention on the two stationary phases are very different in that on the ODS column cavity formation and type A HB interactions determine retention whereas on the β-CD column dipolar and type A HB interactions determine retention. Differences in the retention properties of β-CD and ODS phases are rationalized in terms of types of solute–stationary phase interactions involved in the retention process.

INTRODUCTION

Cyclodextrins (CDs) 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, α-, β- and γ-CD, 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 related applications [1,2]. CDs have also been extensively employed in separation science and their applications in liquid chromatography (LC) have recently been reviewed [3–5].

The driving force of complexation is not yet fully understood, but it is widely accepted that the stabilities of CD inclusion complexes are governed by factors such as hydrogen bonding, hydrophobic interactions, solvation effects and the guest molecule's size and shape. To date only a little work has been done 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 groups on their upper and lower rims, which may undergo hydrogen bonding (HB) interactions with incoming guest molecules. However, HB donor acidity or HB acceptor basicity has not been studied so far. In this paper, we report for the first time the determination of Kamlet–Taft solvatochromic HB donor acidity values (α) [9,10] for α-, β-, and γ-CDs from proton NMR chemical shifts. The α parameter is most conveniently obtained from measurements of the frequency of maximum absorption (ν_{max}) of selected indicators in the solvent of interest [10]. However, electronic absorption spectroscopy could not be employed for the measurement of the α

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values of CDs for the following reason. The most important limitation of application is the lack of a suitable solvent in which CDs show reasonable solubility and an easily measurable shift in ν_{max} occurs on addition of CD. The α value of the solvent must be zero so that HB between the indicator as the HB acceptor and the solvent as the HB donor will not affect the shift in ν_{max}. Dimethyl sulphoxide (DMSO) and N,N-dimethylformamide (DMF) dissolve CDs well and their α values are zero, but in these solvents no appreciable shifts in ν_{max} could be observed when CD was dissolved in the solvent containing an indicator.

In this work, the α values of CDs were conveniently calculated from a correlation of proton NMR chemical shifts of some aliphatic primary and secondary alcohols with their α values. DMSO was chosen as the solvent because the assignments of NMR chemical shifts for CD protons in this solvent were known [11,12]. As DMSO itself is fairly strong HB acceptor base [whose HB acceptor basicity value (β) = 0.76], one has to choose carefully a reference base compound that is a stronger HB acceptor base than the solvent DMSO so that HB interactions between the reference base and alcohol molecules predominate over those between DMSO and alcohol molecules. The size of the reference base should also be small enough to fit entirely into α-CD, whose cavity is the smallest among the three CDs. Hexamethylphosphoramide (HMPA) (β = 1.00), which is the strongest HB acceptor base in the Kamlet–taft scale, meets the above requirements. HB interactions between the acceptor and the donor decreases the electron shielding at the hydrogen of the donor according to the spillover effect [13]. The chemical shift (δ) of a donor proton in the presence of HMPA should then be downfield of that in the solvent alone. The extent of the downfield shift ($\Delta\delta$, ppm) should increase with increasing HB donor acidity of the donor and should be more pronounced for a dilute solution in which the concentration of the reference base is much greater than that of the donor so that isolated donor molecules are surrounded mainly by HMPA molecules. The downfield shifts ($\Delta\delta$) of alcohols thus measured are correlated with their α values, and then the $\Delta\delta$ values of CDs are measured and α values are calculated from this correlation.

Since the introduction of silica-bonded cyclodextrins as high-performance liquid chromatographic stationary phases, they have been widely used in separations of positional, geometric and optical isomers and of simple molecules in both normal- and reversed-phase (RP)modes [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 [14–16]. It is known that for chiral recognition the analyte must fit into the CD cavity as closely as possible [14]. In addition to the size requirement, HB interactions of the analyte with hydroxyl groups of CD are also essential to chiral recognition [15]. It was suggested that complex formation involves HB interactions with the secondary hydroxyls of CD followed by movement into the cavity [16]. Despite the importance of CD–analyte interactions, few studies on the determination of the types and relative strengths of various CD–analyte intermolecular interactions and their effects on the retention behaviour of analytes has been reported. In this work, we examined the retention behaviour of some small molecules on a β-CD-bonded silica stationary phase in RP-LC based on the linear solvation energy relationships (LSERs) [17,18] to deconvolute the types and relative strengths of CD–analyte interactions affecting retention, and compared the results with those obtained on an ODS stationary phase.

Kamlet, Taft and co-workers applied the LSER approach and their solvatochromic parameters, π^* (polarity/polarizability), β (HB acceptor basicity) and α (HB donor acidity) to *ca.* 600 processes [17,18], including a large number of systems of immediate relevance of chromatography, such as Rohrschneider's gas–liquid partition coefficients [19], retention of McReynold's solutes on polymeric silicone oil gas chromatographic phases [20], and retention in normal- [21] and reversed-phase LC [22–25]. According to the LSER formalism, when applied to phase-transfer processes, a general solute or solvent property (SP) can be correlated via the use of three types of terms as follows [17,18]:

$$SP = SP_0 + \text{cavity term} + \text{dipolar term} + \text{hydrogen bonding term(s)} \quad (1)$$

where SP_0 denotes the value of SP when all the three terms in the equation are zero. The cavity

term is usually taken as the product of the solute Van der Waals molar volume (V_I) and the square of the Hildebrand solubility parameter (δ) of the solvent. The dipolar term is the product of the solute π^* and the solvent π^*. The π^* parameter measures a combination of the dipolarity and polarizability of a compound. The hydrogen bonding (HB) terms are written as a cross product of the solute α and the solvent β (type B HB) and the product of the solute β and the solvent α (type A HB). In chromatographic retention, SP in the equation below denotes a logarithmic capacity factor, the subscript 2 designates a solute property and the subscripts s and m denote the stationary and mobile phases, respectively:

$$\log k' = \log k'_0 + M(\delta_s^2 - \delta_m^2)V_{I,2}/100 + S(\pi_s^* - \pi_m^*)\pi_2^* + B(\alpha_s - \alpha_m)\beta_2 + A(\beta_s - \beta_m)\alpha_2 \quad (2)$$

where the coefficients M, S, A and B are the fitting parameters.

When a system with a fixed pair of mobile and stationary phases is considered, eqn. 2 is reduced to

$$\log k' = \log k'_0 + mV_{I,2}/100 + s\pi_2^* + b\beta_2 + a\alpha_2 \quad (3)$$

The coefficients m, s, b and a are obtained by multiple linear regression of $\log k'$ *vs.* the solute parameters. The sign and magnitude of the coefficients measure the direction and relative strength of different types of solute–stationary (or mobile) phase interactions affecting retention for a given pair of mobile–stationary phase conditions. When the capacity factors for a series of solutes measured on two different stationary phase columns using a mobile phase of the same composition are examined, the mobile phase parameters in eqn. 2 (δ_m^2, π_m^*, α_m and β_m) are fixed. Any variations in the coefficients m, s, b and a are then determined only by the variations in the stationary phase properties (δ_s^2, π_s^*, α_s and β_s). The values of the coefficients m, s, b and a can be used to compare the corresponding interaction properties of the stationary phases of interest. This approach has been found useful for the characterization of the chromatographic properties of some stationary phases for use in normal [21] and reversed-phase LC [26,27]. We compared the retention properties of β-CD-bonded silica with those of octadecylsilylsilica (ODS) based on the above approach.

EXPERIMENTAL

Chemicals

α-, β- and γ-CD obtained from Aldrich (Milwaukee, WI, USA) were purified by recrystallization from water and dried under vacuum at 80°C. Methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, DMSO and HMPA obtained from Aldrich were dried and fractionally distilled before use as described [28].

Apparatus

NMR spectra were measured at 25°C using a Varian Model EM-360 60-MHz continuous-wave NMR spectrometer with tetramethylsilane (TMS) as the internal reference.

Procedure

The concentration of HMPA was maintained in large excess ([HMPA] > 3 M) over of the concentration of hydroxyl groups of alcohols or CDs (0.1 M). The chemical shifts of the donor (alcohols and CDs) were measured at concentrations of 0.5, 0.3 and 0.1 M. Changes in the chemical shift in this concentration range were negligible. The shifts for the 0.1 M donor solution were measured throughout the study, as these values can be safely assumed to be the infinite dilution values. Figs. 1 and 2 show the NMR spectra for ethanol and β-CD in DMSO and in a DMSO solution of HMPA, respectively. The OH proton signals for alcohols and CDs appear within the range accessible with HMPA and DMSO. Very small amounts of water and other impurities in DMSO may be responsible for some of the scatter in the data, but we believe that they do not significantly affect the correlation. Fig. 3 shows the plot of α *vs.* measured $\Delta\delta$ values for dichloromethane and some HB aliphatic alcohols. A reasonably good linear relationship is observed between $\Delta\delta$ and α values (r = 0.984, S.D. = 0.017).

RESULTS AND DISCUSSION

In DMSO solution of HMPA, signals only for O_2H and O_3H hydroxyls of CDs could be observed (see Fig. 2). Table I lists measured $\Delta\delta$ and estimated HB donor acidity values for the O_2H and O_3H hydroxyl groups of α-, β- and γ-CDs. The O_2H hydroxyl has a greater HB donor acidity than the O_3H

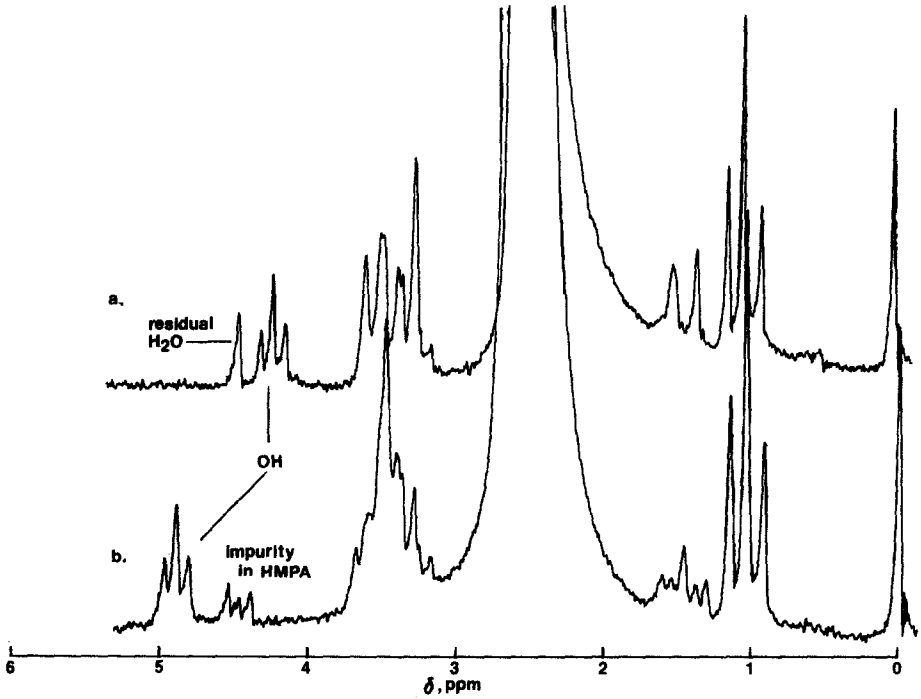

Fig. 1. NMR spectra for ethanol in (a) DMSO and (b) DMSO containing HMPA.

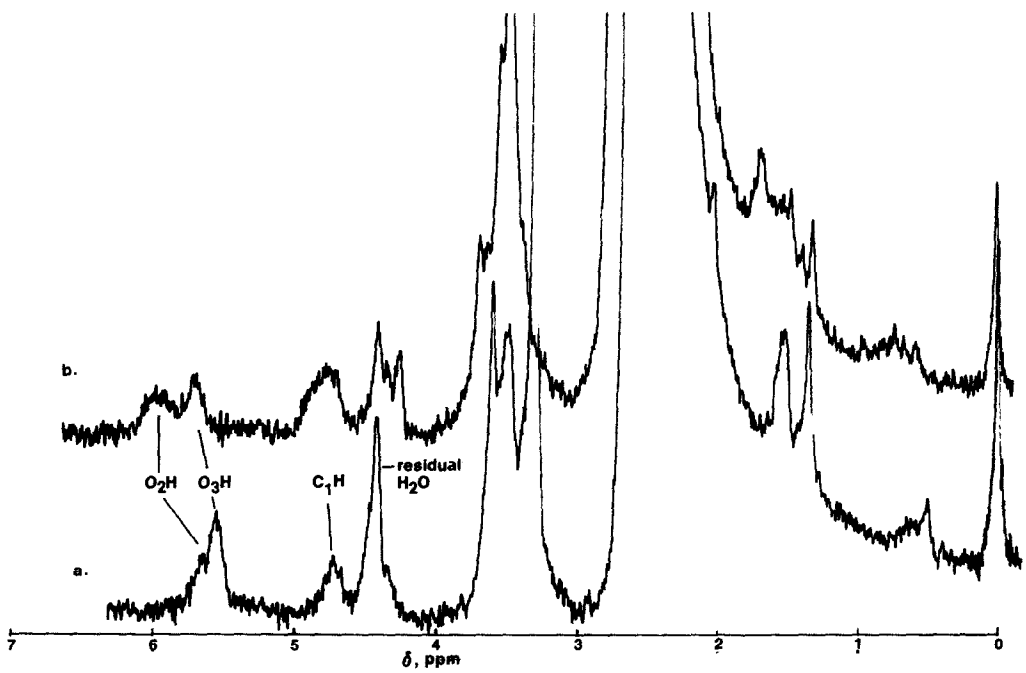

Fig. 2. NMR spectra for β-CD in (a) DMSO and (b) DMSO containing HMPA.

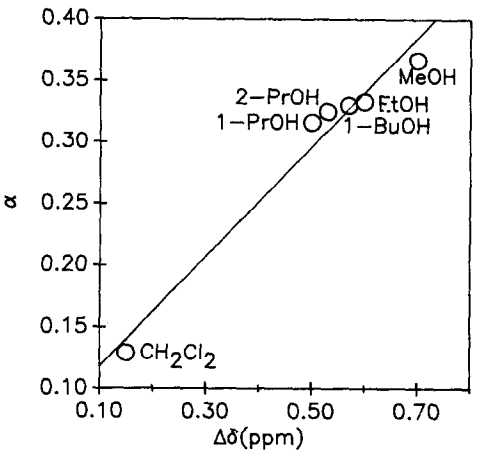

Fig. 3. Plot of HB donor acidity (α) *vs.* $\Delta\delta$. The α values are from Abraham *et al.* [29]. Me = Methyl; Et = ethyl; Pr = propyl; Bu = butyl.

hydroxyl. This is in agreement with the fact that the chemical shift of the O_2H proton is downfield with respect to that for the O_3H proton, indicating that HB interactions with HMPA are stronger for the O_2H hydroxyl. The HB donor acidities of α- and β-CD are the same and greater than that for γ-CD. In view of the fact that the strongest interactions between the guest (HMPA) and CD can occur when the guest is included in the CD cavity with a snug fit [30], a smaller HB donor acidity value for γ-CD is indicative of that the cavity size of γ-CD is too large for HMPA to fit snugly. Overall the HB donor acidities of these secondary hydroxyl groups of CDs are smaller than that for the corresponding hydroxyl group of 2-propanol (α = 0.325 [29]). It is likely that smaller HB donor acidities of CDs than that of the corresponding secondary alcohol are due to the intramolecular hydrogen bond between the O_2H and O_3H hydroxyl groups, which are strong enough to exist even in DMSO [11] and also compete with HMPA in the process of hydrogen bond formation. The HB acceptor basicity of CDs could not be obtained in the solution phase either by electronic absorption spectroscopy or by the present NMR methodology because of the lack of a solvent whose β value is zero.

TABLE I

$\Delta\delta$ (ppm) AND α VALUES FOR CD HYDROXYL PROTONS

Parameter	α-CD		β-CD		γ-CD	
	O_2H	O_3H	O_2H	O_3H	O_2H	O_3H
$\Delta\delta$	0.31	0.15	0.31	0.16	0.22	0.10
α	0.21	0.14	0.21	0.14	0.17	0.12

Determination of the dipolarity/polarizability parameter (π^*) of CDs by the above methodologies in the solution phase is also not feasible for the same reason as for β. The π^* values of CDs may be estimated from the *Py* values [31,32], which are known to represent the polarity of the solvent. The π^* values [33] of some aliphatic alcohols (methanol, ethanol, 1-propanol, 2-propanol, 1-butanol and 2-methoxyethanol) are well correlated with their *Py* values [32], as shown by the following equation:

$$\pi^* = 0.03(\pm 0.03) + 0.43(\pm 0.03)Py \quad (4)$$

$$n = 6,\ r = 0.989,\ \text{S.D.} = 0.01$$

The values of *Py* for CDs have recently been reported [6]. The π^* values calculated from the *Py* values using eqn. 4 are 0.787, 0.428 and 0.431 for α-, β- and γ-CD, respectively. The π^* values for β- and γ-CD are similar but the value for α-CD seems too high. The π^* value of α-CD is believed to be in error as the reported *Py* value of α-CD is known to be erroneous [6]. It has been suggested that pyrene molecules, the probe for use in the determintation of the *Py* value, are too large to fit into the cavity of α-CD [6]. As with the α values for CDs, the π^* values for CDs are lower than those for aliphatic alcohols.

The π^* and α values estimated as above are for free solution CDs. The π^* and α values for bound CDs as in a bonded silica stationary phase would not be the same as the values in solution, as bound CDs are in a different environment owing to microheterogeneity [34,35]. However, it is not expected that the interaction properties of CD will vary much between the free and bound states. Assuming that π^* and α values calculated as above are equally applicable to free or bound CD as in a mobile phase modifier and in bound silica stationary phase, we may compare the solvation ability of CDs. As an example, the solvation ability of β-CD (π^* = 0.43 and α = 0.14–0.21) are more closely approximated by the alkanes (*e.g.*, hexadecane, π^* = 0.08 [36], α = 0.00) than water (π^* = 1.09, α = 1.17 [33]) or

TABLE II

CAPACITY FACTORS[a] AND PROPERTIES OF SOME SOLUTES USED IN MULTIPLE REGRESSION ANALYSES BASED ON THE LSER

Solute	$V_I/100$	π^*	β	α	Log k'	
					β-CD	ODS
Mesitylene	0.769	0.47	0.13	0	−0.678	–
Toluene	0.592	0.55	0.11	0	−0.119	0.785
Benzene	0.491	0.59	0.10	0	0.124	0.497
Anisole	0.630	0.73	0.32	0	−0.237	0.470
Methyl benzoate	0.736	0.76	0.39	0	−0.194	0.410
o-Toluidine	0.660	0.73	0.47	0.13	−0.538	−0.051
m-Toluidine	0.660	0.69	0.51	0.13	−0.538	−0.036
Aniline	0.562	0.73	0.50	0.13	−0.432	−0.229
N-Methylaniline	0.660	0.73	0.47	0.12	−0.409	0.209

[a] Mobile phase: methanol–water (60:40, v/v) [39].

aqueous organic mobile phases ($\pi^* = 1.66$–0.66 [37], $\alpha = 1.17$–0.58 [38]) for solute sizes suitable for the β-CD cavity. This also suggests, according to the LSER (eqn. 2), that the chromatographic properties of β-CD-bonded silica as the stationary phase would be different from those of ODS. Chang *et al.* [39] reported RP–LC capacity factors for a set of solutes on both β-CD-bonded silica and ODS stationary phases in methanol–water mobile phases. We examined these data based on the LSER (eqn. 3) to see how different the two stationary phases are in terms of the type and strengths of interactions with the solutes (see Table II for retention and property data). As cavity formation processes are involved in any transfer processes and β-CD is dipolar and HB donating, we started with regressing capacity factors on the β-CD-bonded silica using the three-parameter equation including the $V_I/100$, π^* and β parameters:

$$\log k' = -0.92(\pm 0.43) - 0.03(\pm 0.39)V_I/100 + 2.77(\pm 0.56)\pi^* - 2.33(\pm 0.31)\beta \quad (5)$$

$n = 9$, $r = 0.964$, S.D. $= 0.088$

Interestingly, the coefficient for the cavity formation term ($V_I/100$) is statistically zero, indicating that the cavity formation process is not affecting the retention of the solutes under study on the β-CD bonded silica. It is not likely that the cavity formation processes truly do not affect the retention process. The model (eqn. 5) may be incomplete because of omission of the solute α parameter. We therefore applied the four-parameter equation including the α parameter:

$$\log k' = 0.53(\pm 1.31) - 0.27(\pm 1.03)V_I/100 + 2.26(\pm 1.68)\pi^* - 1.78(\pm 1.72)\beta - 0.87(\pm 2.68)\alpha \quad (6)$$

$n = 9$, $r = 0.965$, S.D. $= 0.097$

There is no improvement in the goodness of fit and the coefficients for both the $V_I/100$ and α parameter turned out to be statistically zero. This indicates that the two-parameter equation including only π^* and β parameters is appropriate for the representation of retention behaviour on the β-CD bonded phase:

$$\log k' = -0.90(\pm 0.25) + 2.76(\pm 0.48)\pi^* - 2.31(\pm 0.27)\beta \quad (7)$$

$n = 9$, $r = 0.964$, S.D. $= 0.079$

We also checked on the basis of the Ehrenson test [40] whether the resulting three-parameter equation with inclusion of the α term in eqn. 7 improves the fit, and found that the α term is statistically not significant. It should be noted, however, that the data set does not include any HB donor solute stronger than toluidine ($\alpha = 0.13$), so that the dependence on HB donor acidity remains uncertain. The above results indicate that the retention beha-

TABLE III

COMPARISON OF THE COEFFICIENTS IN LSER EQUATIONS FOR RETENTION ON THE β-CD-BONDED SILICA AND ODS STATIONARY PHASES

Column	*m*	*s*	*b*	*a*
β-CD	N.E.[a]	1.45	−1.73	N.E.
ODS	2.52	N.E.	−2.35	N.E.

[a] N.E. = No effect on retention.

viour of the solutes studied on the β-CD bonded phase is well represented by the LSER of eqn. 7. We do not have a firm explanation for the insignificance of the cavity formation term in the retention process on the β-CD-bonded silica. All the analytes studied are of size compatible with the β-CD cavity and it is likely for the transfer process from the mobile phase to the β-CD bonded to silica that cavity formation is not necessary as a cavity for the incoming molecule already exists in β-CD, whereas in other common stationary phases a cavity must be first made for the incoming solute. The CD cavities are known to be hydrophobic [41] and therefore the energetically unfavoured polar–apolar interaction between the included water and the CD cavity is readily substituted without an appreciable expense of energy by the more favoured apolar–apolar interaction between the guest and the CD cavity [42]. However, the above explanation is at best speculative and remains to be confirmed. To this end, retention data for analytes of more varied but still compatible size with the CD cavity should be studied.

Retention data on ODS were examined in a similar fashion to those on the β-CD-bonded silica. The resulting LSER equation is

$$\log k' = -0.46(\pm 0.27) + 2.52(\pm 0.50)V_I/100 - 2.35(\pm 0.22)\beta \quad (8)$$

$$n = 8,\ r = 0.980,\ \text{S.D.} = 0.08$$

As previously observed [22–24], retention on ODS is mainly determined by cavity formation and the solute HB acceptor basicity. The coefficients for the terms in eqns. 7 and 8 are summarized in Table III for comparison. It can be seen that the types of intermolecular interactions affecting retention are different on the two stationary phases and hence there are disparate chromatographic selectivities toward a given set of solutes. On ODS the cavity formation term is the major factor affecting retention whereas on β-CD-bonded silica this term has no effect. On ODS the dipolar interaction term has no effect on retention but on β-CD-bonded silica this term acts to increase the retention. Similar trends in the sign and size of the coefficients in the LSER equations were also observed for retention data in mobile phases with different methanol compositions. As mentioned above, in order for this comparison of the chromatographic properties of the two stationary phases to be validated, a greater number of solutes of widely varying sizes and chemical properties than used in this study must be employed.

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