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Ion-interaction chromatography: a study of the distribution of *n* -alkylammonium ions on an ODS-2 column

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ABSTRACT

The distribution of *n*-alkylammonium ions on a Whatman ODS-2 reversed-phase column was investigated. It was found that only about 24% of the retained *n*-alkylammonium ions act as ion-pairing reagents on the column surface. Approximately 76% of the *n*-alkylammonium ions are electrostatically interacting with the deprotonated surface silanols.

INTRODUCTION

Recently we reported the distribution of *n*-octylammonium ions sorbed on the surface of an octadecylsilane reversed-phase column [1]. The ion-pairing agent was absorbed in at least two forms. The major amount was attached to the silanol groups; the minor fraction was absorbed as the ionic dihydrogen phosphate salt. This find was at variance with previous workers' interpretations which basically assumed that the amount sorbed on the surface was equal in concentration to the amount in the mobile phase [2–5]. Here we focus our attention on the distribution of various *n*-alkylammonium ions on the octadecylsilane reversed-phase column in order to understand separation efficiency.

EXPERIMENTAL

The high-performance liquid chromatography (HPLC) system used in this study consisted of a Perkin-Elmer (P-E) Series 2/2 solvent delivery system equipped with a Rheodyne Model 7125 injector valve (20-μl loop) and a Rheodyne Model 7066 tandem column selector (5 columns and a bypass/flush out tube), a P-E Model LC-75 variable-wavelength detector, and a P-E Model LC Auto control. Recordings of the chromatograms were made on a Fisher Recordall Series 5000. A Whatman Partisil 1025 ODS-2 analytical column with a Whatman CO:PELL ODS guard column was used for the HPLC studies. The ion chromatography system used consisted of a Dionex Series 4000i equipped with a 50-μl loop, conductivity detector and Dionex anion and cation micro-membrane suppressors. Recordings of the chromatograms and data handling were affected with a Spectra-Physics SP 4290 integrator and an Epson Equity +1 computer equipped with a Spectra-Physics Labnet software program. The anion columns used were the analytical HPIC 4S4A and the guard HPIC 4G4A column (both columns were from Dionex). The pH meter was a Fisher Accumet 915 equipped with an AccupHast pH electrode. The mobile phases were prepared using *n*-butylamine, *n*-hexylamine, *n*-octylamine, HPLC-grade 85% phosphoric acid (Aldrich) and water purified with a Milli-Q reagent grade water system.

RESULTS

Adsorption studies

The ion-pairing reagents used in this study were *n*-alkylammonium dihydrogenphosphate salts.

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These salts were used for the following reasons: (1) the elution order of the test anions was found to be independent of the counter anion of the ion-pairing reagent; (2) the dihydrogenphosphate ion resulted in an average retention time for the test ions based on chloride ion (20% longer) and sulfate ion (10% shorter); (3) the dihydrogenphosphate ion can act as a pH buffer for the system. The concentration of the ion-pairing reagents in the mobile phases was approximately 30 m*M* so that the experiments could be completed in a reasonable length of time. Initial studies showed that the breakthrough times were inversely proportional to the ion-pairing reagent concentration. The amount of reagent retained by the column was independent of concentration.

Breakthrough studies were used to measure the amount of ion-pairing reagent sorbed on the column. Prior to each breakthrough experiment, the column was washed with a minimum of twenty void bed volumes of both methanol–water (80:20, v/v) and pure methanol to insure a clean column surface. During the cleaning process the column effluent was monitored with the UV detector set at 210 nm to insure the removal of all absorbed species. The breakthrough chromatograms were obtained by first purging the ODS-2 column with Milli-Q reagent-grade water, then filling the tubing up to the head of the column with the mobile phase and then monitoring the column effluent with the UV detector set at 196 nm which is the absorbance maximum for the *n*-alkylamines in a water matrix. The phosphate ion was measured using ion chromatography. The amount of *n*-alkylamine sorbed on the column surface was calculated by measuring the amount of the amine introduced into the column up to the "breakthrough" time minus the amount of amine found in the column effluent prior to "breakthrough". For the *n*-butyl-, *n*-hexyl- and *n*-octylammonium salts, the mobile phases were prepared by mixing 29.50 ± 0.30 mmol of the *n*-alkylamine with 25.00 ± 0.20 mmol of H_3PO_4 in 1 liter of Milli-Q reagent-grade water resulting in a mobile phase of pH 6.3. The resulting mobile phase contains approximately 22.50 m*M* *n*-alkylammonium dihydrogen phosphate, 2.50 m*M* di-*n*-alkylammonium hydrogenphosphate and 1.85 m*M* *n*-alkylamine. Analyses of the various mobile phases and effluents prior to breakthrough are listed in Table I.

Two points of interest are shown in Table I. First is the decrease in the pH from 6.3 in the mobile phase to 3.7 in the effluent. This decrease in pH was observed by Hansen *et al.* [3] during their studies on the modification of silica with long-chain quaternary ammonium ions. The decrease was attributed to the quaternary ammonium ions displacing the hydrogen ion of the surface silanols on the silica. The second point of interest is the difference in the *n*-alkylammonium ion and dihydrogenphosphate ion in the effluent of the column for all three of the *n*-alkylammonium salts studied.

The column effluent contained more *n*-alkylammonium ion as the chain length increased, but less dihydrogenphosphate anion, indicating that more

TABLE I

ANALYSIS OF THE MOBILE PHASE AND COLUMN EFFLUENT PRIOR TO THE BREAKTHROUGH (IN m*M*)

Whatman ODS-2 column, flow-rate 1 ml/min.

	pH	*n*-Alkyl NH_3^+	*n*-Alkyl NH_2	$H_2PO_4^-$	HPO_4^{2-}	H_3PO_4
n-Octyl						
Mobile phase	6.3	27.50	1.84	22.63	2.51	–
Column effluent	3.7	7.70	–	19.53	–	0.53
n-Hexyl						
Mobile phase	6.3	27.88	1.86	22.56	2.51	–
Column effluent	3.7	5.14	–	19.99	–	0.55
n-Butyl						
Mobile phase	6.3	27.90	1.86	22.10	2.46	–
Column effluent	3.7	5.08	–	20.98	–	0.57

TABLE II

AMOUNTS OF THE VARIOUS SPECIES IN THE SYSTEM THROUGH THE BREAKTRHOUGH (IN mmol)

Whatman ODS-2 column, flow-rate 1 ml/min.

	n-Alkyl NH_3^+	*n*-Alkyl NH_2	$H_2PO_4^-$	HPO_4^{2-}	H_3PO_4
n-Octyl					
Pumped through column	1.96	0.13	1.53	0.17	–
Column effluent	0.58	–	1.33	–	0.04
Retained by column	1.51	–	0.33	–	–
n-Hexyl					
Pumped through column	0.97	0.07	0.82	0.09	–
Column effluent	0.23	–	0.66	–	0.02
Retained by column	0.81	–	0.23	–	–
n-Butyl					
Pumped through column	0.75	0.05	0.59	0.07	–
Column effluent	0.20	–	0.52	–	0.01
Retained by column	0.60	–	0.13	–	–

active ion-pairing reagent was being absorbed on the ODS-2 surface as the chain length increased.

The results of the amount (in mmol) of the various species in the system for the three *n*-alkylammonium salts studied are given in Table II. These data were calculated using the concentrations of the *n*-alkylamine, phosphoric acid, pH and the time of breakthrough [1]. For the *n*-octylammonium salts, 2.09 mmol of the *n*-octylamine and 1.70 mmol of phosphoric acid were used up to the breakthrough point. Of these amounts, only 1.51 mmol of the *n*-octylammonium ion and 0.33 mmol of the phosphate ion were retained on the column. Therefore, the column retained only 72.25% of the *n*-octylammonium ion and 19.41% of the phosphate ion. Using the same analysis for the *n*-hexyl and *n*-butyl salts, it was found that for the *n*-hexyl salts the column retained 77.88% of the *n*-hexylammonium ion and 25.27% of the phosphate ions while for the *n*-butyl reagent, the column retained 75.00% of the *n*-butylammonium ion and 19.70% of the phosphate ion.

TABLE III

AMOUNTS OF THE VARIOUS SPECIES IN THE SYSTEM THROUGH THE BREAKTHROUGH (IN mmol) USING A WHATMAN SILICA COLUMN

	n-Alkyl NH_3^+	*n*-Alkyl NH_2	$H_2PO_4^-$	HPO_4^{2-}	H_3PO_4
n-Octyl					
Pumped through column	0.44	0.03	0.34	0.04	–
Column effluent	0.16	–	0.27	–	0.01
Retained by column	0.31	–	0.10	–	–
n-Hexyl					
Pumped through column	0.47	0.03	0.36	0.04	–
Column effluent	0.16	–	0.30	–	0.01
Retained by column	0.34	–	0.11	–	–
n-Butyl					
Pumped through column	0.47	0.03	0.38	0.04	–
Column effluent	0.16	–	0.30	–	0.01
Retained by column	0.34	–	0.11	–	–

For these three *n*-alkylamines the ODS-2 column retains about 75% of the amines and 22% of the phosphate; however; the amounts of amine decrease from 1.51 mmol:0.81 mmol:0.60 mmol from *n*-octyl:*n*-hexyl:*n*-butyl.

For a reference, the retention of the three *n*-alkylammonium salts was studied on a Whatman Partisil silica column. The results of these experiments are listed in Table III. By dividing the amount of an ion retained by the column by the total amount pumped through the column, the results given in Table III indicate that the retention of three *n*-alkylammonium phosphate reagents on pure silica is essentially the same: 67.3 ± 1.2% for the *n*-alkylammonium ion and 27.5 ± 1.4% for the phosphate ion. The data further indicate that even on a silica column, the *n*-alkylammonium ion adsorbs on the surface in two forms. The major form is attached without dihydrogenphosphate as the neutralizing anion; the minor form has dihydrogenphosphate anion "attached".

Separation studies

To determine the efficiency of various ion-pairing reagents on ODS columns, a series of experiments was carried out using three ion-pairing reagents (*n*-butyl-, *n*-hexyl- and *n*-octylammonium ion) on a Whatman ODS-2 reversed-phase column. The results of these experiments indicated that the *n*-butylammonium ion was not capable of separating the simple anions on the ODS-2 column. All of the test ions (iodate, bromide, nitrate, nitrite and iodide) resulted in a single peak at 3.57 min. However, as shown in Fig. 1, *n*-hexylammonium ion did not separate nitrate and iodide ions, while the *n*-octylammonium ion chromatogram shows fairly good separation of all the test ions. These experiments are summarized in Table IV, which shows a non-linear relation between capacity factor (retention time) and *n*-alkyl chain length at an ion-pairing reagent concentration of 10 m*M*.

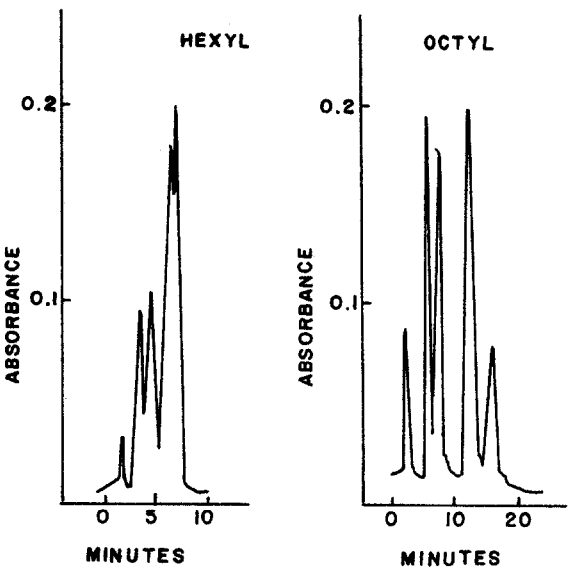

Fig. 1. Chromatograms of (in the order of elution) iodate, bromide, nitrite, nitrate and iodide on an ODS-2 column with *n*-hexylammonium ion (left) or *n*-octylammonium ion (right) as the ion-pairing reagent. Flow-rate 1 ml/min.

TABLE IV

CAPACITY FACTORS OF TEST ANIONS AS A FUNCTION OF THE *n*-ALKYLAMMONIUM ION CHAIN LENGTH ON A WHATMAN ODS-2 COLUMN

Ion-pairing reagent concentration 10 m*M*. Flow-rate 1 ml/min.

	Capacity factor				
	IO_3^-	Br^-	NO_2^-	NO_3^-	I^-
n-Octyl	0.58	0.58	0.58	0.58	0.58
n-Hexyl	0.96	1.24	1.45	1.80	1.98
n-Butyl	1.78	3.16	3.82	5.91	7.54

DISCUSSION

The experimental observations can be summarized as follows: (1) on a Whatman ODS-2 column,

TABLE V

AMOUNTS OF *n*-ALKYLAMMONIUM DIHYDROGENPHOSPHATE RETAINED AS A FUNCTION OF CHAIN LENGTH ON A WHATMAN ODS-2 COLUMN

	n-Alkylammonium dihydrogenphosphate (mmol)	
	Total retained	Corrected for silica retention
n-Octyl	0.33	0.30
n-Hexyl	0.23	0.20
n-Butyl	0.13	0.00

simple inorganic anions cannot be separated using *n*-butylammonium ions, some separation occurs with *n*-hexylammonium ions and acceptable separation occurs with *n*-octylammonium ions, (2) capacity factors, retention, for simple inorganic ions show a non-linear increase as the *n*-alkyl carbon number of the ion-pairing reagent increased from 4 to 8, (3) the Whatman ODS-2 column retains approximately 75% of the *n*-alkylammonium ion and 22% of the phosphate ion that was pumped through the column up to the breakthrough point, (4) the ratio of amounts (mmol) of *n*-alkylammonium ion retained by the ODS-2 column for *n*-octyl:*n*-hexyl:*n*-butyl was 2.51:1.35:1, while the phosphate ratio was 2.54:1.77:1, (5) the amounts (mmol) of *n*-alkylammonium ion and phosphate ion retained by pure silica were essentially independent of the *n*-alkyl chain length.

The equilibria involved are:

$$\text{ODS} + \text{RNH}_3^+ + \text{C}^- \rightleftarrows \text{ODS} \cdots \text{RNH}_3^+ \cdots \text{C}^- \quad (1)$$

$$\text{ODS} \cdots \text{RNH}_3^+ \cdots \text{C}^- + \text{X}^- \rightleftarrows \text{ODS} \cdots \text{RNH}_3^+ \cdots \text{X}^- + \text{C}^- \quad (2)$$

$$-\text{SiOH} + \text{RNH}_3^+ \rightleftarrows -\text{SiO}^- \cdots \text{RNH}_3^+ + \text{H}^+ \quad (3)$$

where ODS is the octadecyl surface of the stationary phase, RNH_3^+ is the mobile phase additive, C^- is the counter ion and X^- is the anion being separated.

The first two equilibria are associated with an ion-interaction type of mechanism. These equilibria indicate that the hydrophobic ion-pairing reagent is being absorbed on the ODS surface, followed by a dynamic ion exchange between the solute anion in the mobile phase and the counter ion of the absorbed ion-pairing reagents. Eqn. 3 accounts for the absorption of the ion-pairing reagent on the surface silanol groups. A typical silica surface is estimated to have between 7 and 9.5 μmol/m^2 of surface silanol groups [6]. The deprotonation reaction, $\equiv\text{SiOH} \rightleftarrows \equiv\text{SiO}^- + \text{H}^+$ is reported to have a pK_a value of 7.1 [7]. At a mobile phase pH of 6.3, a Whatman ODS-2 column would contain approximately 1.45 mmol of deprotonated silanol groups.

If one assumes that the active ion-pairing reagent absorbed on the column surface is in the form of the *n*-alkylammonium dihydrogen phosphate salt, then the amount of phosphate ion retained by the column is equivalent to the ion-pairing reagent on the column surface. The data in Table V show that there is a linear relationship between the total amount of dihydrogenphosphate anion retained by the column and the *n*-alkylammonium ion chain length. Also shown is the situation after subtraction of the dihydrogen phosphate ion sorbed on the silica column surface (Table III) from that on the ODS-2 surface. The additional dihydrogenphosphate ion absorbed on the surface of the ODS-2 column increases from near zero for the *n*-butylammonium ion to approximately 0.2 mmol for *n*-octylammonium ion. The fact that the surface-active *n*-alkylammonium ion increases in concentration as the chain length of the *n*-alkyl group increases clearly accounts for the better separation of the test ions with *n*-octylammonium ion in the mobile phase compared to *n*-hexylammonium ion or *n*-butylammonium ion as the carrier.

Table VI lists the amounts of retained *n*-alkylammonium ion without phosphate counter ion as a function of chain length. The relationship is non-linear on the ODS-2 column but, as shown in Table III, is basically a constant on the silica column. We are forced to conclude that either addition of *n*-alkylammonium ion to the silanol groups on the ODS-2 column is chain length dependent or ion-pairing reagents absorbs on the surface by way of yet another mechanism. We are examining this phenomenon in more detail since it may hold the key to our understanding of the non-linear retention behavior of the test ions shown in Table IV.

In conclusion, (1) of the *n*-alkylammonium ions retained by a Whatman ODS-2 reversed-phase column (for *n*-butyl, *n*-hexyl and *n*-octyl) approximately 76% are electrostatically interacting with the deprotonated surface silanols, (2) the amounts of the *n*-alkylammonium ion electrostatically attached to

TABLE VI

AMOUNTS OF ELECTROSTATICALLY ATTACHED *n*-ALKYLAMMONIUM IONS RETAINED AS A FUNCTION OF CHAIN LENGTH ON A WHATMAN ODS-2 COLUMN

	Amount retained (mmol)
n-Octyl	1.18
n-Hexyl	0.58
n-Butyl	0.47

the column increase exponentially with chain length, (3) only about 24% of the retained *n*-alkylammonium ions act as ion-pairing reagents on the column surface, (4) the amounts of the ion-pairing reagent on the column increase linearly in the ratio of 1:1.77:2.54 for *n*-butyl:*n*-hexyl:*n*-octyl ammonium ions and (5) the reason for the exponential increase in the capacity factors with *n*-alkyl chain length currently is unknown but may be related to the non-linear retention of *n*-alkylammonium ion on the ODS-2 surface.

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