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Comparison of octadecyl-bonded alumina and silica for reversed-phase high-performance liquid chromatography

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SUMMARY

Chromatographic properties of an octadecyl-bonded alumina (ODA) high-performance liquid chromatographic stationary phase are described and compared with those of commonly used octadecylsilica (ODS) stationary phases. Under reversed-phase conditions, selectivity of the ODA phase is shown to be similar to that of ODS, resulting in similar elution orders for most compound mixtures. The higher stability of the ODA material to alkaline solvents and the absence of acidic silanol sites on its surface allow a more efficient separation of organic bases with simpler mobile phases than is generally possible with ODS. Comparison of the correlation of the capacity factors of 31 organic compounds on the ODA and ODS stationary phases indicates a higher degree of hydrogen-bonding solute–stationary phase interactions on ODA than on ODS. Multiple-ring aromatic compounds are more strongly adsorbed on the ODA stationary phase than on ODS. Differences in the retention behavior of benzylamine on the ODA and ODS stationary phases with increasing percentages of methanol in the mobile phase are reported. These are interpreted in terms of the differences in solute–stationary phase interactions on the two phases resulting from the chemical properties of their silica and alumina backbones.

INTRODUCTION

The use of chemically modified silicas as stationary phases in high-performance liquid chromatography (HPLC) is well established. Advances in the understanding of the chemical and physical properties of silica have led to the development of silica-based bonded phases with a wide variety of selectivities and efficiencies. In particular, alkyl-bonded silica stationary phases have gained wide popularity for HPLC separations of low-molecular-weight organic molecules using aqueous mobile phases[1]. Recently, these types of stationary phases have been employed for separations of higher-molecular-weight compounds, such as peptides and proteins[2,3].

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Despite their wide utility, the chemical properties of silica often limit the conditions under which separations of compounds can be attempted. Silica and its derivatives are generally stable only between pH 2 and 8, and thus mobile phases used with silica-based stationary phases are limited to this pH range. This complicates the separations of some compounds (*e.g.* weak organic bases) which are ionized over this pH range. In addition to poor separations caused by equilibrium between ionized and unionized forms of basic solutes, residual acidic silanol groups which are present in varying degrees on the surface of alkyl-bonded silicas can interact with organic bases, often resulting in irreproducible retention and poor peak symmetry[4,5]. Limiting these effects in the analysis of basic compounds on silica-based stationary phases requires a very careful choice of column manufacturer, mobile phase pH, mobile phase additives and sample size[6].

To overcome the problems associated with silica-based stationary phases, there have been many efforts to develop alkyl-bonded HPLC stationary phases which are based on materials that are more chemically stable and contain less chromatographically active sites than silica. Most of these efforts have employed derivatives of either polystyrene–divinylbenzene copolymers or alumina, materials which can be fabricated into microspherical particles of usable diameters and porosities for HPLC applications. Benson and coworkers bonded octadecyl functionalities onto a polystyrene–divinylbenzene polymer matrix, producing a stationary phase (MP-1) with remarkable pH stability (pH 2–14) and superior mass-transfer properties to stationary phases consisting of polystyrene–divinylbenzene copolymers alone[7]. However, retention of compounds on these stationary phases is substantially greater than that obtained with octadecylsilane columns using solvents of similar strength, and high column backpressures can result with certain mobile phases (*e.g.* those containing tetrahydrofuran) due to irreversible swelling of the polymeric particles. Bien-Vogelsang *et al.*[8] coated porous alumina (Spherisorb) with a variety of polymeric films, including polybutadiene and polyoctadecylsilane. Only a few applications of these pH stable stationary phases have been reported[9,10]. However, polymer-coated stationary phases have generally been found to exhibit lower chromatographic efficiency than corresponding monomeric alkyl-bonded materials[11].

Recently, Wieserman and co-workers successfully produced a material in which monomeric octadecyl functionalities are covalently bonded to the surface of a highly porous, microspherical alumina developed specifically for chromatographic applications[12]. Preliminary studies of this "octadecylalumina" (ODA) material demonstrated that it retains the pH stability and permeability of its alumina precursor, while exhibiting chromatographic selectivity similar to standard octadecylsilica (ODS) stationary phases[13]. In this paper, we report some chromatographic properties of this unique alumina-based HPLC stationary phase, and compare them with the properties of the more common silica-based ODS material.

EXPERIMENTAL

Materials

All solvents used were glass distilled, obtained from E.M. Science (Cherry Hll, NJ, U.S.A.). Pharmaceutical compounds were obtained from Sigma (St. Louis, MO, U.S.A.). All other compounds were obtained from Aldrich (Milwaukee, WI, U.S.A.).

Apparatus

The HPLC system consisted of a Perkin-Elmer Series 410 solvent-delivery system, a Rheodyne Model 7125 injector (20-μl loop) and a Perkin-Elmer Model LC-135 diode-array UV–visible detector. Unless otherwise specified, the wavelength monitored was 220 nm. Chromatographic data were recorded and processed on a Perkin-Elmer Omega data system.

The ODA column used in these studies was obtained from Biotage (Cambridge, MA, U.S.A.). It was packed with an experimental (not yet available for purchase) Alcoa Unisphere™ monomeric octadecyl-bonded alumina stationary phase, as described by Wieserman and co-workers[12,13]. The Unisphere alumina particle consists of *ca.* 2000-Å thick platelets bonded together to form 8-μm spherical particles with open, readily accessible inner-platelet macroporosity and inter-platelet microporosity. The following physical measurements were obtained on the Unisphere material before bonding of the octadecyl groups:

Mean particle size	8 μm
Surface area	103 m^2/g
Pore volume	0.295 ml/g
Median pore size of microporosity	11 nm

The bonded phase consists of bound monomolecular octadecyl groups on the surface. The preparation conditions insure that only covalently bound octadecyl groups remain on the surface of the Unisphere particles. No capping reagents were used because of the high loading density of the octadecyl groups and excellent chromatographic performance before capping. The loading of the octadecyl groups were 7.35% (w/w) carbon and 3.4 μmol C_{18}/m^2. The dimensions of the ODA column were 250 mm × 4.6 mm I.D. When this column was used, the mobile phase flow-rate was set at 2 ml/min.

A column packed with unbonded Unisphere alumina was also obtained as a gift from Biotage. The material had the same particle size, surface area and pore volume described above. Column dimensions were 250 mm × 4.6 mm I.D., and the mobile phase flow-rate was set at 2 ml/min.

Two ODS columns were used in these studies: (I) Perkin-Elmer C_{18}, 150 mm × 4.6 mm I.D., 5 μm particle size; mobile phase flow-rate 1 ml/min, and (II) Alltech Econosil C_{18}, 250 × 4.6 mm I.D., 10 μm particle size; mobile phase flow-rate 2 ml/min.

Test mixture analyses

The mixture of substituted anilines used to produce the chromatograms shown in Fig. 1 contained 0.5–1.0 mg/ml of each compound in methanol. Analysis of this mixture on the ODA column (Fig. 1A) was performed using a mobile phase consisting of methanol–0.01 *M* aqueous 4-morpholinopropanesulphonate (MOPS) (50:50), adjusted to pH 7.4 by addition of concentrated sodium hydroxide solution. Analysis of the mixture on ODS column I (Fig. 1B) was performed using a mobile phase consisting of methanol–0.05 *M* aqueous ammonium phosphate buffer (70:30) adjusted to pH 7.4. Chromatographic peak asymmetry factors (Table I) were calculated using the method described by Kirkland *et al.*[14].

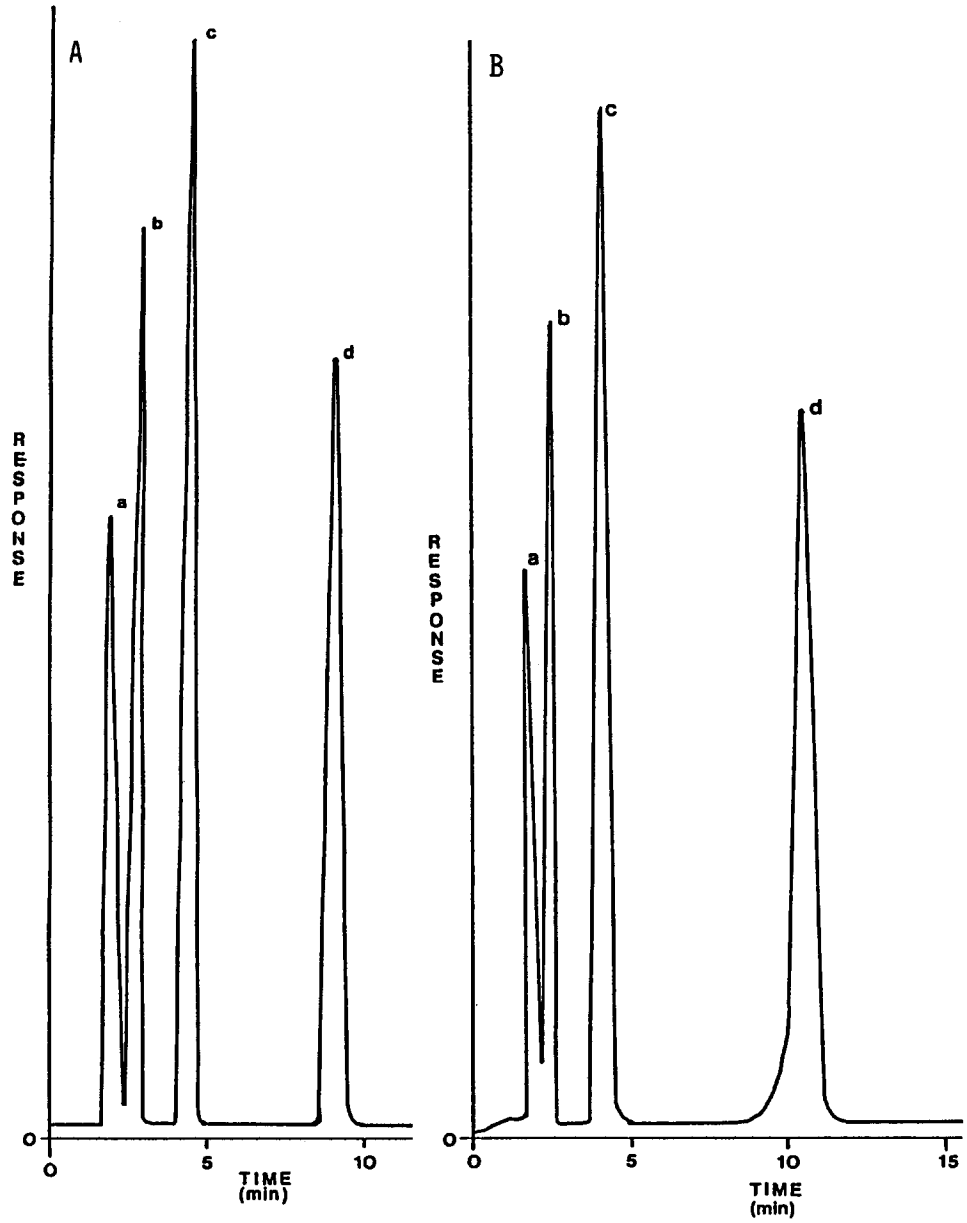

Fig. 1. HPLC chromatograms of aniline derivatives on ODA (A) and ODS (B) stationary phases, using mobile phases buffered at pH 7.4. Detector wavelength = 254 nm. Peaks: a = aniline; b = N-methylaniline; c = N,N-dimethylaniline; d = N,N-diethylaniline.

The mixture of pharmaceutical compounds used to produce the chromatogram shown in Fig. 2 contained 0.2–0.5 mg/ml of each compound in methanol. Analysis of the mixture was performed on the ODA column using a mobile phase consisting of methanol–0.1 *M* aqueous sodium hydroxide (35:65).

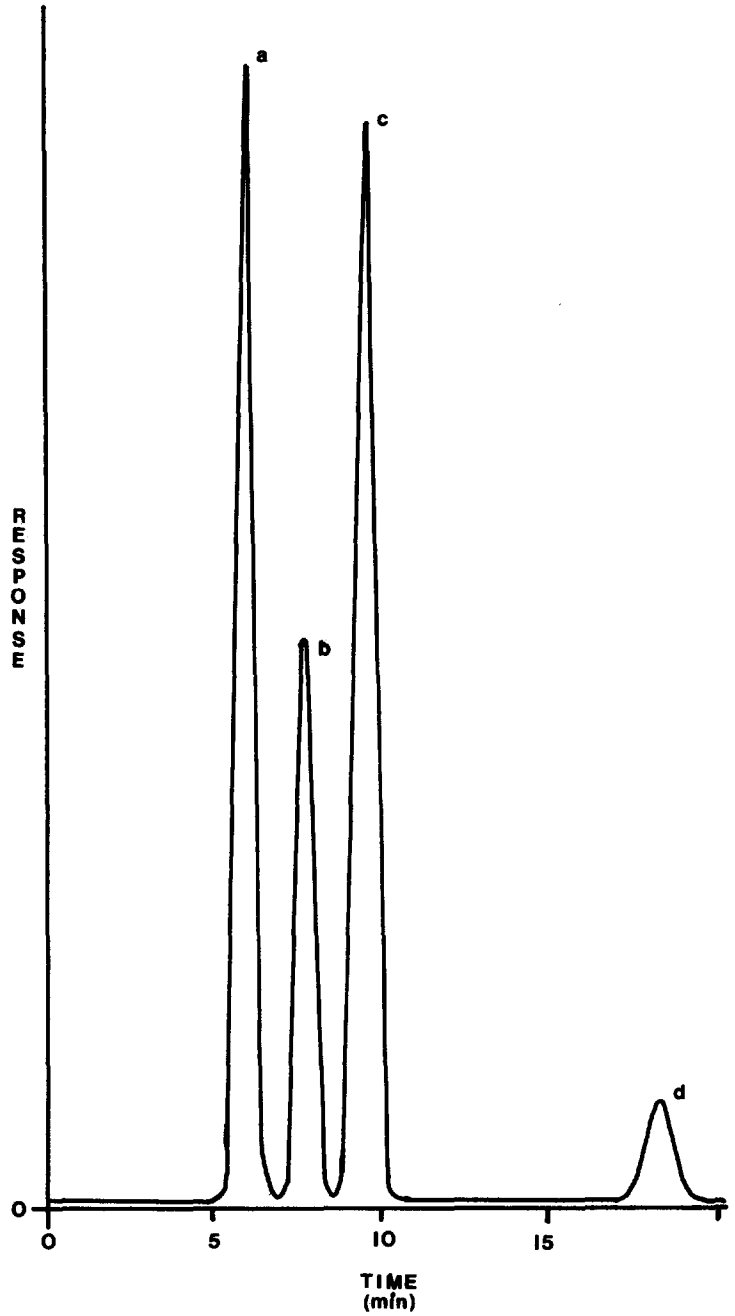

Fig. 2. HPLC chromatogram of a drug mixture on ODA stationary phase using an alkaline mobile phase consisting of methanol–0.10 *M* aqueous sodium hydroxide (35:65). Peaks: a = codeine; b = procaine; c = thebaine; d = cocaine.

TABLE I

CALCULATED ASYMMETRY FACTORS FOR PEAKS IN THE CHROMATOGRAMS OF SUBSTITUTED ANILINE MIXTURE (FIG. 1)

Peak	*Compound*	*Asymmetry Factor (ODS)*	*Asymmetry Factor (ODA)*
a	Aniline	1.00	1.00
b	N-Methylaniline	1.00	1.00
c	N,N-Dimethylaniline	1.25	1.06
d	N,N-Diethylaniline	0.57	0.98

Capacity factor determinations

Retention times for each of the compounds shown in Table II (injected as 1-mg/ml solutions in methanol) were determined on the ODA column using a mobile phase consisting of methanol–0.01 *M* aqueous MOPS (30:70) adjusted to pH 7.4, and on ODS column II using a mobile phase consisting of methanol–0.01 *M* aqueous MOPS (50:50), also adjusted to pH 7.4. The capacity factor, k', of each compound was calculated by the formula $k' = (t - t_0)/t_0$, where t is the compound's retention time and t_0 is the retention time of an unretained substance, determined by injection of a sample of pure methanol.

An attempt was made to evaluate the k' values of anthracene, phenanthrene and biphenyl on the unbonded Unisphere alumina column using the same conditions as that used with the ODA column. However, none of these compounds eluted from the column over a 2-h period.

TABLE II

CHROMATOGRAPHIC CAPACITY FACTORS ON ODS AND ODA COLUMNS

Compound	*Log k' (ODS)*	*Log k' (ODA)*	*Compound*	*Log k' (ODS)*	*Log k' (ODA)*
m-Aminophenol	−0.663	−0.275	Methyl benzoate	0.609	0.861
Benzamide	−0.274	0.044	Trichloroethylene	0.855	1.045
Resorcinol	−0.608	−0.021	Methyl salicylate	0.852	1.252
Aniline	−0.058	0.079	Chlorobenzene	1.071	1.249
Benzyl alcohol	0.088	0.230	Ethyl benzoate	1.007	1.200
Acetanilide	0.031	0.066	1-Naphthol	0.769	1.461
Picoline	0.154	0.016	Toluene	0.946	1.183
Phenol	−0.022	0.373	2-Naphthol	0.705	1.366
Ethyl propionate	0.338	0.230	Bromobenzene	1.142	1.386
Benzonitrile	0.185	0.376	Benzophenone	1.019	1.428
Acetophenone	0.285	0.447	Thymol	1.213	1.625
p-Cresol	0.363	0.729	Naphthalene	1.358	1.641
m-Cresol	0.351	0.692	Biphenyl	1.625	—[a]
Benzene	0.731	0.772	Phenanthrene	1.807	—[a]
Quinoline	0.433	0.655	Anthracene	1.807	—[a]

[a]Did not elute under the chromatographic conditions.

Capacity factor determinations of benzylamine with various percentages of methanol in the mobile phase

The retention times of benzylamine (injected as a 1-mg/ml solution in methanol) were determined on the ODA column and ODS column II using mobile phases ranging from 0–100% methanol and 100–0% aqueous MOPS, adjusted to pH 7.4. Capacity factors were calculated by the equation described above.

RESULTS AND DISCUSSION

General chromatographic properties

Since the monomeric ODA stationary phase is structurally similar to standard octadecylsilane material, the general hydrophobic partitioning mechanisms associated with retention of solutes on ODS stationary phases are also present in ODA. As a result, the selectivity of the alumina-based ODA stationary phase is similar to that of the silica-based ODS. This is demonstrated by the correspondence of elution order in the separation of some substituted anilines obtained on an ODA column (Fig. 1A) and on an ODS column (Fig. 1B).

Despite the relative similarity of the chromatograms of the aniline mixture obtained on the ODS and ODA columns, the following subtle differences in the chromatographic conditions and peak shapes gives an indication of the unique properties of the alumina-based material. Firstly, with this and other separations on the ODA column, it is necessary to avoid the commonly used phosphate buffers in the mobile phase; otherwise, reduced solute retention and unreproducible selectivity can result[15]. This phenomenon, which does not occur with polymer-coated alumina phases, is apparently caused by the displacement of surface octadecyl groups on ODA by phosphate ion, occuring in much the same way as halide ions displace surface hydroxyl groups on underivatized alumina[16]. This problem is easily avoided by substituting for phosphate a popular zwitterionic buffer, MOPS[17]. We have found that the MOPS buffer does not degrade the ODA phase to any measurable extent. Using a mobile phase consisting of this buffer and methanol, the symmetries of the peaks obtained in the chromatogram of the aniline mixture on the ODA column at pH 7.4 were equal or superior to those in the chromatogram of the same mixture on the ODS column, also obtained at pH 7.4 under optimized conditions (see calculated asymmetry factors in Table I).

Despite the incompatibility of the ODA stationary phase with mobile phases containing phosphate buffers at neutral pH values, the material is remarkably resistant to decomposition by hydroxide solutions or other alkaline solvents. This allows the employment of mobile phases at high pH values for suppressing the ionization and thus simplifying conditions for the separation of basic compounds that are ionized over the usable pH range of ODS (*i.e.* pH 2–8). An example of such an application is the separation of some pharmaceutical compounds on ODS using a methanolic mobile phase containing aqueous sodium hydroxide. The chromatogram obtained using this mobile phase (pH > 10) is shown in Fig. 2, and exhibits good separations and peak symmetries. Since the pK_a values of the compounds are all within the range of 7–9 (ref. 18), suppression of ionization using a mobile phase with a pH compatible with an ODS phase is impossible. In fact, the only reported successful separation of these compounds on ODS required a more complicated mobile phase containing ion-pairing reagents[19].

Log k' correlations

Several recent studies have provided definitive evidence that retention of low molecular weight compounds on ODS occurs by a partitioning mechanism. This evidence includes the linear correlation of log k' of organic compounds on ODS with the logarithms of their octanol–water partition coefficients[20] as well as a similar correlations of log k' values with stationary phase carbon densities[21]. However, other studies have demonstrated a small but significant contribution of other types of solute-stationary phase interactions, including those related to hydrogen-bonding[22]. In order to obtain comparative information on the retention mechanisms operating with ODS and ODA stationary phases, the correlation of the k' values of 31 compounds on ODA with those obtained on an ODS column under similar conditions was investigated.

The compounds used for the log k' correlation study and their capacity factors are shown in Table II, and a graph of the data is displayed in Fig. 3. The correlation between log k' values on the two columns is generally good, indicating a general similarity in retention mechanisms for both ODS and ODA. Yet, there are a few differences. The non-elution of biphenyl, anthracene and phenanthrene on ODA under the chromatographic conditions (see Table II) can at least in part be attributed to an adsorption mechanism. These three compounds were also found not to elute from an unbonded Unisphere alumina column using the same mobile phase, which is consistent with the strong adsorption of polycyclic aromatic hydrocarbons (PAHs) on unbonded alumina reported in previous studies[16,23]. This strong adsorption has been attributed to the interaction of electron-rich solutes such as PAHs with electron-deficient aluminum atoms on alumina[16,23,24]. The analogous adsorption effects found with the ODA column suggest a similar accessibility of the PAHs to electron-deficient aluminum atoms in the bonded ODA phase. Surprisingly, the retention of naphthalene is apparently not affected by this adsorption mechanism; it does not have an abnormally high k' value, and it fits well on the overall log k' correlation line (see Table II and Fig. 3). Although the reason for this is not clear, it may be that a greater number of π electrons (as in anthracene) or a greater constitutional flexibility (as in biphenyl) is required before adsorption becomes a significant retention mechanism for solutes on the ODA stationary phase.

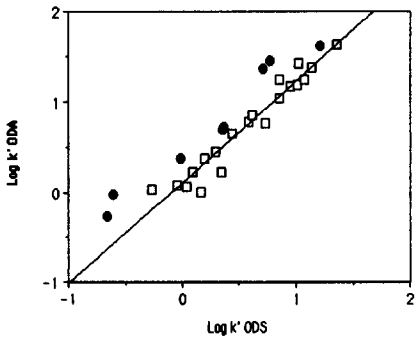

Fig. 3. Plot of the logarithm of the capacity factors (log k') of the compounds shown in Table II on the ODA stationary phase *vs.* the logarithm of their capacity factors on an ODS stationary phase. □ = Non-phenolic compounds; ● = phenolic compounds.

Comparison of log k' correlations obtained for phenolic and non-phenolic compounds on the ODS and ODA columns indicates that the effects of hydrogen bonding and acid–base interactions on retention of compounds on the two types of columns are somewhat different. As shown in Fig. 3, the phenolic compounds exhibit a small but significant deviation from the correlation obtained for other compounds. The data points for the phenols fall above the general ODA–ODS correlation line, indicating that these compounds are retained on ODA to a greater degree than on ODS. Greater relative retention of phenols on unbonded alumina than on silica has been observed previously, and has been atrributed to the greater Brønsted basicity (*i.e.* hydrogen-bond accepting ability) of the alumina's oxygen atoms *vs.* those of silica[16,23,25]. The occurrence a similar difference in the relative retention of phenolic solutes on the ODA vs ODS phases indicates that at least some of these basic oxygen atoms are accessbile to phenolic solutes during their separation on ODA. The data are consistent with an inherently greater basicity of the ODA phase than ODS, corresponding to the previously established greater basicity of unbonded alumina than silica[4,23].

Acid–base interactions and effects of methanol composition in mobile phase

The overt acidity of unbonded silanol groups present in ODS and other silica-based stationary phases can cause interactions between these groups and basic solutes. These interactions are much stronger than those associated with hydrogen bonding discussed earlier, and can interfere with the normal hydrophobic partitioning processes, resulting in loss of chromatographic separation and efficiency[4–6]. As shown in this and earlier studies, acidic sites on alumina have a much lower effect on chromatographic retention than corresponding sites on silica. As a result, interfering interactions with basic compounds are minimal, and separations and chromatographic peak shapes are improved for such compounds at neutral pH values. This is reflected in the greater symmetry (Table I) of the peaks corresponding to the most basic compounds (*i.e.*, N,N-dimethylaniline and N,N-diethylaniline) in the chromatogram of the substituted aniline mixture on the ODA column (Fig. 1A) than of those in the chromatogram of the same mixture on an ODS column (Fig. 1B) using a similare mobile phase.

Comparison of the retention behavior of another weak base, benzylamine, on the ODS and ODA columns also indicates differences in solute–stationary phase interactions occurring on the two materials. Fig. 4 shows a semilogarithmic graph of k' of benzylamine on each column *vs.* the percentage of methanol in the mobile phase. On the ODS column, k' first decreases with methanol composition, reaches a minimum at 50% methanol, and then increases as the percentage of methanol is increased beyond that. Similar relationships between the k' values of weak organic bases and the percentage of methanol in the mobile phase have been reported previously[26,27]. The passage of the k' values of such compounds through a minimum as the fraction of methanol is increased has been attributed to two different effects:

(1) A change in the partitioning mechanism from one dominated by hydrophobic solute expulsion from the aqueous component of the solvent to one dominated by polar solute interactions with the solvent's methanolic component[26,28];

(2) A change in mechanism from one dominated by hydrophobic expulsion to one dominated by interactions of the basic solutes with the acidic silanol sites on the

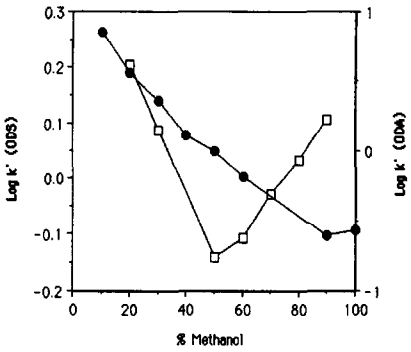

Fig. 4. Plot of the logarithm of the capacity factors of benzylamine on the ODS and ODA columns *vs.* the percentage of methanol in the mobile phase. □ = ODS column; ● = ODA column.

stationary phase[27]. As shown in Fig. 4, the retention behavior of benzylamine on the ODA stationary phase as the percentage of methanol in the solvent is increased is obviously different from that on ODS. Moreover, this difference gives an indication of the dominant mechanisms responsible for the phenomenona observed on both phases. In contrast to that observed on ODS, the k' value of benzylamine continues to decrease as the percentage of methanol in the mobile phase is increased beyond 50%, levelling off only when the compound becomes virtually unretained. The parabolic relationship between capacity factor and methanol composition on the ODS column is not observed on ODA, and therefore it cannot be caused exclusively by a change in the partitioning properties of the solvent alone (effect 1, above), since that would have caused a similar relationship on the ODA column. A change in the partitioning properties of the ODS, or an increase in the relative effect of an interfering retention mechanism on the stationary phase as the methanolic component of the solvent is increased must play a part in establishing the relationship between k' and percentage methanol on ODS. An increasing degree of acid-base interactions between basic solutes and residual silanol sites on the ODS column (or at least a relative increase in their effects on retention) as the amount of methanol in the mobile phase is increased (*i.e.*, effect 2, above) is the most plausible explanation. It accounts for the different retention behavior of benzylamine on the ODA stationary phase, which has no silanol sites and therefore is not subject to this mechanism. Additionally, benzylamine and other weak bases have been previously demonstrated to interact strongly with acidic silanol sites on ODS, especially when methanolic solvents are employed[29]. While this explanation has not been rigorously proven here, the data clearly indicates that the parabolic relationship between k' and methanol percentage for benzylamine and other weak bases found on ODS must at least in part be dependent upon the properties of the stationary phase, and not simply upon those of the mobile phase.

CONCLUSIONS

In this work, the general partitioning mechanisms associated with retention of solutes on a monomeric octadecyl-bonded alumina HPLC stationary phase have been shown to be similar to those of the widely used octadecylsilane phases. The

alumina-based material has a greater stability to alkaline solvents than ODS, and contains no acidic silanol sites which can interfere with hydrophobic partitioning on silica-based phases. All of these features allow the ODA stationary phase to give predictable and efficient separations of some types of compounds (*e.g.* weak bases) using simpler chromatographic conditions than those which are needed to obtain comparable separations on ODS. More research is needed on the chromatographic properties and optimal conditions for separating other compound classes on ODA (*e.g.* phenols and PAHs), which are more strongly retained on the alumina-based phase than on ODS.

Subtle differences in the retention of compounds on ODA and ODS can only be understood in terms of solute interactions with the alumina and silica backbones of these phases (*e.g.* hydrogen bonding). Since neither silica nor alumina are completely inert, it is not likely that such interactions can ever be completely eliminated by modifying chromatographic conditions. Consideration of the nature and extent of these interactions is thus necessary for accurately predicting the retention properties of solutes on either of these types of materials.

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