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High-performance liquid chromatography on silica dynamically modified with hydrazinium derivatives

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ABSTRACT

Chromatographic systems with silica dynamically modified with a series of hydrazine derivatives were studied as a possible alternative to those with chemically bonded stationary phases. The properties of these new systems were compared with those of conventional reversed-phase systems and systems containing silica dynamically modified by cetrimide. As in reversed-phase chromatography on chemically bonded alkylsilicas, solvophobic interactions seem to be the main factor responsible for retention. It is shown that dynamic modification of silica with reagents differing in chemical structure can lead to systems with different selectivity. Betaines and quaternary ammonium and hydrazinium compounds act similarly as modifiers of silica but are very different as ion-pairing agents. The results of experiments performed with modifiers of different hydrophobicity and at various pH values demonstrate that ionic binding is not only mechanism of immobilization on the silica surface. It is shown that the shape of peaks obtained for highly polar and basic solutes is usually better in dynamic modification chromatography than in reversed-phase chromatography on alkylsilicas. Examples of analytical separations of polyfunctional organic substances and some inorganic anions on dynamically modified silica are presented.

INTRODUCTION

High-performance liquid chromatography (HPLC) on dynamically modified silica can be a promising alternative to separations on covalently bonded stationary phases. Owing to its slightly acidic nature, silica can bind organic cations that are added to the mobile phase by way of ion exchange in a certain pH range [1–10]. The organic layer thus generated on the surface can act as a stationary phase in the chromatographic process. Quaternary ammonium salts have so far been the most popular modifiers. It has been shown that dynamic modification of silica with cetrimide (cetyltrimethylammonium bromide) yields chromatographic systems that are very similar to reversed-phase systems with alkylsilicas as stationary phases. One of the advantages of this method is that dynamic modification is a more reproducible procedure than chemical modification and the properties of the systems obtained are only slightly dependent on the type of silica.

It is obvious that the selectivity of systems with dynamic modification should be dependent on the chemical structure of the modifiers. When one modifier can be easily replaced with another, a broad spectrum of selectivity can be obtained using only one column packed with silica. Nevertheless, only a few organic bases have so far been used

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for dynamic modification and no systematic studies on the selectivity of such systems have been published.

The aim of this work was to evaluate the selectivity of a series of new modifiers based on hydrazine derivatives and to demonstrate certain advantages of this separation mode in applied HPLC.

EXPERIMENTAL

Retention was measured in a Gilson HPLC system equipped with a Holochrome spectrophotometric detector set at 254 nm. Dynamic modification was performed on Silasorb 600 silica and Silasorb C_{18} was used as a reference packing (both from Lachema, Brno, Czechoslovakia). Stainless-steel columns (150 mm × 4.6 mm I.D.) were packed by Diagnostikum (Moscow, U.S.S.R.).

The dynamic modifiers included quaternary hydrazinium salts and betaines with different alkyl substituents and functional groups. Cetrimide, the most thoroughly studied modifier, was used as a reference substance. The structures of the modifiers are shown in Fig. 1. The abbreviations used in this work describe the type of modifier ion (Q and B for quaternary salts or betaines, respectively), the nature of basic group (A and H for ammonium and hydrazinium derivatives, respectively) and its hydrophobicity, the number of carbon atoms in the longest alkyl chain in the molecule. For example, the abbreviation for the quaternary salt hexadecyltrimethylammonium bromide is QA16.

The set of test solutes used to characterize the system selectivity included acidic, basic, amphoteric, polar and non-polar substances: isonicotinic acid (PyCOOOH), phenylacetic acid (BzCOOOH), acetanilide (PhNHCOMe), 4-methylpyridine (MePy), acetophenone (PhCOMe), nitrobenzene ($PhNO_2$) and benzene (PhH), where Py = pyridyl, Bz = benzyl, Ph = phenyl and Me = methyl.

The capacity factors, k', were calculated according to the conventional expression:

$$k' = (V_r - V_0)V_0 \qquad (1)$$

where V_r is the retention time of the solute under study and V_0 the retention time of an unretained solute. The problem of the correct choice of unretained solutes for

$(CH_3)_3N^+C_{16}H_{33}Br^-$ (Cetrimide, QA16)

$(CH_3)_2\overset{+}{N}-NHCH_2CH_2COO^-$
C_nH_{2n+1}
n=16 (BH16); n=15 (BH15); n=12 (BH12); n=9 (BH9)

$(CH_3)_2\overset{+}{N}-NHCH_2CH_2CN\ Br^-$ (QH16)
$C_{16}H_{33}$

Fig. 1. Structures of the modifiers studied.

conventional modes of chromatography has been discussed in the literature (see, *e.g.*, refs. 11 and 12). This task is even more difficult for dynamic modification chromatography because the amount of the stationary phase is strongly dependent on the composition of the mobile phase. Therefore, accurate measurements of capacity factors and their correct interpretation are highly problematic in this chromatographic mode. We believe that for practical purposes of retention and selectivity comparison it is allowable to assume V_0 to be constant. V_0 was measured for the silica column gravimetrically. The column was filled with water, then placed in the oven of a gas chromatograph set at 120°C and purged with helium for 3 h. V_0 was calculated from the column weight loss in this experiment. Further heating under the same conditions did not lead to an observable weight loss, indicating that all water except the strongly chemisorbed layer on the surface of silica had been removed.

RESULTS AND DISCUSSION

The course of dynamic modification of silica can be easily monitored during column equilibration with an eluent containing a dynamic modifier. For example, the starting eluent can be a buffer containing a certain amount of methanol or acetonitrile. Under these conditions the system can be regarded as a normal-phase system with a very strong mobile phase. Naturally, relatively non-polar solutes (*e.g.*, benzene) should not be retained in such a system. When the system is switched to an eluent containing a modifier, a non-polar stationary phase is generated and the whole system is converted into a reversed-phase type. The retention of the modifiers under study is very strong, and therefore the modifier is almost completely adsorbed during this process. This is accompanied by an increase in the retention time of the test solute. When the dynamic modification is over, capacity factors remain constant provided that the mobile phase composition is constant (Fig. 2). Such curves can be used to determine the approximate amount of adsorbed modifier. It was found that silica adsorbs about 1.1 μmol/m^2 of BH12 and about 0.9 μmol/m^2 of QH16 from an eluent containing 20% of acetonitrile. The latter result was confirmed by an independent

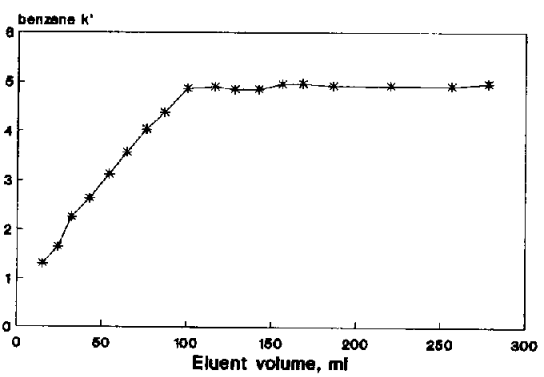

Fig. 2. Equilibration of silica with the dynamic modifier BH12. Mobile phase: 0.01 *M* BH12, 20% acetonitrile in 0.2 *M* acetate buffer (pH 6.0).

TABLE I

CAPACITY FACTORS OF BENZENE

Mobile phase: 0.01 *M* dynamic modifier, 20% acetonitrile in 0.2 M ammonium acetate buffer (pH 6.0).

Column packing	Dynamic modifier	k'
Silasorb C_{18}	None	16.8
	QA16	13.8
	BH16	15.8
Silasorb 600	QA16	8.1
	BH16	13.0
	BH15	12.0
	BH12	5.9
	BH9	0.6
	QH16	10.6

method. The equilibrated column was purged with one volume of water to remove the modifier-containing mobile phase and some packing was removed from the column and dried. Elemental analysis showed the content of carbon to be 11.7%. The content of dynamically immobilized carbon depends primarily on the structure of the modifier and on the concentration of organic solvent. It can reach about 20% in eluents containing no acetonitrile. The mobile phase volume required for complete modification of a given column depends on the concentrations of organic solvent and dynamic modifier. Usually it lies between 50 and 200 ml for 150 mm × 4.6 mm I.D. analytical HPLC columns packed with Silasorb 600 silica.

The retention strength of dynamic modifiers can be evaluated by measuring the k' values of a test solute with a series of mobile phases differing only in the type of dynamic modifier. The data presented in Table I show that with modifiers containing C_{15}–C_{16} alkyl chains the column capacity relative to benzene is slightly lower than that of the chemically bonded alkylsilica column. The system with BH9, containing a C_9 alkyl chain, showed an unexpectedly low retention of benzene.

It is known that retention values in reversed-phase chromatography are dependent on the concentration of organic solvent in the mobile phase. This relationship can be described by several models. We chose the following expression [13]:

$$\log k' = h - p \log C \tag{2}$$

where C is the molar concentration of organic solvent. It has been shown previously [14] in investigations of hundreds of solutes that reversed-phase retention data follow this relationship and the quality of the approximation is not worse than that with other linear models. In contrast, in the experiments on dynamic modification the log k' values did not follow either this relationship (Fig. 3) or other forms of linear models. The typical log k' *vs.* log C curve is very similar to the curve expressing the relationship between the amount of adsorbed modifier and log C. The observed k' values were divided by the amount of adsorbed hydrazine derivative for each concentration of acetonitrile. The resulting values (log k' per 1 mg of the stationary phase) are shown in

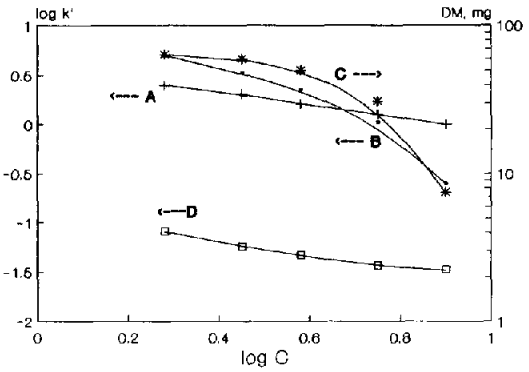

Fig. 3. Influence of acetonitrile concentration in the mobile phase on chromatographic parameters. (A) Capacity factors of benzene in conventional reversed-phase chromatography on Silasorb C_{18}; (B) capacity factors of benzene in dynamic modification chromatography on Silasorb 600 with QA16; (C) amount of adsorbed QA16; (D) capacity factors per 1 mg of adsorbed QA16.

Fig. 3D. The relationship is almost linear and the slope is similar to that in reversed-phase chromatography on Silasorb C_{18} (Fig. 3A). Consequently, the non-linearity of the relationship between log k' and log C is due to the fact that the amount of the active stationary phase (*i.e.*, adsorbed modifier) is not constant in this chromatographic mode but decreases with increasing organic solvent concentration. When the volume fraction of acetonitrile in the mobile phase reaches 40%, the degree of modification becomes negligible and so do the retention values of most solutes.

The selectivity of modifiers was compared in mobile phase consisting of 20% of acetonitrile in 0.2 *M* ammonium acetate buffer at pH 6.0. The concentration of the dynamic modifiers was 0.01 mol/l. The test solutes were arranged in order of increasing retention in conventional reversed-phase chromatography on Silasorb C_{18}. The "selectivity plots" for silica and octadecylsilica are shown in Fig. 4. It can be seen that the steepness of some segments of the plots is not identical for the nine chromatographic systems used, indicating that the introduction of different modifiers really leads to variations in separation selectivity.

The influence of two typical modifiers on the retention of test solutes on octadecylsilica is demonstrated in Fig. 4a. It can be seen that the elution order of basic and neutral solutes is typical of reversed-phase chromatography in the three systems. On the other hand, solvophobic interactions dominating in the reversed-phase mode are not the only significant interactions in the presence of modifiers. It follows from the comparison of plots for no modifier (N) and QA16 that addition of the quaternary ammonium salt leads to a strongly enhanced (20–50 times) retention of acids owing to the ion-pairing effects. Such effects are less clear when octadecylsilica is modified with the hydrazinium betaine BH16; the only difference between plots N and BH16 is the slightly enhanced retention of phenylacetic acid in the dynamically modified system.

A comparison of chromatographic behaviour on silica and octadecylsilica is presented in Fig. 4b. The experiments were performed on octadecylsilica and unmodified silica in the presence of QA16 and BH16. It can be seen that the chemical nature of the sorbent surface is not a crucial factor. The elution order of basic and

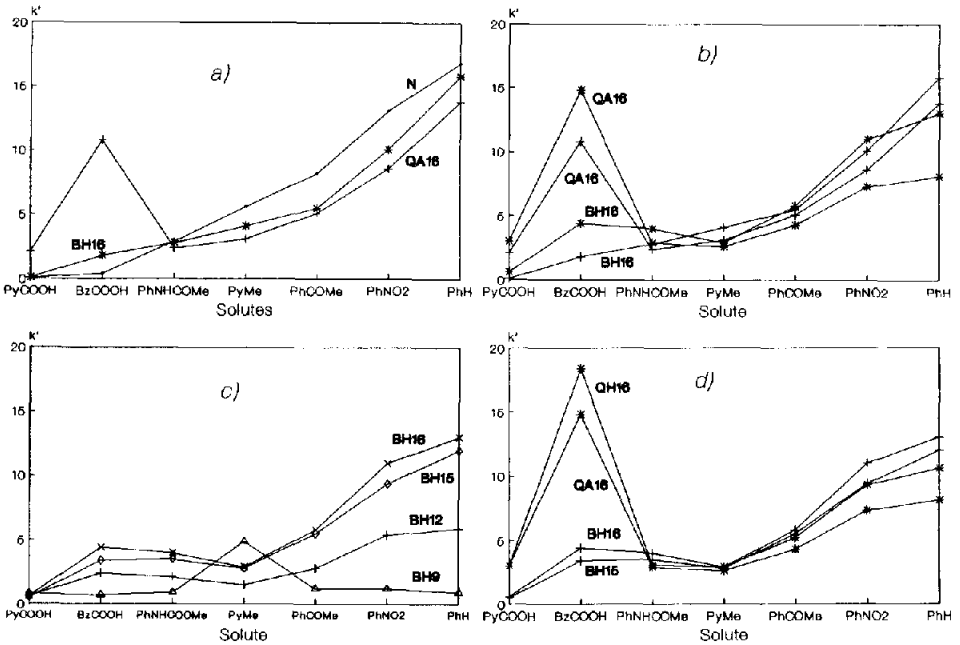

Fig. 4. Selectivity plots. Mobile phase: 0.01 *M* dynamic modifier, 20% acetonitrile in ammonium acetate buffer (pH 6.0). (a) Retention on Silasorb C_{18} with no modifier (N) and with C_{16} ammonium and hydrazinium modifiers; (b) role of sorbents: ∗, silica Silasorb 600; −, octadecylsilica Silasorb C_{18}; (c) role of chain length of hydrazinium betaine derivatives; (d) comparison of properties of (∗) quaternary and (+) betaine modifiers.

neutral solutes on silica dynamically modified with fairly hydrophobic bases was similar to that on the chemically bonded stationary phase, indicating that solvophobic interactions play a dominant role in dynamic modification chromatography similarly to bonded reversed-phase chromatography. Of course, the retention mechanism for acidic solutes in the dynamic modification mode is expected to include ion-pairing effects. This leads to enhanced retention of phenylacetic and isonicotinic acids, as shown in Fig. 4b.

The properties of four hydrazinium betaine modifiers are compared in Fig. 4c. The system capacity with respect to the relatively non-polar acetophenone, nitrobenzene, benzene decreases almost proportionally to the length of the longest carbon chain in the modifier molecules. On the other hand, the retention of most solutes is negligible with the less hydrophobic modifier BH9, containing nine carbon atoms in the longest chain. The only exception is 4-methylpyridine, which is retained even stronger than in the presence of more hydrophobic modifiers. Retention measured on a silica column without modifier showed approximately the same behaviour for all solutes as in the presence of BH9. Consequently, no modification takes place with this substance and the relatively high retention of methylpyridine can be explained by direct interaction of this basic solute with the charged silanols on the surface. This low retention of non-polar solutes with BH9 is unexpected. The degree of modification is believed to be approximately constant for BH16–BH9 because of the very similar

ionogenic properties of these homologues. A possible explanation might be that ion exchange is not the only mechanism responsible for binding of modifier to the silica surface. The ion-exchange binding of the modifier should be suppressed at low pH of the mobile phase because of the negligible extent of silica ionization. No retention should be observed under such conditions. Our experiments with BH16 and a mobile phase of pH 2.5 showed that all the solutes under study still are retained significantly, the capacity factors being about 40% of the values observed at pH 6.0. This is evidence for another mechanism of modifier retention. The polarity of silica is lower than that of the water-rich mobile phase used, and therefore it was assumed that this material can act as a weak reversed-phase sorbent [15]. An example of such a kind of retention of non-ionic solutes, alkylbenzenes from water, has been published previously [16]. We believe that a similar mechanism could be responsible for the sorption of the hydrophobic modifiers under study.

The selectivities obtained with two betaines and two quaternary salts on silica are compared in Fig. 4d. It is clear that the four substances are similar in acting as "reversed-phase dynamic modifiers", whereas the quaternary compounds are much more efficient ion-pairing agents. Obviously, the different properties of these two groups of modifiers can be explained by the presence of a charged carboxylic group in the betaines which partially prevents ion pairing with acidic solutes. Comparison of systems with QH16 and QA16 shows that the quaternary ammonium and quaternary hydrazinium fragments have very similar effects on retention and selectivity towards the test solutes (this may not be the case for groups of closely related substances). The presence of an additional $-NH(CH_2)_2CN$ fragment in the molecule of modifier QH16 does not influence the selectivity very much. Consequently, the hexadecyl group is the main fragment responsible for selectivity.

Certain features of HPLC with dynamic modification are important from a practical point of view. It has been observed that the column efficiency for neutral and acidic solutes is not lower than the efficiency of modern columns for reversed-phase chromatography. It is known that many extremely polar basic solutes show poor

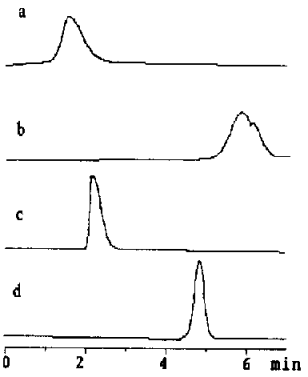

Fig. 5. Chromatograms of pyrazine-2,5-dicarboxylic acid on Silasorb C_{18} (a–c) and Silasorb 600 (d). Mobile phases: (a) 0.01 *M* sodium dodecylsulphate, 2.5% acetic acid, 97.5% water; (b) 1% tetrabutylammonium phosphate in water (pH 2.5); (c) and (d) 0.01 *M* QA16 and 23% acetonitrile in 0.2 *M* acetate buffer (pH 6.0).

peak symmetry and efficiency. The addition of buffers, salts or ion-pairing agents to the mobile phase is not a universal solution to the problem and in many instances the peak shape and symmetry remain unsatisfactory. One of the advantages of dynamic

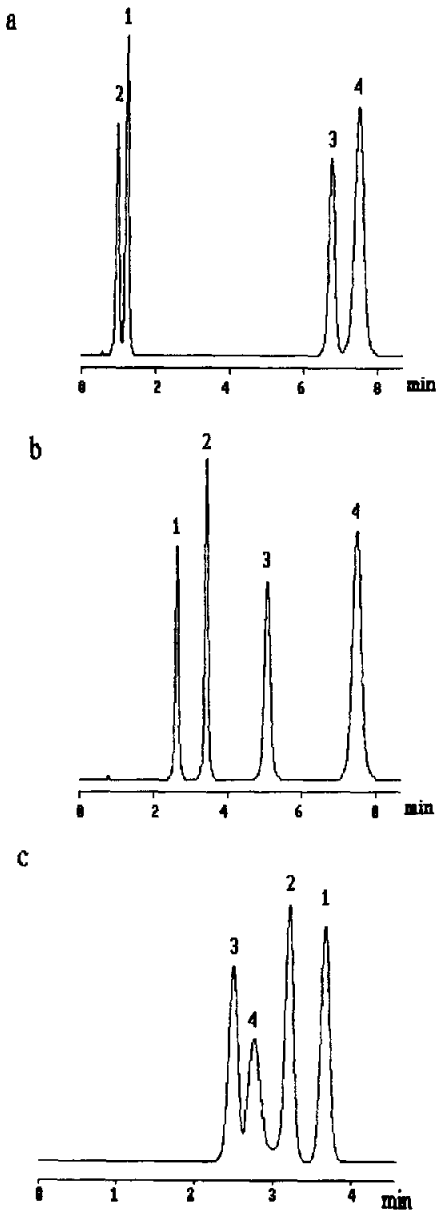

Fig. 6. Chromatography of antibiotics and intermediates. 1 = Aminocephalosporanic acid; 2 = aminopenicillanic acid; 3 = cephalexin; 4 = ampicillin. Mobile phase: (a) 5% acetonitrile in 0.2 *M* acetate buffer (pH 6.0); (b) 0.01 *M* QA16, 10% acetonitrile in 0.2 *M* acetate buffer (pH 6.0); (c) 0.01 *M* QH16, 7% acetonitrile in 0.2 *M* acetate buffer (pH 6.0). Columns: (a) Silasorb C_{18}; (b) and (c) Silasorb 600.

modification chromatography is that the column efficiency is usually higher and the peak shape is better than on bonded non-polar stationary phases. This is probably due to the less rigid binding of alkyl chains to the silica matrix and the greater flexibility of stationary phase molecules compared with the bonded stationary phase. An example is the chromatography of pyrazine-2,5-dicarboxylic acid. This highly hydrophilic solute is not retained from the aqueous buffers traditionally used as eluents in reversed-phase chromatography. Addition of the ion-pairing agent sodium dodecylsulphate results in a slight increase in retention, but the peak of the solute is still unacceptably wide (Fig. 5a). Enhanced retention of this solute was achieved by addition of quaternary ammonium modifiers to the mobile phase. Nevertheless, the peak shape remained unsatisfactory when octadecylsilica was used as the column packing (Fig. 5b and c). Consequently, chemically bonded alkylsilica cannot be the optimum sorbent for some classes of solutes. The use of a column packed with unmodified silica resulted in an increased retention volume and simultaneously improved markedly the efficiency and peak symmetry for this solute (Fig. 5d). Similar improvements in peak shapes were observed for many basic solutes. These observations support the opinion that poor peak shape in bonded reversed-phase chromatography in some instances can be explained not by the presence of residual silanol groups on the surface of sorbent but by steric inaccessibility of such groups [17]. This effect may be especially important in the HPLC of multifunctional bases.

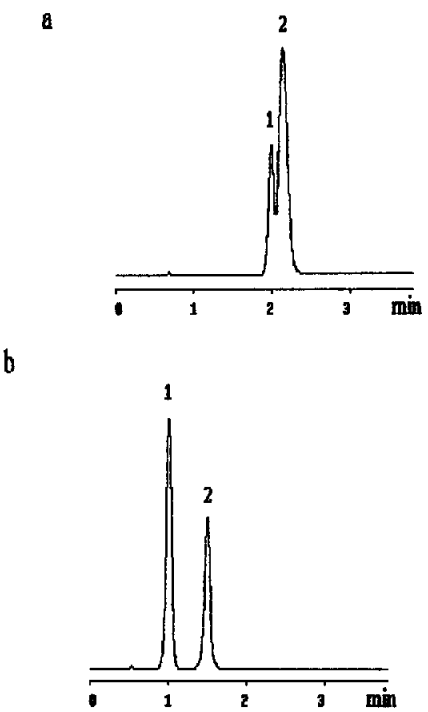

Fig. 7. Separation of (1) uracil and (2) 5-fluorouracil: (a) on Silasorb C_{18}, mobile phase 0.2 *M* acetate buffer (pH 6.6); b on Silasorb 600, mobile phase 0.01 *M* QH16 in 0.2 M acetate buffer (pH 6.6).

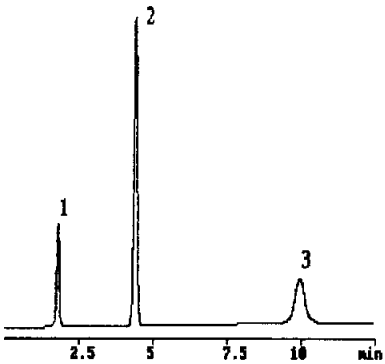

Fig. 8. Separation of anions: 1 = acetate; 2 = nitrite; 3 = nitrate. Mobile phase: 0.037 *M* BH16, 3% acetonitrile, 97% 0.04 *M* phosphate buffer (pH 6.9). Column: Silasorb 600.

HPLC in systems dynamically modified with hydrazinium derivatives offers additional possibilities of selectivity control in the separation of some mixtures of practical interest. Two examples are shown in Figs. 6 and 7.

Another area of possible application of hydrazinium derivatives as dynamic modifiers is anion analysis. A possible mechanism of sorption in this simple procedure involves the formation of a dynamically coated layer of the stationary phase and its interaction with the ion pair consisting of the solute anion and modifier cation. A chromatogram for the separation of some UV-absorbing anions is shown in Fig. 8. It is obvious that the column efficiency and peak symmetry are better than those typically observed in ion chromatography.

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