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## SURFACE SILANOLS IN SILICA-BONDED HYDROCARBONACEOUS STATIONARY PHASES

### I. DUAL RETENTION MECHANISM IN REVERSED-PHASE CHROMATOGRAPHY

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#### SUMMARY

“Irregular” retention behavior of crown ethers in reversed-phase chromatography with silica-bonded hydrocarbonaceous stationary phases is interpreted by using a dual binding model. It is assumed that retention is caused not only by the usual solvophobic interactions but also by “silanophilic” interactions between the elute and the accessible silanol groups at the surface of various alkyl-silica bonded phases. Minima observed in the dependence of the retention factor of dibenzo-18-crown-6 on the composition of methanol-water mixtures used as the eluent are in agreement with the theory. The model also correctly predicts the temperature dependence of retention under various conditions as far as the nature of the stationary phase and the eluent composition are concerned. Thus curved Van 't Hoff plots of the experimental data are attributed to the sufficiently dissimilar enthalpies involved in the solvophobic and silanophilic binding. It is likely that minima exhibited by plots of retention factor against eluent composition in reversed-phase chromatography are in most cases manifestations of the countervailing effect of the solvophobic and silanophilic forces on retention.

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#### INTRODUCTION

Reversed-phase chromatography (RPC) has become the dominant branch of high-performance liquid chromatography (HPLC) over the last few years. Yet, physico-chemical phenomena responsible for retention on silica-bonded hydrocarbonaceous stationary phases, which are employed in RPC almost exclusively, have not been fully understood. In earlier studies from our laboratory<sup>1</sup> it was assumed that the stationary phase surface consists of a uniform greasy layer of covalently bound alkyl ligates and the solvophobic theory<sup>2</sup> was employed to treat quantitatively the role of the eluent in determining retention behavior on such “inert”, non-polar stationary phases. Indeed, under many practical conditions in RPC, particularly when binary aqueous eluents lean in organic solvent are employed, retention behavior and selectivity are governed mainly by solvent effects<sup>3-5</sup>.

Besides inert hydrocarbonaceous ligates, however, the abundant silanol groups at the alkyl-silica stationary phase surface<sup>6</sup> may be accessible to elute molecules particularly when the hydro-organic eluent is lean in water<sup>7,8</sup>. Analysis of a large number of retention data<sup>9</sup> has also suggested that silanol groups at the stationary phase surface may be responsible for departure from the retention behavior expected on the basis of the solvophobic theory. In fact, good chromatographic practice may require masking of surface silanols by doping the eluent with an aliphatic amine in order to reduce tailing of certain peaks<sup>10,11</sup>. Generally, surface silanols ubiquitous in hydrocarbonaceous bonded phases are considered undesirable<sup>12-15</sup> although no detailed study has focused so far on their role in determining retention behavior, column efficiency and longevity. As a result of surface heterogeneity, retention on bonded stationary phases may be determined by elute interactions not only with organic ligates but also with accessible silanol groups. Indeed, in a detailed investigation of tailing reducers Sokolowski and Wahlund<sup>16</sup> used a two-site adsorption model and postulated that surface silanols may be responsible for tailing of cationic elutes.

The present study shows that surface silanols also play a major role when large molecules having polar functions are chromatographed by using water-lean binary eluents in RPC. A simple model is developed to account for dual binding mechanism on the assumption that the magnitude of elute retention can be determined not only by solvophobic (hydrophobic) interactions but also by reversible binding involving polar silanolic sites at the surface. For convenience, the term *silanophilic* interactions will be used to denote binding mechanism with the participation of silanol groups. Experimental results obtained with crown ethers show that the dependence of the retention factors on eluent composition and temperature is consistent with the predictions of the theory.

## THEORY

It is assumed that the elute can independently bind in two different ways to the surface of a given stationary phase which contains covalently bound alkyl functions and silanol groups. One of the mechanisms is purely solvophobic (hydrophobic); the other is silanophilic because the elute is believed to interact with silanol groups as well as with other alkyl chains. Assuming that the respective equilibrium constants are  $K_1$  and  $K_2$ , the retention factor,  $k$ , can be expressed as the sum of the retention factors for solvophobic,  $k_1$ , and silanophilic,  $k_2$ , binding as

$$k = \varphi_1 K_1 + \varphi_2 K_2 = k_1 + k_2 \quad (1)$$

where  $\varphi_1$  and  $\varphi_2$  are the phase ratios of the column appropriate for the hydrocarbonaceous ligates and silanols, respectively. The phase ratio is conveniently defined as moles of accessible binding sites per unit volume of mobile phase that is explored by the elute in the column.

### *Effect of eluent composition*

Many studies have examined the effect of eluent composition on retention in RPC and found that in the case of "regular retention behavior" the logarithm of the retention factor is linearly dependent on the volume fraction of water,  $\psi$ , in binary

hydro-organic eluents<sup>17,18</sup>. Consequently, we may express the solvophobic retention factor as

$$k_1 = A \exp(B\psi) \quad (2)$$

where  $A$  and  $B$  are, respectively, the intercept and the slope of linear  $\ln k$  vs.  $\psi$  plots obtained in RPC when silanophilic interactions or secondary equilibria<sup>5</sup> do not cause departure from regular behavior.

In chromatography with polar adsorbents such as silica the retention factor has been related to the composition of a binary eluent<sup>19,20</sup> by

$$k_2 = (C + D\psi)^{-1} \quad (3)$$

where  $C^{-1}$  is the retention factor obtained with the less polar solvent as the eluent,  $\psi$  is the volume fraction of the more polar eluent component and  $D$  is believed to be a characteristic constant of the chromatographic system.

Substitution of eqns. 2 and 3 into eqn. 1 yields the following relationship for the dependence of the retention factor on the composition of the eluent

$$k = A \exp(B\psi) + (C + D\psi)^{-1} \quad (4)$$

Since the first term increases whereas the second term decreases with increasing  $\psi$ , eqn. 4 predicts a concave  $\log k$  vs.  $\psi$  plot with a minimum for certain sets of parameters.

#### Effect of temperature

The temperature dependence of both limiting retention factors,  $k_1$  and  $k_2$ , is expected to follow the Van 't Hoff equation<sup>1,5,17,18</sup> *i.e.*

$$k_1 = \varphi_1 \exp \left\{ \frac{-\Delta H_1^0}{RT} + \frac{\Delta S_1^0}{R} \right\} \quad (5a)$$

and

$$k_2 = \varphi_2 \exp \left\{ \frac{-\Delta H_2^0}{RT} + \frac{\Delta S_2^0}{R} \right\} \quad (5b)$$

where  $T$  is the absolute temperature,  $R$  is the gas constant,  $\Delta H_1^0$  and  $\Delta S_1^0$  are the enthalpy and entropy changes for solvophobic interactions, respectively and  $\Delta H_2^0$  and  $\Delta S_2^0$  are the respective silanophilic retention enthalpy and entropy changes. Thus the enthalpy change for the overall chromatographic retention process,  $\Delta H^0$  is given by

$$(-R) \frac{d \ln k}{d(1/T)} = \frac{\varphi_1 \Delta H_1^0 \exp \left( \frac{-\Delta H_1^0}{RT} + \frac{\Delta S_1^0}{R} \right) + \varphi_2 \Delta H_2^0 \exp \left( \frac{-\Delta H_2^0}{RT} + \frac{\Delta S_2^0}{R} \right)}{\varphi_1 \exp \left( \frac{-\Delta H_1^0}{RT} + \frac{\Delta S_1^0}{R} \right) + \varphi_2 \exp \left( \frac{-\Delta H_2^0}{RT} + \frac{\Delta S_2^0}{R} \right)} \quad (6)$$

which can be simplified to

$$\Delta H^0 = \frac{k_1 \Delta H_1^0}{k} + \frac{k_2 \Delta H_2^0}{k} \quad (7)$$

According to eqns. 6 and 7, dual retention mechanism leads to non-linear Van 't Hoff plots when  $k_1$  and  $k_2$  are of commensurable magnitude except in the unlikely situation of  $\Delta H_1^0 = \Delta H_2^0$ .

The asymptotic values of eqn. 6 for  $1/T \rightarrow 0$  and  $1/T \rightarrow \infty$  can be evaluated for three cases:  $\Delta H_1^0 > \Delta H_2^0$ ;  $\Delta H_1^0 = \Delta H_2^0 = \Delta H^0$  and  $\Delta H_1^0 < \Delta H_2^0$ . Their respective values when  $1/T \rightarrow \infty$  are  $\Delta H_2^0$ ,  $\Delta H^0$  and  $\Delta H_1^0$ ; when  $1/T \rightarrow 0$  they are  $(\Delta H_1^0 + \gamma \Delta H_2^0)/(1 + \gamma)$ ,  $\Delta H^0$  and  $(\Delta H_1^0 + \gamma \Delta H_2^0)/(1 + \gamma)$ , where  $\gamma = \varphi_2/\varphi_1 \exp [(\Delta S_2^0 - \Delta S_1^0)/R]$ . In each of the three cases the slope of Van 't Hoff plots increases or remains constant as the temperature decreases. Consequently, eqn. 6 predicts concave Van 't Hoff plots when  $k_1$  and  $k_2$  are of commensurable magnitude under conditions of the experiment and  $\Delta H_1^0 \neq \Delta H_2^0$ .

## EXPERIMENTAL

### Materials

Dibenzo-18-crown-6 (DB18C6) was obtained from Eastman (Rochester, NY, U.S.A.); 18-crown-6 and dibenzo-24-crown-8 (DB24C8) were from Strem Chemicals (Newburyport, MA, U.S.A.). All other chemicals were purchased from Chem Service (West Chester, PA, U.S.A.). Acetonitrile, methanol, tetrahydrofuran, ethyl acetate, methylene chloride and *n*-hexane were "distilled in glass" from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Distilled water was prepared with a Barnstead distilling unit.

### Instruments and columns

The chromatographic system consisted of a Model FR-30 pump (Knauer, Berlin, G.F.R.), a Model 7010 sampling valve (Rheodyne, Berkeley, CA, U.S.A.) having a 20- $\mu$ l sample loop and a Model FS-970 fluorometric detector (Kratos-Schoeffel, Westwood, NJ, U.S.A.) with excitation wavelength and emission filter cut-offs set at 218 and 300 nm, respectively, in tandem with a Kratos-Schoeffel Model 770 variable-wavelength UV detector operated at 210 nm. Chromatograms were obtained with a Model 56 dual-pen recorder (Perkin-Elmer, Norwalk, CT, U.S.A.). The column temperature was controlled by recirculating water through an insulated stainless-steel jacket from Model K-2/R thermostatted water bath (Messgeraete-Werk, Lauda, G.F.R.). Columns packed with 10- $\mu$ m Partisil silica, 10- $\mu$ m Partisil ODS and 10- $\mu$ m Partisil ODS-2, all 250  $\times$  4.6 mm I.D., were supplied by Whatman (Clifton, NJ, U.S.A.). A 150  $\times$  4.6 mm I.D. column packed with 5- $\mu$ m Hypersil ODS (Shandon Southern Products, Runcorn, Great Britain) was also employed.

The dimensions of the column packed with macroreticular hydrocarbonaceous polymers (cross-linked polystyrene) were 150  $\times$  4.6 mm I.D. Styragel was obtained from Waters Assoc. (Milford, MA, U.S.A.). Amberlite XAD-2 (Rohm & Haas, Philadelphia, PA, U.S.A.) was ground by using Model Pulverisette 0 micro-pulveriser (Tekmar, Cincinnati, OH, U.S.A.) and the resulting powder was sedimented in methanol to obtain a particle size fraction of 5–15  $\mu$ m. Each particulate polymer was slurry packed into the column.

### Procedures

Columns packed with octadecyl-silica (ODS) were first conditioned by washing

sequentially with 200 ml of methanol, 30 ml of both tetrahydrofuran and ethyl acetate, 100 ml of methylene chloride and 200 ml of *n*-hexane. The flow-rate was approximately 2 ml/min and during methylene chloride wash the column was heated to 70°C for 30 min. Thereafter the washing procedure was carried out in the reversed sequence and the methanol wash was continued until a stable baseline was observed at 210 nm at maximum detector sensitivity. After another 200 ml of methanol was passed through the column, DB18C6 was repetitively injected until the retention times were reproducible. In subsequent experiments the column was equilibrated with methanol-water mixtures of increasing water content and the retention of DB18C6 or DB24C8 was measured. Retention times were evaluated from the position of peak maxima on the chromatogram and the mobile phase hold-up time was obtained by injecting fructose and measuring the retention time of the corresponding peak. The analysis of the chromatographic data was performed with a Model PDP 11/10 computer (Digital Equipment, Maynard, MA, U.S.A.) and a non-linear least squares regression analysis program written in BASIC language was used for parameter estimation.

## RESULTS AND DISCUSSION

We have investigated the effect of silanophilic interactions in RPC by using the crown ether DB18C6 as the "elute probe" for the following reasons. First, its retention factor measured with octadecyl- or octyl-silica, exhibits a concave dependence on the composition of hydro-organic eluents as illustrated in Fig. 1. This retention behavior resembles that reported for amino compounds<sup>21-25</sup> and polar solutes<sup>26</sup> known to interact with surface silanol groups under similar conditions. Second, the effect of silanophilic interactions can be investigated under conditions where neither the elute nor the silanol groups are ionized. Third, retention factors of

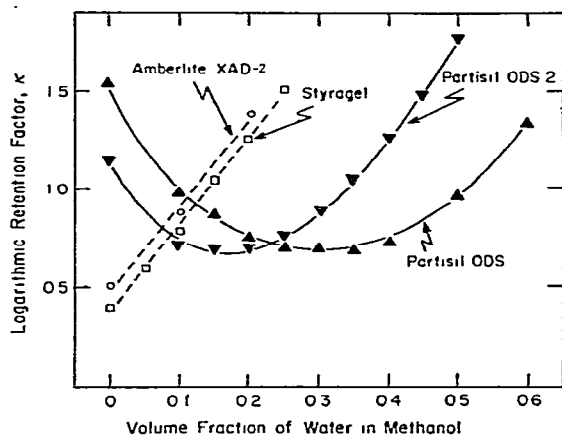


Fig. 1. Plots of the logarithmic retention factor of DB18C6 against the volume fraction of water in the methanol-water mixture used as the eluent. Dashed lines represent results obtained with macroreticular polystyrene columns:  $\circ$ , Amberlite XAD-2; and  $\square$ , Styragel. Solid lines were calculated from eqn. 4 with the parameters listed in Table I.

DB18C6 on ODS can be accurately measured with eluents encompassing a wide range of solvent composition. Fourth, the elute can readily be detected in the effluent spectrophotometrically or fluorometrically.

In other cases investigated earlier in our laboratory irregular retention behavior, *i.e.*, non-linear  $\kappa^*$  vs. eluent composition plots, in RPC with hydro-organic eluents was found to arise from solvent effects<sup>4,5,18</sup>. Indeed, the minimum shown on the plot of retention factor against eluent composition for DB18C6 in Fig. 1 could be interpreted theoretically in several ways. It might, for instance, be explained within the hermeneutics of the solvophobic theory<sup>1</sup> or on the basis of elute solubility in the mobile phase<sup>27,28</sup>. Consequently, the evocation of silanophilic interactions and the use of the dual retention mechanism to treat the results shown in Fig. 1 is meaningful only if DB18C6 exhibits regular retention behavior over a wide composition range of hydro-organic eluents when a hydrocarbonaceous stationary phase void of silanol groups is employed. On the other hand, non-linear dependence of the retention factor on the eluent composition with a non-siliceous chromatographic system would strongly suggest that the observed behavior is due to solvent effects.

According to the linear  $\kappa$  vs.  $\psi$  plots shown also in Fig. 1, DB18C6 exhibits regular retention behavior over a wide composition range of methanol-water mixtures when columns packed with Styragel or Amberlite XAD-2 are used. Similar results were obtained with acetonitrile-water mixtures. In contradistinction both  $\kappa$  vs.  $\psi$  plots for DB18C6 obtained from measurements using the two ODS columns are concave with minima as discussed above and seen in Fig. 1. Examination of the two sets of data strongly suggests that (i) solvent effects do not cause departure from the regular linear dependence of  $\kappa$  on  $\psi$  and (ii) silanol groups at the surface are responsible for the observed irregular retention behavior.

In Fig. 2 the reciprocal retention factors of DB18C6 and DB24C8, as measured on "naked" silica, *i.e.*, plain silica gel, are plotted against the composition of the methanol-water mixture used as the eluent. It is seen that the linear relationship observed generally upon the "normal" use of silica gel as the stationary phase<sup>19</sup> holds also when an aqueous eluent is employed, at least under the conditions described here.

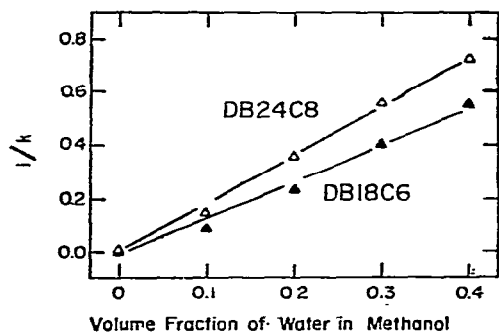


Fig. 2. Plots of reciprocal retention factors measured on "naked" silica against the composition of the aqueous eluent rich in methanol.

\* The decimal logarithm of retention factor,  $\log k$ , is denoted by  $\kappa$  in agreement with earlier convention<sup>17</sup>.

The results suggest, therefore, that eqn. 3 appropriately describes the dependence of the silanophilic retention factor,  $k_2$ , on the composition of the eluent used in RPC.

The results presented above give sufficient support to the dual retention model so that it appears appropriate to examine experimental data in the light of the theory.

#### Temperature dependence of retention

In Fig. 3 Van 't Hoff plots are illustrated for the retention factor of DB18C6, measured under various conditions. The results show that in plain methanol or when the methanol or acetonitrile concentration is 90% (v/v) in the hydro-organic eluent linear plots are obtained. On the other hand, increasing the water content of the mobile phase gives rise to a concave curvature in the Van 't Hoff plot as predicted by the dual sorption model according to eqn. 7. As the mechanism of retention with plain organic solvent is expected to be different from that with plain aqueous eluent, there should be a gradual change in the retention mechanism when  $\psi$  goes from zero to unity, which yields a non-linear dependence of  $\kappa$  on the reciprocal temperature. Indeed, as shown in Fig. 3, the Van 't Hoff plots of data obtained on Hypersil ODS column are first linear, then curved and subsequently linear again as the volume fraction of water in methanol changes from 0.1 to 0.50. In view of earlier studies<sup>5</sup> as well as the discussion of eqns. 6 and 7 above, linear portions of the plots shown in Fig. 3 are expected to reflect conditions under which only one of the mechanisms dominates the retention process.

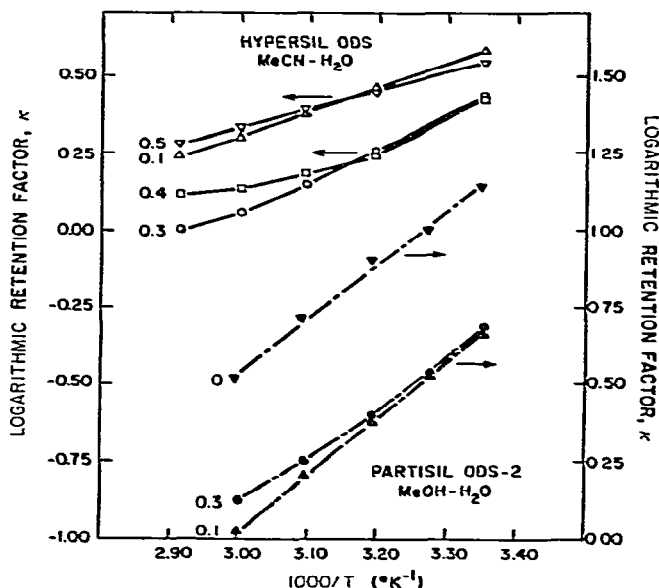


Fig. 3. Van 't Hoff plots illustrating the dependence of the logarithmic retention factors of DB18C6 on the reciprocal absolute temperature. Open symbols indicate data obtained with Hypersil-ODS and aqueous acetonitrile as the eluent containing 0.1 ( $\Delta$ ), 0.3 ( $\circ$ ), 0.4 ( $\square$ ) and 0.5 ( $\nabla$ ) volume fraction of water. Data represented by solid symbols were obtained with Partisil ODS and aqueous methanol as the eluent containing 0.0 ( $\blacktriangle$ ), 0.1 ( $\blacktriangledown$ ) and 0.3 ( $\bullet$ ) volume fraction of water.

TABLE I  
 PARAMETERS OF EQN. 4 FOR THE RETENTION FACTOR OF DB18C6 IN REVERSED-PHASE CHROMATOGRAPHY WITH AQUEOUS METHANOL AS THE ELUENT; THE ENTHALPIES AND RANGES OF PARAMETER  $A$  USED IN THE CALCULATIONS ARE ALSO GIVEN

	$k^*$	$\Delta H^0$ <sup>a</sup> (cal/mole)	$r^{***}$	Range of $A^{\dagger}$	Value of parameters of eqn. 4 at 25°C			
					A	B	C	D
'Naked' silica (Partisil)	48.0	-8797 ± 290 <sup>**</sup>	0.999	—	—	—	—	—
Octadecyl-silica (Partisil ODS)	32.8	-8433 ± 656	0.998	0-3.56	0.0405 ± 0.0095	10.150 ± 1.350	0.0305 ± 3.02 · 10 <sup>-3</sup>	0.74 ± 0.750
Octadecyl-silica (Partisil ODS-2)	13.9	-7356 ± 1500	0.994	0-5.00	0.234 ± 0.102	10.468 ± 0.987	0.0732 ± 5.50 · 10 <sup>-4</sup>	1.244 ± 0.333

\* Measured with plain methanol at 25°C.

\*\* Enthalpy change used for  $\Delta H^0$  in eqn. 7.

\*\*\* Correlation coefficient of Van 't Hoff plots.

† Evaluated from eqn. 7 with the assumption that  $\Delta H^0 = 0$  when plain methanol was used as the mobile phase at 25°C.



### Parameter estimation

The four parameters of eqn. 4, which expresses the dependence of  $k$  on  $\psi$  at fixed temperature, were evaluated from the data points shown in Fig. 1, by using a non-linear least square analysis and the values thus obtained are shown in Table I. The curves calculated by using these parameters are also shown in Fig. 1 and it is seen that the fit is satisfactory. In order to substantiate that the values thus obtained for the parameters  $A$  and  $C$  fall indeed in the proper range, eqn. 7 was also employed to estimate these parameters as described below.

When the eluent is a plain less-polar solvent, *i.e.*,  $\psi = 0$ , eqns. 1 and 4 yield for the solvophobic and silanophilic retention factors  $k_1$  and  $k_2$ , the following expressions

$$k_1 = A \quad (8a)$$

and

$$k_2 = C^{-1} \quad (8b)$$

In the estimation of the parameters  $A$  and  $C$  we may take advantage of the observation that for many non-polar eluents the enthalpy for solvophobic retention,  $\Delta H_1^0$ , on hydrocarbonaceous bonded phases is close to zero with eluents containing a plain organic solvent such as acetonitrile<sup>17</sup>. Assuming that this also applies to essentially solvophobic retention of DB18C6 under the conditions employed here, we may evaluate the parameters  $A$  and  $C$  from eqn. 7 via eqns. 8a and 8b provided the enthalpy of silanophilic retention,  $\Delta H_2^0$ , is known.

In order to calculate  $\Delta H_2^0$  for DB18C6 its retention factors were measured with neat methanol as the eluent at different temperatures on a silica column packed with "naked" silica (10- $\mu$ m Partisil) and on two ODS columns packed with 10- $\mu$ m Partisil ODS and 10- $\mu$ m Partisil ODS-2 having 5% and 16% carbon load, respectively. All three sets of data yielded linear Van 't Hoff plots with a correlation coefficient of 0.994 or higher and the enthalpy values thus obtained together with their 95% confidence limits are given in Table I. The  $\Delta H^0$  values calculated from retention data obtained with plain methanol on the three columns are considered statistically indistinguishable. The value appropriate for the naked silica was taken as  $\Delta H_2^0$  in eqn. 7 to calculate the range for the parameters  $A$  and  $C$ . The results in Table I show that the values obtained for  $A$  and  $C$  from the least square fit of retention data measured at various eluent compositions fall within the range predicted via eqn. 7 from data measured at different temperatures.

As seen in Table I the values of  $B$  obtained with the two octadecyl-silicas, Partisil ODS and Partisil ODS-2 are quite similar. This is expected when the same eluent is used and the retention is governed by a solvophobic interaction of the elute with long-chain alkyl ligates of the bonded phases. According to solvophobic theory<sup>1</sup> the value of  $B$  is expected to increase with the molecular contact area between the elute and the hydrocarbonaceous surface upon binding to the stationary phase. For small aromatic compounds, Snyder *et al.*<sup>29</sup> have reported  $B$  values in the range 4-9, whereas we found that  $B$  is about 10 as expected for a comparatively large molecule like DB18C6. We may note that parameter  $B$  evaluated for a standard elute can be used as a convenient measure of the eluotropic strength of the organic solvent component of the eluent.

### Comparison of chromatographic systems

Comparison of the values of  $A$  and  $C$  listed in Table I (note that  $A$  and  $C^{-1}$  are equal to the limiting retention factors  $k_1$  and  $k_2$  obtained with neat organic solvent as the eluent in RPC) suggests that 0.12 and 1.7% of the retention factor can be attributed to hydrophobic interactions when plain methanol is used as the eluent with Partisil ODS and ODS-2, respectively. The results presented in Table I therefore imply that DB18C6 is essentially retained by silanophilic interactions on both stationary phases when neat methanol is the mobile phase. Predominance of silanophilic interactions when neat methanol is the eluent can also be inferred from the comparison of the retention factors obtained with DB18C6 on the three columns listed in Table I. The retention factor is largest with "naked" silica (10- $\mu$ m Partisil), which is expected to have the highest "phase ratio" for surface silanols. On the other hand, octadecyl-silica with the highest carbon load (10- $\mu$ m Partisil ODS-2) is presumed to have the lowest accessible silanol concentration at the surface, and consequently yields the smallest retention factor.

Table I shows that for Partisil ODS the value of parameter  $D$  is smaller than for Partisil ODS-2, which has a lower silanol concentration at the surface. According to eqn. 3,  $D$  is equal to the slope of the  $1/k_2$  vs.  $\psi$  plot and, thus, measures the decrease in retention due to silanophilic interactions upon increasing the concentration of the more polar solvent component of the eluent. In fact, parameter  $D$  could be considered as the product of parameter  $C$  and the binding constant of the more polar eluent component to the silanol groups at the stationary phase surface<sup>30</sup>. As the binding constant is expected to be about the same for silanophilic binding of water to the surface of the stationary phases under consideration, the  $D/C$  ratio should also be the same. The data listed in Table I for Partisil ODS and ODS-2 appear to support indeed this interpretation.

A comparison of the eluent compositions at which the retention factor is minimum can also shed light on the relative significance of the silanophilic interactions observed with different stationary phases. As seen in Fig. 1 the minimum of the  $\kappa$  vs.  $\psi$  plot for the data obtained on Partisil ODS occurs at a water concentration in the eluent higher than that which gives a minimum for the data obtained with Partisil ODS-2. This behavior is expected because of the relatively high concentration of accessible residual silanols at the surface of the former stationary phase.

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