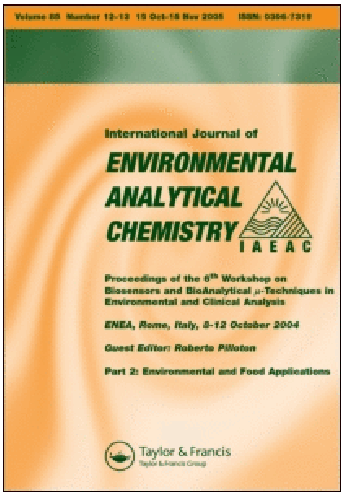

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Metal-Loaded Silicas for On-Line Coupling of Enrichment Pre-Column and Analytical Columns

M. -A. Bagnoud[a]; W. Haerdi[a]
[a] Department of Inorganic, Analytical and Applied Chemistry, University of Geneva, Geneva 4, Switzerland

To cite this Article Bagnoud, M. -A. and Haerdi, W.(1990) 'Metal-Loaded Silicas for On-Line Coupling of Enrichment Pre-Column and Analytical Columns', International Journal of Environmental Analytical Chemistry, 38: 2, 97 — 104
To link to this Article: DOI: 10.1080/03067319008026918
URL: http://dx.doi.org/10.1080/03067319008026918

Intern. J. Environ. Anal. Chem., Vol. 38, pp. 97–104
Reprints available directly from the publisher
Photocopying permitted by license only

METAL-LOADED SILICAS FOR ON-LINE COUPLING OF ENRICHMENT PRE-COLUMN AND ANALYTICAL COLUMNS

M.-A. BAGNOUD and W. HAERDI

Department of Inorganic, Analytical and Applied Chemistry, University of Geneva, 30, Quai E. Ansermet, 1211 Geneva 4, Switzerland

The preparation of two metal-loaded silicas is presented. The first one, iron-8-hydroxyquinoline silica (Si-Ox-Fe) is used in a small pre-column for selective enrichment of catechol derivatives. The other one, copper-cyclam silica (Si-Cy-Cu) acts as stationary phase for outer-sphere ligand exchange chromatography. The on-line coupling of these two silicas makes the HPLC analysis of acid derivatives of catechol in urine more specific and the analysis can be done without any pretreatment of the sample.

KEY WORDS: Sample handling, trace analysis, metal-loaded silica.

INTRODUCTION

During the past twenty years, HPLC has become a very powerful and extended tool for the separation and determination of organic compounds. However, the analysis of trace components in natural and biological fluids needs pretreatment for selectively extracting and enriching them. The on-line coupling of liquid–solid extraction on a pre-column packed with a suitable sorbent followed by elution in an analytical column[1–3] allows automation and avoids contamination risks and losses of compounds (Figure 1). Great selectivity can be achieved depending on the nature of the extracting sorbent.

Table 1 gives the energies of the possible interactions between solutes and stationary phases. The usual reversed and normal phases show mainly weak and very common Van der Waals and hydrogen bond interactions with organic compounds. Because of the high dielectric constant of water, electrostatic interactions are reduced by a factor of 80 when they take place in aqueous media, which is mainly the case with natural and biological fluids.

Selectivity can be improved by using metal-loaded silicas (MLS). Table 2 shows that each transition metal has a preference for a particular organic compound, generally Lewis bases to which it binds. The use of such silicas as solid–liquid extractants would therefore need very tiny pre-columns since interaction are very strong.

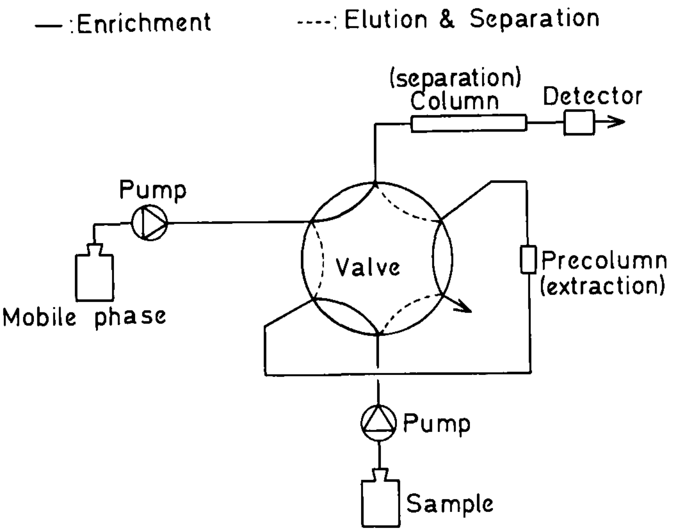

Figure 1 Assembly for on-line coupling of extraction pre-column with chromatographic column.

Table 1 Energy values of the different kinds of interaction that can take place between compounds.

Interaction	*Energy [kcal/mole]*
Covalent	100–300
Ionic	50–200
Metal–ligand	10–50
Hydrogen bond	5–10
Dipole–dipole	3–10
Dipole-induced dipole	2–6
Dispersion	1–5

Table 2 Affinities of metals for organic Lewis bases.

Metal	*Lewis bases*
Hg(II)	Carboxylic acids, phenols, unsaturated chains, S-containing cpds
Ag(I)	Unsaturated chains containing cpds
Cu(II)	Amines, carboxylic acids, phenols, amino-acids, unsaturated, S-containing
Cd(II), Zn(II)	Amines, S-contg, P-contg
Fe(III)	Carboxylic acids, amines, S-containing Phenols, catechols

APPLICATIONS OF MLS

Earlier we presented[4] the behaviour of metal-loaded silicas charged with transition metals toward different classes of compounds. We concluded that those which allow the metal to keep free complexing sites are best suited for enrichment purposes. Examples are given by a Pt-loaded 2-amino-1-cyclopentene-1-dithiocarboxylic acid modified silica which strongly retained aniline[5] or a Hg-8-hydroxyquinoline phase that selectively enriched 2-mercaptobenzimidazole.[6] In our laboratory, Cu-loaded-bis-dithiocarbamate (Si-bis-DTC-Cu)[7] and dialkyl-dithiocarbamate (Si-dial-DTC-Cu)[8] have been used for the enrichment of catecholamines in urine followed by on-line separation on C_8 analytical column.

When the metal is entirely complexed on the silica such as in the Co-loaded ethylene-diamine silica[10–12] or as in the Cu-loaded cyclam silica (Si-Cy-Cu),[4] inner-sphere complexation is no longer possible. Outer-sphere ligand exchange chromatography will therefore be a better use for these stationary phases.

We will not describe here all the mechanisms of chromatography on Si-Cy-Cu[13] but this stationary phase has proven to induce a certain selectivity for organic compounds possessing a negatively ionisable group, typically carboxylic acids. Figure 2 shows the separation of a family of acid indoles using Si-Cy-Cu as stationary phase. L-tryptrophan, a zwitterionic indole, and tryptamine, a neutral molecule at this pH, are almost eluted with minimum dead volume. The chromatogram illustrates the powerful resolution obtained for compounds varying only by a few atoms.

STRATEGY FOR THE COUPLING OF TWO MLS

If we now look at figure 1, we can imagine the introduction of selectivity at different steps of the whole process: 1) the stationary phase of the pre-column can be a powerful selective extracting material, 2) as we just mentioned with Si-Cy-Cu, stationary and mobile phases of the separation column can induce a certain selectivity, and 3) the nature of the detector or the introduction of a post-column derivatisation device is a good way to improve the selectivity of the whole process.

We already made reference to the use of iron-loaded 8-hydroxyquinoline silica (Si-Ox-Fe; Ox stands for oxine, another name of 8-hydroxyquinoline) as selective extractor for catecholamines. The catechol group is in fact the main interacting function with the iron atom because a stable chelate is formed. Therefore catecholamines were subsequently selected from other catechol derivatives by using the following chromatographic conditions: ion-pairing chromatography in the acidic mobile phase containing lauryl sulfate as ion pairing agent and C_8 stationary phase. Adrenaline, noradrenaline and dopamine of untreated urine samples could be directly extracted, preconcentrated and separated by on-line elution with electrochemical detection.[14]

Bearing these results in mind we thought that by coupling the same Si-Ox-Fe pre-column with a Si-Cy-Cu analytical column, we would be able to specifically recover carboxylic acid derivatives of catechol such as 3, 4-dihydroxy-benzoic (3,

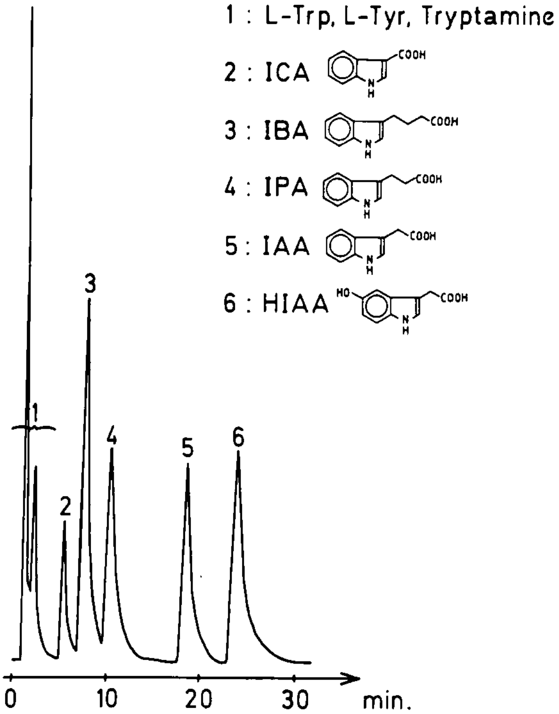

Figure 2 Direct injection of indole derivatives (1 μg each in 20 μl) onto Si-Cy-Cu chromatographic column (20 cm × 4 mm I.D.). Mobile phase: 0.2 M acetate buffer pH = 5.0/MeOH 20:80 (v/v) from 0 to 10 minutes then 50:50 from 10 to 17 minutes. UV-detection at 280 nm. ICA: indole-3-carboxylic acid; IBA: indole-3-butyric acid; IPA: indole-3-propionic acid; IAA: indole-3-acetic acid; HIAA: 5-hydroxyindole-3-acetic acid.

4-DHBA), 3, 4-dihydroxy-phenyl-acetic (3, 4-DHPA) and 3, 4-dihydroxy-mandelic (3, 4-DHMA) acids present in urine.

We first measured breakthrough volumes, V_b, of these three organic compounds on the Si-Ox-Fe pre-column. Results for pure solution of these compounds in the presence of 0.1 M $NaClO_4$ showed V_b greater than 30 ml (>70 ml for 3, 4-DHBA and >60 for 3, 4-DHMA). The results obtained were disappointing considering that under the same chromatographic conditions as for the separation of indoles on the Si-Cy-Cu column peaks were very large for compounds containing a catechol moiety. Big peaks were not observed with analogous compounds with one of their hydroxy group substituted by a methoxy group. Hydrogen bonding with residual silanol groups of the column could be responsible for the interaction. Peak enlargements were reduced only by lowering the pH of the chromatographic mobile phase. Consequently we sought an acid mobile phase which would enable us to elute the enriched compounds from the Si-Ox-Fe pre-column and separate them on the Si-Cy-Cu analytical column.

EXPERIMENTAL

a) Synthesis of Si-Ox-Fe: The synthesis was done according to the procedure described by Shahawn and Jezorek.[15] 10 g of silica (Polygosil 60–10, Macherey-Nagel, particle size 10 μm) were dried at 150 °C for one night under reduced pressure (15 mmHg). 4 ml of 3-aminopropyl-triethoxy-silane (Fluka) and 36 ml of dry toluene were added. The mixture was refluxed fro 210 minutes under nitrogen atmosphere and then filtered. At the end of the reaction, the silica was washed with dry toluene, methanol, and acetone and dried at 80 °C for 24 hours under reduced pressure.

10 g of the silica was then added to a solution containing 10 g of p-nitrobenzoile chloride, 10 ml of triethylamine and 50 ml of chloroform. The mixture was refluxed under nitrogen atmosphere for 50 hours, filtered, and washed with chloroform, methanol and acetone. Then the silica was suspended in 100 ml of 4.6 10^{-2} M $Na_2S_2O_4$ solution and refluxed for 60 minutes. The mixture was then filtered and washed with hot ethanol and water.

A solution of 2% $NaNO_2$ in 2 M HCl solution was added to the silica. The mixture was cooled to 0–3 °C and shaken for 30 minutes. The filtered silica was washed with ice water and immediately added to a 2% oxine (8-hydroxyquinoline, Merck) solution in ethanol. The mixture was shaken for 30 minutes again, filtered, and the resulting red-coloured oxine silica (Si-Ox) thoroughly washed with ethanol, 0.1 M HCl and water and dried at 120 °C overnight under reduced pressure.

The silica thus obtained was stored in a refrigerator (4 °C). Iron loading was performed by agitating 200 mg of Si-Ox with 1 mmole of $Fe(NO_3)_3$ in about 50 ml of water for 30 minutes. Filtration and abundant rinsing (until no more coloration was observed when potassium thiocyanate is added to the eluate) was required to eliminate the excess of iron.

b) Synthesis of Si-Cy-Cu: Synthesis described previously for Si-Cy-Cu[16] has been modified. 5 g of silica (Nucleosil 50-5, Macherey-Nagel, particle size 5 μm) were dried at 150 °C for one night under reduced pressure (15 mmHg). 50 ml of dry toluene, 7 ml of 3-glycidyloxy-propyl-trimethoxysilane (Fluka) and 105 μl of triethylamine (Fluka) were added. After refluxing for 4 hours under nitrogen atmosphere, the silica was filtered and washed with 70 ml of dry toluene, 100 ml of acetone and 100 ml of ether.

200 ml of water containing 0.2 g of 1, 4, 8, 11-tetraazacyclo-tetradecane (cyclam, Fluka) and 1 g of silanized silica were stirred for 48 hours at ambient temperature. Filtration and washing with water, methanol and acetone eliminates the excess of cyclam.

The loading of copper was carried out by suspending 100 mg of cyclam silica in 10 ml of 0.01 M copper sulfate solution and stirring for 30 minutes. The modification consisted of using an aqueous instead of a chloroform solution of cyclam. This results in a higher fixation of cyclam at the surface of the silica. The quantity of copper retained is therefore increased from 80 to 200 μM per gram of silica.

c) Filling of the columns: The empty pre-column (10 × 2 mm I.D.) designed in our laboratory was filled by injecting a suspension of the silica in water with a syringe.

The analytical column (20 cm × 4 mm I.D.) was filled by pumping the suspension of 2.5 g of Si-Cy-Cu in 10 ml of CCl_4 with a Haskell pump (Chromatom, Touzart & Matignon) and flushing it with hexane (P = 400 bars) for at least half an hour.

d) Enrichment step: The Si-Ox-Fe pre-column was first conditioned with 10 ml of 0.1 M $NaClO_4$ (1 ml/min) (Pump = Kontron LC 414-T). 5 ml of urine was then pumped into the pre-column. Larger volumes are not recommended because the chloride present in urine tends to reduce the breakthrough volumes of catechol derivatives on the pre-column.[9] It is very important to wash the enriched species with 2 ml of 0.1 M $NaClO_4$ and 2 ml of methanol. This clean up eliminates compounds that are absorbed on the oxine-iron silica and eluted at the beginning of the chromatogram.

e) Elution and separation step: The mobile phase consists of a mixture of 4% CH_3COOH in water and methanol in the ratio 20:80 (v/v) for the separation of 3, 4-DHBA and 3, 4DHPA, and 80:20 for the determination of 3, 4-DHMA. The elution time (time of coupling between the pre-column and the analytical column) was 2 minutes (Pump = Varian LC-5000). Contact of Si-Ox-Fe with the acid mobile phase tends to destroy it. Therefore the pre-column must be refilled after each analysis. This is not a big drawback because this operation needs only 3 to 4 minutes and the cost of silica synthesis is very low.

RESULTS AND DISCUSSION

Figure 3 shows the results obtained for the same sample of urine analysed by the described method using UV and electrochemical detectors. We see that, as selectivity is dependent on the interactions between the pre-column and the analytical column, a weakly specific detection such a 280 nm UV-detection is sufficient.

The composition of the mobile phase is very critical. It must be acidic to elute the enriched compounds in the smallest possible volume but an excess of acid would reduce the retention time and the compounds would appear as a big peak at the beginning of the chromatogram. Methanol allows the separation of analogous compounds and changes the retention times too. Hence its proportion in the mobile phase plays a very important role.

The time of elution depends on the strength of the eluent of the chromatographic mobile phase. Compared to the direct injections on the analytical column, the described method gives 84% recoveries for 3, 4-DHBA and 3, 4-DHPA. 3, 4-DHMA is much more retained on Si-Ox-Fe and Si-Cy-Cu than the other two catechol derivatives are. So elution and separation need a higher proportion of acid in the mobile phase.

CONCLUSIONS

Metal-loaded silicas are very effective for liquid–solid extractions or ligand exchange chromatography, depending on the type of complexing ligand they

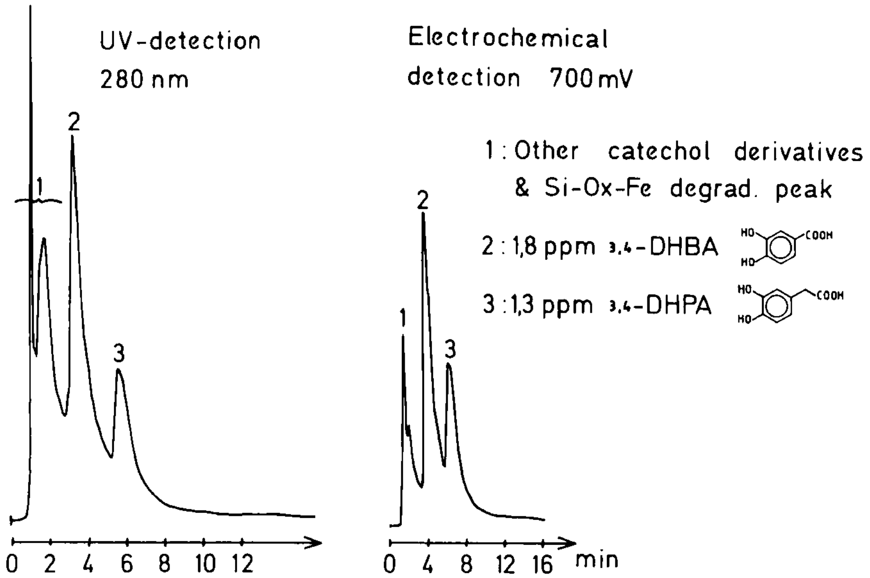

Figure 3 A comparison of UV (280 nm) and electrochemical (700 mV vs Ag/AgCl ref. electrode) detectors chromatograms obtained using 5 ml of urine extracted on Si-Ox-Fe pre-column (10 × 2 mm I.D.) followed by on-line elution and chromatography on Si-Cy-Cu separation column (20 cm × 4 mm I.D.). Mobile phase: CH_3COOH 4% in water/MeOH 20:80 (v/v).

possess at their surface and the transition metal they are loaded with. The introduction of a metal induces a better selectivity for organic compounds that are able to act as Lewis bases toward transition metals. So these silicas are just an additional tool in liquid–solid extraction because such compounds are generally not strongly retained on reversed or normal phase silicas. Outer-sphere ligand exchange chromatography is preferred for metal-loaded silicas in which all sites of the metal are irreversibly complexed by another ligand. A certain selectivity is induced by this type of complexation. Hence, the coupling of two different MLS, one acting as enrichment pre-column and the other as chromatographic stationary phase, makes the method very specific for compounds whose properties correspond to the interactions selected by both metal-loaded silicas. A complex matrix such as urine may be analysed without any previous treatment.

Acknowledgements

The authors wish to thank R. Haering for his technical assistance in the elaboration and the designing of the pre-column.

References

1. R. W. Frei and U. A. Th. Brinkman, *TrAC, Trends Anal. Chem.* **1**, 45 (1981).

2. H. P. M. Van Vliet, Th. C. Bootsman, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.* **185,** 483 (1979).
3. R. W. Frei, *Swiss Chem.* **6,** 55 (1984).
4. M. A. Bagnoud, J. L. Veuthey and W. Haerdi, *Chimia* **12,** 432 (1986).
5. C. E. Goewie, P. Kwakman, R. W. Frei, U. A. Th. Brinkman, W. Maasfeld, T. Seshadri and A. Kettrup, *J. Chromatogr.* **284,** 73 (1984).
6. M. W. F. Nielen, R. Bleeker, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.* **358,** 393 (1986).
7. J. L. Veuthey, M. A. Bagnoud and W. Haerdi, *J. Chromatogr.* **393,** 51 (1987).
8. J. L. Veuthey and W. Haerdi, submitted to publication.
9. A. A. Descombes and W. Haerdi, submitted to publication.
10. F. K. Chow and E. Grushka, *J. Chromatogr.* **185,** 361 (1979).
11. F. K. Chow and E. Grushka, *Midl. Macromol. Monogr.* **7,** 301 (1980).
12. M. Sinibaldi, V. Carunchio, A. Messina and C. Corradini, *Annali di Chimica* (*Roma*), **74,** 175 (1984).
13. M. A. Bagnoud and W. Haerdi, to be published.
14. A. A. Descombes, personal communication.
15. C. J. Shahawn and J. R. Jezorek, *J. Chromatogr.* **256,** 39 (1983).
16. J. L. Veuthey, M. A. Bagnoud and W. Haerdi, *Intern. J. Environ. Anal. Chem.* **26,** 157 (1986).