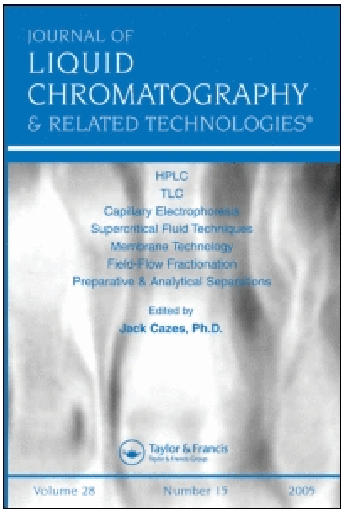

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THE EFFECT OF SILANOL MASKING ON THE RECOVERY OF PICLORAM AND OTHER SOLUTES FROM A HYDROCARBONACEOUS PRE-ANALYSIS EXTRACTION COLUMN

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ABSTRACT

The recoveries of picloram, picloram-methylester, hexazinone, benzene, and acetophenone from aqueous samples were studied using a commercially available hydrocarbonaceous pre-analysis extraction cartridge, both with and without tetrabutylammonium hydrogen sulfate (TBAHS) in the eluent. Extraction efficiency was found to be dependent on sample loading volume. The results suggest a mixed mechanism of retention involving both "silanophilic" and "hydrophobic" interactions in the absence of tetrabutylammonium ion. The ability of TBAHS to mask surface silanol groups and/or ion-pair with counterionic solutes is invoked to explain the observations. Chromatograms of the solutes obtained on a C_{18} bonded analytical column in both the presence and absence of TBAHS are also presented.

INTRODUCTION

Some organic compounds are efficiently removed from solution by contact with an adsorbent via adsorption trapping (1). Silica gel bonded with hydrocarbonaceous ligands, i.e. reversed-phase material, is a potentially useful adsorbent for extraction and concentration of organic compounds from aqueous samples. The sample adsorption/desorption process from hydrocarbonaceous stationary phase material consists of three steps: (a) an organic solvent followed by aqueous prewash, (b) charging the sample to the column from an aqueous matrix, and (c) sample desorption involving the elimination of low or high polarity contaminants which may or may not precede removal of the compound of interest. Retention for this process is reflected by the percent recovery or efficiency.

However, chromatographic effects attributed to unmasked silanol groups in reversed-phase analytical columns (2-6) may also affect the recovery of adsorbate from reversed-phase preanalysis extraction columns. Several manufacturers now offer C_{18} bonded phases in convenient-to-use disposable cartridges, but these products vary as to the extent of free silanol groups remaining in the bonded phase material.

The presence of solvent-accessible silanol groups in chemically bonded stationary phases for reversed-phase liquid chromatography (RPLC) can lead to a dual retention mechanism (2-4). Horvath et al. (5,6) postulate that both "hydrophobic" and "silanophilic" interactions occur. Depending on the manner in which a hydrocarbonaceous bonded phase is produced (7-9), some silanol groups will remain unreacted due to steric inhibition; furthermore, SiOH groups may be reformed in the work-up procedure if bifunctional or trifunctional modifers are used (10,11). These silanol groups can produce undesirable effects on column stability, retention, and peak symmetry due to the reduced hydrophobic character of the surface (2,3).

The technique of "capping" accessible silanol groups with a small silane such as trimethylchlorosilane has been used to improve retention characteristics (3,4). Nondek and Vyskocil (12) demonstrated that the concentration of hydroxyl groups could be greatly reduced by this procedure. Protonated alkylamines (6,13) and lipophilic quaternary ammonium salts (6) have been added to the mobile phase in RPLC to reduce the silanophilic influence upon retention by preferential adsorption of these charged species on residual silanol sites.

The previously reported work has dealt with the problem of silanol groups remaining on reversed-phase material used in analytical columns. The purpose of this paper is to report results observed when TBAHS was used to minimize silanophilic interactions in solute adsorption/desorption from a commercially available C_{18}-bonded pre-analysis extraction column.

MATERIALS AND METHODS

Apparatus

The liquid chromatograph consisted of a Waters (Milford, MA) Model 6000A solvent pump, Model 710B intelligent sample processor, Model 440 UV absorbance detector, and a Houston Instrument (Austin, TX) recorder. Hypodermic syringes with Luer Lok tips were purchased from Becton-Dickson (Rutherford, NJ) and a Filtrator from Fisher Scientific (Pittsburgh, PA).

Reagents and Chemicals

Spectrophotometric grade acetonitrile (ACN) and glacial acetic acid (HOAc) were obtained from Burdick and Jackson Laboratories, Inc. (Muskegon, MI) and J.T. Baker Chemical Co. (Phillipsburg, NJ), respectively. Water was processed with a Bion Exchange system purchased from Pierce Chemical Company (Rockford, IL). Tetrabutylammonium hydrogen sulfate (TBAHS) and acetophenone were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI), and benzene from Fisher Scientific. Picloram (99.0%) and picloram-methyl ester (99.7%) were obtained from the Dow Chemical Company (Midland, MI) and hexazinone (analytical standard, 99+%) from E.I. DuPont de Nemours & Co. (Wilmington, DE).

Chromatographic Procedures

SEP-PAK C_{18} cartridges were purchased from Waters Associates, Inc. House vacuum (530 mm Hg) was used to pull the samples through the reversed-phase cartridges. Preparation of the cartridges and charging of the sample was accomplished by using a side arm filtering flask. For component elution, a Fisher Filtrator was used to collect the sample directly into a 10.0 mL volumetric flask. Individual sample solutions of acetophenone, benzene, picloram, picloram-methyl ester and hexazinone were prepared by adding 1.0 uL, 2.5 uL, 4.3 ug, 57.5 ug and 13.0 ug respectively to the appropriate volume of 4% acetic acid with or without 10 mM TBAHS. The following procedures were used for sample concentration of C_{18} SEP-PAK cartridges:

1) Prewash of cartridges with 5 mL ACN followed by 10 mL of 4% HOAc or 4% HOAc/10 mM TBAHS.
2) Loading of sample in 1.0 mL of 4% HOAc or 4% HOAc/10 mM TBAHS.
3) Desorption by 9.0 mL of 25% HOAc or 25% HOAc/10 mM TBAHS.
4) Samples were then diluted to 10.0 mL and analyzed by RPLC.

Two Ultrasphere ODS (5 μm, spherical porous particle) columns (1UE732N and 1UE747N) were obtained from Altex Scientific, Inc. (Berkeley, CA) and used in this study. The analytical column was preceded by a guard column, 7 cm by 2.1 mm i.d. dry packed with Whatman CO:PELL ODS (30-38 μm). After equilibration, mobile phase solvent mixtures were degassed in an ultrasonic bath. The mobile phase consisted of mixtures of water, acetic acid, and acetonitrile with or without the presence of 10 mM TBAHS, at a flow rate of 1.5 mL/min. The ultraviolet detector was operated at 254 nm.

Calculations

Determinations of retention times and integration of peak areas were performed by a Perkin-Elmer Model Sigma 10B data handling system (Norwalk, CT). Direct comparison of peak areas with external standards was used to determine sample concentration.

RESULTS AND DISCUSSION

The recovery of the herbicide picloram from aqueous environmental samples following concentration by hydrocarbonaceous extraction cartridges is of current interest in this laboratory. Bushway (14) demonstrated quantitative recoveries of carbaryl and 1-naphthol following trace enrichment of one liter water samples by passage through C_{18} SEP-PAK cartridges. Beier and Greenblatt (15) recovered up to 80% of some lacinilines and cadalenes by extraction and concentration of one liter aqueous samples with SEP-PAK C_{18} cartridges. However when attempts were made to effect the trace enrichment of large volume water samples containing picloram using SEP-PAK C_{18} cartridges, recoveries were found to be very dependent upon sample loading volume. A profile of the dependence of percent recovery upon sample loading volume for picloram (Fig. 1) shows that less than 50% recovery was obtained for a sample throughput of 25 mL. A similar non-linear dependence of extraction efficiency upon sample loading volume was demonstrated by Saner et al. (16) for some simple aromatic solutes and Nyagah (17) for the recovery of the herbicide pyrazon from SEP-PAK C_{18} cartridges.

Since silanophilic interactions have been implicated in chromatography on analytical reversed-phase columns, the participation of silanol groups in the recovery of adsorbate from hydrocarbonaceous cartridges is also possible. To establish the nature of this contribution, TBAHS was chosen as a silanol masking agent

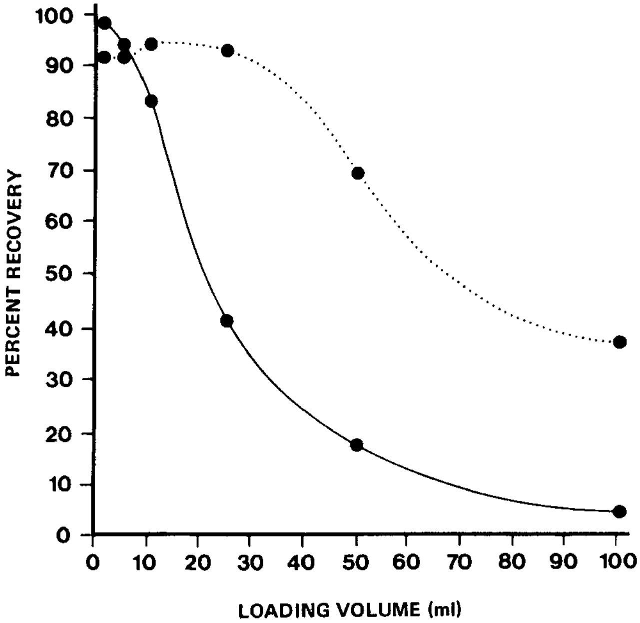


FIGURE 1. Percent recovery of picloram as a function of sample loading volume either with (•••) or without (—) TBAHS.

(6). The recovery profile for picloram as a function of sample volume was obtained with TBAHS (10 mM) added to each of the three steps of the adsorption/desorption process and is shown in Fig. 1. In the presence of TBAHS, the recovery of picloram was still curvilinear and dependent upon sample loading volume, but the efficiency was much improved for sample volumes greater than 10 mL.

To elucidate the results observed during the determination of picloram, the percentage recovery of other solutes (Fig. 2) representing diversity in polarity was studied as a function of the loading volume of the sample. All experiments were conducted under optimal conditions for picloram recovery for purposes of comparison and may not produce the best recovery for the other solutes. Acetic acid (4%) was used for pH control to enhance the retention of picloram (pKa=1.97) (18) by ion suppression (19). Acetic acid (25%) was optimal for the desorption of picloram. Experiments were performed both with and without 10 mM TBAHS in the prewash, sample loading, and desorption eluents. When samples containing TBAHS in the desorption solvent were analyzed on a C_{18} analytical column, the HPLC eluent was also modified with 10 mM TBAHS. The presence of TBAHS produced a slower drip rate from the cartridges, as well as a slightly higher back pressure in the chromatograph, presumably due to an increase in viscosity.

For the non-polar solute benzene, the alteration of the recovery plot upon addition of a silanol masking agent should be minimal. Indeed, the offset of the plots in Fig. 3B is likely due to the slight increase in the carbon coverage of the surface from adsorbed TBAHS resulting in a small increase in extraction efficiency. The maxima observed toward low loading volumes in Fig. 3 are curious, and may be dependent upon the manner in which the data were collected. The aqueous wash of the cartridge prior to charging of the sample may have been insufficient to remove all of the adsorbed acetonitrile.

Acetophenone represents a moderately polar, non-ionizable solute. The recovery of acetophenone versus loading volume is

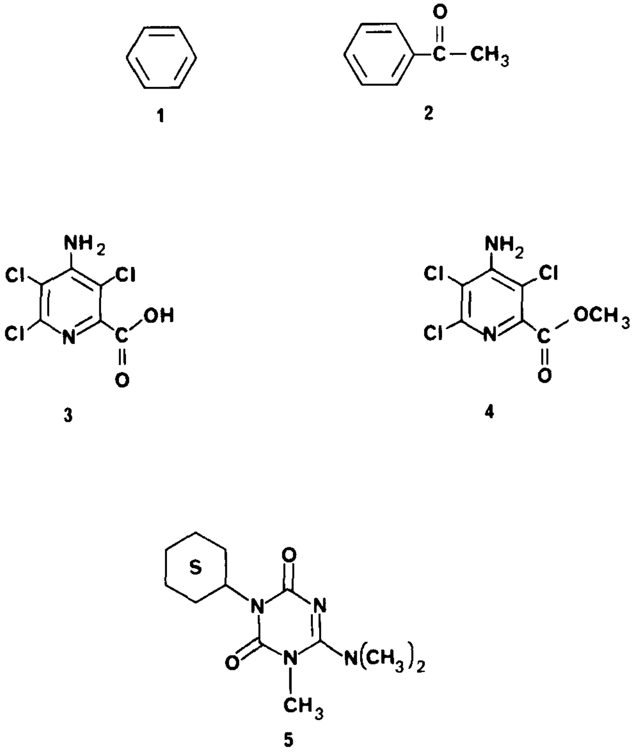


FIGURE 2. Compounds included in this study: (1) benzene, (2) acetophenone, (3) picloram (4-amino-3,5,6-trichloropicolinic acid), (4) picloram-methyl ester, and (5) hexazinone (3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4-(1H, 3H)-dione).

illustrated in Fig. 3A. At sample loading volumes greater than 22 mL, the recovery of acetophenone was greater without the silanol masking agent. In the absence of TBAHS, hydrogen bonding between surface silanol groups and the polar carbonyl function in acetophenone enhances the overall adsorption to the stationary phase and thereby increases the resultant recovery. Therefore, a dual retention mechanism, i.e. silanophilic as well as hydrophobic, is not always detrimental to an analysis.

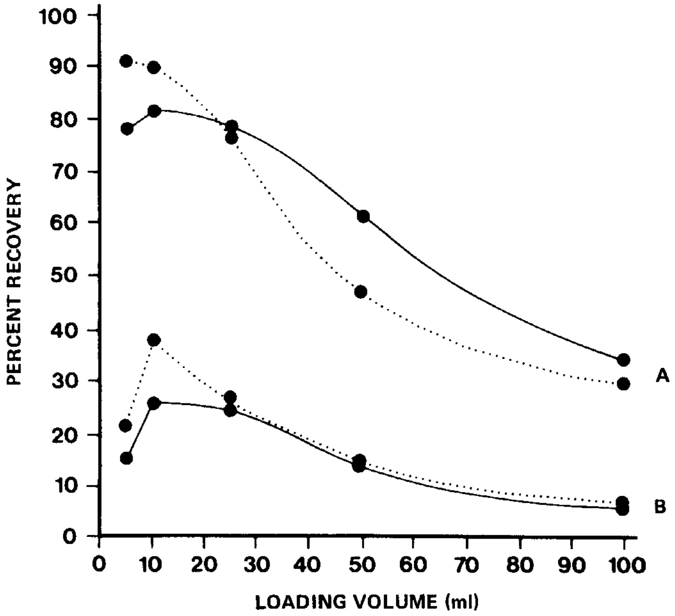


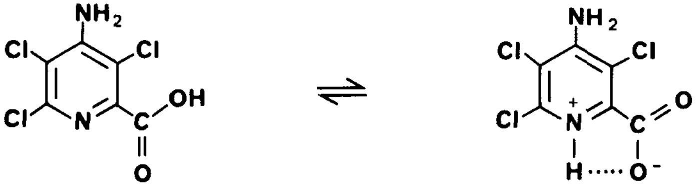
FIGURE 3. Percent recovery as a function of sample loading volume for (A) acetophenone and (B) benzene, either with (•••) or without (—) TBAHS.

The chromatographic consequences of masking silanol groups with TBAHS are two-fold. The number of electrical charges on the bonded phase surface (20) is reduced by the adsorption of the tetrabutylammonium moiety on unreacted silanol groups. The interaction also slightly increases the total hydrocarbonaceous content of the stationary phase.

Tanaka _et al_. (2) have shown on C_{18} bonded analytical columns that relative to benzene, retention of toluene decreased and retention of acetophenone increased with increased exposure to silanol groups. Cooke and Olsen (3) illustrated the capacity factor changes (d=decreased, i=increased) for acetophenone (d), nitrobenzene (d) benzene (i), and toluene (i) after the end-capping of a C_{18} analytical column. These changes in capacity factor are analogous to the changes in efficiency observed for benzene and acetophenone in these recovery experiments.

Results obtained on analytical columns (2) suggest that silanols interact most with functional groups made up of electron rich atoms, such as hydroxyl, carbonyl, nitrile, and nitro moieties, and therefore are expected to exert the greatest effect upon compounds containing such groups. Even the most commonly used organic modifiers for the mobile phase, methanol and acetonitrile, are also competing for adsorption on silanol sites (2) with methanol apparently being bound in preference to acetonitrile (6).

For ionizable compounds even greater effects would be expected upon masking the electrically charged surface with TBAHS. Picloram, 3A, can exist as: (a) the neutral molecule, (b) the conjugate acid (formed by protonation of the base functionality), (c) the conjugate base (due to ionization of the carbonyl group), and (d) the zwitterion, 3B, (18,21). Zwitterion formation is also observed in other pyridine carboxylic acids (22). Intramolecular hydrogen bonding helps to reinforce structure 3B. This complicates measurement of the acid dissociation constant for picloram. Reported pKa values range from 2.8 to 4.1. The most reliable value seems to be that of Osteryoung and Whittaker (18) who report a pKa' value of 1.97 for picloram, representing a combined equilibrium constant for the dissociation of both the neutral and zwitterionic forms. The pKa for the amine group of picloram is estimated at -2.0 to -1.3 (18), so the concentration of the conjugate acid is negligible under the experimental conditions used here. In 4% acetic acid (pH=2.5), some equilibrium exists between



3A 3B

the neutral molecule, the zwitterion, and the conjugate base. The presence of negative sites on the bonded phase surface greatly reduces retention (and therefore recovery) by inhibiting interaction with the bonded phase ligand (C_{18}).

The possible contribution of ion-pair formation to the improvement of the recovery observed for picloram cannot be ignored. Strong acids can be retained throughout the usable pH range for bonded phase materials (pH2-8) by ion-pairing with lipophilic quaternary ammonium salts. Complexation with the tetrabutylammonium counterion (in the mobile phase or at the bonded phase surface) would increase the hydrophobicity of the picloram anion and increase retention on a reversed-phase adsorbent.

In order to pursue this problem, the dependence of recovery upon loading volume was plotted for the methyl ester of picloram, 4, in Fig. 4. Zwitterion formation is blocked in the ester and ion-pairs with TBAHS would not occur. Fig. 4 demonstrates the improvement in recovery of picloram-methyl ester due to silanol masking. The comparison of Figs. 1 and 4 cannot be expected to be additive, because the methyl group not only blocks ionization, but also contributes its own hydrophobicity. The conclusion then, is that the improvement in the recovery of picloram (Fig. 1) is probably due to a combination of silanol masking and ion-pairing, while the improvement for the methyl ester (Fig. 4) is attributed to silanol masking alone.

The recent results of Nyagah (17) in developing a recovery analysis for the herbicide pyrazon (5-amino-4-chloro-2-phyenyl-3-pyridizone) lend further support to these observations. The recovery of pyrazon from C_{18} cartridges was shown to be dependent upon the concentration of NaCl in the sample. Nyagah found that NaCl added to water samples containing pyrazon greatly improved the recovery of this solute from SEP-PAK C_{18} cartridges, and proposed that the added salt decreased the water solubility of pyrazon thereby increasing adsorption onto the C_{18} bonded ligand. Palusova et al. (23) have shown that the addition of sodium chloride to water containing pyrazon increased the yield of this

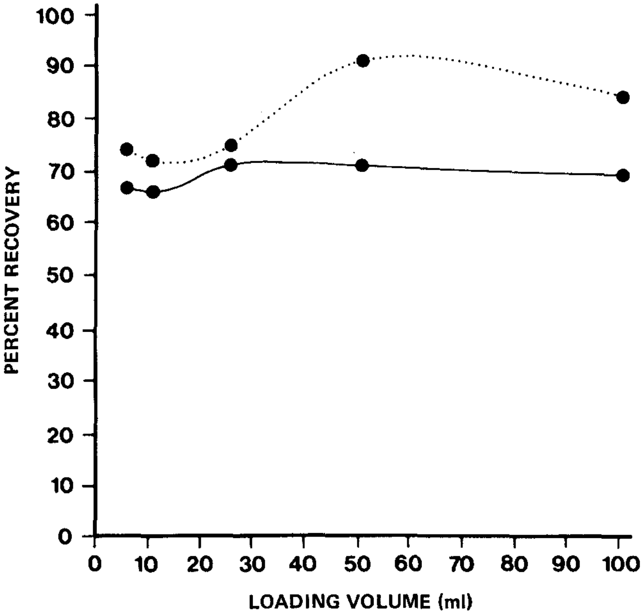


FIGURE 4. Percent recovery of picloram-methyl ester as a function of sample loading volume either with (•••) or without (——) TBAHS.

herbicide in the organic solvent because this decreased the solubility of the extracting solvent in water. The quantitative recovery of pyrazon from 500 mL water samples containing 25% NaCl demonstrated by Nyagah (17) may actually be another example of silanol masking. Saturation of the silanol groups on ODS (Octyldecasilyl) silica columns by inorganic salts added to the eluent was first established by Knox and Jurand (24). The ability of a potassium phosphate buffer to attenuate the untoward effects of the Donnan equilibrium and ion exclusion due to electrical charges on the bonded phase surface of analytical columns has been shown (25). In this lab, the mean values for the extraction of 800 mL water samples containing picloram demonstrated no difference by Student's t (P>0.20) whether the sample was acidified to pH2 with H_2SO_4 or acidified with H_3PO_4 and NaCl (4%) added prior to extraction with diethyl ether. Andrews and Good (26) tested the recovery of pesticides from various types of water samples with C_{18} Bond Elut columns and found that recovery improved for 6 of the 7 compounds examined when distilled water samples (100 mL) were compared to water samples containing salt or up to 10% methanol. These results are compatible with the argument presented here as both methanol (6) and inorganic salts (24) adsorb on silanol sites.

The ionic species of picloram is negatively charged in 4% acetic acid, while the dimethylamino group of hexazinone, 5, is protonated resulting in a positive charge. This positive charge should effect a reduction in hexazinone recovery. However, the increase in recovery found for hexazinone upon addition to TBAHS (Fig. 5) suggests that the effects of silanol masking outweigh the expected reduction in recovery due to like charge competition.

A chromatogram of the five solutes further emphasizes the positive effects of silanol masking in this instance. The analytical column used in this experiment was end-capped by the manufacturer, therefore solvent-accessible silanol groups are minimal. Alteration in the chromatogram of the solutes compared in this study upon the addition of TBAHS is shown in Fig. 6. Table 1 lists the solute capacity factors for the chromatograms in Fig. 6. Each k' value represents the average of four injections. The chromatograms were recorded using the same solvent batch, half of which had been made 10 mM in tetrabutylammonium hydrogen sulfate. The capacity factors for benzene, acetophenone, and picloram-methyl ester were each decreased approximately 3% by the addition of TBAHS. The retention of acetophenone and picloram-methyl ester relative to benzene, as given by the α_X value, did not change however. Since these three diverse solutes behaved similarly, there appear to be no observable SiOH interactions in this analytical column.

The capacity factor of picloram nearly doubled when TBAHS was added to the eluent while k' for hexazinone decreased by about

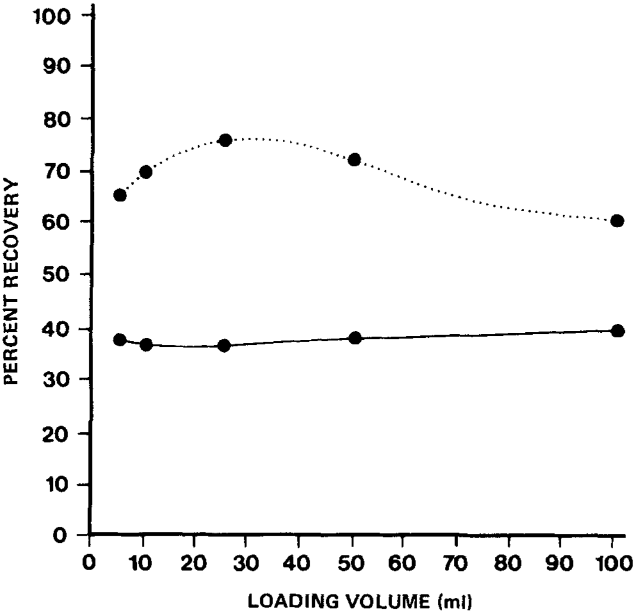


FIGURE 5. Percent recovery of hexazinone as a function of sample loading volume either with (•••) or without (—) TBAHS.

TABLE 1

Experimentally Determined Capacity Factors, k', on Ultrasphere ODS (1UE747N)

$\alpha_x = k'_x / k'_{benzene}$

	4% Acetic Acid/Acetonitrile (70:30)			
	No Added TBAHS		TBAHS (10 mM)	
Solute (x)	k'	α_x	k'	α_x
picloram	1.13	0.10	2.03	0.19
hexazinone	3.51	0.32	2.77	0.26
acetophenone	4.42	0.40	4.25	0.40
picloram-methyl ester	6.88	0.63	6.67	0.63
benzene	10.93	1.00	10.60	1.00

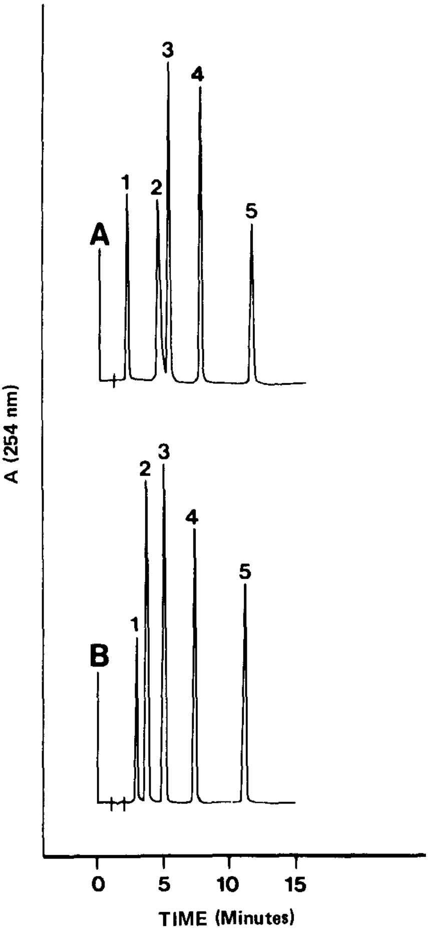


FIGURE 6. RPLC elution on Ultrasphere ODS (1UE747N) in 4% acetic acid/acetonitrile (70:30): (A) without TBA, (B) containing 10 mM TBA. Peaks: (1) picloram, (2) hexazinone, (3) acetophenone, (4) picloram-methyl ester, and (5) benzene.

TABLE 2

Summary of Chromatographic Results Produced by Addition of TBAHS (10 mM).

Solute	Sep-Pak Recovery	Analytical Column Retention
benzene	increased	slight decrease
acetophenone	decreased	slight decrease
picloram	increased	increased
picloram-methyl ester	increased	slight decrease
hexazinone	increased	decreased

20%. Since there seems to be little or no interaction attributable to silanol groups in this column, the observed increase for picloram must be the result of ion-pair formation between picloram and the TBAHS added to the mobile phase. Based on this result and those for picloram-methyl ester, it appears that the improvement in recovery for picloram observed in Fig. 1 is due to both silanol masking and ion-pair formation. The reduction in k' for hexazinone is produced by like-charge competition between the hexazinone and TBAHS moieties. Table 2 summarizes the results of both the extraction column and analytical column studies.

CONCLUSIONS

The heterogeniety of covalently bonded hydrocarbonaceous stationary phases can lead to a mixed mechanism of retention. The potential problem of residual silanol groups is common to all hydrocarbonaceous bonded materials whether used as analytical columns or preconcentration cartridges. End-capping appears to successfully reduce the number of solvent-accessible silanol groups in reversed-phase sorbents, but may not be economically feasible for pre-analysis extraction columns. Silanol masking with lipophilic quaternary ammonium ions is a viable alternative.

For complicated molecules having a diversity of functional groups, the interactions become more complex and masking SiOH groups may or may not improve recovery. Ion-pairing techniques, already well developed for use in reversed-phase analytical columns, can be used to improve extraction efficiency from hydrocarbonaceous recovery columns.

REFERENCES

1. Ogan, K., Katz, E., and Slavin, W., J. Chromatogr. Sci., 16, 517(1978).
2. Tanaka, N., Goodell, H., and Karger, B. L., J. Chromatogr., 158, 233(1978).
3. Cooke, N. H. C., and Olsen, K., J. Chromatogr. Sci., 18 512(1980).
4. Ogan, K., and Katz, E., J. Chromatogr., 188, 115(1980).
5. Nahum, A., and Horvath, C., J. Chromatogr., 203 53(1981).
6. Bij, K. E., Horvath, C., Melander, W. R., and Nahum, A., J. Chromatogr., 203, 65(1981).
7. Colin, H., and Guiochon, G., J. Chromatogr., 141, 289(1977).
8. Karch, K., Sebestian, I., and Halasz, I., J. Chromatogr., 122, 3(1976).
9. Rehak, V., and Smolkova, E., Chromatographia, 9, 219(1976).
10. Halasz, I., Anal. Chem., 52, 1393A(1980).
11. Roumeliotis, P., and Unger, K. K., J. Chromatogr., 149, 211(1978).
12. Nondek, L., and Vyskocil, V., J. Chromatogr., 206, 581(1981).
13. Sokolowski, A., and Wahlund, K. -G., J. Chromatogr., 189, 299(1980).
14. Bushway, R. J., J. Chromatogr., 211, 135(1981).
15. Beier, R. C., and Greenblatt, G. A., J. Liq. Chromatogr., 4, 515(1981).

16. Saner, W. A., Jadamec, J. R., Sager, R. W., and Killeen, T. J., Anal. Chem., 51, 2180(1979).

17. Nyagah, G., J. Chromatogr. Sci., 19, 500(1981).

18. Osteryoung, J., and Whittaker, J. W., J. Agric. Food Chem., 28, 95(1980).

19. Bildlingmeyer, B. A., J. Chromatogr. Sci., 18, 525(1980).

20. Berendsen, G. E., Shoenmakers, P. J., de Galan, L., Vigh, G., Varga-Puchony, Z., and Inczedy, J., J. Liq. Chromatogr., 3, 1669(1980).

21. Saha, J. G., and Gadallah, L. A., J. A. O. A. C., 50, 637(1967).

22. Abramovitch, R. A., Ed., Pyridine and Its Derivatives, Supplement Part Three, John Wiley & Sons, New York, N. Y., 1974, p. 281.

23. Palusova, O., Sackmauerova, M., and Madaric, A., J. Chromatogr., 106, 405(1975).

24. Knox, J. H., and Jurand, J., J. Chromatogr., 142, 651(1977).

25. Wells, M. J. M., and Clark, C. R., Anal. Chem., 53, 1341(1981).

26. Andrews, J. S., and Good, T. J., Am. Lab., 14 (4), 70(1982).