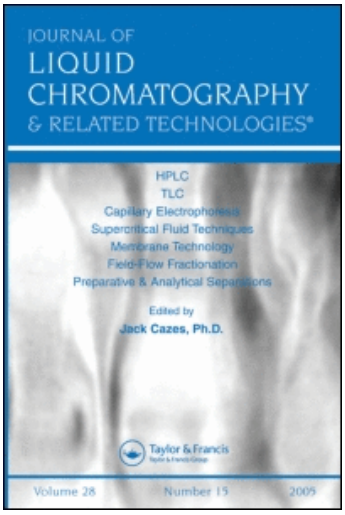

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HIGH PERFORMANCE MICELLAR LIQUID CHROMATOGRAPHY WITH SILICA MICROPARTICLES HAVING SURFACE-BOUND CATIONIC SURFACTANT MOIETIES. I. COMPARISON WITH OCTADECYLSILICA AND APPLICATIONS TO THE SEPARATION OF DANSYL AMINO ACIDS, HERBICIDES, AND CATECHOLAMINES

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ABSTRACT

The usefulness of silica microparticles having surface-bound cationic surfactant ligands in reversed phase chromatography (RPC) and micellar liquid chromatography (MLC) was examined, and the influence of surfactant type on retention and separation selectivity was studied. Different selectivities were observed by using silica microparticles having surface-bound cationic surfactant ligands, such as n-octadecyldimethyl(propylsilyl)ammonium groups ($C_{18}N^+(Me)_2Pr$) when compared to a C_{18}-silica column in the separation of dansyl amino acids (Dns-AA), herbicides and catecholamines by RPC and MLC. The presence of surface-bound charged moieties affected the amount of surfactant adsorbed onto the surface of the stationary phase from a given micellar mobile phase, which in turn influenced the retention behavior of neutral and charged species, thus leading to a unique chromatographic selectivity.

INTRODUCTION

Reversed phase chromatography (RPC) using alkyl bonded phases is the most frequently used technique for the separation of non-volatile compounds [1]. The use of

secondary chemical equilibria in RPC including micellar liquid chromatography (MLC) has greatly enlarged the scope of applications of RPC. In fact, MLC has proved to be a powerful analytical tool [2-6] since its introduction by Armstrong and Henry [2] in 1980.

Thus far, the majority of MLC separations have been carried out on traditional C_{18}-silica stationary phases [7-9] and to a lesser extent on the less hydrophobic cyanopropyl-silica [10] and phenyl bonded stationary phases [11]. Although it is widely recognized that in all modes of HPLC the nature of the stationary phase largely affects retention and selectivity, little or no attempts have been made to introduce novel stationary phases specially designed for MLC. In addition, since under most circumstances the stationary phases used in MLC are dynamically coated by the surfactant thus forming *in situ* surfactant-modified stationary phases, it becomes obvious that the nature of the stationary phase has a major role in determining selectivity and resolution among the separated analytes.

Thus, the aim of this paper is to introduce a novel stationary phase and to report on its utility in the HPLC separation of species of biological and environmental interests, including dansyl amino acids, catecholamines and some representative herbicides. The stationary phase described here is based on silica whose surface is chemically bonded with a cationic surfactant moiety, namely an octadecyldimethyl(propylsilyl)ammonium function. The results are compared to those obtained on the traditional C_{18}-silica stationary phase under otherwise identical conditions.

EXPERIMENTAL

Reagents

n-Octadecyldimethyl[3-(trimethoxysilyl)propyl]ammonium chloride, n-octadecyldimethylchlorosilane were obtained from Hüls America (Bristol, PA, U.S.A.). Analytical grade sodium phosphate monobasic was from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Isopropanol (HPLC grade) used to make the mobile phase solution was from

Table 1. Structure, CMC and nature of surfactants used in this study.

surfactant	structure	CMC (mM)	nature
SDS	$CH_3(CH_2)_{11}-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-O^-N_a^+$	8.2[a]	anionic
Em	$CH_3(CH_2)_{11}-\overset{\overset{CH3}{\|}}{\underset{\underset{CH_3}{\|}}{N^+}}-CH_2-COO^-$	1.8[b]	zwittergent, pH > 6 cationic, pH < 6

a. 25 °C.
b. 23 °C.

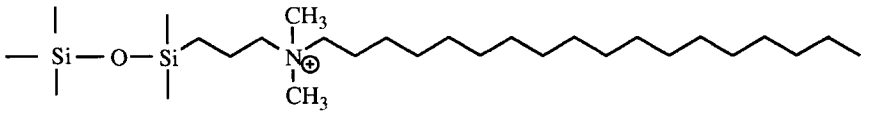
Fisher Scientific or Baxter (McGaw Park, IL, U.S.A.). Reagent grade isopropanol used in column packing was from EM Science (Cherry Hill, NJ, U.S.A.). The surfactants sodium dodecyl sulfate (SDS) and Empigen BB (N-dodecyl-N,N-dimethyl-glycine) (Em) were purchased from Calbiochem (LaJolla, CA, U.S.A.). The structure, CMC and nature of each surfactant is shown in Table 1. Dansyl-L-amino acids (Dns-AA) and catecholamines were obtained from Sigma (St. Louis, MO, U.S.A.). Herbicides were form Chem service (West Chester, PA, U.S.A.).

Apparatus

The chromatograph was assembled from an LDC-Milton Roy (Riviera Beach, FL, U.S.A.), Model CM4000 solvent delivery pump with a variable wavelength detector SpectroMonitor 3100, a Rheodyne (Cotati, CA, U.S.A.) Model 7125 sampling valve with a 20-µl sample loop, and a C-R5A Chromatopac integrator from Shimadzu (Columbia, MD, U.S.A.). The detection wavelength was set at 230 nm for herbicides, 245 nm for urea herbicides and 254 nm for all the other solutes. Home-made 100 x 4.6 mm I.D. C_{18} and $C_{18}N^+(Me)_2Pr$ columns were used.

Preparation of Stationary Phases

Typically, 5.0 g of Zorbax microspherical silica of 4.6 μm mean particle diameter and 150 Å mean pore diameter were suspended in 50 mL of dry DMF in a round-bottom flask. To this mixture, 7.0 mL of n-octadecyldimethyl[3-(trimethoxysilyl)propyl]ammonium chloride were added, and the suspension was stirred with a paddle stirrer. The reaction was performed at 120°C for 27 hours. The structure of the resulting stationary phase is given by



The C_{18}-silica stationary phase was prepared in a similar manner using 2.6 g of n-octadeclyldimethylchlorosilane, 5.0 g silica and 50 mL toluene. The suspension was heated at 125°C and stirred for 72 hours. In both cases, the resulting stationary phase was washed with acetone and methanol several times and then air dried at room temperature.

Elemental Analysis and Surface Coverage of Stationary Phases

The % C, H and N for C_{18}-silica and $C_{18}N^+(Me)_2Pr$-silica stationary phases were determined by elemental analysis at Galbraith Laboratories, Inc. (Knoxville, TN, USA). The % C and H for C_{18}-silica were 7.28 and 1.38, respectively, while the % C, H and N for $C_{18}N^+(Me)_2Pr$-silica were found to be 8.64, 1.71 and 0.53, respectively. These amounts when converted to surface coverage yielded 2.1 μmoles ligands/m^2 of silica for both silica bonded stationary phases.

Column Packing

The above stationary phases were packed from an isopropanol slurry at 8000 psi using a Shandon column packer (Keystone scientific, Bellefonte, PA, U.S.A.).

Isopropanol was used as the solvent for stationary phase suspension and as the packing solvent. All columns were 100 x 4.6 mm I.D., No. 316 stainless steel tubes (Alltech Associates, Deerfield, IL, U.S.A.). Column end fittings were also No. 316 stainless steel fitted with 0.5-μm frits and distributor disks from Alltech Associates.

Procedures

Micellar mobile phases were prepared by dissolving the appropriate amounts of surfactants and NaH_2PO_4 in water and then adjusting the pH to the desired value. For gradient elution runs, two mobile phases, A and B, were prepared for each set of experiments. They contained the same amounts of sodium phosphate buffer and surfactant, but solvent B contained 50% (v/v) isopropanol (2-PrOH). Throughout this study, two different gradient profiles were utilized. Gradient I consisted of 15 min at linearly increasing 2-PrOH concentration from 20% solvent B (i.e., 10% v/v 2-PrOH) to 100% solvent B (i.e., 50% v/v 2-PrOH) while gradient II was performed for 15 min at linearly increasing 2-PrOH concentration from 30% solvent B (i.e., 15% v/v 2-PrOH) to 50% solvent B (i.e., 25% v/v 2-PrOH). All mobile phase solutions were filtered through a S/P™ filter paper grade 360, qualitative from Baxter (McGaw Park, IL, U.S.A.).

RESULTS AND DISCUSSION

Since different types of interactions (such as electrostatic and hydrophobic) and competing equilibria are operating in MLC, it is obvious that the nature and concentration of the surfactant and the type of the stationary phase, as well as the concentration and type of the organic modifier in the mobile phase have profound effects on retention and selectivity [12, 13].

The retention behavior of dansyl amino acids (Dns-AA), catecholamines and herbicides on a $C_{18}N^+(Me)_2Pr$ column were examined under various conditions using

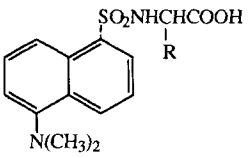
hydro-organic eluents with or without micellar phases. In the following sections, the results of these studies are discussed and compared to those obtained on a C_{18} column under the same operating conditions.

Comparison of C_{18} and $C_{18}N^+(Me)_2Pr$ Stationary Phases

a) Hydro-organic eluents. Dns-AA, and typical herbicides were used as the test solutes to compare the stationary phase having surface-bound cationic surfactant ($C_{18}N^+(Me)_2Pr$) to the traditional C_{18} sorbent in terms of retention, selectivity and the overall elution pattern. As shown in Experimental, both sorbents had the same surface coverage in terms of ligands, i. e., 2.1 μmole ligands/m^2 of silica.

Figure 1a and b shows chromatograms of Dns-AA obtained on $C_{18}N^+(Me)_2Pr$ and C_{18} columns, respectively, by using hydro-organic eluents at pH 2.5. Different elution orders of the solutes, i.e., change in selectivity, can be observed on the $C_{18}N^+(Me)_2Pr$ column when compared to the C_{18} column under otherwise identical elution conditions. In addition, the $C_{18}N^+(Me)_2Pr$ column afforded higher peak capacity than the traditional C_{18}-silica column, meaning that the number of resolved peaks is higher. This may be due in part to the fact that with the $C_{18}N^+(Me)_2Pr$ column, electrostatic interaction are superimposed on hydrophobic interaction.

The general chemical structure of the Dns-AA is given by



where R is the side chain group. According to studies on the ionization of Dns-AA [14, 15], the pK_a value of the dimethylamino group of Dns-AA is between 3.0 and 4.0 regardless of the ionic properties of the side chain group of the amino acids. The amino group adjacent to the sulfonyl group of the dansyl moiety has a pK_a value of 11.7, which

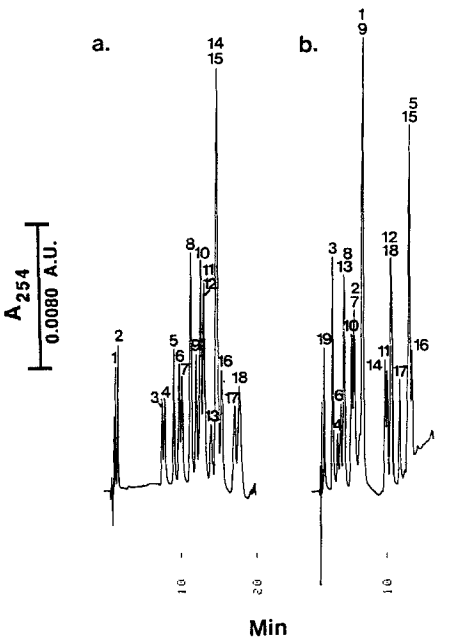
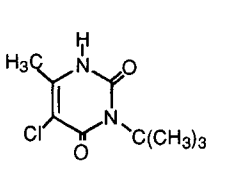


FIGURE 1. Chromatograms of Dns-AA obtained on $C_{18}N^{+}(Me)_2Pr$ column (a) and C_{18} column (b). Columns, 100 x 4.6 mm ID. 15 min linear gradient from 10 to 50% (v/v) 2-PrOH in 20.0 mM sodium phosphate, pH 2.5. Flow rate, 1.0 mL/min. Solutes: 1, arginine; 2, lysine; 3, asparagine; 4, glutamine; 5, tyrosine; 6, serine; 7, threonine; 8, glutamic acid; 9, alanine; 10, glycine; 11, proline; 12, valine; 13, aspartic acid; 14, methionine; 15, isoleucine; 16, leucine; 17, phenylalanine; 18, tryptophan; 19, cysteic acid.

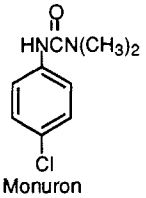
will become negatively charged only at an extreme alkaline pH. The pK_a value of the α-carboxyl group of each Dns-AA is around 4.8 [15]. At the pH of the experiment (i. e., pH = 2.5), the dimethyl amino group is positively charged (fully protonated), the amino group adjacent to the sulfonyl group is uncharged and the α-carboxyl group of the derivative is neutral. As expected, on the C_{18} column (see Fig. 1b), the Dns-AA with nonpolar side chains were eluted after polar amino acids and they emerged out of the column in the order of increasing hydrophobic character of the side chain, i.e., glycine < alanine < valine < leucine. The elution order of the Dns-AA with polar side chains was influenced by the polarity of the solute. Less hydrophobic and charged amino acids, e.g., cysteic acid,

eluted first. Asparagine and glutamine each carrying a side chain acetamido group (polar groups) and a short alkyl chain eluted thereafter. Although lysine and arginine are doubly positively charged, they were more retarded due to their relatively stronger hydrophobic character.

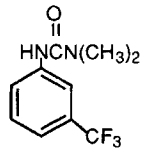
In the case of the $C_{18}N^{+}(Me)_2Pr$ column (see Fig. 1a), the surface of which is positively charged, cysteic acid whose net charge is zero, but it has a negatively charged group (a sulfonic acid group) at the pH of the experiment could not be eluted because of strong electrostatic interaction with the positively charged stationary phase. The doubly positively charged solutes, i.e., arginine and lysine, eluted first due to their stronger electrostatic repulsion from the positively charged stationary phase. In summary , on the $C_{18}N^{+}(Me)_2Pr$ column under investigation, most solutes exhibited longer retention time



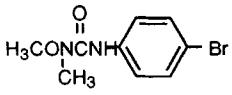
Terbacil



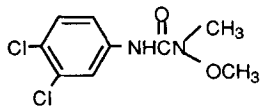
Monuron



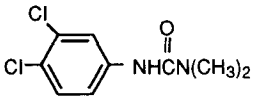
Fluometuron



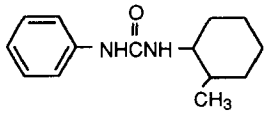
Metobromuron



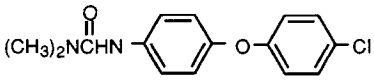
Linuron



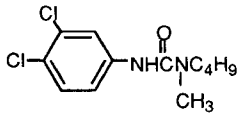
Diuron



Siduron



Chloroxuron



Neburon

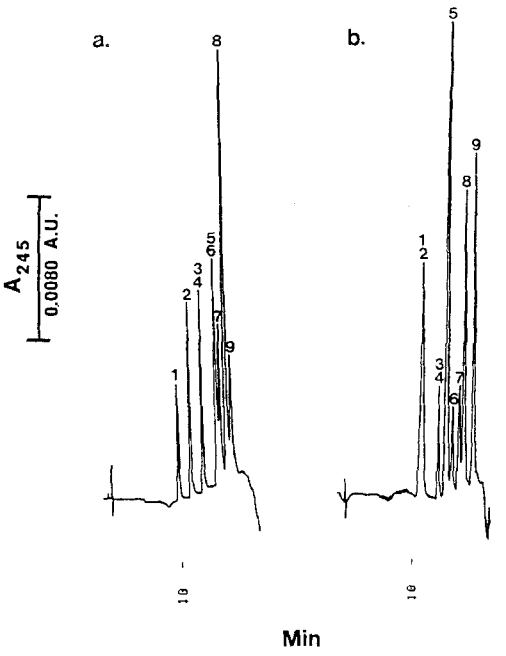
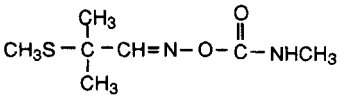


FIGURE 2. Chromatograms of urea herbicides obtained on $C_{18}N^+(Me)_2Pr$ column (a) and C_{18} column (b). Mobile phase pH is 4.0. Other conditions as in Fig. 1. Solute: 1, terbacil; 2, monuron; 3, fluometuron; 4, metobromuron; 5, diuron; 6, linuron; 7, siduron; 8, chloroxuron; 9, neburon.

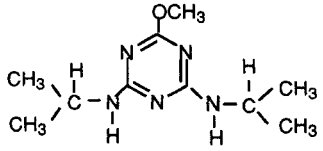
and in turn better separation than on the C_{18} column. In addition, the $C_{18}N^+(Me)_2Pr$ column exhibited a unique selectivity toward the Dns-AA.

Figure 2a and b shows the chromatograms of 9 urea herbicides, namely terbacil, monuron, fluometuron, metobromuron, diuron, linuron, chloroxuron and neburon (for structures, see below), obtained on both $C_{18}N^+(Me)_2Pr$ and C_{18} columns at pH 4.0, respectively. It can be seen that the analysis time is shorter on the $C_{18}N^+(Me)_2Pr$ column because of the weaker interaction between these nonionic species and the mildly hydrophobic $C_{18}N^+(Me)_2Pr$ stationary phase. In addition, different selectivities were observed between terbacil and monuron, and between linuron and diuron.

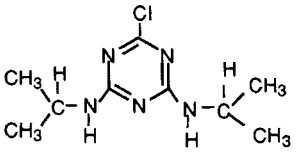
In another set of experiments, seven herbicides including aldicarb, prometon, propazine, prometryne, diazinon, parathion and 2,4-D butyl ester (for structures and pK_a values, see below) can be separated with baseline resolution on the $C_{18}N^+(Me)_2Pr$ column (Fig. 3a), while only five solutes can be resolved on the C_{18} column with different elution order and selectivity (Fig. 3b). It should be noted that prometon and 2,4-D butyl ester, prometryne and diazinon coeluted on the C_{18} column. On the $C_{18}N^+(Me)_2Pr$ column, with the exception of 2,4-D butyl ester which has larger retention time, all other solutes moved down the column much faster than on the C_{18} column, especially prometryne and prometon, because these solutes are slightly positively charged at pH 4.0, thus undergoing repulsion from the $C_{18}N^+(Me)_2Pr$ stationary phase.



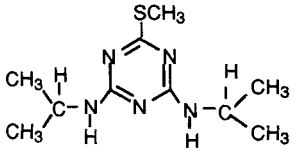
Aldicarb



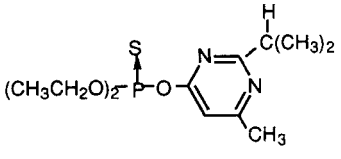
Prometon
pK_a = 4.2



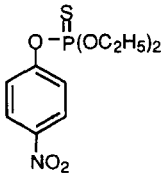
Propazine
pK_a = 1.85



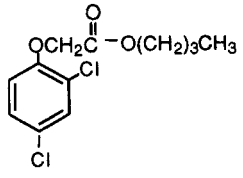
Prometryne
pK_a = 4.05



Diazinon



Parathion



2,4-D butyl ester

In summary, the $C_{18}N^+(Me)_2Pr$ stationary phase under investigation has different interactions with solutes than the C_{18} column, thus providing a method to improve

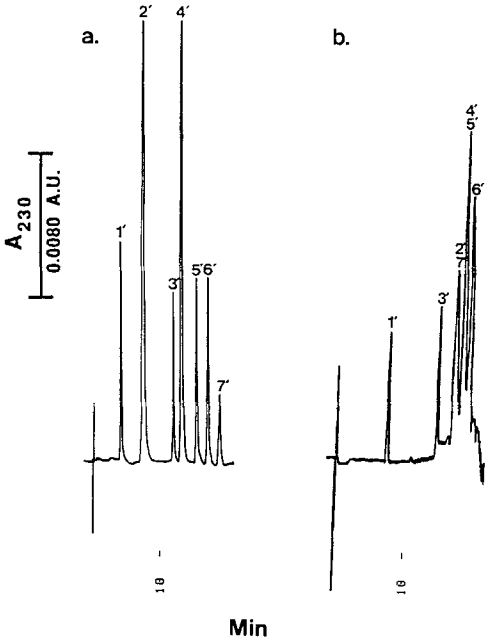


FIGURE 3. Chromatograms of herbicides obtained on $C_{18}N^+(Me)_2Pr$ column (a) and C_{18} column (b). Conditions as in Fig. 2. Solutes: 1', aldicarb; 2', prometon; 3', propazine; 4', prometryne; 5', diazonin; 6', parathion; 7', 2,4-D butyl ester.

separation and selectivity of some ionic and nonionic compounds. The difference in selectivity exhibited by the $C_{18}N^+(Me)_2Pr$ column may be attributed to the superimposition of polar interactions over nonpolar association of the solutes with the positively charged, hydrophobic ligand of the stationary phase.

b) Micellar hydro-organic (i. e., hybrid) eluents. When Empigen (Em) was added to the hydro-organic mobile phase at pH 2.5, the retention of various Dns-AA solutes under investigation decreased slightly on the $C_{18}N^+(Me)_2Pr$ column when compared to the retention observed with the hydro-organic mobile phase but without the surfactant (i. e., without Em); compare Fig. 4a to Fig. 1a. This reduction in retention is the result of electrostatic repulsion between equally charged solute and surfactant-modified stationary

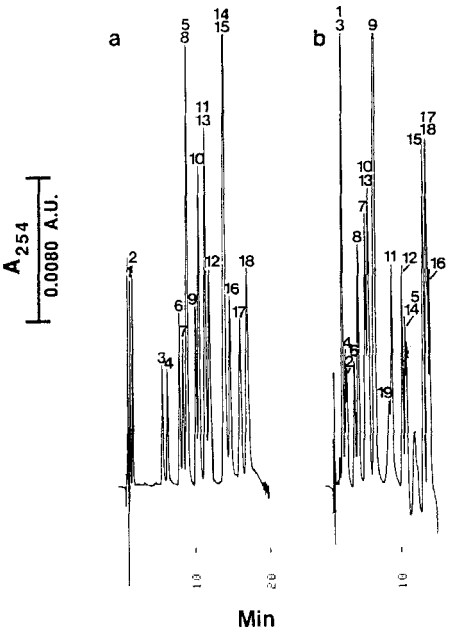


FIGURE 4. Chromatograms of Dns-AA obtained on $C_{18}N^{+}(Me)_2Pr$ column (a) and C_{18} column (b). Columns, 100 x 4.6 mm ID. 15 min linear gradient from 10 to 50% (v/v) 2-PrOH in 20.0 mM sodium phosphate containing 5.0 mM Empigen, pH 2.5. Flow rate, 1.0 mL/min. Solutes: 1, arginine; 2, lysine; 3, asparagine; 4, glutamine; 5, tyrosine; 6, serine; 7, threonine; 8, glutamic acid; 9, alanine; 10, glycine; 11, proline; 12, valine; 13, aspartic acid; 14, methionine; 15, isoleucine; 16, leucine; 17, phenylalanine; 18, tryptophan; 19, cysteic acid.

phase, i.e., a stationary phase with adsorbed surfactant molecules on its surface. At pH 2.5, both the surfactant and the Dns-AA are positively charged. The degree of reduction in retention varied among the various solutes and was largely dependent on the hydrophilic-hydrophobic balance of the solute. The retention of Dns-AA solutes of relatively large hydrophobicity such as tryptophan was not as much affected as that of weakly hydrophobic solutes such as serine and asparagine. The difference in the degree of repulsion/hydrophobic interaction may explain why the elution order of valine/aspartic acid and serine/tyrosine were reversed when the Em surfactant was added to the mobile phase (compare Fig. 4a to Fig. 1a).

Using the same hydro-organic mobile phase (as in the preceding experiment) containing Em but raising the pH to 6.0, all Dns-AA with nonpolar side chains could not be eluted and only some of the Dns-AA with polar side chains, i.e., serine, threonine, asparagine, glutamine, tyrosine, lysine and arginine, were eluted. For the amino acids that eluted at pH 6.0, their retention times were longer than at pH 2.5. This indicates that the electrostatic interaction between each dansyl amino acid and the stationary phase is stronger at higher pH. At higher pH values, i.e., pH 6.0, the carboxyl group of Dns-AA is negatively charged and has a stronger electrostatic interaction with the surfactant modified-stationary phase. Under this condition, the hydrophobic Dns-AA with nonpolar side chains underwent both hydrophobic and electrostatic interactions, and as a result they were retarded longer. This dual interaction mechanism provided even stronger interaction when the solute possessed two carboxyl groups (e.g., aspartic and glutamic acids) and as a result they could not be eluted. At a lower pH, i.e., pH 2.5, however, where the Dns-AA are positively charged, electrostatic repulsion from the modified-stationary phase overshadowed hydrophobic interaction, and were eluted in shorter time.

Figure 4b illustrates the chromatogram of Dns-AA obtained on a C_{18} column under the same mobile phase conditions as in Fig. 4a. With the C_{18} column, the surfactant (i.e., Em) is adsorbed onto the stationary phase, thus forming a surfactant modified-stationary phase. Under this condition, and comparing to a C_{18} column that was used with a hydro-organic mobile phase (Fig. 1b), the doubly positively charged solutes, such as lysine and arginine eluted earlier because of the electrostatic repulsion from the surfactant modified-stationary phase. Also, tyrosine, glutamine and asparagine eluted earlier due to their repulsion and weak hydrophobicity. On the other hand, hydrophobic amino acids such as phenylalaline and tryptophan were retained more (Fig. 4b). When comparing to a $C_{18}N^{+}(Me)_2Pr$ column that was eluted with an Em micellar mobile phase (Fig. 4a), most of the Dns-AA yielded reduced retention time on the C_{18} column except tyrosine and lysine. Cysteic acid which can not be eluted on a $C_{18}N^{+}(Me)_2Pr$ column could be eluted here. As can be seen in Fig. 4, the $C_{18}N^{+}(Me)_2Pr$ column provided a better overall

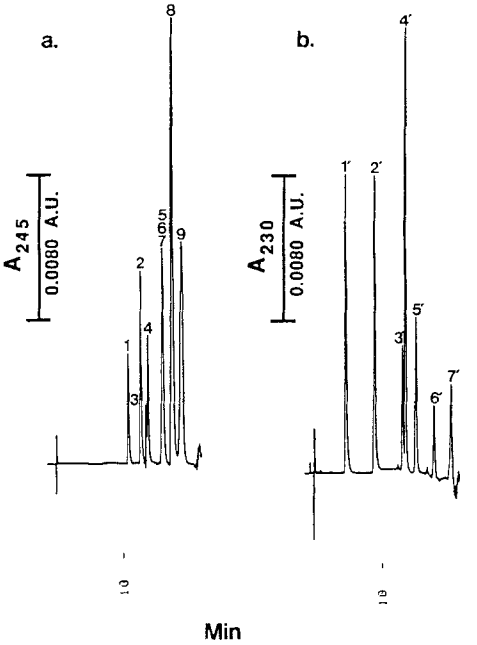


FIGURE 5. Chromatograms of urea herbicides (a) and other herbicides (b) obtained on $C_{18}N^+(Me)_2Pr$ column. Mobile phase pH is 4.0. Other conditions are as in Fig. 4. Solutes in (a): 1, terbacil; 2, monuron; 3, fluometuron; 4, metobromuron; 5, diuron; 6, linuron; 7, siduron; 8, chloroxuron; 9, neburon. Solutes in (b): 1', aldicarb; 2', prometon; 3', propazine; 4', prometryne; 5', diazonin; 6', parathion; 7', 2,4-D butyl ester.

resolution between the various solutes. In fact, 15 Dns-AA were baseline resolved on the $C_{18}N^+(Me)_2Pr$ column as opposed to only 14 Dns-AA partially resolved on the C_{18} column. This may be attributed to the higher coating of the C_{18}-silica surface with the Em surfactant as compared to the $C_{18}N^+(Me)_2Pr$-silica surface, thus rendering the former column more repulsive than the latter. The higher binding of the Em surfactant to the C_{18}-silica surface may be explained by the absence of similarly charged moieties as in the case of the $C_{18}N^+(Me)_2Pr$ column, since both stationary phases (i.e., C_{18} and $C_{18}N^+(Me)_2Pr$) possess the same surface coverage with ligands.

Figure 5a and b displays the chromatograms of herbicides obtained on a $C_{18}N^+(Me)_2Pr$ column using hydro-organic eluents containing Em at pH 4.0. When

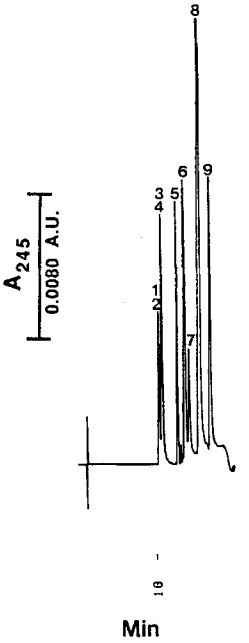


FIGURE 6. Chromatogram of urea herbicides obtained on C_{18} column. Mobile phase pH is 4.0. Other conditions are as in Fig. 4. Solutes: 1, terbacil; 2, monuron; 3, fluometuron; 4, metobromuron; 5, diuron; 6, linuron; 7, siduron; 8, chloroxuron; 9, neburon.

compared to Fig. 2a, the retention of urea herbicides shown in Fig. 5a was little or not affected since these solutes are nonionic. Also, using the same column and mobile phase, the herbicides shown in Fig. 5b and 3a behaved similarly regardless of the presence or absence of Em in the hydro-organic eluent. At this pH, the weakly basic herbicides (prometon and prometryne) were only slightly ionized and did not undergo extensive electrostatic repulsion with the micelle and/or the surfactant modified-stationary phase. On the C_{18} column and using Em as the mobile phase micelles (see Fig. 6), all urea herbicides were less retained when compared to the case of the C_{18} column without the Em surfactant present in the mobile phase. However, the surfactant has no effect on the elution order (compare Fig. 6 to Fig. 2b). When compared to the case of the $C_{18}N^{+}(Me)_2Pr$ column,

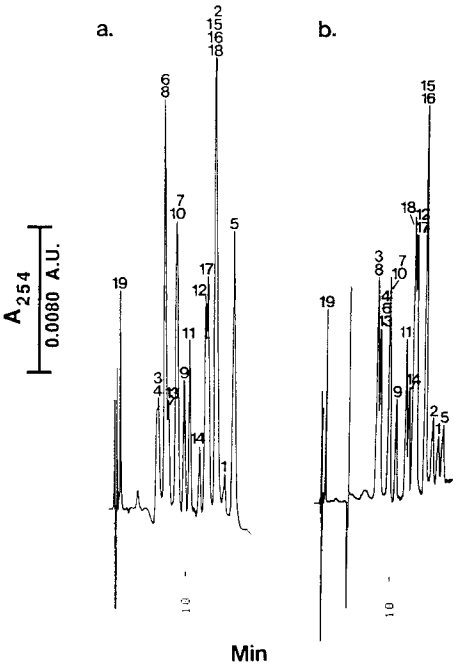


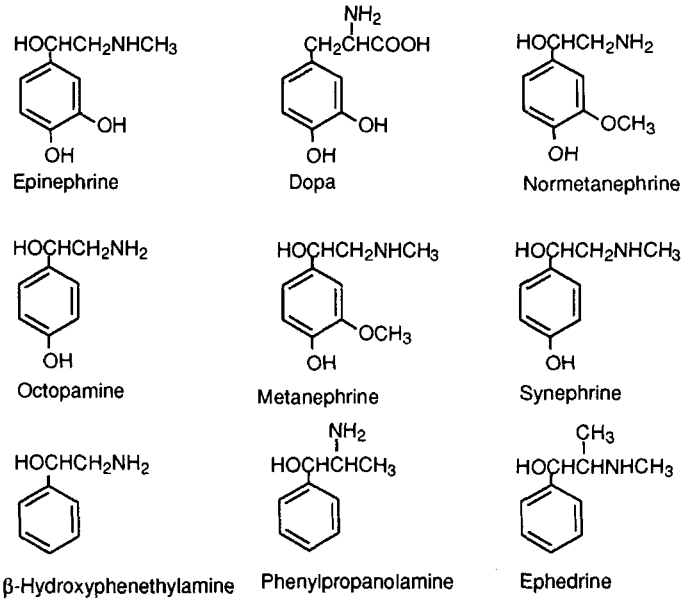
FIGURE 7. Chromatograms of Dns-AA obtained on $C_{18}N^+(Me)_2Pr$ column (a) and C_{18} column (b). Columns, 100 x 4.6 mm ID. 15 min linear gradient from 10 to 50% (v/v) 2-PrOH in 20.0 mM sodium phosphate containing 20.0 mM SDS, pH 2.5. Flow rate, 1.0 mL/min. Solutes: 1, arginine; 2, lysine; 3, asparagine; 4, glutamine; 5, tyrosine; 6, serine; 7, threonine; 8, glutamic acid; 9, alanine; 10, glycine; 11, proline; 12, valine; 13, aspartic acid; 14, methionine; 15, isoleucine; 16, leucine; 17, phenylalanine; 18, tryptophan; 19, cysteic acid.

the presence of the Em surfactant has different effect on the retention and selectivity of urea herbicides on the C_{18} column (compare Fig. 6 to Fig. 5a).

On the other hand, polar and highly positively charged catecholamines (structures are shown below) could not be retained on the $C_{18}N^+(Me)_2Pr$ column.

With a $C_{18}N^+(Me)_2Pr$ column and adding SDS instead of Em to the mobile phase, the Dns-AA behaved differently (see Fig. 7a). Under this condition, the positively charged quaternary ammonium groups of the stationary phase form ion-pairs with the oppositely charged SDS. In addition, the surfactant is adsorbed onto the stationary phase thus

yielding an SDS-modified stationary phase. At pH 2.5, dansyl amino acids with strong side chain acidic groups will repulse from the negatively charged SDS-modified stationary phase, and therefore their retention will be reduced. This is the case of cysteic acid whose strong sulfonic acid group is ionized at any pH. On the other hand, the retention of the doubly positively charged Dns-AA such as lysine and arginine increased and they were retarded much longer in the presence than in the absence of the SDS micellar phase, compare Fig. 7a to Fig. 1a.



On a C_{18} column, and in the presence of SDS in the hydro-organic eluent, the elution order is almost the same as with a $C_{18}N^+(Me)_2Pr$ column but with different selectivity. An exception is that tryptophan eluted earlier on a C_{18} column (see Fig. 7b).

Using a $C_{18}N^+(Me)_2Pr$ column, urea herbicides terbacil and fluometuron eluted faster while siduron and neburon eluted slower when SDS was present as compared to the absence of SDS, so that fluometuron was separated from metobromuron and the elution order of siduron and chloroxuron was reversed (compare Fig. 8a to Fig. 2b). Prometon

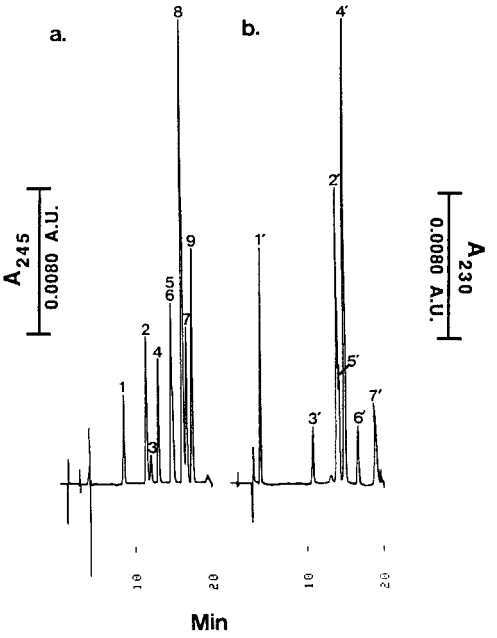


FIGURE 8. Chromatograms of urea herbicides (a) and other herbicides (b) obtained on $C_{18}N^{+}(Me)_2Pr$ column. Column, 100 x 4.6 mm ID. 15 min linear gradient from 10 to 50% (v/v) 2-PrOH in 20.0 mM sodium phosphate containing 20.0 mM SDS, pH 2.5. Flow rate, 1.0 mL/min. Solutes in (a): 1, terbacil; 2, monuron; 3, fluometuron; 4, metobromuron; 5, diuron; 6, linuron; 7, siduron; 8, chloroxuron; 9, neburon. Solutes in (b): 1', aldicarb; 2', prometon; 3', propazine; 4', prometryne; 5', diazonin; 6', parathion; 7', 2,4-D butyl ester.

and prometryne solutes each carrying a positive charge migrated much slower due to the electrostatic attraction to the surfactant-modified stationary phase and eluted after propazine and diazinon, respectively (Fig. 8b). The overall separation of 16 herbicides is illustrated in Fig. 9 whereby 12 of them were resolved.

On a C_{18} column, nine urea herbicides were completely separated using SDS as the micellar mobile phase (see Fig. 10a). As can be seen not only the retention time of each urea herbicides was reduced, but also monuron and metobromuron were separated from terbacil and fluometuron, respectively, when compared to the case without SDS (see Fig.

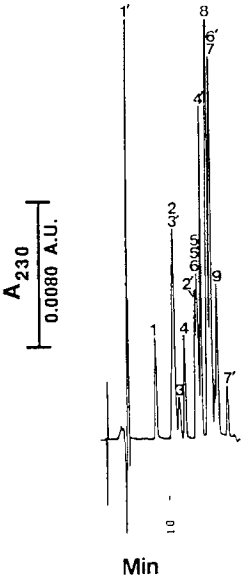


FIGURE 9. Chromatogram of all herbicides obtained on $C_{18}N^+(Me)_2Pr$ column. Conditions are as in Fig. 8. Solutes : 1, terbacil; 1', aldicarb; 2, monuron; 2', prometon; 3, fluometuron; 3', propazine; 4, metobromuron; 4', prometryne; 5, diuron; 5', diazonin; 6, linuron; 6', parathion; 7, siduron; 7', 2,4-D butyl ester; 8, chloroxuron; 9, neburon.

2b). When comparing to a $C_{18}N^+(Me)_2Pr$ with an SDS micellar mobile phase, the retention of diuron and chloroxuron were reduced even more, so that diuron was separated from linuron and chloroxuron was separated from siduron, respectively.

Another set of seven herbicides (namely aldicarb, prometon, propazine, prometryne, diazinon, parathion and 2,4-D butyl ester) was chromatographed on a C_{18} column (Fig. 10b) in the presence of an SDS micellar mobile phase. The weakly ionized cationic herbicides prometon and prometryne were retained for longer time because they underwent electrostatic interactions with the oppositely charged surfactant-modified stationary phase. When compared to the result on a $C_{18}N^+(Me)_2Pr$ column (Fig. 8b), the selectivity was completely different. All solutes were more retained except 2,4-D butyl ester, and in addition, the $C_{18}N^+(Me)_2Pr$ column afforded better overall separation.

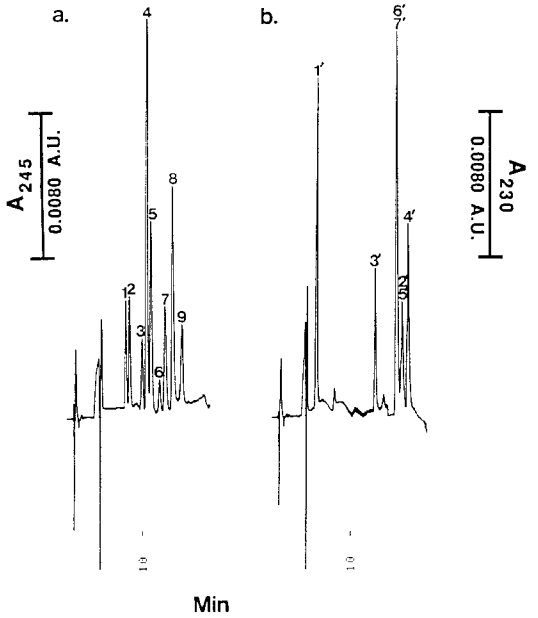


FIGURE 10. Chromatograms of urea herbicides (a) and other herbicides (b) obtained on C_{18} column. Conditions are as in Fig. 7. Solutes in (a): 1, terbacil; 2, monuron; 3, fluometuron; 4, metobromuron; 5, diuron; 6, linuron; 7, siduron; 8, chloroxuron; 9, neburon. Solutes in (b): 1', aldicarb; 2', prometon; 3', propazine; 4', prometryne; 5', diazonin; 6', parathion; 7', 2,4-D butyl ester.

However, when all the 16 herbicides were chromatographed on a C_{18} column, the overall resolution was better than on a $C_{18}N^{+}(Me)_2Pr$ column (see Fig. 11) since the C_{18} column seems to better segregate the urea herbicides from the other herbicides, so that less overlapping of peaks occurred.

In another instance where the C_{18} column under investigation seems to afford superior separation is with polar catecholamines (see Fig. 12). This reflects the larger hydrophobicity of the C_{18} column. In fact, in the presence of SDS as the micellar mobile phase, catecholamines including epinephrine, dopa, normetanephrine, octopamine, metanephrine, synephrine, β-hydroxyphenethylamine, phenylpropanolamine and ephedrine, can be retained and separated using gradient elution (see Fig. 12b). The elution

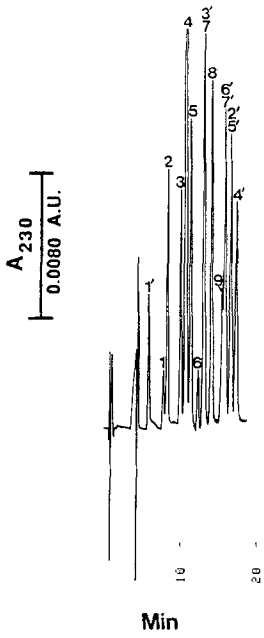


FIGURE 11. Chromatogram of all herbicides obtained on C_{18} column. Conditions are as in Fig. 7. Solutes : 1, terbacil; 1', aldicarb; 2, monuron; 2', prometon; 3, fluometuron; 3', propazine; 4, metobromuron; 4', prometryne; 5, diuron; 5', diazonin; 6, linuron; 6', parathion; 7, siduron; 7', 2,4-D butyl ester; 8, chloroxuron; 9, neburon.

order depends on the hydrophobic character of the solutes. The solutes with two polar hydroxyl groups on their benzene ring, i.e., epinephrine and dopa, eluted first. The solutes without hydroxyl group on their benzene rings, i.e., phenylpropanolamine and ephedrine, eluted last. Catecholamines which could not be retained on a $C_{18}N^+(Me)_2Pr$ column in the absence of SDS in the mobile phase, they were retained on this column in the presence of SDS as the micellar mobile phase (see Fig. 12a). However, since the $C_{18}N^+(Me)_2Pr$ column is less hydrophobic than the C_{18} column, the overall resolution among the catecholamine solutes was less satisfactory on the former column than on the latter.

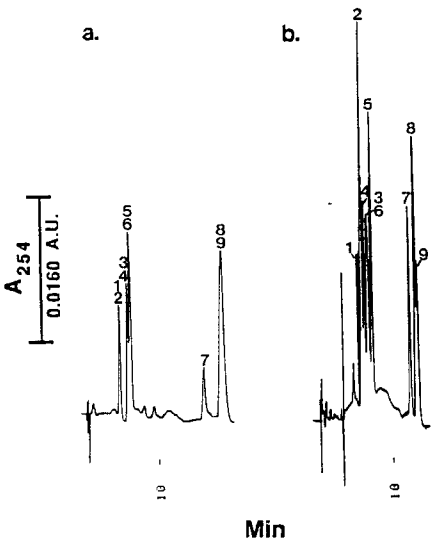


FIGURE 12. Chromatograms of catecholamines on $C_{18}N^+(Me)_2Pr$ column (a) and C_{18} column (b). Columns, 100 x 4.6 mm ID. 15 min linear gradient from 15 to 25% (v/v) 2-PrOH in 20.0 mM sodium phosphate containing 20.0 mM SDS, pH 2.5. Flow rate, 1.0 mL/min. Solutes: 1, dopa; 2, epinephrine; 3, normetanephrine; 4, octopamine; 5, metanephrine; 6, synephrine; 7, β-hydroxyphenethylamine; 8, phenylpropanolamine; 9, ephedrine.

CONCLUSIONS

The possibility of using silica microparticles having surface bound cationic surfactant ligands has been examined. Better separation can be obtained on the $C_{18}N^+(Me)_2Pr$ column for Dns-AA and herbicides. The presence of micelles in the mobile phase has a great influence on the chromatographic selectivity depending on the nature of the micelle. For the Dns-AA, the Em surfactant seems to give a better separation selectivity than the SDS surfactant on the $C_{18}N^+(Me)_2Pr$ column. Conversely, for urea herbicides, it seems that SDS yielded a better separation than Em on the C_{18} column .

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