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Publisher *Taylor & Francis*

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Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597273>

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Online publication date: 31 May 2001

To cite this Article Watson, R. C. , Shaw, P. N. , Ritchie, H. J. , Ross, P. and Barrett, D. A.(2001) 'EFFECT OF TRACE METAL CONCENTRATION ON CHROMATOGRAPHIC PERFORMANCE OF ODS BONDED SILICAS WITH MODIFIED METAL CONTENT', *Journal of Liquid Chromatography & Related Technologies*, 24: 9, 1253 – 1273

To link to this Article: DOI: 10.1081/JLC-100103446

URL: <http://dx.doi.org/10.1081/JLC-100103446>

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EFFECT OF TRACE METAL CONCENTRATION ON CHROMATOGRAPHIC PERFORMANCE OF ODS BONDED SILICAS WITH MODIFIED METAL CONTENT

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ABSTRACT

Three batches of 5 μm spherical silica particles with differing added amounts of iron were manufactured and each of these was bonded with n-dimethyloctadecylsilyl (ODS) followed by end-capping with trimethylsilyl (TMS) groups. The concentration of twelve trace metals in the untreated silicas and in the bonded silicas was measured by ICP-AES. Iron was confirmed to be present at different concentrations in each of the silicas: 300 ppm ('high Fe'), 191 ppm ('medium' Fe), and 132 ppm ('low Fe') in the base silicas, and the same pattern was also observed in the bonded silicas. The addition of iron during manufacture displaced other metals from the silicas, notably titanium aluminium. Following

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refluxing of the silicas in dilute hydrochloric acid, a substantial decrease in trace metal content was observed for most metals apart from aluminium and titanium, which showed very little reduction (<5%).

A range of neutral, acidic, basic, and metal chelating test solutes was used to examine the chromatographic performance of each bonded phase, and these results were then correlated with the trace metal contents of the bulk stationary phases. Neutral solutes were unaffected by trace metal content. Metal chelating solutes also showed no correlation with the iron content of the silicas, probably due to the total metal content between the phases being similar. However, the chromatographic retention and peak shape of weak bases and weak acids did correlate with iron, aluminum and titanium concentrations in the ODS bonded silicas. This study confirmed that individual rather than total metal concentration can affect the chromatographic behaviour of an ODS silica phase.

INTRODUCTION

It is well-established that traces of various metals can become incorporated into the matrix of chromatographic silica depending on the process used to manufacture the silica and the nature of the silica substrate.^{1,2} Bonded phases derived from silicas with a high trace metal content often suffer from a poor chromatographic performance towards weak organic bases and metal chelating solutes.² This poor performance, characterised chromatographically by the strong adsorption of basic analytes resulting in 'tailed' peaks, has been attributed, either directly or indirectly, to the presence of trace metals in the chromatographic matrix.^{3,4} Much of the work undertaken on the role of metals in liquid chromatography has focused on the total metal content of the bulk bonded silica phase, with a consensus emerging that a 'low metal' or pure silica bonded phase is likely to have a better performance for the analysis of weak bases.^{2,5-7}

The nature of the metallic species responsible for the degradation of chromatographic performance observed in high trace metal content silicas is less well studied. It has been shown by *ab initio* calculations and from experimental measurements, that certain metal oxides (eg aluminium, magnesium) present in the silica matrix can enhance the acidity of adjacent silanols.^{8,9} It is believed that these highly acidic isolated surface silanols are responsible for the well-known ion-exchange interactions with positively-charged solutes, which result in the poor performance of bonded silicas towards ionised bases.^{3,4} Several studies have shown that surface bound iron can degrade chromatographic performance of a

variety of solutes (especially peptides and proteins) resulting in irreversible adsorption and drastic changes in peak shape.¹⁰⁻¹²

In this paper, an attempt is made to investigate the potential role of a single metal (iron) on chromatographic performance of a bonded silica. Different concentrations of iron are incorporated into three batches of a single base silica to produce three octadecylsilane (ODS) bonded phases, differing only in the amount of added iron. A comparison is made of the concentration of iron and eleven other trace metals before and after acid rehydroxylation of the silicas, and chromatographic performance is correlated with the concentration of individual metals in each of the manufactured phases.

EXPERIMENTAL

Synthesis of the ODS Silica Materials

Three batches of microparticulate base silica were prepared by the gelation of the same colloidal silica under controlled conditions. The batches had three different concentrations of Fe, equivalent to 340 ppm, 180 ppm, and 93 ppm added. After emulsification, washing, and sintering, the base silicas were classified to ensure the particle size distribution was within the normal specification (4.5-5 μm average). Samples were taken at this stage for determination of metal content by ICP-AES. The three iron-treated silica batches were then modified by boiling in water, followed by treatment with 0.1% HCl, to produce the rehydroxylated base silicas used in the production of *n*-alkyl-bonded silicas. 5 μm ODS-bonded silicas were prepared from the batches of hydrated base silicas using *n*-dimethyloctadecylsilyl chloride.

The ODS chlorosilane (40% v/w in toluene) was reacted with 10.0 g of the base silica with 10% w/w of base, and refluxed for 6 h. The reaction product was dried in a vacuum oven for 12 h, allowed to cool, washed in hexane (100 mL), acetone (100 mL), acetone-water 50/50 (100 mL), and acetone (100 mL) respectively. The final products were oven dried at 80°C prior to use. A portion of each of the batches was endcapped using trimethylchlorosilane (TMS) under similar conditions to the initial *n*-alkyl bonding procedure. These final products (ODS and ODS/TMS) were analysed for metal content by ICP-AES.

The pore size and pore volume of the silica particles was determined using mercury porosimetry (Micrometrics 9300 Analyzer), and the surface area was measured by BET single point analysis (Micrometrics Flowsorb 2300). The carbon loading (% C) for the silicas was obtained from an EC-12 Carbon Determinator (Leco Corporation, St. Joseph, MI, USA). Table 1 shows the measured pore size, pore volume, surface area, and % C for each of the batches of silica. The bonded silicas were slurry-packed into 10 cm x 4.6 mm i.d. stainless

Table 1. Surface Area, Pore Size, Pore Volume, and Bulk % Carbon of All Batches of Silica

Batch of Silica	Surface Area (m ² /g)	Pore Size (Å)	Pore Volume (cm ³ /g)	% Carbon
Base silica High Fe	166.5	121.9	0.59	0.15
Base silica Medium Fe	170.34	127.5	0.60	0.11
Base silica Low Fe	175.0	127.6	0.64	0.13
ODS silica High Fe	127.0	107.0	0.38	8.30
ODS silica Medium Fe	119.1	111.0	0.43	8.58
ODS silica Low Fe	121.1	113.3	0.46	8.63
ODS/TMS silica High Fe	106.4	101.9	0.36	9.26
ODS/TMS silica Medium Fe	111.0	104.1	0.40	9.68
ODS/TMS silica Low Fe	114.7	105.6	0.39	9.81

steel columns using acetone as a packing and slurry solvent, at a pressure of 8000 psi. Prior to the chromatographic testing, the columns were conditioned with methanol: water (70:30).

Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) Analysis

Hydrofluoric acid (40%) was Aristar grade and supplied by Fisons Ltd (Loughborough, UK). Concentrated nitric acid (99.999 + % purity) was supplied by Sigma Chemicals Ltd. All elemental standards were prepared from atomic absorption grade standards and were supplied by either BDH Ltd (Poole, UK) or Aldrich Chemical Company Ltd (Poole, UK). British Chemical Standard high purity silica (BCS-313 silica) was used for the validation of the ICP-AES methodology and was supplied by the LGC (Teddington, UK). All other reagents were of analytical grade or better.

The metals chosen for investigation were zinc, cobalt, nickel, iron, chromium, magnesium, copper, titanium, calcium, aluminium, sodium, and potassium. A sample of microparticulate silica (approximately 0.25 g) was transferred to a TeflonTM digestion vessel. A solution of hydrofluoric acid (40%, 8 mL) and concentrated nitric acid (2 mL) was added and the vessel was agitated to ensure wetting of the sample. The sealed and capped digestion vessel was placed into a microwave digestion system (MSD-81D microwave, CEM Ltd) and was digested on 100% power for 5 min, then 50% power for a further 5 min.

Samples were analysed as replicates; the coefficient of variation and standard deviations were calculated by the system software. Each metal concentra-

tion was weighted to account for the carbon loading of the particular silica to obtain a true metal concentration per gram of silica. The reproducibility of the method was assessed from the inter- and intra- day precision of the main elements of interest using a well characterised British Chemical Standards high purity silica. The inter-day coefficients of variation were found to be Fe (1.6%), Mg (4.5%), Ti (8.2%), Ca (3.9%), and Al (5.7%); the intra-day coefficients of variation were Fe (1.2%), Mg (1.3%), Ti (1.9%), Ca (2.9%), and Al (1.0%). Limits of detection for all elements were calculated using a value of three times the standard deviation of the instrumental noise.

Chromatography

The HPLC system consisted of a pump (Gilson Medical Electronics Ltd, Villiers le Bel, France, Model 302), a mixer (Gilson Model 811), a manometric module (Gilson Model 8020), an automated injector (Gilson Model 231) with diluter (Gilson Model 401), and a UV detector (Gilson Model 116). The bonded silica materials were packed into columns with dimensions 100 mm x 4.6 mm i.d. The column was maintained at 30°C in a column oven (Jones Chromatography Ltd.). The system was controlled using Gilson 713 software. De-ionized water was obtained from an Elgastat water purification system (Elga Ltd, High Wycombe, UK). The mobile phases were prepared from HPLC grade methanol (Fisons Ltd, Loughborough, UK): 0.05 M phosphate buffer adjusted to pH 7 (20:80) for all test probes, except for the weak acid chromatographic probes, where the mobile phase was the same composition but was buffered to pH 3.0. The flow rate was 1.0 mL.min⁻¹, the injection volume was 20 µL, and the detection wavelength was 251 nm for all the solutes.

The chromatographic probe compounds were 8-hydroxyquinoline, acetylacetone (metal chelators); benzoic acid, salicylic acid, and diethylbarbituric acid (weak acids); pyridine, 3-amino pyridine, 3-ethylpyridine (weak bases), toluene, and diethyl-*m*-toluamide (neutral). Toluene was used in our studies as a neutral analyte to establish the hydrophobic character of the stationary phases through its interactions with the bonded alkyl chains. Diethyl-*m*-toluamide has been reported as a polar marker for silanol activity.¹³ The substituted pyridines were chosen for their recognised ability to interact with ionised surface silanols on reversed phase ODS materials.¹⁴ Benzoic acid, salicylic acid, and diethylbarbituric acid are all organic weak acids, the latter two having weak metal chelating activity.

8-Hydroxyquinoline and acetylacetone are known to have strong interactions with trace metals and their retention is dependent on metal content of the chromatographic phase.^{3,15,16} Each probe compound was subjected to chromatographic analysis (n=6), and the average retention factor and asymmetry values for

each peak were calculated. Solutions of each solute were made up in the mobile phase and used at concentrations of $10 \mu\text{g.mL}^{-1}$, except for 8-hydroxyquinoline ($100 \mu\text{g.mL}^{-1}$), salicylic acid ($100 \mu\text{g.mL}^{-1}$), and benzoic acid ($200 \mu\text{g.mL}^{-1}$). Retention factor (k) and peak asymmetry factor were measured from replicate ($n=6$) injections of each individual test compound on each column evaluated.

The hold-up time (t_0) was taken as the time from injection to the moment when the trace solvent disturbance crossed the. The peak asymmetry factor was calculated at 10% of full peak height on expanded-scale chromatograms.

RESULTS

ICP-AES Metal Analysis of Base Silicas and Bonded Phases

The ICP-AES data for the bulk trace metal concentrations of the underivatized base silicas are given in Table 2(a) and Figure 1 (a). The metals in highest concentration were sodium (75-78 % (w/w) of total metal concentration), aluminium (8.7-11.5%w/w), iron (3.3-9.1%w/w), and titanium (1.7-3.1% w/w). The three batches of base silica were designated 'high Fe', 'medium Fe', and 'low Fe', respectively, according to the additional iron added during manufacture. The measured iron content of these three batches, respectively, were 300 ppm (9.1% w/w), 191 ppm (5.9% w/w), and 132 ppm (3.3% w/w), in agreement with the designations. There were unexpected changes in the concentrations of titanium (55→73→121 ppm; 1.7→2.2→3.1% w/w) and aluminium (286→335→452 ppm; 8.7→10.3→11.5% w/w), which both increased in concentration as the iron decreased in the three batches. The reason for these changes in titanium and aluminium concentrations was not clear, but may have been due to the added iron displacing the titanium and aluminium from the silica during the process of manufacture.

The concentrations of the remaining trace metals ranged between 0.6 and 64 ppm, and showed no consistent trends with respect to the added iron. However, weak correlations were observed in the order 'medium' – 'low' – 'high' Fe batches for several of the metals present in very low concentrations: nickel, chromium, copper, and potassium. The metal content of the silicas determined in this study are in broad agreement with a number of previous reports of trace metal levels in 'high sodium' chromatographic silicas prepared from silica sols.¹¹⁻¹³

The ICP-AES results showed that the overall metal content of the silicas was reduced following the process of manufacture and bonding (Tables 2(b) and 1(c), Figures 1(b) and 1(c)). The depletion of metals from silica is known to occur at the rehydroxylation stage when the silica is refluxed in dilute mineral acid.¹ The ODS bonding process itself is unlikely to affect the metal content, and indeed, no further reduction in individual or overall trace metal concentration was

Table 2. ICP-AES Determination of Trace Metal Concentration in 'High', 'Medium', and 'Low' Fe Content Silicas. (a) Underivatised (Base) Silicas, (b) ODS Bonded Silicas, (c) ODS Bonded and End-Capped Silicas. Data Are the Mean of Two Determinations

	Metal Concentration (ppm)											
	Zn	Co	Ni	Fe	Cr	Mg	Cu	Ti	Ca	Al	Na	K
<i>(a) Base Silica Batches</i>												
High Fe	8	0.8	17	300	32	32	3	55	50	286	2481	37
Medium Fe	7	0.6	7	191	14	30	2	73	58	335	2500	28
Low Fe	3	0.7	11	132	24	17	3	121	38	452	3081	64
<i>(b) ODS Silica Batch</i>												
High Fe	4	0.6	14	147	20	22	2	52	37	272	1816	35
Medium Fe	3	0.3	5	105	8	21	1	64	47	295	1829	41
Low Fe	2	0.3	3	47	9.5	12	2	109	27	408	2211	58
<i>(c) ODS Endcapped Silica Batch</i>												
High Fe	5	0.6	14	155	20	25	2	54	45	260	1792	37
Medium Fe	4	0.4	6	104	8	18	1	64	37	295	1907	30
Low Fe	3	0.3	4	49	10	12	3	108	28	408	2223	56

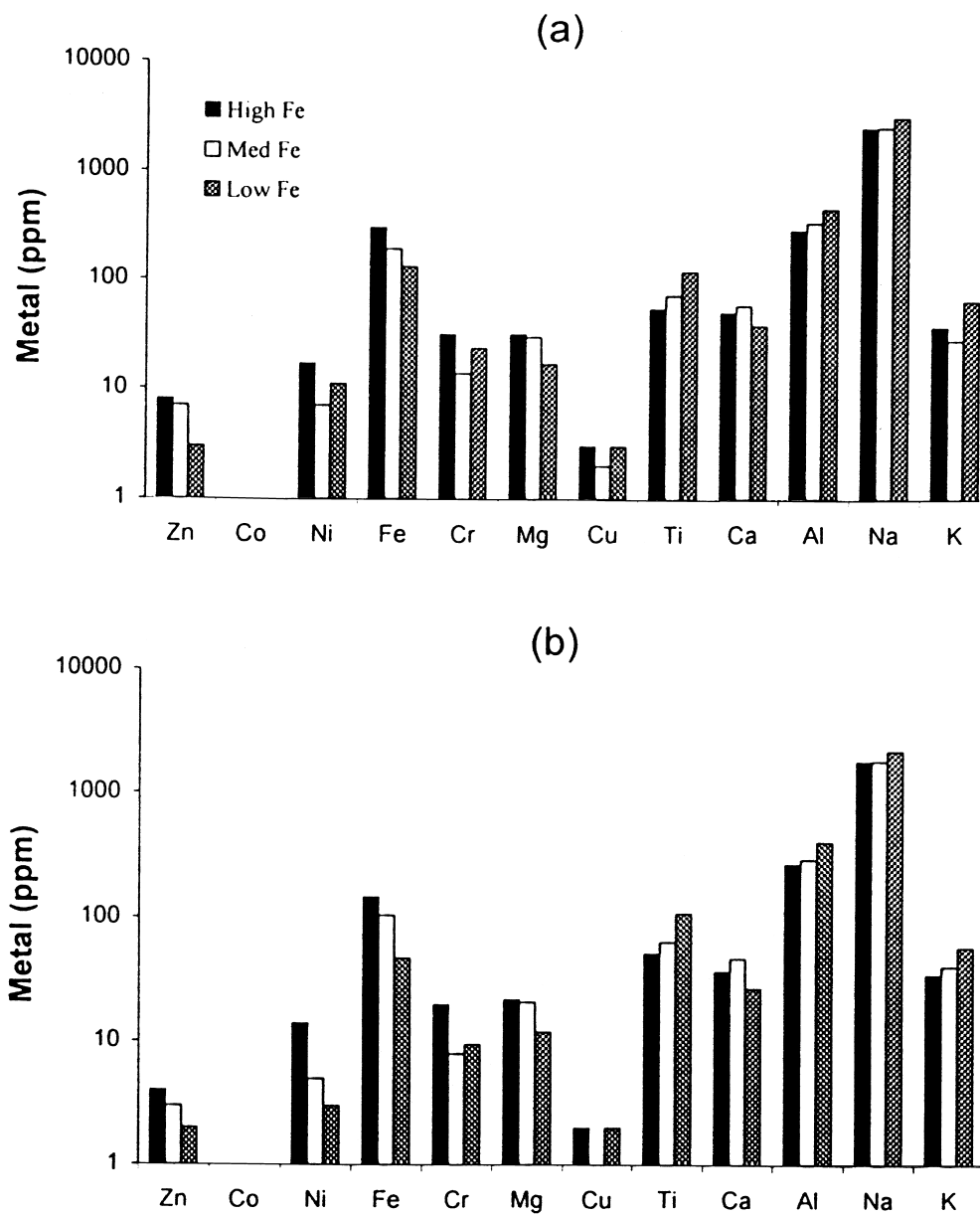


Figure 1. Metal concentrations determined by ICP-AES for high, medium, and low Fe content silicas. a) Underivatized (base) silicas; b) ODS bonded silicas; and c) ODS bonded and endcapped silicas.

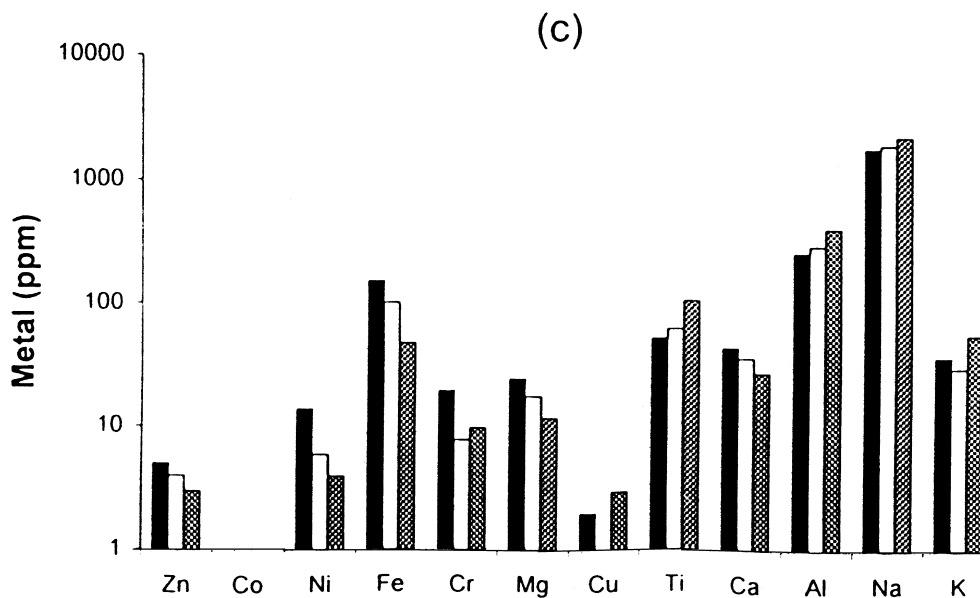


Figure 1. Continued.

observed after endcapping the phases (Tables 2(b) and 1(c)). The effect of the acid rehydroxylation process on metal content is examined in more detail (Figures 2(a) and 2(b)) for the 'high Fe' batch by plotting the ppm reduction and % reduction for each of the trace metals. The decrease in total ppm bulk metal concentration was dominated, as expected, by the more abundant metals (sodium and iron), but small reductions in concentration were also observed for all other metals.

As a proportion of the total reduction in metal ppm content following rehydroxylation, sodium (865 ppm, 75.6% w/w) and iron (153 ppm, 17.4% w/w) were the main contributors, with the remainder of the metals together, contributing only 6.0%. Examining the changes in the % decrease in concentration of each individual metal, it can be seen that the majority of the metals decreased in concentration by 25-50%, irrespective of their starting ppm concentration in the silica. The exception to this observation was the much smaller decrease (less than 5%) seen for titanium, aluminium, and potassium (Figure 2(b)), which increased the relative proportions of each of these metals in the silica.

Similar results have been reported in other studies on the removal of trace metals by acid treatment of silica.^{17,18} Aluminium and titanium are known to be more tightly bound into the silica matrix, and there is strong evidence to suggest that mixed oxides with SiO₂ as the major component and titanium or aluminium

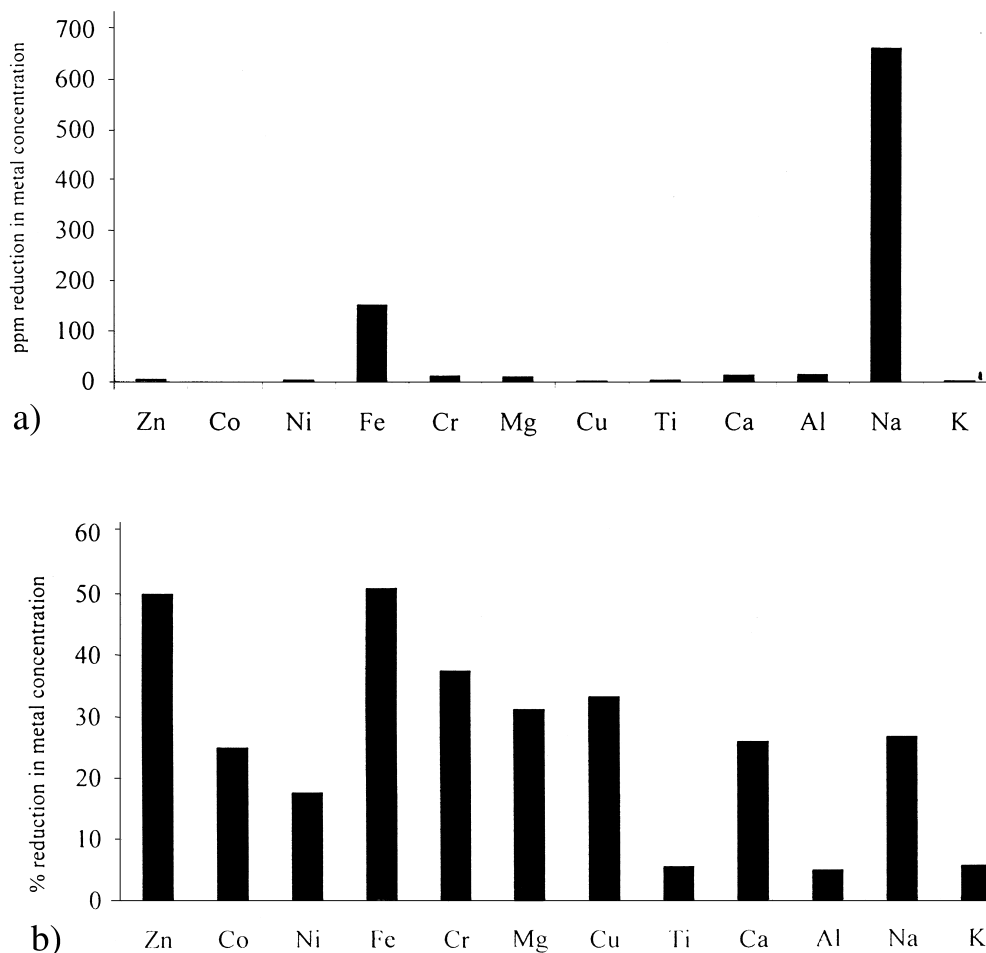


Figure 2. a) Ppm reduction in bulk metal content following acid rehydroxylation and bonding for 'high' Fe batch. b) Percent reduction in bulk metal content following acid rehydroxylation and bonding for 'high' Fe batch.

as the minor component will form highly acidic centers.¹⁹ Clearly, this observation has significance for chromatographic performance of the phases, where the increased proportions of aluminium and titanium following acid treatment may result in the generation of an increased number of unwanted acidic silanols.

In the ODS bonded phases, the consistent differences observed in the respective metal content of the 'low', 'medium', and 'high' Fe base silicas was maintained (Table 2(b), Figure 1(b): iron (147→10547 ppm; 6.14.3→1.6% w/w),

titanium (52→64→109 ppm; 2.0→2.6→3.8% w/w), and aluminium (272→295→408 ppm; 11.2→12.1→14.1% w/w), but there was still no significant trend for sodium (1816→1829→2211 ppm; 75.0→75.6→76.5% w/w). There were also differences between the batches for some of the trace metals present in lower concentration, such as potassium, nickel, and magnesium, but these did not always coincide with the 'low', 'medium', and 'high' Fe designations. The endcapping process produced no significant changes in either the total metal content or in the concentration of individual metals (Table 2(c), Figure 1(c)).

Chromatographic Evaluation of Bonded Phases

The retention factors and asymmetry factors determined for the probe analytes were used to characterise the chromatographic performance of the bonded stationary phases (Tables 3 and 4, Figures 3 and 4). All the non-endcapped phases were found to have approximately the same k values for toluene, the neutral marker solute. After endcapping of the three phases, the retention factors for toluene increased by a similar amount for all three phases. The k values for toluene and certain other analytes were extremely high, but were necessary to permit retention data to be obtained for the more weakly retained analytes using the same mobile phase organic percentage. With these strongly retained analytes,

Table 3. Retention Factors for Test Solutes on Columns Packed with ODS and ODS Endcapped Silicas from High, Medium and Low Fe Batches

Test Solute	Retention Factor					
	ODS Silica Batches			Endcapped ODS Silica Batches		
	High Fe	Med Fe	Low Fe	High Fe	Med Fe	Low Fe
Acetylacetone	3.1	3.2	3.5	2.5*	2.7	2.67*
8-Hydroxyquinoline	91.5*	76.4	89.8*	65.5	69.5	68.0
Pyridine	13.0	13.3	15.3*	4.6	5.2	5.7
3-Aminopyridine	6.9	8.0	11.34	2.1	2.5	3.1
Ethylpyridine	72.1	73.4	78.4	31.5	32.6	33.7
Diethyltoluamide	42.7	44.5	42.0	35.3*	35.8	36.5
Salicylic Acid	13.0	13.6	12.4	21.8	21.6	21.7
Benzoic Acid	14.7	15.6	14.3	23.5	23.2	24.4
5,5-Diethylbarbituric Acid	5.6	5.5	5.5	7.1	7.1	7.3
Toluene	66.8	66.8	66.7	106.1	104.3	104.4

Data presented as a mean for at least three determinations.

*Denotes a single determination.

Table 4. Asymmetry Factors for Test Solutes on Columns Packed with ODS and ODS Endcapped Silicas from High, Medium and Low Fe Batches

Test Solute	Asymmetry Factor					
	ODS Silica Batches			Endcapped ODS Silica Batches		
	High Fe	Med Fe	Low Fe	High Fe	Med Fe	Low Fe
Acetylacetone	2.7	2.3	2.1	1.5*	1.8	1.5*
8-Hydroxyquinoline	4.4*	4.6	†	†	†	1.4
Pyridine	9.1	8.4	8.0*	12.8	14.3	14.1
3-Aminopyridine	19.0	30.7	30.6	19.5	22.9	23.7
Ethylpyridine	21.5	20.5	12.5	15.9	21.9	17.3
Diethyltoluamide	1.2	1.1	0.9	1.1	1.1	1.0
Salicylic Acid	1.6	1.3	1.2	8.4	3.1	2.5
Benzoic Acid	1.3	1.0	1.0	2.8	1.7	1.3
5,5-Diethylbarbituric acid	1.3	1.1	1.0	1.3	1.2	1.1
Toluene	0.6	0.6	0.6	0.5	0.5	0.5

Data presented as a mean for at least three determinations.

† Unable to determine retention time due to strongly adsorbed peak.

* Denotes a single determination.

care was taken to collect sufficient data to obtain an accurate value for peak asymmetry.

The weak bases chromatographed very poorly with broad asymmetric peaks, but this was not unexpected since the relatively high metal content of the base silica would be expected to give substantial silanol interactions with basic solutes. End-capping of the stationary phase increased the retention of all the neutral/unionised test compounds, but reduced the retention of all the basic solutes. These observations demonstrated that the packed columns behaved in a reproducible manner, and gave retention behaviour typical of ODS bonded phases derived from silica containing a relatively high trace metal content.

Correlation Between Trace Metal Composition and Chromatographic Retention Behaviour

The presence of metals in the phases has been shown to be detrimental to the separation performance towards certain solutes, although the individual metals responsible for this effect are only poorly characterised at present.¹⁹⁻²² ICP-AES analysis of the bulk stationary phases has given an accurate measure of the total metal content, but the technique does not give any indication of the location

of the metals in the silica matrix. Only those metals located on or near the silica surface will be able to assert any effect on the chromatographic behaviour.

Hence, any attempt to correlate bulk metal content with chromatographic performance should be treated with caution, since the extent of binding of individual metals to the chromatographic surface is unknown. With this caveat in mind, it is possible to consider some tentative correlations between metal content and retention behaviour observed on the ODS bonded columns from the 'low', 'medium', and 'high' Fe batches.

Comparing the 'low', 'medium', and 'high' iron containing ODS bonded phases, the retention of most of the test solutes is essentially independent of metal concentration for 8-hydroxyquinoline, acetylacetone, diethyltoluamide, diethylbarbiturate, salicylic acid, benzoic acid, and toluene (Table 3, Figure 3(a)). Only the basic analytes (pyridine, 3-aminopyridine, ethylpyridine) appear to have their retention related to the batch of silica, and all show a consistent correlation between k and the high, medium, and low Fe content of the silicas.

The lack of observed retention changes for the known metal chelators, acetyl acetone and 8-hydroxyquinoline, probably reflected the fact that the total metal content actually varies little between the three batches with the high (>75%) sodium content dominating. Studies by Verzele et al.⁴ showed that the retention of acetylacetone changed according to the overall metal content of a phase, with low metal phases showing little retention and high metal phases giving retained and poorly shaped peaks. Clearly, the two metal chelator probes, acetylacetone and 8-hydroxyquinoline, were not sufficiently sensitive to distinguish the smaller changes in individual metal content of the phases used in this study. Diethyltoluamide is known as a sensitive silanol probe, which interacts with polar (but unionised) silanols on the silica surface.¹³

The lack of retention change of diethyltoluamide between the three phases suggests that the silica surfaces of the three batches have a similar silanol content, presumably because they all underwent the same rehydroxylation process with dilute acid and had identical conditions for the bonding of the stationary phases. The ODS/TMS phases illustrate the same trends as observed with the ODS phases, with a predictable increase in k of the neutral/unionised solutes and a decrease in k of the basic solutes as discussed earlier (Figure 3(b)).

Peak symmetry data can provide another useful method of correlating bulk metal content with chromatographic interactions. The extent of peak tailing indicated by the asymmetry factor is usually an indication of secondary interactions of the analyte with the stationary phase, typically seen in the extensive peak tailing observed for ionised bases interacting with ionised silanols in reversed-phase chromatography on silicas containing acidic silanols.

The peak asymmetry data for each of the solutes is presented in Figures 4(a) and (b), and is more revealing of relationships between the analytes and the different batches of silica than the retention data alone. The weak bases (pyri-

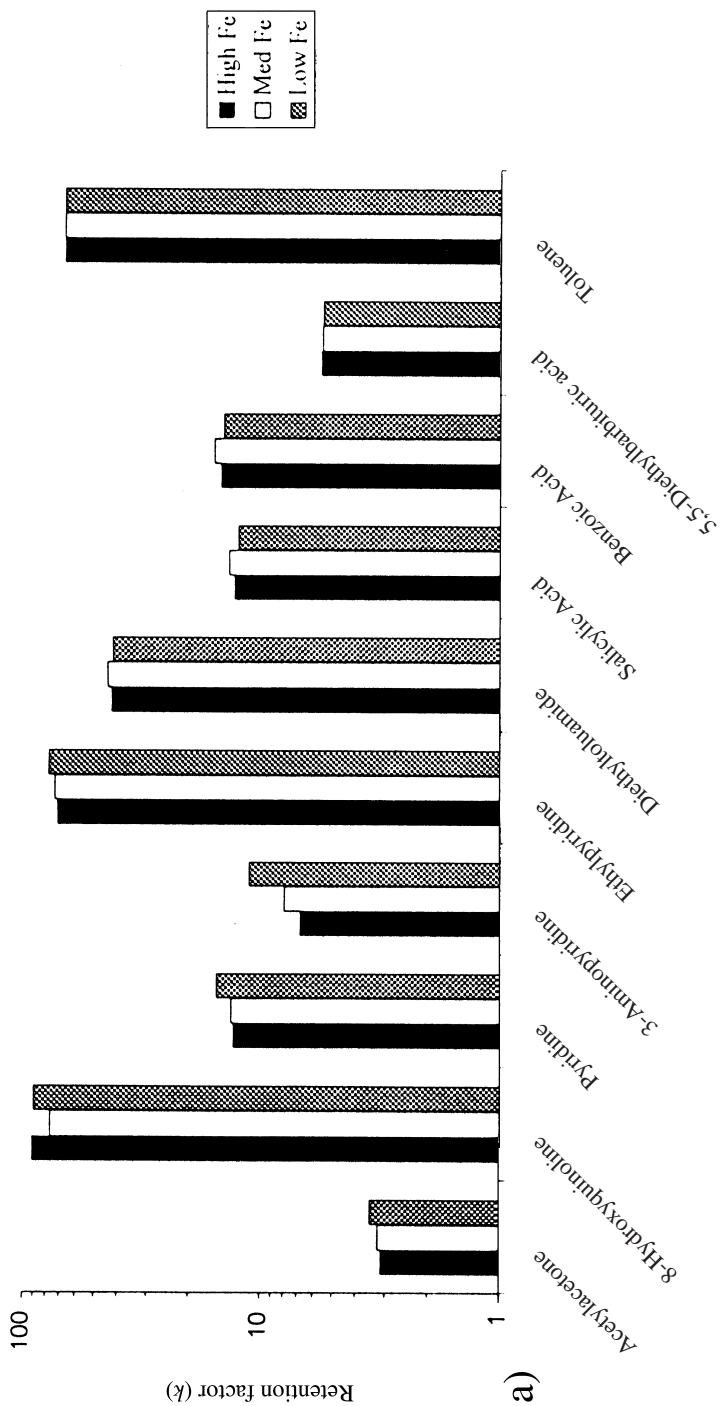
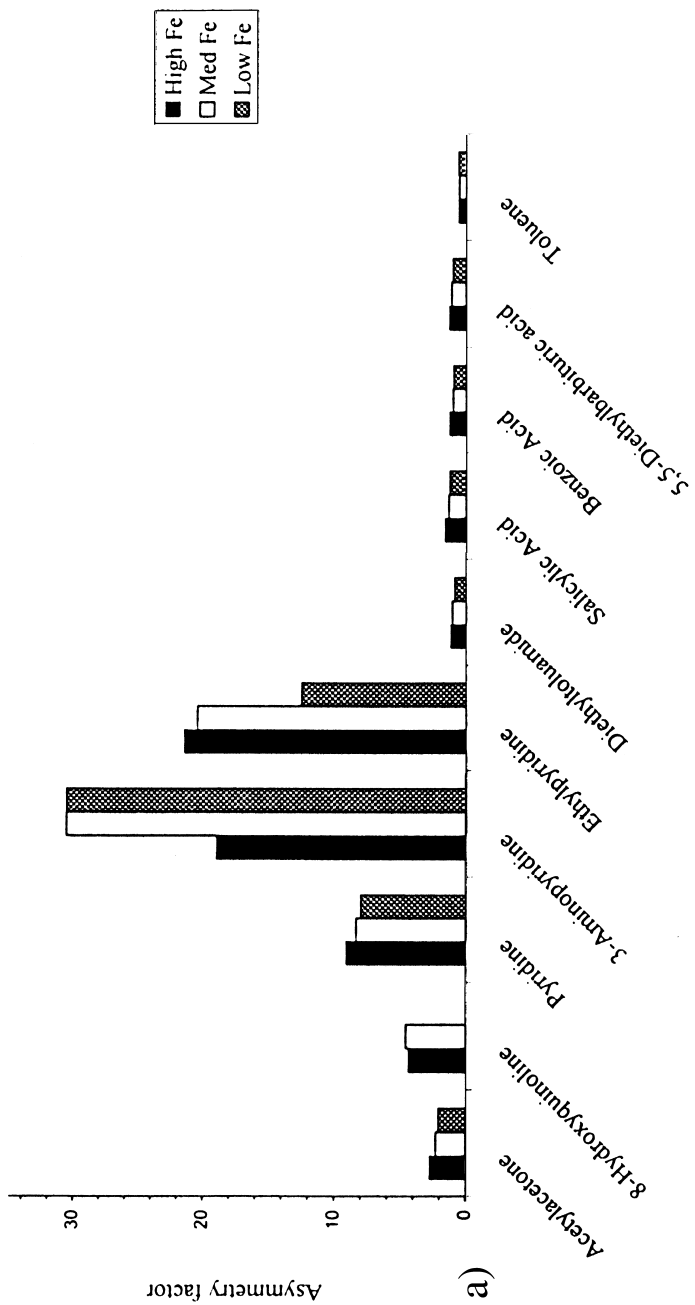




Figure 3. a) k values for test solutes on high, medium, and low Fe ODS bonded silicas. b) k values for test solutes on high, medium, and low Fe encapped ODS bonded silicas.



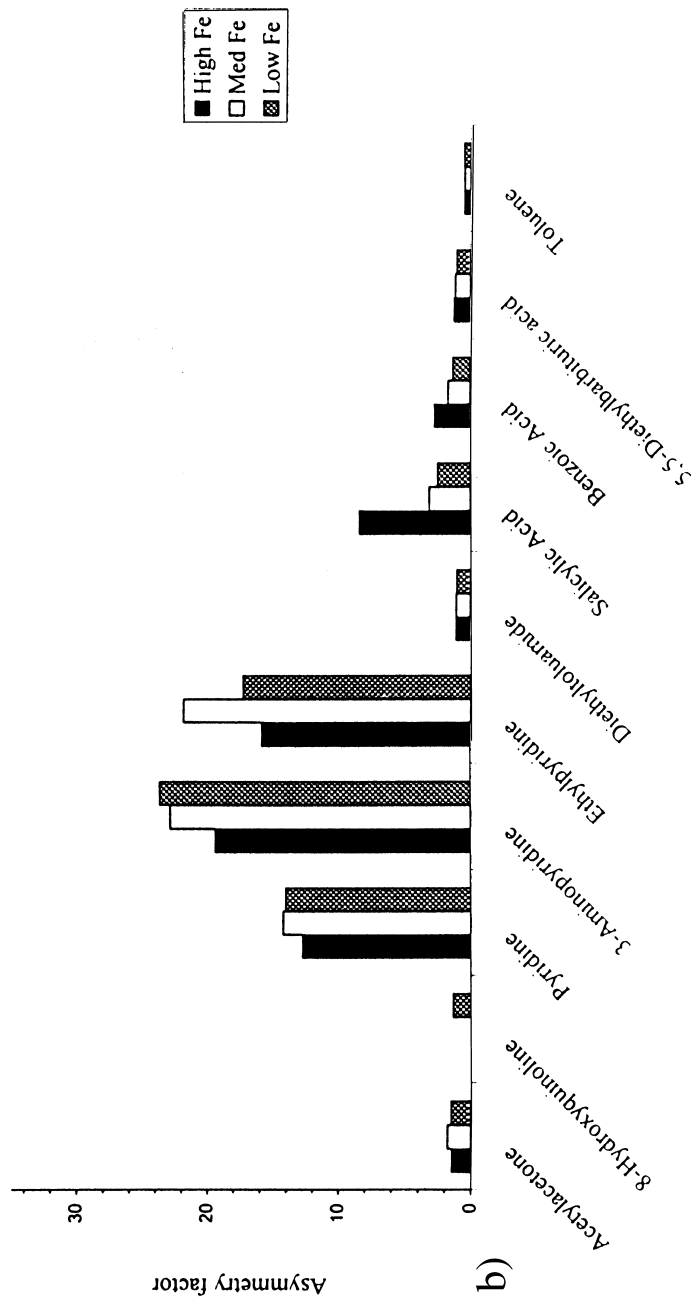


Figure 4. a) Asymmetry values for test solutes on high, medium, and low Fe ODS bonded silicas. b) Asymmetry values for test solutes on high, medium, and low Fe encapped ODS bonded silicas.

dine, 3-aminopyridine and ethylpyridine) gave badly tailed asymmetric peaks on all the ODS phases, and showed little or no improvement in symmetry on the end-capped phases (Table 4).

Peak asymmetry values for the bases changed with the different batches of silica, but not in such a predictable manner as observed with the retention data. The chromatographic performance of all the phases was noticeably poorer for the weak bases than the other classes of analytes, but was typical of phases based on silicas with a high starting metal content.³ The weak acids, salicylic acid, benzoic acid and, to a lesser extent, diethylbarbituric acid, also showed a good correlation of their peak asymmetry values (particularly on the endcapped phases) with the high, medium, and low Fe batches, although the opposite trend was observed compared with the bases. This was an unexpected observation and might be explained by the weak metal chelating properties of these acids.

As with the retention data, the neutral analytes, toluene and diethyltoluamide and the unionised weak acid, diethylbarbiturate, showed no consistent change in asymmetry factor between the batches of silica. 8-Hydroxyquinoline produced inconsistent peak asymmetry between injections, and some of the results are not reported for this reason. Acetylacetone peak asymmetry showed a weak correlation with the phase, but this was not consistent between the ODS and the ODS/TMS.

The correlation of chromatographic behaviour with the concentrations of iron, aluminium, and titanium in the bonded phases, suggests that one or more of these trace metals is responsible for at least some of the poor performance of the phases towards bases. It is possible to speculate that the role of the added iron may be to adsorb and, hence, block the activity of acidic silanols, and this is supported by a previous observation of added Fe³⁺ ions reducing adsorption of amines on the silica surface.²³ This accords with the observation of lowered retention of amine analytes in this study, which can be explained by a reduction of ion-exchange retention. However, an alternative explanation is that the higher aluminium/titanium concentrations result in more adsorptive sites due to the generation of acidic silanols from mixed oxides with the silica matrix, which in turn cause poor chromatography of the amines. In this study, it is not possible to distinguish between these two mechanisms.

CONCLUSIONS

The work reported has demonstrated the feasibility of investigating the role of individual metals in the retention of solutes in liquid chromatography, and revealed some potentially interesting correlations between metal content and chromatographic behaviour of acidic and basic analytes. It was confirmed, that individual rather than total metal concentration can affect the chromatographic

behaviour of an ODS silica phase, and it was possible to tentatively identify the individual metals responsible as a combination of iron, aluminium, and titanium. The addition of iron during manufacture of the phases also displaced other metals, notably aluminium and titanium. The large background of sodium and other trace metals in the silica used was also a complicating factor, which prevented the effective use of the metal sensitive probe solutes, acetyl acetone and 8-hydroxyquinoline.

ACKNOWLEDGMENTS

Mr. R.C. Watson was supported by a UK Biotechnology and Biological Science Research Council postgraduate studentship.

REFERENCES

1. Nawrocki, J.; Buszewski, B. Influence of Silica Surface-Chemistry and Structure on the Properties, Structure and Coverage of Alkyl-Bonded Phases for High-Performance Liquid-Chromatography. *J. Chromatogr.* **1988**, *449*, 1-15.
2. Nawrocki, J. The Silanol Group and its Role in Liquid Chromatography. *J. Chromatogr. A*, **1997**, *779*, 29-71.
3. Kohler, J.D.; Chase, D.B.; Farlee, R.D.; Vega, A.J.; Kirkland, J.J. Comprehensive Characterisation of Some Silica-Based Stationary Phases for High-Performance Liquid Chromatography. *J. Chromatogr.* **1986**, *352*, 275-305.
4. Verzele, M.; Dewaele, C. Stationary Phase Characterisation in High-Performance Liquid Chromatography. *J. Chromatogr.* **1981**, *217*, 399-404.
5. Dorsey, J.G.; Cooper, W.T. Retention Mechanisms of Bonded-Phase Liquid-Chromatography. *Anal. Chem.* **1994**, *66*, A857-A867.
6. Nawrocki, J.; Silica Surface Controversies, Strong Adsorption Sites, Their Blockage and Removal. Part I. *Chromatographia* **1991**, *31*, 177-192.
7. Nawrocki, J. Silica Surface Controversies, Strong Adsorption Sites, Their Blockage and Removal. Part II. *Chromatographia* **1991**, *31*, 192-205.
8. Heidrich, D.; Volkman, D.; Zurawski, B. The Place of SiOH Groups in the Absolute Acidity Scale from *ab initio* Calculations. *Chem. Phys. Lett.* **1981**, *80*, 60-63.
9. Sadek, P.C.; Koester, C.J.; Bowers, L.D. The Investigation of the Influence of Trace Metals in Chromatographic Retention Processes. *J. Chromatogr. Sci.* **1987**, *25*, 489-493.

10. Sadek, P.C.; Carr, P.W.; Bowers, L.D.; Hadded, L.C. A Radiochemical Study of Irreversible Protein Loss on High-Performance Liquid Chromatography Column Frits. *Anal. Biochem.* **1985**, *144*, 128-131.
11. Shih, Y-T.; Carr, P.W. Silanization of Stainless Steel Frits for Use in Trace Metal Analysis by High-Performance Liquid Chromatography. *Talanta* **1981**, *28*, 411.
12. Cramer, S.M.; Nathanael, B.; Horvath, C. High-Performance Liquid Chromatography of Deferoxamine and Ferroxamine: Interference by Iron Present in the Chromatographic System. *J. Chromatogr.* **1984**, *295*, 405-411.
13. Walters, M.J.; Classification of Octadecyl-Bonded Liquid-Chromatography Columns. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 465-469.
14. Barrett, D.A.; Brown, V.A.; Shaw, P.N.; Davies, M.C.; Ritchie, H.; Ross, P. Characterisation Of a Range OF Alkyl-Bonded Silica HPLC Stationary Phases: Chromatographic Behaviour OF Neutral, Acidic and Basic Test Solutes. *J. Chromatogr. Sci.* **1996**, *34*, 146-156.
15. Verzele, M.; Dewaele, C. The Evaluation of Reversed-Phase High-Performance Liquid Chromatography Packing Materials. *Chromatographia* **1984**, *18*, 84-86.
16. Schuddinck, G.; Verzele, M. Avoidance of Trace-Metal Activity with Micro-Liquid Chromatography in the Analysis of Hop and Beer Bitter Acids. *J. Chromatogr.* **1987**, *407*, 159-169.
17. Köhler, J.; Chase, D.B.; Farlee, R.D.; Vega, A.J.; Kirkland, J.J. Comprehensive Characterisation of Some Silica-Based Stationary Phases for High-Performance Liquid Chromatography. *J. Chromatogr.* **1986**, *352*, 275-305.
18. Verzele, M.; De Potter, M.; Ghysels, J. Trace Elements in HPLC Silica Gel. *J. High Resolut/Chromatogr. Commun.* **1979**, *2*, 151-153.
19. Busewski, B. Influence of Silica Gel Surface Purity on the Coverage Density of Chemically Bonded Phases. *Chromatographia* **1992**, *34*, 573-580.
20. Tanabe K. in *Catalysis, Science and Technology*, Anderson, J.R., Boudart, M., Eds.; Springer-Verlag: Berlin, 1981; 231.
21. Ohtsu, Y.; Shiojima, Y.; Okumura, T.; Koyama, J-I.; Nakamura, K.; Nakata, O.; Kimata, K.; Tanaka, N. Performance of Polymer-Coated Silica C-18 Packing Materials Prepared from High-Purity Silica-Gel - the Suppression of Undesirable Secondary Retention Processes. *J. Chromatogr.* **1989**, *481*, 147-157.
22. Kimata, K.; Tanaka, N.; Araki, T. Suppression of the Effect of Metal Impurities in Alkylsilylated Silica Packing Materials. *J. Chromatogr.*, **1992**, *594*, 87-96.

23. Pfleiderer, B.; Bayer, E. Silanediol Groups of the Silica-Gel Nucleosil - Active-Sites Involved in the Chromatographic Behavior of Bases. *J. Chromatogr.* **1989**, *468*, 67-71.

Received November 6, 2000

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Accepted January 3, 2001