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LIQUID CHROMATOGRAPHIC SEPARATION OF SEVERAL CLASSES OF ACIDIC AND BASIC AROMATIC COMPOUNDS ON SILICA-BONDED 8-QUINOLINOL STATIONARY PHASES

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SUMMARY

Silica-bound 8-quinolinol (QSG) phases were used for the liquid chromatographic separation of various substituted aromatic compounds including acids, anilines, sulfa drugs, nitrogen heterocyclics and positional isomers. Some iron(III)- and copper(II)-loaded QSG materials were used in addition to non-loaded phases. Aqueous acetate–organic eluents were employed. Two different synthetic routes were used to prepare the QSG phases, resulting in different chromatographic behavior. Those phases obtained via an initial aminopropylsilane surface modification favor acidic compounds, while QSG prepared by an initial nitrobenzamide silylation exhibits greater retention of basic analytes. Phases of relatively low 8-quinolinol content exhibited better resolution and, in some cases, greater retention than higher-capacity materials.

INTRODUCTION

For high-performance liquid chromatography (HPLC), today mostly nonpolar alkyl-chain stationary phases and polar water–organic mobile phases are employed, the so-called reversed-phase mode. Other stationary phases are commercially available, however, such as the nitrile, phenyl and alkylamine columns which permit additional interactions between the analyte and stationary phase such as dipole, π–π and hydrogen bonding. These columns can be employed in either the reversed-phase or normal-phase (non-polar eluent) mode.

Several selective stationary phases[1,2], as well as a chelating phase[3], have been recently employed for the separation of organic analytes. In our laboratory we have been assessing the general utility of selective phases, especially silica-bound chelating-agent materials, for both metal-ion[4,5] and organic analyte[6] separations. Bonded chelating-agent materials differ from most commercially available phases and others commonly studied in that they are usually multi-site, multi-mode materials. The chelating phases discussed in this report employ azo-coupled 8-quinolinol attached to the silica surface by an organic backbone.

The selectivity of these phases can be enhanced by loading the chelating moiety

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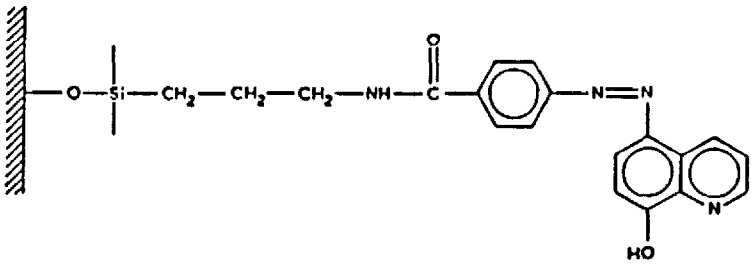


8-Quinolinol silica gel (QSG)

with an appropriate metal, and operating the column in the ligand-exchange mode. We have used iron(III)-loaded QSG as a ligand-exchange phase for the separation of phenols[6], and other workers have successfully employed the secondary chemical equilibria of bound metal ions in the separation of various organic "ligands"[7–9].

The present manuscript reports on the use of QSG phases prepared by two different synthetic methods, at both relatively high and low coverages, with and without loaded metal, for separation of several classes of organic species, including organic acids, phenols, aromatic amines, nitrogen heterocycles and sulfa drugs. The intent of this study was to demonstrate the versatility of these phases. Only minimal effort was made to optimize mobile phase composition and other chromatographic parameters. Indeed, one of the silica supports was a preparative-grade material from which, at best, only modest efficiency was to be expected. The drawback of multi-site phases, namely the efficiency loss which may occur, is recognized, but the intent is that, for samples not needing great resolving power, the added versatility will be beneficial in separating widely different analyte types.

EXPERIMENTAL

Apparatus

Most of the chromatographic studies were performed on a Spectra-Physics SP-8000 gradient liquid chromatograph with either a Beckman Model 153 or a Kratos Model 770 detector operated at 254 nm. Some runs employed a Waters Model 501 pump with a Gilson HM Holochrome detector, or an IBM LC-9533 system. A Haskel pneumatic pump (Model 26980-4) was used to slurry-pack the columns.

Spectroscopic studies employed a Hitachi 100-80 for UV–VIS or a Perkin-Elmer Model 272 for atomic absorption studies.

Reagents and solutions

The stationary phase support materials used were Adsorbosil-LC, a preparative-grade, 10-μm diameter, 70-Å pore, 480-m^2/g silica from Applied Science (now Alltech); and Polygosil 60-10, a 10-μm diameter, 60-Å pore, 500-m^2/g silica made by Machery-Nagel and supplied by Rainin. The reagents used in the syntheses, and suppliers, have been previously documented[4–6,10].

Water used for this work was deionized, passed through an activated-carbon column, a mixed-bed ion-exchange column (Barnstead) and then distilled from an

all-glass apparatus and stored in a plastic bottle for no more than a few days (to minimize degradation). Mobile phases were prepared using reagent-grade methanol. Acetate buffers were made with ultrapure acetic acid (Baker "Instra-Analyzed") and HPLC-grade sodium acetate (Baker). All mobile phases were filtered through 0.45-μm membrane filters. Samples were obtained from several suppliers and used as received. Sample concentrations were about $1 \cdot 10^{-4}$ M, in 100% methanol or methanol–water (50:50), and were also filtered before use.

Stationary phase synthesis

Two different approaches to the production of 8-quinolinol silica gels were employed in this study. Method I involves the silylation of silica with 3-aminopropyltriethoxylane, subsequent amidization with *p*-nitrobenzoyl chloride, reduction of the nitro group with sodium dithionite, conversion to the diazonium salt in nitrous acid, and finally coupling to 8-quinolinol. Details of this procedure have been previously published[11].

Method I was used for the Adsorbosil supports, which were prepared originally as part of an earlier study[6]. Two preparations of method-I QSG were accomplished, each starting from a different batch of parent aminopropyl silica gel. The first used an excess of dissolved reactants over silica-bound groups for all reactions, resulting in a QSG stationary phase with about 180 μmol/g of copper(II) uptake capacity[6], and, by implication, 180 μmol/g of 8-quinolinol groups[12]. This batch of material had about 650 μmol/g of aminopropyl groups on the surface at the initial silylation, as determined by copper(II) extraction.

The second batch of method-I QSG was intended to be of lower capacity. While the initial silylation produced about 500 μmol/g of bound aminopropyl groups, the final 8-quinolinol content of this phase was limited by using a substoichiometric amount of sodium dithionite in the reduction of the *p*-nitrobenzamide silica gel (NBSG) to the aminobenzamide material (ABSG). Subsequent reactions employed excess reagents. The copper(II) uptake of this QSG was about 40 μmol/g. These materials will be referred to as QSGI-180 and QSGI-40, signifying their preparative approach and metal-uptake capacity.

Method II employs triethoxysilylpropyl-*p*-nitrobenzamide silylation, and eliminates the lengthy amidization step of method I[10]. After the initial silylation to produce the propyl-substituted NBSG, all subsequent reactions are identical to those of method I. The Polygosil was modified using this second procedure. The NBSG prepared by method II was shown by elemental nitrogen determination to have about 225 μmol/g of nitrobenzamide groups bound to the surface. Two batches of QSG were prepared from this method-II NBSG, again a low- and relatively high-capacity material. The QSGII-110 was prepared with reagent excess at each step. The QSGII-56 was prepared by adding a limited amount (a few drops) of a dilute 8-quinolinol solution in 95% ethanol to the diazonium-salt silica gel slurried in 50 ml of ethanol.

The materials produced by both methods were deactivated by "end-capping" residual silanol groups with trimethylchlorosilane (5% solution in dry toluene, 5 h reflux) at the NBSG stage. The method-II NBSG phase was very hydrophobic after the capping reaction, and had to be "wet" with methanol before it could be slurried into aqueous dithionite solution for the reduction to ABSG[10].

Capacity determination

The numbers of aminopropyl and 8-quinolinol groups were determined by batch copper(II) extraction[11], assuming 2-to-1 ligand-to-metal stoichiometry for the former and 1-to-1 for the latter[12]. Atomic absorption spectroscopy (AAS) was used to quantitate the copper(II). Elemental analyses were performed by Desert Analytics (Tucson, AZ, U.S.A.).

Column packing

Column blanks were 25 cm long and either 4.6 mm I.D., stainless steel or 4.0 mm I.D., glass-lined stainless steel (Scientific Glass Engineering). All columns were packed at about 6000 p.s.i., by an upward slurry-packing technique[13], with packing pressure maintained for about 30 min. Methanol was used as both the slurry and packing solvent.

Column metal loading

Packed QSG columns were first washed briefly with pH 1 nitric acid, rinsed with water, and equilibrated with pH 2.5 nitric acid. After filling the pump and tubing with the pH 2.5, 0.01 *M* metal-ion solution, the column was attached and the metal-ion solution was pumped through the column at 1 ml/min. Iron(III) breakthrough was determined visually by testing the effluent with potassium thiocyanate. Effluent from copper-loading runs was mixed (postcolumn) with $3 \cdot 10^{-5}$ *M* 4-(2-pyridylazo)resorcinol (PAR)[4,5] and passed through a detector set at 510 nm. These breakthrough values gave a check on the batch copper(II) capacity determination. A further check was obtained by stripping the metal ion off the column with pH 1 nitric acid after use, and determining the amount of metal ion by AAS.

General chromatographic conditions

Mobile phases were helium-degassed for about 20 min. Columns were equilibrated for 30–45 min, longer if a pH change was involved. Equilibration was assumed to have been attained when effluent pH matched that of the mobile phase and when a steady baseline was observed. Flow-rates were from 1 to 2 ml/min. Sample volumes were either 55, 20 or 10 μl, depending on which chromatographic system was in use. All injections were done at least twice, and all runs were replicated on different days to be sure retention data were consistent. Anomalous data were rerun several times over a period of days or weeks as a further check on reproducibility. Chromatographic runs were made at ambient temperature, and detectors were set at 254 nm.

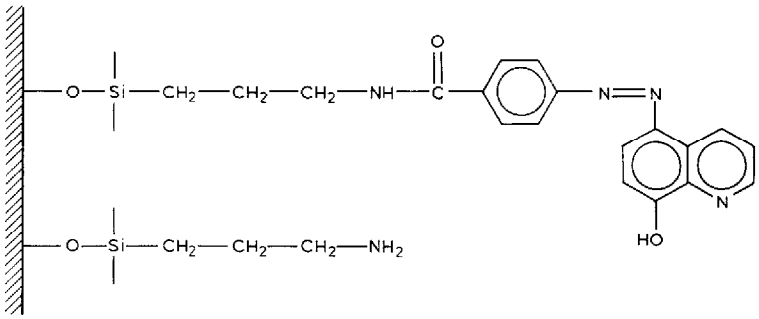
RESULTS AND DISCUSSION

An earlier study from this laboratory which employed a QSG stationary phase for the separation of phenols[6] has been extended to several additional classes of organic analytes. Two different silica support materials and two different surface-modification chemistries were employed for stationary phases. The preparation and characteristics of these materials are treated below.

Stationary phase considerations

Two stationary phases prepared by method I and two by method II were used in

this study. The method-I materials had comparable initial silylation coverages, 500 and 650 μmol/g of aminopropyl groups[12] for QSGI-40 and QSGI-180, respectively. All subsequent reactions of the higher-capacity method-I material were performed in an excess of reagents, but only about 180 μmol/g of coupled 8-quinolinol groups resulted, presumably due to steric constraints at the amidization reaction with the bulky *p*-nitrobenzoyl chloride and/or the coupling reaction with the even larger 8-quinolinol[10,11,14]. These observations suggest that unreacted aminopropyl groups may remain using the method-I approach, as shown below. Some of these may be present in an oxidized form unreactive to metal ions[10–12] and *p*-nitrobenzoyl chloride, but may provide a site which interacts with acidic analytes. The relatively low method-II NBSG coverage (see below), implies steric constraints also for the nitrobenzamide silylation reaction.



Both routes to QSG employed "end capping" with trimethylchlorosilane at the NBSG stage of the synthesis. Phases produced by method-II silylation probably contain a greater number of trimethylsilyl groups than those produced by method I, as the initial silylation yielded only about 225 μmol/g surface coverage as compared to 500–700 μmol/g for the aminopropyl silylation, leaving a greater number of unreacted silanols[10]. In any case, other studies have shown that only about half of the original silanols can be reacted, even with the smallest silane (*e.g.* ref. 15, p. 278), and coverage decreases as the size of the silane increases.

These QSG phases contain sites which make possible several types of retention mechanisms. Dispersion interactions may occur at the propyl chain and aromatic portions, as well as at the trimethylsilyl groups. The aromatic groups also permit π–π interactions, while the amide, azo and quinolinol portions of the bound species allow for polar and hydrogen-bonding interactions[16]. If the heterocyclic nitrogen atom is protonated, anion exchange interactions can also occur. Additionally, interactions at any unreacted aminopropyl, nitrophenyl and silanol, as well as diazonium salt decomposition sites are possible. The presence of all these types of interaction sites provides great versatility, but also may degrade column efficiency[15], as is seen in the chromatographic data which follow.

TABLE I

EFFECT OF pH, IRON LOADING AND IONIC STRENGTH ON RETENTION OF ORGANIC ACIDS

Compound	pK_a	*Capacity factor, k'*						
		pH 3.1[a]			*pH 4.8*[b]			
		QSGI-180Fe	*QSGI-180*	*QSGII-110*	*QSGI-180Fe*	*QSGI-180*	*QSGII-110*	*QSGI-180Fe*[c]
p-Hydroxybenzoic acid	4.48	1.13	0.8	0.32	1.80	1.4	0.27	1.36
o-Hydroxybenzoic acid	2.97	1.20	–	–	1.73	–	–	1.36
Hydrocinnamic acid	4.69	1.6	1.0	0.35	3.01	2.5	0.36	1.73
Vanillic acid	4.62	1.73	–	–	2.27	–	–	1.73
Benzoic acid	4.19	1.93	–	–	2.48	–	–	1.82
m-Coumaric acid	4.4	2.27	–	–	4.0	–	–	3.1
o-Coumaric acid	4.61	2.27	–	–	5.1	–	–	3.3
Gentisic acid	2.97	2.73	–	–	19.0	–	–	–
p-Coumaric acid	4.72	2.9	1.6	0.53	3.9	–	0.45	2.73
Cinnamic acid	4.45	3.5	2.4	0.55	5.2	5.2	0.45	4.0
p-Methoxycinnamic acid	4.54	5.75	3.0	0.94	8.1	7.8	0.64	5.36
p-Methylcinnamic acid	4.56	6.0	–	–	7.64	–	–	5.0
2,4,5-Trimethoxycinnamic acid	–	7.0	3.8	1.8	10.4	9.0	1.3	7.0
3-Furoic acid	4.5	1.3	1.4	0.33	1.7	2.8	0.04	1.0
2-Furoic acid	3.6	6.0	9.0	0.41	2.7	6.4	0.09	1.1

[a] Mobile phase: water–methanol–1 *M* acetic acid (25:50:25) (apparent pH 3.1).
[b] Mobile phase: water–methanol–0.1 *M* pH 4 acetate buffer (25:50:25) (apparent pH 4.8).
[c] Same mobile phase as [b] except 0.075 *M* in $NaClO_4$.

TABLE II

EFFECT OF pH, IRON AND COPPER LOADING ON RETENTION OF SUBSTITUTED ANILINES

Compound	pK_b	*Capacity factor, k'*						
		Water-methanol (55:45)[a]				*Water–methanol–0.1 M pH 5 acetate buffer (45:45:10) (apparent pH 5.5)*		
		QSGII-110	*QSGI-180*	*QSGI-180Fe*	*QSGI-180Cu*	*QSGI-180*	*QSGI-180Fe*	*QSGI-180Cu*
p-Anisidine	8.70	6.81	0.48	1.32	0.19	0.7	1.18	1.56
p-Toluidine	8.92	4.0	0.29	1.65	0.17	0.85	1.5	1.5
m-Toluidine	9.30	1.05	0.36	1.65	0.27	–	1.5	1.5
Aniline	9.38	1.31	0.24	1.18	0.25	0.57	0.87	1.0
m-Anisidine	9.52	0.87	0.56	1.28	0.56	–	1.18	1.2
o-Anisidine	9.53	1.5	0.56	1.73	0.41	0.7	1.5	1.57
o-Toluidine	9.58	2.05	0.36	1.5	0.19	–	1.3	1.1
p-Chloroaniline	10.0	0.72	1.11	2.68	1.06	1.2	2.3	2.0
m-Chloroaniline	10.5	0.56	1.11	2.78	1.28	1.2	2.4	2.0
m-Nitroaniline	11.5	0.36	1.27	3.57	1.86	1.6	3.25	2.4
p-Nitroaniline	13.0	0.41	1.67	4.9	2.28	2.1	4.5	3.1
o-Nitroaniline	14.2	0.56	1.72	5.4	2.36	2.4	4.7	3.3

[a] Apparent pH 4.2.

Organic acids

The QSGI-180 columns used for the phenol study[6] were also employed in the present investigation for aromatic carboxylic acids, as were the method-II stationary phases. Results similar to those of the phenol study were obtained, in that the iron(III)-loaded QSGI-180 column exhibited greater retention than the non-loaded column for all the benzene-based acids at both pH 3.1 and 4.8 (Table I). A curious exception to this is that the furoic acids exhibited decreased retention on the iron-loaded column relative to the nonloaded.

Several acids were also run on the non-loaded QSGII-110 column at pH 3.1 and 4.8. Retention was much smaller on this method-II Polygosil phase than on the method-I Adsorbosil column. An increase in pH from 3.1 to 4.8 produced slightly *lower* k' values on the QSGII-110, compared to at least *doubled* retention on the QSGI-180 column. This behavior difference can be ascribed to the different surface-modification chemistries. The Adsorbosil-based method-I phase appears to display a relatively "basic" surface, possibly due to the unreacted aminopropyl groups suggested above. The Polygosil-based method-II material displays more typical "reversed-phase" character with reduced retention of acid analytes at higher pH values. As the pK_a values of most of the acids used as analytes were about the same (in the 4–5 range) no correlation of k' with acid strength emerged from this data. A typical separation of several organic acids on QSGII-110 at pH 2.9 with an acetic acid-methanol mobile phase is shown in Fig. 1.

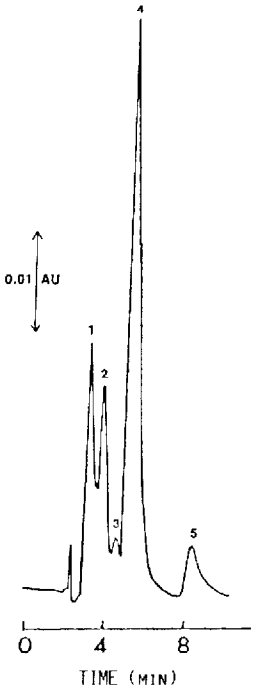


Fig. 1. Separation of cinnamic acids on a QSGII-110 column. Mobile phase: water–methanol–1 *M* acetic acid (25:40:35) (apparent pH 2.9); sample size 20 μl; flow-rate 1.5 ml/min. Peaks: 1 = hydrocinnamic acid; 2 = *p*-coumaric acid; 3 = cinnamic acid; 4 = *p*-methoxycinnamic acid; 5 = 2,4,5-trimethoxycinnamic acid.

Nitrogen-containing analytes

Several substituted anilines were run on both metal-loaded and non-loaded method-I columns, with retention increasing with decreasing base strength of the analyte (Table II). The presence of iron increased retention from two- to five-fold over the non-loaded column, the copper only slightly, if at all. Retention was lower on the iron-loaded column in the acetate mobile phase than in water–methanol. The copper-loaded column exhibited the opposite behavior. This difference in behavior may be due to the greater competitive interaction of the acetate ion with iron(III) than with copper(II)[6].

The anilines were also run on the QSGII-110 column in water–methanol (55:45), yielding striking differences compared to the QSGI-180 column (Table II). First, elution order is reversed on the Polygosil-based method-II column; and second, the range of k' values is much greater than for the Adsorbosil-based method-I phase. These results also imply that a different mechanism operates on the QSGI phases than on the QSGII.

Although peak shapes for the anilines on the copper-loaded and non-loaded columns were acceptable, the iron produced noticeable tailing. For example, asymmetry values in acetate mobile phase for *m*-chloroaniline were about 1.4 on both the copper-loaded and the non-loaded QSGI-180 column but 2.3 on the iron-loaded phase. Aniline itself had a value of 2.9 on the iron phase. Similar results were obtained for naphthalene, a neutral probe analyte. Retention was greater on the iron-loaded columns than on the non-loaded, but efficiency was badly degraded by the iron. For example, on the iron-loaded QSGI-180 column in 100% methanol ($k' = 0.3$) the asymmetry factor was 1.7, while in methanol–water (80:20) ($k' = 0.8$) the asymmetry was 4.0. These results imply competing mechanisms with different mass-transfer rate

TABLE III

EFFECT OF COPPER LOADING, pH AND BUFFER CONCENTRATION ON CAPACITY FACTORS OF SULFA DRUGS ON QSGI AND QSGI-Cu COLUMNS

NR = Not retained.

Compound	pK_a	*Capacity factor, k'*					
		QSGI-180		*QSGI-180Cu-80*		*QSGI-180Cu-100*	
		pH 6.35[a]	*Unbuffered*[b]	*pH 6.35*[a]	*pH 6.35*[c]	*pH 6.35*[a]	*pH 5.0*[d]
Sulfathiazole	7.2	NR	1.4	1.93	3.86	7.57	3.57
Sulfamerazine	6.9	NR	1.14	1.28	2.14	4.7	2.43
Sulfadiazine	6.4	NR	1.0	1.28	2.28	4.7	2.14
Sulfapyridine	8.4	NR	1.14	0.86	1.21	2.14	1.28
Sulfisoxazole	4.9	NR	2.43	0.71	1.43	1.28	0.43
Sulfabenzamide	4.6	1.14	6.14	0.71	0.57	1.28	1.57
Sulfacetamide	5.4	NR	1.28	–	–	0.43	0.43
Sulfanilamide	10.4	NR	1.28	–	–	0.14	0.14

[a] Methanol–0.1 *M* pH 5 acetate buffer (70:30) (apparent pH 6.35).
[b] Methanol–water (45:55).
[c] Methanol–0.1 *M* pH 5 acetate buffer–water (70:10:20) (apparent pH 6.35).
[d] Methanol–0.1 *M* pH 4 acetate buffer (70:30) (apparent pH 5.0).

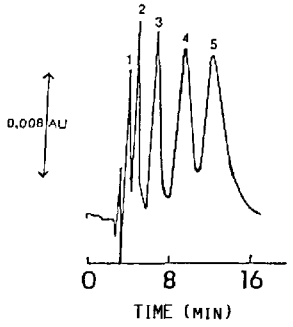


Fig. 2. Separation of sulfa drugs on QSGI-180Cu, about 80 μmol/g copper(II) content. Mobile phase: water–acetonitrile–0.1 *M* pH 5 acetate buffer (50:40:10) (apparent pH 5.5); sample size 10 μl; flow-rate 1 ml/min. Peaks: 1 = sulfanilamide; 2 = sulfacetamide; 3 = sulfapyridine; 4 = sulfamerazine; 5 = sulfathiazole.

constants, as found by Kunzru and Frei[17] for amine separation on cadmium-impregnated silica gel.

The QSG phases also were employed for the separation of a number of sulfa drugs. Separation of these analytes is somewhat difficult because of their wide range of pK_a values, hydrophobicity, structures and functional groups[18–21]. Both QSGI-180 and QSGII-110, with and without copper(II), were used as stationary phases for these

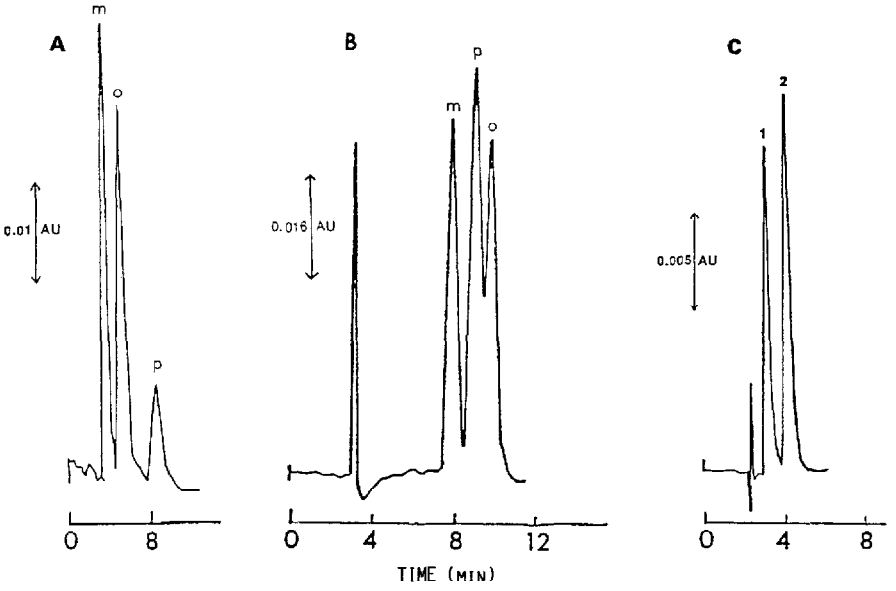


Fig. 3. Separation of positional isomers on QSG phases. (A) Toluidine isomers on a QSGII-110 column in water–methanol (50:50) mobile phase; 20 μl sample size; 1.8 ml/min flow-rate. (B) Nitroaniline isomers on a QSGI-40 column in water–methanol (55:45) mobile phase; 10 μl sample size; 1 ml/min flow-rate. (C) 3-furoic (1) and 2-furoic acid (2) on a QSGII-110 column in water–methanol–1 *M* acetic acid (25:50:25) (apparent pH 3.1) mobile phase; 10 μl sample size; 1.5 ml/min flow-rate.

analytes. Most of the sulfa drugs were unretained in acetate mobile phases on both columns in the absence of copper, while on the copper-loaded columns these solutes exhibited significant retention, with larger k' values at higher pH, lower buffer concentration, and greater copper loading (Table III). It appears that the greatest contribution to sulfa drug retention is interaction with chelated copper(II), as qualitatively similar behavior was observed on the copper-loaded method-I and method-II columns, rather than the opposite behavior found for the acids and anilines. A typical separation of several sulfa drugs is shown in Fig. 2.

Both the QSGI and QSGII phases were employed for the separation of positional isomers of anisidine, toluidine, nitroaniline, and furoic acid. The toluidine and anisidine isomers elute in different order on the two phases (Table II), again highlighting the apparently different character of the materials prepared by these two methods. Further, even though the elution order of the furoic acids is the same on the two phases, the very large k' value for the 2-furoic isomer on the QSGI phase is striking, and cannot be accounted for by differences in the amount of 8-quinolinol alone (Table I). Chromatograms of some isomers are shown in Fig. 3.

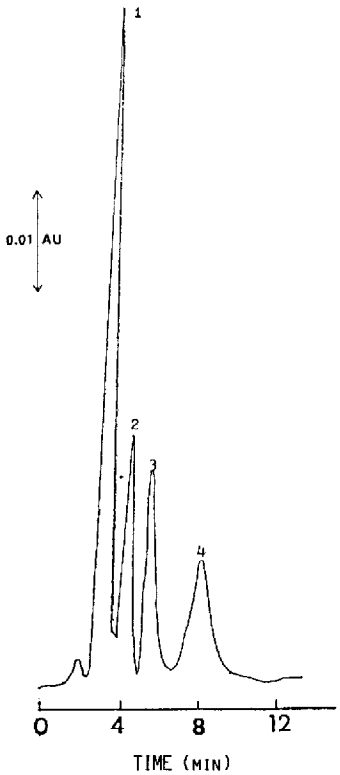


Fig. 4. Separation of some nitrogen-heterocyclic compounds on a QSGI-180Fe column. Mobile phase: water–methanol–0.1 *M* acetic acid (50:40:10) (apparent pH 4.0). Peaks: 1 = cytosine; 2 = uracil; 3 = thymine; 4 = purine.

A number of nitrogen-heterocyclic bases were run on the QSG and iron-loaded QSG phases, including various purine and pyrimidine bases and structurally related compounds. Separation on iron-loaded QSGI-180 of a mixture of uracil, thymine, cytosine and purine is illustrated in Fig. 4. Like the aniline-bases these analytes displayed opposite pH effects on the two classes of stationary phases.

Stationary phase capacity effects

Several classes of analytes were compared on the low- and high-capacity columns prepared by methods I and II. Significantly enhanced resolution was obtained on the QSGI-40 compared to the QSGI-180 column for phenols as well as anilines (Fig. 5). More surprising, however, is the fact that *larger k'* values were obtained on the *lower*-capacity material (Table IV). The effect is most pronounced for the carboxylic acids, less so for the phenols and aromatic amines.

Although the two method-II columns available for high- and low-capacity comparison did not have as large an 8-quinolinol difference as the method-I columns, some efficiency improvement was still found for the QSGII-56 column compared to the QSGII-110. However, retention was dramatically *larger* on the *lower*-capacity than the higher-capacity column for the aromatic amines (Table IV), some of the stronger bases being irreversibly retained in water–methanol (55:45).

Several factors may be at work here. With fewer 8-quinolinol groups present there is less steric crowding on the low-capacity phases allowing the bonded moieties to

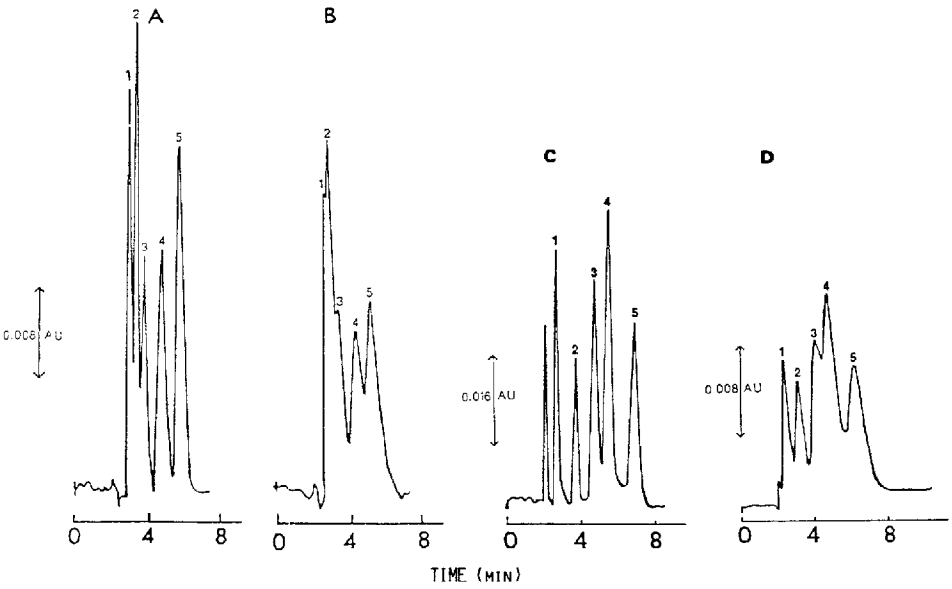


Fig. 5. Effect of stationary phase capacity on separation of organic analytes. Five phenols on QSGI-40 (A) and QSGI-180 (B) in a water–methanol–1 *M* acetic acid (25:50:25) mobile phase (apparent pH 3.1). Peaks: 1 = phenol; 2 = *p*-cresol; 3 = *o*-chlorophenol; 4 = *p*-nitrophenol; 5 = 2,4-dichlorophenol. Five anilines on QSGI-40 (C) and QSGI-180 (D) in a water–methanol–0.1 *M* pH 4.0 acetate (45:45:10) mobile phase (apparent pH 4.6). Peaks: 1 = *p*-anisidine; 2 = *o*-anisidine; 3 = *p*-chloroaniline; 4 = *m*-nitroaniline; 5 = *o*-nitroaniline.

TABLE IV

EFFECT OF STATIONARY PHASE CAPACITY ON RETENTION OF ORGANIC ANALYTES

NR = Not retained.

Compound	*Capacity factor, k'*			
	QSGI-40	*QSGI-180*	*QSGII-56*	*QSGII-110*
Acids[a]				
Hydrocinnamic acid	2.6	1.0	0.2	0.35
Cinnamic acid	6.6	2.4	0.4	0.55
p-Methoxycinnamic acid	8.1	3.0	0.6	0.94
p-Hydroxybenzoic acid	1.8	0.8	–	–
2-Furoic acid	35.0	9.0	NR	0.41
3-Furoic acid	4.2	1.4	NR	0.33
Phenols[a]				
Phenol	0.5	0.4	–	–
p-Cresol	0.7	0.5	–	–
o-Chlorophenol	1.0	0.8	–	–
p-Nitrophenol	1.8	1.0	–	–
2,4-Dichlorophenol	2.1	1.7	–	–
Amines[b]				
p-Toluidine	0.37	0.4	>12.0	4.0
o-Anisidine	0.87	0.4	14.2	1.5
m-Toluidine	0.5	0.5	>15.0	1.05
m-Anisidine	0.5	0.3	>24.0	0.87
p-Chloroaniline	0.9	0.8	4.8	0.72
m-Chloroaniline	1.0	0.8	1.8	0.56
p-Nitroaniline	1.5	1.3	0.8	0.41

[a] Mobile phase: water–methanol–1 *M* acetic acid (25:50:25) (apparent pH 3.1).

[b] Mobile phases: QSGI columns: water–methanol–0.1 *M* pH 4 acetate buffer (40:50:10) (apparent pH 4.8); QSGII columns: water–methanol (55:45).

be more "mobile" and accessible, and more rapid phase transfer to occur. The efficiency-degrading effect of too much stationary phase liquid is well known in gas chromatography, and has also been demonstrated for a bonded phase in liquid chromatography[22].

Once more, opposite behavior was observed for the method-I and method-II phases, with acids exhibiting the greatest k' enhancement on the low-capacity QSGI phase, bases on the low-capacity QSGII column. These facts again imply that there are basic sites underlying the backbone on the QSGI bonded material and that they are more accessible with fewer 8-quinolinol groups present. Steric effects do seem to be a factor on the higher-capacity materials, however, because the aniline bases are also more retained on the lower-capacity QSGI phase.

In general, then, greater affinity for acidic analytes was found on the method-I materials, and greater retention of basic species on the method-II phases, the reasons for which remain unclear. We plan to systematically prepare and study the materials which result from these two synthetic routes to better understand the nature of the modified surface and the mechanisms of interaction for the various classes of analytes.

Nevertheless, from the present and earlier results, it would seem that the multi-site QSG materials described in this study offer attractive versatility, as they can be used to separate metal ions[4]; "as is" for a variety of classes of organic analytes; and loaded with metal ions in the more selective ligand-exchange mode[6]. These phases do appear to be less efficient than single-mechanism materials[16]. Indeed, the efficiency of the separations in this study (5000–8000 plates per meter) is lower than typically found on non-polar alkyl phases. It may be that the complexity of the phases which yields the versatility precludes high efficiency, and that this trade-off "goes with the territory". However, as mentioned above, this was a preliminary study, even using preparative-grade silica for some columns. Efficiency optimization was not the goal of the work. We plan further study, especially of the more promising low-capacity phases, to see if plate counts can be increased to more acceptable levels. Nevertheless, these materials do appear to have some utility as multipurpose liquid chromatographic phases.

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