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Chromatographic stability of silica-based aminopropyl-bonded stationary phases

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ABSTRACT

Causes of frequently observed changes in the properties of columns packed with silica modified with primary amino groups were investigated chromatographically, and it was confirmed that the initial instability of new columns appears to be due to the gradual desorption of the respective silane. Further changes in the chromatographic properties during the operation ensue from deactivation of the surface with impurities capable of reacting with the primary amino group. The main source of these impurities is the mobile phase. Simple prevention can be achieved with a protective column packed with the same sorbent and placed between the pump and the injection valve, which allows the column to be used for several months without changes in its chromatographic properties.

INTRODUCTION

Silica modified with chemically bonded primary amino groups is one of the most widely used column packings for the high-performance liquid chromatography (HPLC) of sugar mixtures [1]. In addition to this main field of application, it is also of advantage in some other instances [2]. A frequent, though by no means unanimous, objection to its application is the insufficient chemical stability and the short lifetime of the columns. There is a broad range of views between such extremes, from uselessness of the packing owing to its decomposition, on the one hand [3], and a considerable durability in the case of proper treatment, on the other [4].

Gradual dissolution of the primary amino phase matrix in aqueous mobile phases explains its settling in the column and the decrease in efficiency [5]. Moreover, this sorbent occupies a special position among the various bonded phases, because it is a chemically reactive material [1,2]. The primary amino group may react with many impurities from the mobile phase or the sample and may become deactivated, which in turn may cause changes in the chromatographic properties, *i.e.*, capacity factors and selectivity. In contrast to deactivation by sorption, where in most instances conditions can be found for the desorption of impurities, the amino phase may be regenerated only exceptionally, if the reacting impurity is known [6].

Knowledge regarding the chromatographic stability of columns packed with primary amino phases is incomplete [7]. Changes in the content of the stationary phase and in the dissolution of the matrix can be followed by employing suitable analytical procedures [8,9] or spectral analysis [10]. It has been shown [11] that dissolution of the silica matrix takes place also with bonded phases, especially at lower pH. The bonded groups protect the matrix; the extent of this protection depends on the size and length of the organic groups and on the bonding chemistry. Hence the gradual dissolution of the silica gel matrix in water-containing mobile phases should be accepted as a fact [12], but the essential features for practical application are a sufficiently long lifetime and the chromatographic stability of the columns.

For this reason, this study considered with the chromatographic stability of the sorbent from the practical point of view, *i.e.*, with the effect of changes in the content of the stationary phase due to washing in water on chromatographic properties in a non-aqueous medium and in the separation of sugar mixtures. Using identical chromatographic conditions, consequences of the chemical deactivation of the sorbent's surface by impurities reacting with the primary amino group were established.

EXPERIMENTAL

Materials

Two types of silica were used (mean particle diameter d_p = 10 μm) with chemically bonded primary amino groups: Separon SIX NH$_2$ (Laboratory Instruments, Prague, Czechoslovakia; present manufacturer, Tessek, Prague, Czechoslovakia) and LiChrosorb NH$_2$ (E. Merck, Darmstadt, Germany), specific surface area 480 and 300 m^2/g, respectively. Xylose, arabinose, fructose, glucose, saccharose, maltose, lactose, nitrobenzene, *o*-, *m*- and *p*-chloronitrobenzene and acetonitrile (all analytical-reagent grade) were obtained from Fluka (Buchs, Switzerland) and toluene and heptane (technical and analytical-reagent grade) from Lachema (Brno, Czechoslovakia).

Apparatus, columns and procedures

The liquid chromatograph consisted of a VCM 300 reciprocal membrane pump (CSAS Development Works, Prague, Czechoslovakia), a stop-flow injection valve made at the Institute of Macromolecular Chemistry, an R 401 differential refractometer (Waters Assoc., Milford, MA, U.S.A.) and a Servogor 2S recorder (Goerz Electro, Vienna, Austria). Stainless-steel columns made at the Institute of Macromolecular Chemistry (100 × 6 mm I.D.) and glass columns (150 × 3.3 mm I.D.) (CGC system; Laboratory Instruments, present manufacturer, Tessek) were packed by the slurry technique at 40 MPa with methanol–dioxane as the slurry liquid. The content of amino groups was determined by acid–base titration with perchloric acid in anhydrous acetic acid [13]. The sorbents were washed by stirring or boiling a 2% suspension for 2 h in water.

RESULTS AND DISCUSSION

To investigate the hydrolytic stability of a amino-bonded stationary phase, one must be sure that the surface is covered with really chemically bonded groups. In practice, it is usual to regard as chemically bonded those compounds or groups which

cannot be extracted [14] (usually by a series of solvents at the boiling point). With modification of silica gel with γ-aminopropyltriethoxysilane, which is a basic compound (a 5% aqueous solution has pH = 10.7), the reagent was found to be strongly sorbed on the surface [10,15]. It is possible that during the preparation of the amino-bonded phase in dry organic solvents (in order to prevent the formation of a polymeric layer) the resulting sorbent contains [15] unreacted sorbed silane, which is gradually washed out only if the chromatographic run is taking place in an aqueous solution.

To elucidate these relationships further, changes in the content of the bonded phase and primary amino groups during washing of the amino phases in cold water and after boiling in water, when deeper changes such as oxidation of the amino group can be expected, were investigated by elemental analysis. The results are summarized in Table I. It can be seen that washing in cold water is followed by a decrease in the content of the organic phase and amino groups in both sorbents, which suggests that mainly adsorbed silane is being washed out [10,15]. No further decrease in the C, H, N and NH_2 content was found, within experimental error, after the second wash in cold water.

TABLE I

CHANGES IN THE CONTENT OF THE STATIONARY PHASE AFTER WASHING FOR 2 h IN WATER: COMPARISON BETWEEN SEPARON SIX NH_2 AND LICHROSORB NH_2

Sample	Conditions	Elemental analysis			NH_2 content	
		C (%)	H (%)	N (%)	mmol/g	μmol/m^2
LiChrosorb NH_2	Original	3.5	0.94	1.04	0.85	2.83
1	25°C	2.92	0.81	0.89	0.67	2.23
2	100°C	2.47	0.72	0.79	0.558	1.86
Separon SIX NH_2	Original	5.45	1.40	2.06	1.29	2.69
1	25°C	4.98	1.32	1.78	1.16	2.42
2	100°C	3.55	1.07	1.18	0.56	1.17
3	100°C	3.50	1.01	1.09	0.53	1.11

After boiling in water, the decrease in the content of the organic phase and amino groups in both sorbents continues. The results indicate that both sorbents behave very similarly during washing; the only difference is a lower content of the organic phase in LiChrosorb NH_2, which may be due to the different specific surface area of the starting silicas, as follows from the content of amino groups related to the surface area in Table I. The surface coverage is also very similar with both packings, and may be compared with its maximum value [15] of 4.7 μmol/m^2. From the values of the C/N ratio of the lost material after extraction at 25°C (LiChrosorb NH_2 3.9, Separon SIX NH_2 1.8) and at 100°C (LiChrosorb NH_2 4.1, Separon SIX NH_2 2.2), is possible to conclude that hydrolysed silane is washed out (the C/N ratios of the unhydrolysed and hydrolysed silane are 7.7 and 2.6, respectively). The differences between the two packings at both temperatures probably lie within the experimental error of the Perkin-Elmer 2400 C,H,N analyzer used ($\pm$ 0.3%) owing to the low content of carbon and especially of nitrogen. Therefore, no further conclusions can be

drawn. Different layers of ^{14}C-labelled γ-aminopropylsilane were found in sorption studies on Pyrex glass [16]. Three layers were described; one of them could be removed by a cold water rinse, the second was extractable by hot water and the remainder was non-removable. Although the experimental conditions and aim of these experiments were different, there is a strong similarity with our experiments.

In order to compare variously washed variants, Separon SIX NH_2 was used and chromatographic tests were carried out with toluene–nitrobenzene and o-, m- and p-chloronitrobenzene mixtures in anhydrous heptane (Table II). Compared with Separon SIX NH_2, the original LiChrosorb NH_2 has lower values of the capacity factors k' and separation factor ($\alpha = k'_2/k'_1$) for the selected pairs of compounds undergoing separation. The k' values of nitrobenzene increased after washing at 25°C with Separon SIX NH_2 while the efficiency decreased (the asymmetry of the peak increased); this again suggests that the mainly sorbed silane has been washed out and that further silanol groups capable of interaction with molecules of nitrobenzene have been made accessible [15]. Nitrobenzene was used as a silanol marker; a higher retention indicates increased silanol activity [17]. The k' values of the individual chloronitrobenzenes also increased after washing in cold water, whereas the selectivity between the m- and p-isomers decreased. On boiling in water there was a distinct change in the chromatographic properties: at a considerably decreased content of the amino groups the k' values of all three chloronitrobenzenes decreased to about half the original value, and the k' value of nitrobenzene also decreased. Moreover, the p- and m-isomers remained virtually unseparated. It is likely, therefore, that boiling in water does indeed bring about deeper changes on the sorbent's surface (oxidation of amino groups, or even splitting of the ligand's bonds) than a mere washing out of the mainly unbound silane. The origin of retention changes after the hot water extraction is unclear; some transformation and/or loss of primary amine functionality resulting in

TABLE II

CHROMATOGRAPHIC COMPARISON BETWEEN LICHROSORB NH_2 AND SEPARON SIX NH_2 WITH VARIOUSLY WASHED VARIANTS OF SEPARON SIX NH_2 IN ANHYDROUS HEPTANE

Sorbent	k'					α (m-/p-isomer)
	Nitrobenzene	Toluene	Chloronitrobenzenes			
			p-	m-	o-	
LiChrosorb NH_2	1.76	0.16	1.36	1.83	3.97	1.35
Separon SIX NH_2, original	2.83	0.23	1.79	2.63	6.05	1.47
Separon washed I[a]	4.82	0.32	3.09	4.04	9.68	1.31
Separon washed II[b]	3.03	0.22	2.25	2.33	4.65	1.03
Separon washed III[c]	4.78	0.35	2.99	3.85	9.80.	1.29

[a] Water, 25°C.
[b] Boiled in water.
[c] Washed wit 2.5 l of acetonitrile–water (80:20) with a protective column packed with Separon SIX NH_2.

increased hydrophobicity of the surface may be suggested as one possible explanation. Good resolution of m- and p-chloronitrobenzene seems to be typical of amino phases; the more hydrophobic cyanoethylated silica with a similar content of organic phase exhibits a lower retention in comparison with a corresponding amino-phase and separates m- and p-isomers much more poorly [18].

The last line in Table II is the most interesting; after washing of the column with 2.5 l of acetonitrile–water followed by transfer into anhydrous heptane, both the k' and the α values of the original Separon SIX NH$_2$ virtually coincide with those obtained after washing of the sorbent with cold water. Consequently, the changes in retention observed with new columns [7,8] with the amino phase can also be explained [15] mainly by washing out of the sorbed silane.

In Table III it can be seen that washing of these sorbents affects their properties in the most common application, $i.e.$, separation of sugar mixtures, to a much smaller extent. The difference between the original LiChrosorb NH$_2$ and Separon SIX NH$_2$ consists only in lower k' values; the same applies after boiling in water. Hence, it can be concluded that, unlike the separation of isomeric chloronitrobenzenes, a considerably lower content of amino groups in the analysis of sugars is also sufficient for maintaining a satisfactory selectivity.

TABLE III

CHROMATOGRAPHIC COMPARISON BETWEEN LICHROSORB NH$_2$ AND SEPARON SIX NH$_2$, ON THE ONE HAND, AND VARIOUSLY WASHED VARIANTS OF SEPARON SIX NH$_2$, ON THE OTHER, IN THE ANALYSIS OF A MIXTURE OF SUGARS IN THE MOBILE PHASE ACETONITRILE–WATER (80:20, v/v)

Sorbent	k'							α		
	1 xylose	2 arabinose	3 fructose	4 glucose	5 saccharose	6 maltose	7 lactose	2/1	4/3	7/6
LiChrosorb NH$_2$	1.12	1.30	1.64	2.25	4.20	5.77	6.35	1.16	1.37	1.10
Separon SIX NH$_2$	1.55	1.89	2.29	3.11	5.66	7.77	8.97	1.22	1.36	1.15
Separon washed I[a]	1.22	1.47	1.78	2.38	4.31	5.81	6.70	1.20	1.34	1.20
Separon washed II[b]	0.92	1.14	1.71	1.84	3.28	4.38	5.28	1.24	1.31	1.20

[a] Water, 25°C.
[b] Boiled in water.

In the usual application of columns packed with Separon SIX NH$_2$ washed in water, changes in the k' values were observed also after prolonged use. After the columns had been emptied there was usually a higher content of the organic phase and a lower content of primary amino groups. To ensure that the cause is really impurities fixed at the beginning of the sorbent column, we used two glass CGC columns packed with fresh Separon SIX NH$_2$ which before and after being connected in series had been tested with nitrobenzene in analytical-reagent grade heptane containing 0.05% of isopropanol (Table IV). Subsequently, analytical-reagent grade heptane was replaced with technical grade heptane and the test mixture was injected repeatedly. It appeared that the k' value of nitrobenzene increased continuously depending on the amount of the mobile phase that had passed through the joined

TABLE IV

EFFECT OF IMPURITIES FROM MOBILE PHASES ON THE CHROMATOGRAPHIC BEHA-
VIOUR OF THE CGC SYSTEM IN ANALYTICAL-REAGENT GRADE HEPTANE CONTAIN-
ING 0.05% OF ISOPROPANOL

Column	k'(nitrobenzene)		
	1^a	2^b	3^c
I	1.14	1.93	1.17
II	1.10	1.14	1.10
I + II	1.31	1.85	1.36

[a] Freshly packed columns.
[b] After using 75 ml of technical grade heptane as the mobile phase (see text).
[c] After 3 weeks of chromatographic analysis of sugars; column re-equilibrated in heptane after methanol–dioxane (1:1) wash.

columns until it reached 1.85 at a total amount of the mobile phase of 75 ml. At the same time, the colour of the sorbent on the inlet side of the first column changed, while in Table IV it can be seen that the properties of the second column remained the same. All attempts at washing out (i.e., desorption in the sense of ref. 14) failed. Another pair of columns connected in a series and packed with an identical charge of Separon SIX NH$_2$ was used for about 3 weeks for the chromatography of sugar mixtures in the mobile phase [19] acetonitrile–phosphate buffer (pH 5.9) (80:20, v/v) to prevent glycosylamine formation, with a protective column also containing Separon SIX NH$_2$ placed between the pump and injection valve. Table IV shows that the subsequent test of both the connected and separate columns by using nitrobenzene in heptane containing 0.05% of isopropanol gave k' values which were virtually identical with those obtained for freshly packed columns.

The results suggest that deactivation is indeed due to the chemically bonded impurities from the mobile phase. To these, air oxygen should also be added if the mobile phase has not been helium degassed. The amino group can be protected from oxidation [20] and some other reactions [21] by conversion into a salt, but it is necessary to check whether this change would affect the separation because, e.g., after the conversion of the amino phase into the sulphate form separation of anomers of the respective sugars occurs [22]. Also, with the purest (HPLC-grade) solvents, where transparency to UV radiation is the priority requirement, one cannot expect all impurities able to deactivate the amino group to have been removed.

CONCLUSIONS

Causes underlying the frequently observed changes in the chromatographic behaviour of amino-bonded phases are of twofold in nature: in new columns they are mainly due to desorption of the sorbed silane, whereas on further use the amino groups are deactivated by chemical reactions with impurities, especially those from the mobile phase. In isocratic separations of sugars a protective column with the amino-bonded phase between the pump and the injection valve provides simple pre-

vention against the effect of impurities in the solvents. If reactive impurities may also occur in the samples, a precolumn can be suitably added and to prevent reaction with aldoses the mobile phase should be buffered [19]. At the same time, such a protective column represents a saturation column [9], which also prevents dissolution of the matrix, and the column can be applied for several months without any changes in its chromatographic properties.

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