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# Novel, highly deactivated reversed-phase for basic compounds

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## SUMMARY

Silica has proven invaluable as a support material for reversed-phase high-performance liquid chromatography. However, contributions of residual acidic silanols lead to poor chromatography of basic compounds. This paper demonstrates the utility of a new, highly deactivated silica-based reversed-phase packing material for the study of a series of weak and strong organic bases. Correlation between retention times on the column and octanol–water partition coefficients at pH 7.0 is demonstrated. Also shown are the results of further experiments in which we investigated the retention characteristics of basic, acidic and neutral compounds by the new deactivated phase.

## INTRODUCTION

Silica-based reversed-phase high-performance liquid chromatography (RP-HPLC) has become the method of choice for a large proportion of liquid chromatographic separations. Many of the benefits of RP-HPLC can be attributed to the silica itself, which lends mechanical stability, narrow distribution of pore and particle sizes, and chemical reactivity for easy modification of the surface silanol groups. The latter characteristic of silica provides numerous possibilities for stationary phases.

Unfortunately this reactivity, although the key to the success of silica supports, is also in part the source of its limitations. Even under optimal known conditions, only partial alkylation of the silanols is accomplished, leaving an excess of unreacted groups which can strongly interact with and adsorb basic organic analytes. The kinetics of this adsorption/desorption are slow relative to the other chromatographic processes[1] and produce broad, tailing peaks which are difficult to quantify and can interfere with closely eluting compounds.

A great deal of research has gone into the quantitation of residual silanols, as witnessed by the large variety of techniques used. Methods employed for this study have included: complexation with copper-amine compounds[2], methane or ethane release after reaction with methyl- or ethyl-lithium compounds[3], deuterium exchange[4], surface pH[5,6], $^{29}Si$ cross-polarization magic angle spinning NMR, proton spin-counting solid-state NMR, diffuse-reflectance infrared Fourier-transform

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(DRIFT), thermogravimetric and elemental analysis[7,8], Raman spectroscopy[9,10], behavior of various basic test probes[11–15], titration with sodium hydroxide[16] and gas-phase titration with amines[17] or diethylketone[18].

In addition, it has been suggested that the silica surface has different kinds of silanol groups, *e.g.* free (isolated), associated (vicinal), geminal, etc., having different physical/chemical activities and affecting differently the chromatographic results[3,19].

To limit the negative effect of such groups on reversed-phase supports, special treatment of the surface before bonding and better bonding procedures were recommended. For general discussions of silanol research see Unger[3], Engelhardt and Müller[5], Nawrocki and Buszewski[20], Bayer and Paulus[21] and Iler[22].

The problem of reactive silanols is most apparent in the chromatography of amines, especially those which are sterically less hindered and thus are able to penetrate to the surface and interact with the silanol groups. To diminish the effect of the silanols, bulky ligands were synthesized to make the underlying silanols less accessible to basic compounds[23,24]. Other investigators formed a protective polymer on the silica surface and showed improved chromatographic results[25,26]. Nevertheless, even with such improvements, silanol suppression techniques such as using low pH or high ionic strength mobile phases, addition of amines, etc., are still required. In this respect, it should be mentioned that non-silica-based HPLC supports (*e.g.* cladded

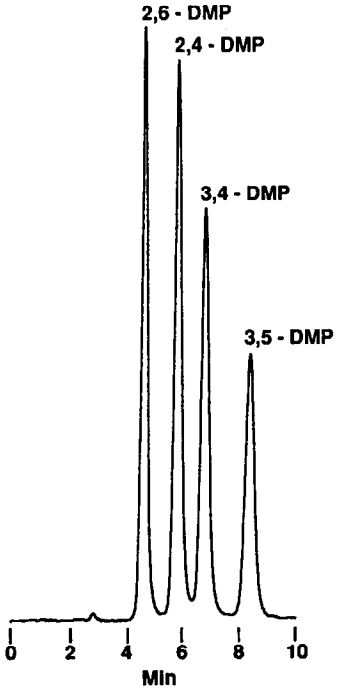



Fig. 1. Dimethylpyridine (DMP) derivatives. Column, 50 mm × 4.6 mm Suplex pKb-100 (5-$\mu$m packing); mobile phase, acetonitrile–25 m*M* aqueous potassium phosphate, pH 7.0 (2:98); detection, 254 nm UV, 0.16 a.u.f.s.; sample, 5 $\mu$l (0.5 $\mu$l/ml of each compound in mobile phase buffer); flow-rate, 2 ml/min; temperature, 35°C.

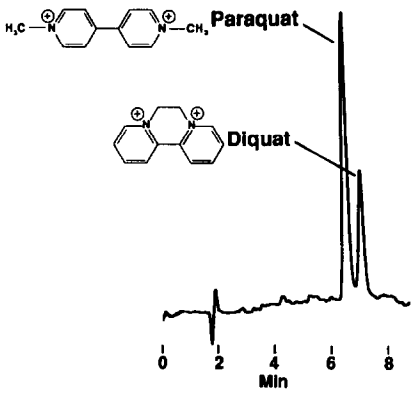



Fig. 2. Quaternary ammonium compounds paraquat and diquat. Column, 150 mm × 4.6 mm Suplex pKb-100 (5-$\mu$m packing); mobile phase, acetonitrile–(25 m$M$ potassium phosphate, 5 m$M$ decylsulfonate, pH 3.0) (30:70); detection, 254 nm UV, 0.008 a.u.f.s.; sample, 10 $\mu$l (paraquat 4 $\mu$g/ml, diquat 10 $\mu$g/ml in mobile phase buffer); flow-rate, 1 ml/min; ambient temperature.

alumina, polymeric resins, and graphitized carbon) show potential but have their own limitations.

Recently[27,28] a new HPLC material was introduced that possesses the benefits of silica-based RP but minimizes the adverse silanol reactivity by attaching chemically competing nucleophilic groups close to the silica surface. Using such a reversed-phase material, the usual chromatographic performance of non-polar analytes is extended to basic probes. The result is that analyses of bases can be performed at neutral pH, low ionic strength, and without amino modifiers. An example of the utility of the phase for the separation of dimethylpyridine (lutidine) isomers is seen in Fig. 1. Even the more potent quaternary bases such as paraquat and diquat are easily resolved (see Fig. 2).

This novel reversed-phase column, Suplex™ pKb-100, was used to study a series of predominantly monofunctional organic bases which differ in the ability of the basic nitrogen to interact with the surface silanols[29–31]. Correlation between chromatographic retention and octanol–water partition coefficients was determined and asymmetry factors were correlated to the p$K_a$ values of the different bases.

We also studied how the retention of these basic compounds, as well as acidic and neutral species, is affected by changes in the mobile phase conditions. Since silanols are effectively excluded from the retention process, the retention is primarily the result of altering the polar/hydrophobic nature of the sample molecule and of concurrent partitioning changes in the ligated stationary phase.

This report should serve as a demonstration of the potential use of the new deactivated reversed-phase in the study of organic bases.

## EXPERIMENTAL

### *Chromatography*

Chromatography was carried out on a Spectra-Physics (San Jose, CA, U.S.A.) liquid chromatographic system consisting of an SP8800 gradient pump, SP8780 autosampler and SP4290 integrator interfaced with LABNET/RS-232 connected to

a Kratos (Ramsey, NJ, U.S.A.) Spectroflow 757 variable-wavelength UV detector or on a Hewlett-Packard (Avondale, PA, U.S.A.) HP-1090 liquid chromatograph equipped with an HP-1040A diode array detector, autosampler, column oven, HP-85B PC system controller and an HP-3392A integrator/recorder.

The HPLC columns used were Suplex pKb-100 or Supelcosil™ LC-8-DB (both 150 × 4.6 mm, 5-μm particles having 100-Å pores) and were obtained from Supelco, Bellefonte, PA, U.S.A. In some experiments short columns (50 × 4.6 mm) were used to reduce the run time and conserve mobile phase. Analytical columns were protected by 0.5-μm in-line frit filters.

Analytical columns used in the deactivated column comparison study were as follows: Ultracarb ODS-20 (150 × 4.6 mm, 5 μm, Phenomenex, Rancho Palos Verdes, CA, U.S.A.); Zorbax $R_x$ (150 × 4.6 mm, 5 μm, Mac-Mod Analytical, Chadds Ford, PA, U.S.A.); SynChropak SCD-100 (150 × 4.6 mm, 5 μm, Keystone Scientific, State College, PA, U.S.A.); Capcell Pak C18-SG (250 × 4.6 mm, 5 μm, Shiseido, Tokyo, Japan); Hibar LiChrosorb RP-Select B (250 × 4 mm, 5 μm, E. Merck, Darmstadt, F.R.G.); Chemcosorb 5-ODS-H (150 × 4.6 mm, 5 μm, Chemco Scientific, Osaka, Japan); Deltabond Octyl (150 × 4.6 mm, 5 μm, Keystone Scientific); Chromega γ-$C_{18}$ (250 × 4.6 mm, 5 μm, Fisher Scientific, Fair Lawn, NJ, U.S.A.) and Rexchrom ODS (150 × 4.6 mm, 5 μm, Regis, Morton Grove, IL, U.S.A.).

All solvents were of HPLC grade and were obtained from Accusolv Anachemia (Champlain, NY, U.S.A.). Deionized water was prepared in-house on a Milli-Q system (Millipore, Milford, MA, U.S.A.). HPLC-grade potassium hydrogen phosphate and phosphoric acid were purchased from Fisher Scientific. Triethylamine (TEA) was obtained from Kodak (Rochester, NY, U.S.A.).

Samples for chromatography were obtained from Sigma (St. Louis, MO, U.S.A.) or Aldrich (Milwaukee, WI, U.S.A.). Stock solutions of standards were made in acetonitrile and were diluted in mobile phase buffer (25 m*M* aqueous potassium phosphate, pH 7.0) before use. Mobile phase and other chromatographic conditions appear in the figure captions.

Capacity factors ($k'$) were measured by the equation:

$$k' = (t_R - t_0)/t_0 \tag{1}$$

where $t_R$ is the retention time of the compound of interest and $t_0$ is the column void time measured using the solvent disturbance peak obtained when trace amounts of methanol were injected onto the column.

The asymmetry factor ($AF_{10}$) was calculated by drawing a perpendicular line from the apex of the peak to the baseline and measuring the front ($A$) and back ($B$) widths of the peak at 10% height:

$$AF_{10} = B/A \tag{2}$$

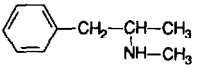
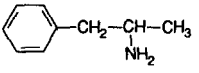
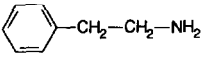
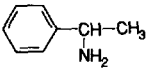
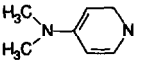
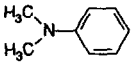
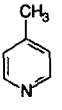
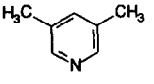
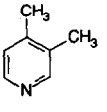
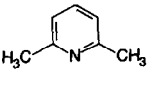
Insight into the significance of capacity and asymmetry factors can be found in ref. 32.

The Foley–Dorsey method[33], which utilizes statistical moments, was used to measure the column efficiency since skewed peaks were often involved. The method uses the equation:

$$N = \frac{41.7\left(\frac{t_R}{w}\right)^2}{\frac{B}{A + 1.25}} \tag{3}$$

TABLE I

STRUCTURES OF COMPOUNDS USED IN THIS STUDY

| *Compound No.* | *Name* | *Structure* |
|---|---|---|
| 1 | Benzene |  |
| 2 | Methamphetamine |  |
| 3 | Amphetamine |  |
| 4 | β-Phenethylamine |  |
| 5 | α-Phenethylamine |  |
| 6 | 4-Dimethylaminopyridine |  |
| 7 | N,N-Dimethylaniline |  |
| 8 | Pyridine |  |
| 9 | 4-Methylpyridine |  |
| 10 | 3,5-Dimethylpyridine |  |
| 11 | 3,4-Dimethylpyridine |  |
| 12 | 2,6-Dimethylpyridine |  |

*(Continued on p. 362)*

TABLE I *(continued)*

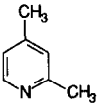
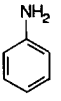
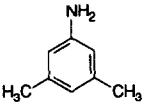
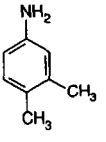
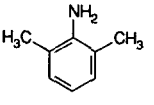
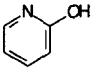
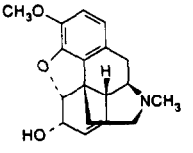
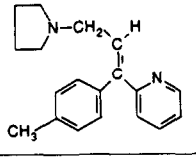
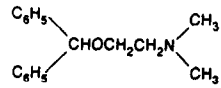
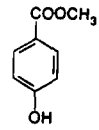
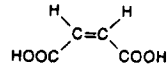
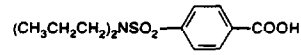
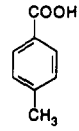
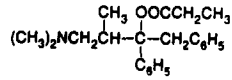
| *Compound No.* | *Name* | *Structure* |
|---|---|---|
| 13 | 2,4-Dimethylpyridine |  |
| 14 | Aniline |  |
| 15 | 3,5-Dimethylaniline |  |
| 16 | 3,4-Dimethylaniline |  |
| 17 | 2,6-Dimethylaniline |  |
| 18 | 2-Hydroxypyridine |  |
| 19 | Codeine |  |
| 20 | Triprolidine |  |

TABLE I *(continued)*

| *Compound No.* | *Name* | *Structure* |
|---|---|---|
| 21 | Diphenhydramine |  |
| 22 | Methylparaben |  |
| 23 | Maleic acid |  |
| 24 | Probenecid |  |
| 25 | *p*-Toluic acid |  |
| 26 | Propoxyphene |  |

where $N$ is the efficiency in plates per column and $w$ is the peak width at 10% height. The efficiency values in plates per column were divided by the column length to give efficiency in plates per meter.

All values of $k'$, $N$, and $AF_{10}$ were the means of at least two injections for which the measurements differed by less than 2%. Each experiment was repeated and the results verified on a different column run on the alternate chromatographic system. The structures of compounds used in this paper are shown in Table I.

*Selected organic bases*

The compounds chosen are a sampling of hindered and unhindered, primary, secondary, tertiary and heterocyclic amines. A hindered amine is one in which bulky alkyl groups are substituted near the basic nitrogen limiting its interaction with the silica surface.

TABLE II

RETENTION ($k'$) AND ASYMMETRY ($AF_{10}$) VALUES FOR SOME ORGANIC BASES

Conditions: column, 50 × 4.6 mm Suplex pKb-100; mobile phase, acetonitrile–25 m*M* aqueous potassium phosphate, pH 7.0 (2:98); flow-rate, 2 ml/min; temperature, 35°C. n.a. = Not available.

| *Compound No.*[a] | *Name* | $pK_a$[b] | *Concentration* | $k'$ | *log* $P_{o/w}$ | $AF_{10}$ |
|---|---|---|---|---|---|---|
| 1 | Benzene | – | 7 μl/ml | 24.3 | 2.10 | 0.97 |
| 2 | Methamphetamine | 10.2 | 50 μg/ml | 4.77 | n.a. | 1.72 |
| 3 | Amphetamine | 10.0 | 50 μg/ml | 3.57 | 1.66 | 1.31 |
| 4 | β-Phenethylamine | 10.0 | 50 μg/ml | 1.96 | 1.41 | 1.58 |
| 5 | α-Phenethylamine | 9.2 | 1 μl/ml | 1.95 | n.a. | 1.43 |
| 6 | 4-Dimethylaminopyridine | 9.6 | 60 μg/ml | 0.92 | 1.34 | 3.12 |
| 7 | N,N-Dimethylaniline | 5.15 | 0.1 μl/ml | 66.2 | 2.36 | 1.05 |
| 8 | Pyridine | 5.25 | 1 μl/ml | 2.71 | 0.64 | 1.51 |
| 9 | 4-Methylpyridine | 5.68 | 1 μl/ml | 7.79 | 1.27 | 1.93 |
| 10 | 3,5-Dimethylpyridine | 6.15 | 0.5 μl/ml | 26.2 | n.a. | 1.43 |
| 11 | 3,4-Dimethylpyridine | n.a. | 0.5 μl/ml | 20.6 | n.a. | 1.57 |
| 12 | 2,6-Dimethylpyridine | n.a. | 0.5 μl/ml | 13.1 | 1.68 | 1.70 |
| 13 | 2,4-Dimethylpyridine | 6.99 | 0.5 μl/ml | 17.1 | n.a. | 1.77 |
| 14 | Aniline | 4.63 | 1 μl/ml | 5.23 | 0.90 | 1.00 |
| 15 | 3,5-Dimethylaniline | n.a. | 1 μl/ml | 42.8 | n.a. | 1.05 |
| 16 | 3,4-Dimethylaniline | n.a. | 50 μg/ml | 37.4 | n.a. | 1.02 |
| 17 | 2,6-Dimethylaniline | 3.55, 3.95 | 1 μl/ml | 34.3 | n.a. | 1.11 |
| 18 | 2-Hydroxypyridine | 0.75 | 50 μg/ml | 0.81 | −0.58 | 1.17 |

[a] From Table I.

[b] $pK_a$ Values from refs. 31, 34 and 35.

The $pK_a$ values reported in Table II were obtained from the literature[31,34,35]. Some $pK_a$ values were not available. Octanol–water partition coefficients were obtained from Hansch and Leo[36]. Benzene was used as a non-ionic probe to obtain the $AF_{10}$ value which excludes the silanol contribution.

Dilute solutions of each base were made in mobile phase buffer. The mobile phase used in this study was acetonitrile–25 m*M* aqueous potassium phosphate pH 7.0 (2:98). The very low organic concentration resulted in high $k'$ values for some compounds, but the use of 50-mm columns kept the total analysis time low, while also reducing solvent consumption. The short columns also enabled the concurrent study of amines with drastically different $k'$ values. The mobile phase conditions in this study (pH 7, low ionic strength, no amino additives) ensured that residual silanols are not artificially suppressed by the mobile phase, but by the bonded phase of the Suplex pKb-100. (The $pK_a$ of the silanol group is *ca.* 4.5.)

## RESULTS AND DISCUSSION

In this study, we attempted to determine the extent of suppressed activity of the silica gel matrix in the new type of reversed-phase column. We evaluated the relationship between the peak symmetry of the bases and their $pK_a$ values, as well as the correlation between the octanol–water partition coefficient and retention time.

TABLE III

PEAK SHAPE OF CODEINE[a] ON SILANOL-DEACTIVATED HPLC COLUMNS

Conditions: columns, 150 or 250 mm × 4.6 mm (5-μm packings). Columns were further described in the Experimental section. Mobile phase: acetonitrile–25 m*M* aqueous potassium phosphate, pH 6.2; flow-rate, 1 or 2 ml/min; ambient temperature.

| *Column* | $k'$[b] | $AF_{10}$ | $N$[c] |
|---|---|---|---|
| Suplex pKb-100 | 2.22 | 1.26 | 11 130 |
| LiChrosorb RP Select B | 1.86 | 2.13 | 3500 |
| SCD-100 | 1.84 | 3.00 | 6330 |
| Deltabond octyl | 2.37 | 3.93 | 1200 |
| Rexchrom ODS | 2.10 | 5.16 | 270 |
| Zorbax $R_x$ | 2.58 | 6.0 | 870 |
| Capcell Pak C18 | 1.62 | n.m.[d] | n.m. |
| Chemcosorb 5 ODS H | 2.44 | n.m. | n.m. |
| Chromega $\gamma$-$C_{18}$ | 2.29 | n.m. | n.m. |
| Ultracarb ODS 20 | 2.15 | n.m. | n.m. |
| Supelcosil LC-18-DB | 2.15 | n.m. | n.m. |

[a] Compound 19 from Table I, $pK_a$ = 8.21.
[b] Acetonitrile–buffer ratio adjusted to maintain $k'$ within a narrow range.
[c] Plates/m measured by Foley–Dorsey method[33].
[d] $AF_{10}$ and $N$ not measurable because of extremely long time for peak to return to baseline.

Finally, as no silanol suppressing additives were needed in the mobile phase, we determined how selectivity between basic, acidic, and neutral analytes was affected by altering mobile phase conditions.

In Table II there is a list of primarily organic bases, their $pK_a$ values, capacity ($k'$) and asymmetry factors ($AF_{10}$) on a Suplex pKb-100 column using acetonitrile–25 m*M* aqueous potassium phosphate pH 7.0 mobile phase (2:98) at 35°C. As mentioned, at this pH residual silanol groups should be fully effective.

Even though the new reversed-phase column shows a significant improvement in peak symmetry in comparison to some other commercial deactivated reversed-phases (see Table III), "silanol effects" are still observed, as indicated by peak asymmetry (see Table II). Peak asymmetry of the bases depends, in part, on their $pK_a$ values. Compounds 10–13, for example, are dimethyl-substituted pyridine isomers. The asymmetry factors increase with $pK_a$ values in the order 1.43, 1.57, 1.70 and 1.77 for compounds 10, 11, 12 and 13, respectively. Two of the $pK_a$ values are not reported, but could be approximated from the inductive effect of substitution in the *ortho*-, *meta*- and *para*-positions on the basicity of the compounds. We assumed that compound 12 has a $pK_a$ value comparable to that of compound 13, as one additional *ortho*- replaces a *para*-substituent. Compound 11, a *m,p*-isomer, should have a $pK_a$ between 6.15 and 6.99 in comparison to an *m,m*- and *o,p*-analogue, respectively.

On the other hand, not only $pK_a$ but also steric factors in the close vicinity of the basic nitrogen contribute to the "silanol effect", as can be seen for compound 6 *vs.* compounds 2–5. Here, an unhindered, protonated planar $sp^2$ nitrogen is compared to a protonated $sp^3$ nitrogen. Compound 6, with a $pK_a$ of 9.6, has an asymmetry factor of 3.12 *vs.* 1.31–1.72 for compounds 2–5 which have $pK_a$ values between 9.2 and 10.2.

TABLE IV

EFFECT OF TRIETHYLAMINE (TEA) CONCENTRATION ON PEAK SHAPE AND RETENTION OF BASES ON DEACTIVATED PHASES

Conditions: columns, 150 × 4.6 mm, 5 μm, mobile phase for Suplex pKb-100, acetonitrile–25 m*M* aqueous potassium phosphate (0–0.14%, v/v, TEA), pH 6.0 (22:78); mobile phase for LC-8-DB, acetonitrile–25 m*M* aqueous potassium phosphate (0–0.147%, v/v, TEA), pH 3.0 (40:60); flow-rate, 2 ml/min; temperature, 35°C.

| *Compound* | *Suplex pKb-100* | | | *LC-8-DB* | |
|---|---|---|---|---|---|
| | *TEA (%)* | $AF_{10}$ | $k'$ | $AF_{10}$ | $k'$ |
| Triprolidine (compound 20)[a] | 0 | 1.23 | 3.83 | 8.48 | 5.18 |
| | 0.02 | 1.20 | 3.65 | 5.73 | 2.15 |
| | 0.08 | 1.24 | 3.61 | 2.93 | 1.61 |
| | 0.14 | 1.21 | 4.02 | 2.58 | 1.44 |
| Diphenhydramine (compound 21)[a] | 0 | 1.25 | 4.44 | 3.60 | 2.31 |
| | 0.02 | 1.16 | 4.23 | 3.14 | 2.11 |
| | 0.08 | 1.10 | 4.19 | 2.35 | 1.74 |
| | 0.14 | 1.19 | 4.62 | 2.30 | 1.65 |

[a] From Table I.

It should be mentioned that, when working with these and other organic compounds in an aqueous mobile phase, the sample concentration must be carefully considered. A ten-fold increase in concentration of the pyridine derivatives (from 0.5 to 5 μl/ml in a 5-μl injection) caused a doubling of the asymmetry factor.

For regular organic bases, the contribution to reversed-phase retention due to "silanol effects" is negligible or small on the Suplex pKb-100 in comparison to a Supelcosil LC-8-DB column, as can be seen from Table IV. Adding TEA, a common silanol suppressor, to the mobile phase-reduced asymmetry and retention on the Supelcosil LC-8-DB column, while leaving asymmetry unaffected and retention even slightly increased on the Suplex column. A possible explanation for this increase is reduced solubility of the basic analyte in the mobile phase upon the addition of TEA.

It is of interest that hydrophobic-related retention and distribution coefficients between a water–octanol layer for the same bases are highly correlated[37]. A plot of log $k'$ *vs.* log $P_{o/w}$, the octanol–water partition coefficient, is described in Fig. 3. There is a good correlation between the retention time and the partition coefficient, except for compounds 3, 4 and 6. In the pH 7.0 buffered mobile phsae, these three compounds are completely charged (protonated) and are driven into the polar mobile phase, while at their unbuffered pH (identical with their $pK_a$), the compounds would show a greater tendency towards the octanol phase in an octanol–water mixture. On the other hand, the $pK_a$ values of the majority of the bases are similar, and at pH 7.0 all are in the free base form. Thus, for these compounds, there is good correlation between the two techniques ($r = 0.98$).

*Effect of pH*

A benefit of diminished silanol activity is the ability to better use pH as a tool for

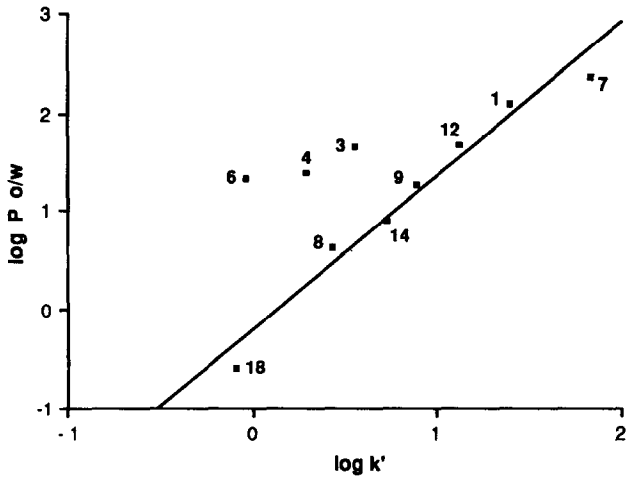



Fig. 3. Log $P_{o/w}$ (octanol–water partition coefficient) *vs.* log $k'$, retention on a Suplex pKb-100 column. Conditions and compounds as in Tables I and II.

improving resolution. Whereas in the analysis of bases on traditional RP-HPLC columns it is necessary to work at low pH to suppress silanols, the Suplex phase expands the workable pH range. For example, Table V shows the effect of pH of a 25 m*M* potassium phosphate buffer on retention on a Suplex pKb-100 column. Retention of amines 3, 4, 6 and 14 increases with increasing pH. The neutral compound, methylparaben, is unaffected while that of three organic acids (probenecid, maleic and *p*-toluic acids) decreases. The percent of acetonitrile was adjusted to maintain all $k'$ values within the 0–7 range.

By increasing the pH, the extent of ionization of basic compounds is decreased, hence increasing the compound's hydrophobic character and increasing its retention

TABLE V

EFFECT OF pH OF A 25-m*M* POTASSIUM PHOSPHATE MOBILE PHASE ON RETENTION BY SUPLEX pKb-100

Conditions: column, 150 × 4.6 mm Suplex pKb-100; mobile phase, acetonitrile–25 m*M* potassium phosphate (ratio indicated in table); flow-rate, 2 ml/min; temperature, 35°C.

| *Compound* | | *% $CH_3CN$* | *k'* | | | |
|---|---|---|---|---|---|---|
| *No.* | *Name* | | *pH 7.01* | *pH 5.50* | *pH 3.49* | *pH 2.06* |
| 4 | β-Phenethylamine | 2 | 3.55 | 1.21 | 0.58 | 0.35 |
| 3 | Amphetamine | 2 | 6.20 | 2.78 | 1.55 | 0.59 |
| 14 | Aniline | 2 | 5.00 | 4.69 | 0.35 | 0 |
| 6 | 4-Dimethylaminopyridine | 2 | 3.28 | 0.94 | 0 | – |
| 22 | Methylparaben | 25 | 2.88 | 3.05 | 2.85 | 2.78 |
| 23 | Maleic acid | 2 | 0.09 | 5.41 | 32.0 | – |
| 24 | Probenecid | 35 | 1.49 | 9.20 | 27.0 | – |
| 25 | *p*-Toluic acid | 25 | 0.91 | 9.19 | 14.5 | – |

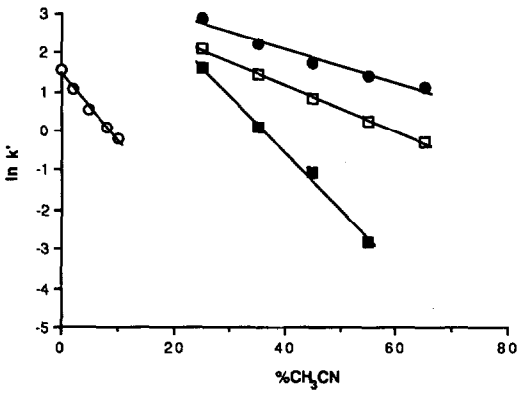



Fig. 4. Effect of acetonitrile concentration on retention of acidic, basic and neutral compounds. Column, 150 mm × 4.6 mm Suplex pKb-100 (5-μm packing); mobile phase, acetonitrile–25 m*M* potassium phosphate, pH 6.0; flow-rate, 2 ml/min; temperature, 35°C. □ = *p*-Toluic acid; ○ = amphetamine; ■ = propoxyphene (p$K_a$ 10.0); ● = benzene.

by a reversed-phase mechanism. The opposite effect occurs for an acid, being more hydrophobic at lower pH. Retention of a neutral species, without an ionizable moiety in this pH range, does not show a pH dependence. It is important to mention that peak shape for these compounds was not significantly affected by the pH over the investigated range.

*Effect of organic modifier*

Retention on the Suplex pKb-100 stationary phase (acid, base and neutral species) correlates with the organic modifier concentration of the mobile phase in a predictable manner (see Fig. 4). The excellent linearity between ln $k'$ and acetonitrile concentration demonstrates that the column operates by a reversed-phase mechanism, even with very basic compounds.

## CONCLUSION

A new silica-based RP-HPLC packing material, Suplex pKb-100, which shows a high level of silanol deactivation, was used for the study of organic bases at pH 7. The higher pH range could be essential for improving resolution of organic bases and cannot be used on conventional RP-HPLC columns due to excessive tailing. Retention times were found to correlate with lipophilic character as measured from octanol–water partition coefficients. Results of the retention study indicate that selective, predictable retention behavior of acidic and neutral species is possible as well. A reversed-phase mechanism was verified by the linear relationship between ln $k'$ of acidic, neutral and basic species and concentration of organic modifier in the mobile phase.

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