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Chromatographic effects of residual amino groups on liquid chromatographic stationary phases derived from aminopropyl-silica gel

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ABSTRACT

Low-capacity phenylazo-8-quinolinol-silica gel (QSG) stationary phases were made by two different procedures. The first began with an aminopropyl-silica gel (APSG), which was converted to nitrobenzamide-silica gel (NBSG) by amidization with *p*-nitrobenzoyl chloride. The second began with the direct preparation of the NBSG using nitrobenzamidesilane. Retention data for several organic analytes were obtained on APSG and on the NBSG and QSG phases made by both procedures. Anomalously high capacity factors were obtained for *p*-nitrobenzoic acid on the NBSG material obtained from APSG, indicating the presence of unreacted aminopropyl anion-exchange sites. Only after reamidization of the NBSG using more vigorous conditions did the retention data approach that on the NBSG made by the second method. Even then, the opposite order of elution of *p*-nitrobenzoic and benzoic acids on the two NBSG and QSG phases indicated that vestiges of amino groups remained on the materials made from the APSG. Several chromatographic and chemical characterization methods confirmed the presence of residual aminopropyl groups.

INTRODUCTION

Chemical modification of surfaces by means of silane coupling agents is employed in many areas of commerce such as the textile, coatings and material composites industries [1,2]. In science and technology, silane-modified solids such as electrodes [2–4], heterogeneous catalysts [2–5] and gas and liquid-chromatographic stationary phases [6,7] are being increasingly employed.

Aminopropylsilane is a commonly used surface-modification reagent. Many organic and biochemical species are directly or indirectly "coupled" to the surface in question via a covalent bond with the amino group at the end of the propyl spacer arm [8], and therefore understanding and control of alkylamine surface chemistry [9] is very important. In fact, one of the early uses of this silane coupling agent was for the immobilization of enzymes on silica gel [10]. Aminopropylsilane has also been used for the production of silica gel-bonded "amino" columns for carbohydrate separations and for the parent modified silica gel from which a variety of stationary phases have been produced [8], including chiral phases [11].

In most of these situations little attention, if any, has been given to whether the amio group is fully reacted with the species with which it is to be coupled. A few studies have found, however, that some of the original amino groups did remain unreacted, for example, in the production of picramidopropyl-silica gel [12,13] or silica gel-immobilized cyclodextrins [14], a stationary phase on which anomalously large retention of organic acids was noticed.

Aminopropyl-silica gel (APSG) has been used in our laboratory for some time [15–17] as the parent material from which propylamido-substituted phenylazo-8-quinolinol-silica gel (QSG) is made [18]. The amino group is reacted with *p*-nitrobenzoyl chloride to yield nitrobenzamide-silica gel (NBSG). The nitro group is reduced to aminobenzamide-silica gel (ABSG) from which the diazonium

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salt-silica gel (DSSG) is made. This material is then coupled with 8-quinolinol to give the QSG phase. Several years ago, we developed a shortened route to QSG which employs triethoxysilylpropyl-*p*-nitrobenzamide to yield NBSG in one step on reaction with silica gel. Subsequent reactions are the same as for the longer method. We had assumed that the final QSG was the same whether APSG or NBSG was the parent material [19] until two columns made by each method were used to separate a series of organic analytes [20]. In that work, organic acids were found to have substantially longer retention times on the columns which were made from aminopropyl-silica gel than on those made from the nitrobenzamide material. We speculated that residual basic amino groups were responsible for the anomalously large retention of the organic acids [20]. In a more recent study, QSG phases made from both parent species were employed for the separation of mixed-class analytes, including inorganic anions. Very high retention values for the anions were obtained on the APSG-derived QSG [21]. It appeared likely that residual protonated amino groups were acting as anion exchangers for both inorganic anions and ionized organic acids [21].

The purpose of this study was to confirm these suspicions and to show that stationary phases produced from aminopropyl parent materials may well exhibit chromatographic results traceable to analyte interaction with residual amino or protonated amino groups, even at low coverages. In this study packing materials produced from both aminopropyl- and nitrobenzamide-silica gel were chromatographically compared, and the presence of residual amino groups was confirmed for phases produced from APSG.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Beckman (Fullerton, CA, USA) Model 100-A pump and Model 153 single-wavelength (254 nm) detector. Nitrate analyte was monitored on an ISCO (Lincoln, NE, USA) Model V^4 detector at 210 nm. Columns, packing procedures and general chromatographic conditions have been described previously [16,17,20].

Spectroscopic studies employed a Varian (Sunnyvale, CA, USA) DMS-100 spectrometer for UV–VIS and a Perkin-Elmer (Norwalk, CT, USA) Model 272 for flame atomic absorption spectrometric work.

Silylations employed a Coulter Ultrasonic (Hialeah, FL, USA) Model CE-12 80-W ultrasonic cleaning bath.

Reagents and solutions

The stationary phase support material was Adsorbosil-LC, a preparative-grade, 10-μm diameter, 60-Å pore, 480 m^2 g^{-1} irregular silica from Applied Science (Alltech) (Deerfield, IL, USA). Fines were removed by stirring about 13 g of the material into 250 ml of water and drawing off the top 100 ml of slurry with a 100-ml pipet after 15 min of settling. The procedure was repeated twice, with 100 ml of fresh water added after each removal of fines.

Water was purified with a Barnstead (Dubuque, IA, USA) NANOpure four-cartridge system using feed water doubly deionized by the departmental system. High-performance liquid chromatographic (HPLC)-grade methanol was used for all chromatography and ACS-grade methanol for synthetic and column-packing work. Acetate buffers were made from HPLC-grade sodium acetate and Instra-Analyzed acetic acid, both from J. T. Baker (Phillipsburg, NJ, USA). Analytes were of analytical-reagent grade and about 10^{-3}–10^{-4} *M* solutions were made up in methanol or mobile phase. Analytes and mobile phases were filtered through 0.45-μm membrane filters.

Triethylamine and *p*-nitrobenzoyl chloride were of Puriss grade (>99.5%) from Fluka (Ronkonkoma, NY, USA). 3-Aminopropyltriethoxysilane (Hülls/Petrarch, Bristol, PA, USA) was distilled before use at 3 Torr and 69.5°C. Trimethylsilylimidazole (TMSI) (Hülls/Petrarch) was used as received. All other reagents were of ACS grade and used as received from various suppliers.

Stationary phase synthesis

The 8-quinolinol-modified silica gels (QSG) were made by two different routes. The first (method I) begins with an aminopropyl-modified material (APSG), which is converted into nitrobenzamide-silica gel (NBSG) by amidization of the propylamine with *p*-nitrobenzoyl chloride [15,18,22]. The NBSG is subsequently converted into an amino-

benzamide-silica gel (ABSG) by sodium dithionite reduction of the nitro group. A diazonium salt-silica gel (DSSG) is next prepared from the ABSG in nitrous acid and the DSSG is coupled to 8-quinolinol in ethanol solution. NBSG and QSG phases derived from APSG parent material (synthetic method I) are designated by the suffix I.

Method II employs triethoxysilylpropyl-*p*-nitrobenzamide silylation of silica gel to eliminate the lengthy amidization step and yield NBSG directly [19]. Subsequent reactions are the same as for method I. Stationary phases prepared from NBSG made by method II are designated by the suffix II.

The APSG synthesis was performed in hexane, and used ultrasound rather than the usual refluxing approach [23]. About 13 g of sized Adsorbosil was wet with 60 ml of dried hexane and then 0.28 g of distilled aminopropylsilane was added with a syringe. The flask was placed in an ordinary laboratory ultrasonic cleaning bath and sonicated for 2 h at about 48°C. The resulting APSG was washed with hexane and methanol and cured in a vacuum oven at 85°C for 3 h. The 0.28 g of silane used would yield a 100-μmol g^{-1} APSG if fully reacted.

A solution of 80 ml of dried chloroform containing 0.44 g of *p*-nitrobenzoyl chloride and 0.4 g of triethylamine was added to about 6.8 g of APSG. The mixture was sonicated for 14 h. The resulting NBSGI was washed with chloroform, methanol, 1 *M* hydrochloric acid and methanol. The residual silanol sites on the NBSGI were end-capped using hydroxyl group-selective TMSI. About 80 ml of a 5% (v/v) hexane solution of TMSI were mixed with the NBSGI and sonicated for 3 h. The capped NBSGI was washed with hexane, *n*-propanol and methanol.

The capped NBSGI was reacted a second time with *p*-nitrobenzoyl chloride to convert aminopropyl groups more completely to the amide. About 60 ml of chloroform containing 0.9 g of the benzoyl chloride and 1 ml of triethylamine were added to about 6 g of the capped NBSGI and sonicated for 5 h, then an additional 1 g of the acid chloride and 1 ml of triethylamine were added and sonication was continued for another 10 h. This re-amidized NBSGI was washed with chloroform, methanol, 0.5 *M* hydrochloric acid, water and methanol. Subsequent reactions to yield QSGI were performed as described previously [15,18,22].

NBSGII was prepared from 5 g of sized Absorbosil to which 60 ml of a hexane solution of 0.23 g of recrystallized nitrobenzamide-silane were added [21]. The mixture was sonicated for 4 h. The NBSGII was washed with hexane, acetone and methanol and cured in a vacuum oven at 90°C for 8 h. The theoretical coverage of the silica by nitrobenzamide groups based on the above amounts is 130 μmol g^{-1}. The NBSGII was capped as described above. Subsequent reactions to produce QSGII were performed as described previously [15,18,22].

Capacity determination

Determination of nitrogen by elemental analysis of APSG, NBSG and QSG was performed by Desert Analytics (Tucson, AZ, USA).

Batch extraction of copper(II) by APSG and QSG was performed as described previously [15–17,24], with copper determination by atomic absorption spectrometry.

The coverage of silica by aminopropyl groups was also determined by reaction with salicylaldehyde to produce the silica-bound Schiff's base [8,9] with UV determination of unreacted reagent at 326 nm. A 1–2-fold measured excess of $1.0 \cdot 10^{-2}$ *M* salicylaldehyde in 100% ethanol was added to 0.1 g of APSG and the mixture was shaken for 30 min. The yellow Schiff's-base product was filtered off and the filtrate and ethanol washings were diluted to the range $1 \cdot 10^{-4}$–$4 \cdot 10^{-4}$. The UV absorbance of this and of the original solution was measured and the amount of bound reagent determined by difference. The calibration line had a correlation coefficient of 0.9999 and a slope of $3.98 \cdot 10^{-3}$ l mol^{-1} cm^{-1} (zero intercept).

The capacity of QSG phases was also obtained by determining the amount of 8-quinolinol that remained after the coupling reaction with DSSG. A measured (1–2-fold excess) of a $2.0 \cdot 10^{-2}$ *M* 8-quinolinol solution in 95% ethanol was used for the coupling reaction. The QSG was filtered off and washed with ethanol. The combined filtrate and washings were diluted to the range $2.0 \cdot 10^{-4}$–$5 \cdot 10^{-4}$ *M* for UV determination at 309 nm. The difference between the original and final 8-quinolinol amounts gave a value for the QSG quinolinol coverage. The calibration line in the concentration range above had a correlation coefficient of 0.99999 and a slope of $2.66 \cdot 10^{-3}$ l mol^{-1} cm^{-1} (zero intercept).

RESULTS AND DISCUSSION

The chromatographic behavior of QSG phases prepared from APSG differed from that prepared from NBSGII parent material in two separate studies in this laboratory. Organic acids with pK_a values of 3–5 were found by deBot *et al.* [20] to exhibit capacity factors 3–10 times larger on APSG-derived QSGs than on NBSGII-derived phases of comparable 8-quinolinol coverage. Thompson and Jezorek [21] observed k' values for chloride, bromide, perchlorate and nitrate to be ten times larger on QSGI than on QSGII. These QSG phases had the same copper(II) uptake capacity, again indicating comparable 8-quinolinol coverage.

The pH of the aqueous–organic mobile phases in these two studies ranged from 3 to 5, as measured in the mobile phase itself. Primary akylamines in homogeneous solution exhibit pK_b values around 3–4, while the bound aminopropl groups of ASPG appear to be weaker bases, with pK_b values around 6–7 [24]. Nevertheless, any residual aminopropyl groups which did exist on the method I-derived phases used in these two studies were probably protonated under the mobile phase conditions employed.

In the present study, two QSG phases of similar 8-quinolinol coverage were prepared, one by method I and the other by method II, and chromatographic comparisons of these phases and of APSG, NBSGI and NBSGII were made. The APSG coverage was observed to be 100 μmol g^{-1} by elemental analysis and about 80 μmol g^{-1} based on copper (II) extraction (assuming a 2:1 copper to amino ligand stoichiometry) [24–26]. NBSGI (made from the above APSG) had an original coverage by benzamide groups of 50 μmol g^{-1}, and about 70 μmol g^{-1} after the second amidization reaction, based on elemental nitrogen analysis. NBSGII (made directly with nitrobenzamide-silane) had a benzamide coverage of about 60 μmol g^{-1} by elemental analysis. QSGI (APSG parent material) exhibited a quinolinol coverage of 89 μmol g^{-1} by elemental analysis, 99 μmol g^{-1} by UV determination of unreacted 8-quinolinol and 116 μmol g^{-1} by copper(II) extraction (assuming a 2:1 copper to 8-quinolinol stoichiometry [27]). QSGII (NBSGII parent material) exhibited a coverage of 51 μmol g^{-1} by elemental analysis, 37 μmol by 8-quinolinol UV determination and 58 μmol g^{-1} by copper(II) extraction. Although it is very likely that high-capacity APSG may not react completely with *p*-nitrobenzoyl chloride to give NBSG because of steric constraints, it is reasonable to consider that complete reaction of a low-capacity APSG would routinely occur. Therefore, we deliberately prepared a low-capacity APSG phase (ca. 100 μmol g^{-1}) to see if, even then, residual amino groups remained after the usual synthesis procedure. We used organic chromatographic analytes that were neutral, basic and acidic, and methanol–aqueous acetate mobile phases from pH 4 to 6 to observe the pH dependence of analyte retention.

Various analytes were run on the 100 μmol g^{-1} APSG ("amino") column. Capacity factors for aniline, benzoic acid and *p*-nitrobenzoic acid as a function of pH are shown in Fig. 1. The difference in behavior of the two acids is probably due to their differing acidities. The aqueous pK_a values of benzoic and *p*-nitrobenzoic acid are 4.2 and 3.6, respectively. Therefore, in the pH 4.2 mobile phase, most

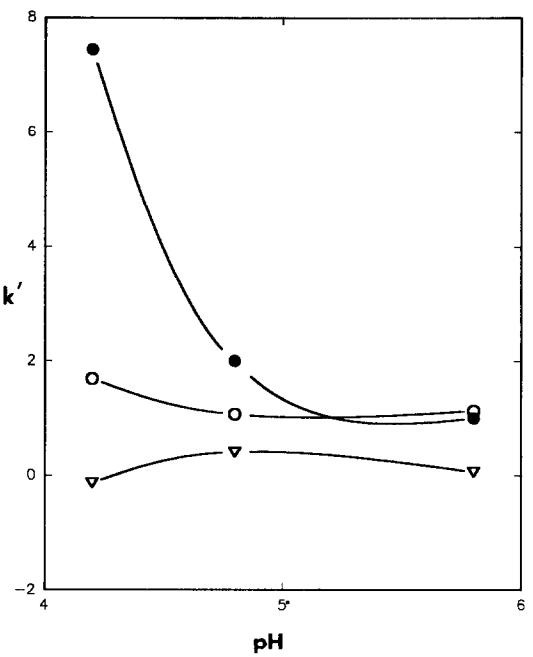

Fig. 1. Capacity factor on the APSG column as a function of pH. Mobile phases: methanol–water–0.1 *M* acetate buffer (50:25:25); pH 3.4 acetate buffer yielded apparent mobile phase pH of 4.2, pH 4.0 buffer an apparent pH of 4.8 and pH 5.0 buffer an apparent pH of 5.8. ● = *p*-Nitrobenzoic acid; ○ = benzoic acid; ▽ = aniline.

of the *p*-nitrobenzoic acid is ionized and much or most of the benzoic is neutral. The anionic form of the *p*-nitrobenzoic acid interacts via an anion-exchange mechanism with the protonated amino groups. The decrease in k' for *p*-nitrobenzoic and the absence of a k' increase for benzoic acid with a pH increase, even though it is more ionized at higher pH, is due to a competitive anion-exchange process with acetate ions in the mobile phase. Based on the aqueous pK_a value of acetic acid of 4.8, most of the acetate species are ionized at pH 5.8. At pH 4.2 most are protonated and therefore neutral. The anion-exchange behavior of the *p*-nitrobenzoic acid and the acetate competition for exchange sites at higher pH were further illustrated by obtaining k' values of the two acids and the neutral phenol species, still at pH 5.8, but at a five-fold lower acetate concentration (0.0046 *M*) than was used for the data in Fig. 1 (0.023 *M*). The expected lack of ionic strength dependence for phenol was indeed found, but k' for the two acids approximately doubled, from 3.3 to 7.9 for benzoic and from 3.1 to 7.5 for *p*-nitrobenzoic acid. Neutral species such as phenol or aniline and positively charged species such as the anilinium ion, which may exist at the lower pH values employed here, clearly show little or no pH or ionic strength dependence, but the benzoic acid–*p*-nitrobenzoic acid pair is a sensitive indicator of anion-exchange interactions in the pH region used here.

Similar tests for anion-exchange interactions were used with the NBSG phases. One column was packed with NBSGI. This material was made from the APSG discussed above using the usual threefold excess of *p*-nitrobenzoyl chloride. A second column was filled with NBSGII which, of course, can contain no residual amino groups. Capacity factors on NBSGI for several analytes at three pH values are given in Table I; k' values for benzoic acid, *p*-nitrobenzoic acid and aniline are plotted *versus* pH in Fig. 2A. Similar data on the NBSGII column (*ca.* 60 μmol g^{-1}) also are given in Table I and in Fig. 2B. The analytes exhibit typical reversed-phase behavior on the NBSGII phase, that is, a small k' decrease for the acids due to increased ionic character and mobile phase solubility at the higher pH values. The anomalously high k' for *p*-nitrobenzoic acid on NBSGI indicates the presence of unreacted aminopropyl groups. This was confirmed by the same experiment as was used with the APSG phase, namely repeating the chromatography on both NBSG columns at pH 5.8, but in a mobile phase with a fivefold lower acetate concentration. No change in k' was observed for any analyte on the NBSGII column, but the two test acids exhibited larger k' values in the less ionic mobile phase on the NBSGI column; the benzoic acid k' changed from 0.4 at the higher to 0.7 at the lower acetate concentration, while the *p*-nitrobenzoic k' changed from 0.5 to 1.0. These results again indicate an anion-

TABLE I

RETENTION OF SEVERAL ANALYTES ON NBSGI AND NBSGII IN THREE MOBILE PHASES

All mobile phases: methanol water 0.1 *M* acetate buffer (50:25:25); pH 3.4 acetate buffer yielded an apparent mobile phase of 4.2, pH 4.0 buffer an apparent pH of 4.8 and pH 5.0 buffer an apparent pH of 5.8.

Analyte	Capacity factor, k'					
	NBSGI[a]			NBSGII		
	pH 4.2	pH 4.8	pH 5.8	pH 4.2	pH 4.8	pH 5.8
Aniline	0.20	0.33	0.40	0.73	0.60	0.67
2-Chloroethylbenzene	1.87	1.80	1.87	5.13	5.27	5.20
Naphthalene	2.07	2.07	2.13	5.40	5.53	5.53
Phenol	0.47	0.47	0.47	0.93	0.93	1.00
Benzoic acid	1.40	1.07	0.40	1.33	0.67	0.33
p-Nitrobenzoic acid	4.67	1.87	0.47	0.93	0.53	0.20

[a] Coverage by nitrobenzamide groups is about 50 μmol g^{-1} based on nitrogen determination; derived from APSG in Fig. 1.

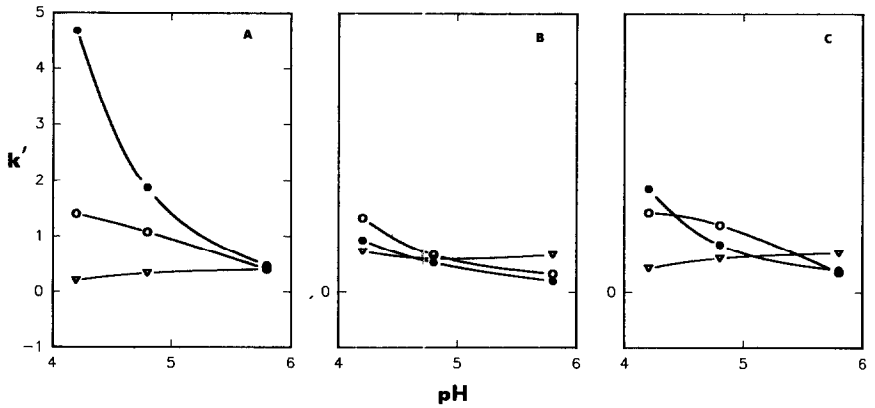

Fig. 2. Capacity factor on the NBSG columns as a function of pH. (A) NBSGI column; (B) NBSGII column; (C) NBSGI column after second amidization reaction. Mobile phases as in Fig. 1. ● = *p*-Nitrobenzoic acid; ○ = benzoic acid; ▽ = aniline.

exchange interaction on NBSGI, the phase made from APSG parent material.

This conclusion was confirmed by some characterization studies on the packing materials themselves, as discussed later, and by two other chromatographic experiments. First, nitrate ion was used as an analyte on both NBSG phases, exhibiting a k' of about +0.5 on NBSGI but about −0.4 on NBSGII (possibly owing to an ion-exclusion mechanism causing elution before the solvent peak). The retention of nitrate also indicates an anion-exchange interaction on the NBSGI. Some of this phase was reacted a second time with *p*-nitrobenzoyl chloride, but with a tenfold instead of the threefold excess of acid chloride that has been used previously [16,18,22]. The results are shown in Fig. 2C. The large decrease in k' for *p*-nitrobenzoic acid clearly indicates that most of the residual amino groups were amidized in this second reaction. However, the fact that benzoic acid still exhibited a k' smaller than that of the stronger *p*-nitrobenzoic acid implies that some residual amino groups remain. The more highly ionized acid is more soluble in the aqueous mobile phase, and should be less retained, as it is on NBSGII (Fig. 2B). A tenfold decrease in the acetate concentration in the mobile phase resulted in no k' change at pH 4.8 for aniline, phenol or even benzoic acid; *p*-nitrobenzoic acid still exhibited an increase in k' of about 0.4, indicating again that some vestiges of anion-exchange behavior remained even after re-amidization of the original NBSGI. Again, no k' change for any analyte on NBSGII was found as a result of the decrease in mobile phase acetate concentration.

The capacity factors of all analytes were less than unity on both QSGI and QSGII, probably as a result of partial loss of trimethylsilyl capping groups and their retentive capacity in the 2 *M* hydrochloric acid used in the diazotization reaction. Little or no pH dependence was observed for the several analytes used, with the exception of the two test acids. Again, both benzoic and *p*-nitrobenzoic acid exhibited a decrease in k' with increase in pH on both columns, as expected, owing to the solubility increase (Fig. 3). However, although the k' values are small, that for *p*-nitrobenzoic is still larger than that of benzoic acid on QSGI, but k' is smaller on QSGII, as reversed-phase behavior would predict. Again, residual aminopropyl groups are probably the cause of the opposite elution order of benzoic and *p*-nitrobenzoic acids on the two QSG phases.

A control experiment was run to determine whether residual amino groups could in fact survive the harsh reaction conditions of the diazonium salt preparation and so remain on a QSG phase. It has been suggested that aliphatic amines can be diazotized, but that the product is not stable and decomposes by losing a molecule of nitrogen [28]. Others claim that, below pH 3, reaction with nitrous acid does not occur [29]. We subjected a 60 μmol g^{-1} APSG sample to the diazonium salt reaction (sodium nitrite in 2 *M* hydrochloric acid) and then added the product to an alcoholic 8-quinolinol solution.

Columns were packed with both the original and

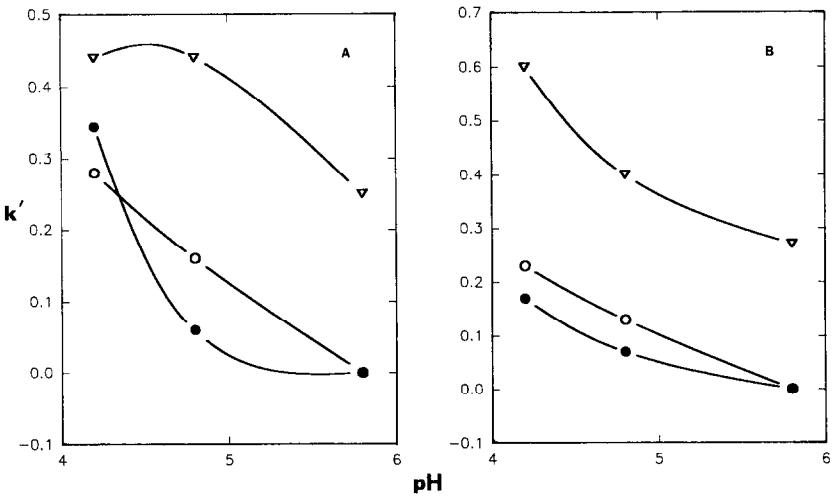

Fig. 3. Capacity factor on the QSG columns as a function of pH. (A) QSGI column; (B) QSGII column. Mobile phases as in Fig. 1. ● = *p*-nitrobenzoic acid; ○ = benzoic acid; ▽ = aniline.

treated APSG and several chemical tests were also run on these materials. Capacity factors for several analytes are given in Table II. These indicate little or no loss of amino groups. Copper(II) and salicylaldehyde [8,9] uptake reactions and elemental analysis also indicated that most of the amino groups remained. Copper uptake was 23 and 21 μmol g^{-1} of APSG, salicylaldehyde uptake was 65 and 43 μmol g^{-1} and nitrogen elemental analysis results were 0.15% and 0.16% on the original and treated APSG, respectively. UV spectrophotometry of the 8-quinolinol solution before and after the coupling reaction indicated that essentially no quinolinol ligand was coupled; indeed, only a slight pinkish tint of the diazotized, 8-quinolinol-treated APSG was observed, whereas the yellow silica-bound Schiff's base was evident on reaction of the treated APSG with salicylaldehyde. It appears that under these reaction conditions the aminopropyl groups do not form diazonium salts but do survive the diazotization reaction and remain on QSG phases.

Because of the low capacity of the stationary phases used here, chemical tests to detect unreacted aminopropyl groups on the NBSG and, especially, QSG, were not conclusive. Elemental analysis for nitrogen gave values of 0.14, 0.21, 0.24 and 0.48 %N for APSG, NBSGI, NBSGI after the second reaction with *p*-nitrobenzoyl chloride and QSGI, respectively. These results imply phase capacities of about 100, 50 and 70 μmol g^{-1} of bonded groups

TABLE II

RETENTION DATA ON ORIGINAL AND TREATED APSG COMPARED WITH QSGI

Mobile phase: methanol–water–0.1 *M* acetate buffer (pH 3.4) (50:25:25) (apparent pH 4.2).

Analyte	Capacity factor, k'		
	Original APSG	Treated APSG	QSGI
Benzoic acid	3.2	2.5	0.28
p-Nitrobenzoic acid	12.5	12.1	0.34
Phenol	0.0	0.0	0.19
Aniline	0.0	0.0	0.44

for APSG, NBSGI and the re-amidized NBSGI, indicating that 50 and 30 μmol g^{-1} of NH_2 groups remained on the two NBSGI materials. Chromatographic capacity factors of *p*-nitrobenzoic acid on the above three materials are about 8, 5 and 1, respectively, however, indicating that most of the amine groups have been amidized after the second reaction. The nitrogen determination has a standard deviation of 0.02–0.05% absolute [30], and is clearly not very reliable for low-capacity phases such as these.

It would appear from this study that following literature directions for the amidization of alkylamines may well leave unreacted amino groups which can affect later chemistry or chromatography. This is alsmost certainly what happened in the two previous studies in our laboratory [20,21]. Precautions need to be taken to mininize this possibility if alkylamino materials are used as parent species to couple some group to a solid surface. Longer reaction times and more concentrated reactant species might be used, and the resulting product should be checked for residual amino groups. Alternatively, if an azo-coupled material is desired, the amide (NBSG) can be obtained with silylpropyl-*p*-nitrobenzamide [19], eliminating the possibility of residual amino groups altogether.

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