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# Note

## Argentation liquid chromatography of polynuclear aromatic hydrocarbons on a silver(I)-loaded mercaptopropyl silica gel stationary phase

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One of the more intensively investigated areas of high-performance liquid chromatography (HPLC) today involves the development of selective stationary phases to complement the general nature of non-polar reversed-phase materials. One facet of this effort has employed secondary chemical equilibria involving metal ions to effect greater selectivity in the separation of analyte ligands. The metal ion can be included in the mobile phase[1] or immobilized on the stationary phase[2,3]. This approach is generally called ligand-exchange chromatography (LEC), as an analyte ligand of interest is exchanged for a mobile phase solvent or buffer ligand in the coordination sphere of the metal ion[4].

An interesting and useful subset of LEC is argentation chromatography, where silver(I) is the metal ion employed[5]. The selective interaction of silver ion with nitrogen- and sulfur-containing species and unsaturated compounds is exploited in this approach. The relative merits of incorporating the silver ion in the stationary phase *versus* including it in the mobile phase have been discussed[1]. The major disadvantages of the mobile phase approach seem to be possible column deterioration and the build-up of metallic silver on the chromatographic system. Silver nitrate has been incorporated in the silica gel matrix, but bleeding of the metal from the column is a severe problem. Frei and co-workers[6] deposited insoluble silver halides onto silica gel and obtained some good separation of nitrogen-containing species, but interaction with unsaturated species was too weak to be useful due to the low coverage by the silver halide. Furthermore, with this approach, uneven deposition may leave residual silanols exposed and lead to mixed chromatographic mechanisms and poor efficiency. The use of high-capacity strong cation-exchange resins to bind the silver ion tightly minimizes the low coverage and bleeding problems, but these phases are not useable in the presence of buffers or other ion-containing eluents as silver will be lost by exchange with mobile phase cations. Walton[7] has discussed the use of ion-exchange materials as LEC supports.

This paper reports our initial results with mercaptopropyl silica gel (MPSG) as a stationary phase for argentation chromatography. This material appears to solve the bleeding and low-capacity problems which have been annoyances in the past use of this interesting and selective LC mode. As shown below, silver(I) is very tightly held to the sulfur site and thus provides a very stable stationary phase. Also, as coverage of the silica by the mercaptopropyl group can be made relatively high,

sufficient silver ion can be loaded to provide strong interaction with unsaturated compounds. Several polynuclear aromatic hydrocarbon (PAH) compounds were used for the initial chromatographic characterization of this stationary phase.

EXPERIMENTAL

*Apparatus*

Two chromatographic systems were used, a Beckman Model 324 consisting of two Model 100-A pumps and a Hitachi Model 100-10 detector, or a Beckman Model 110-A pump and Model-153 detector. Both systems employed 20-$\mu$l sample loops and a 254-nm detector wavelength. All columns were 25 cm × 4.0 mm I.D. glass-lined stainless steel (SGE, Austin, TX, U.S.A.) and were slurry packed using a Model-26980-4 pneumatic pump (Haskel, Burbank, CA, U.S.A.) according to the method of Manius and Tscherne[8]. An isopropanol–methanol (70:30) mixture was used as the slurrying medium, and methanol as the packing liquid. Columns were packed at 6000 p.s.i. for about 45 min.

*Reagents and solutions*

Water used for solutions was purified with a 3-cartridge system consisting of two deionizers and a charcoal filter (Milli-Q, Millipore, Bedford, MA, U.S.A.) or with a doubly deionized-distilled water system constructed in our laboratory[9]. The PAH test samples were about $10^{-7}$ $M$ and dissolved in either methanol or hexane. All solvents were either ACS Reagent or HPLC grade. Several silica gels were used in this study: a TLC-grade, 10–50-$\mu$m, 60-Å pore, 550-m$^2$/g material (type HR, E. Merck) for metal extraction and stoichiometry studies; Adsorbosil, a 10 $\mu$m, preparative grade silica with 70-Å pores and a 480 m$^2$/g surface area (Applied Science, Deerfield, IL, U.S.A.); 5 $\mu$m LiChrosorb Si 100 silica with 100-Å pores and a 300-m$^2$/g surface area (E. M. Science); and 5 $\mu$m Hypersil WP-300 (Shandon Southern, Sewickley, PA, U.S.A.) with 300-Å pores and a 60-m$^2$/g surface area. The 3-mercaptopropyltrimethoxysilane was obtained from either Petrarch Systems (Bristol, PA, U.S.A.) or Dow-Corning (Midland, MI, U.S.A.). It was stored in a refrigerator and used as received. Toluene used in the syntheses was dried over 4-Å molecular sieves.

*Syntheses*

The MPSGs were prepared by first heating the silica at about 120°C to remove adsorbed water; cooling under vacuum; refluxing with a toluene solution of 3-mercaptopropyltrimethoxysilane; filtering off the MPSG; washing it with toluene, methanol and/or acetone; and curing the solid at about 80°C under vacuum for several hours. Specifics for each preparation are given in Table I. Approximately a two-fold excess of silane to available silica –OH groups was used for the Adsorbosil material, nearly a twenty-fold excess for the LiChrosorb, and a 180-fold excess for the Hypersil. No real difference in surface coverage was observed for the Adsorbosil and LiChrosorb materials, these values being about 1.4 and 1.5 $\mu$mol/m$^2$ respectively. The coverage of the Hypersil-based MSPG, however, was about 2.9 $\mu$mol/m$^2$. Some of the fines were removed from the Adsorbosil-based MPSG by stirring up the material in water and pouring off the liquid after 10 min. This was repeated twice. All the MPSG materials were white or slightly off-white. If the MPSG-Ag materials were left out

TABLE I

SYNTHESIS CONDITIONS AND FINAL METAL-UPTAKE CAPACITY FOR MPSG PHASES

| Substrate material | Mass (g) | Volume toluene, silane (ml) | Reflux time (h) | Metal uptake (μmol/g) | Ligand coverage*** (μmol/m²) |
|---|---|---|---|---|---|
| TLC silica | 50 | 200, 5 | 2 | 400* | 0.7 |
| Adsorbosil | 20 | 175, 10 | 2.5 | 650** | 1.4 |
| LiChrosorb | 6 | 80, 20 | 5.5 | 440** | 1.5 |
| Hypersil | 4 | 80, 14 | 5 | 175** | 2.9 |

   * Based on mercury(II) extraction; carbon determination gave 383 μmol/g[1].
  ** Based on silver(I) extraction.
*** Assuming ligand: metal stoichiometry (1:1).

in the light for several months they turned tan or dirty yellow, but MPSG itself remained white.

*Capacity determination*

Generally a 100–200-mg sample of MPSG was mixed with a 20–50 fold excess of silver nitrate solution and stirred for 30 min. The solid was filtered off and washed with water. Washings and filtrate were combined and unextracted silver ion was determined by atomic absorption spectrometry (AAS). The capacity of the TLC silica-based MPSG was obtained by extraction of mercury(II). Unextracted mercury was determined by displacement titration with Mg-EDTA and Erichrome Black T indicator.

In some cases an acid digestion of the silver-loaded MPSG was performed. Three 5-ml aliquots of concentrated nitric acid were added to 100–200 mg of the MPSG-Ag and the mixture was heated for several min after each addition to free the silver ion, which was determined by AAS.

*Loading of silver(I) on MPSG*

To about 4 g of MPSG 6 ml of 1 $M$ silver nitrate solution and 10–20 ml of water were added. The mixture was stirred for about 20 min. The solid was filtered off and washed with water and then methanol.

*Extraction studies*

About 200 mg of MPSG were placed in a 100-ml beaker and 30 ml of water and 0.04 mmol of the desired metal ion were added. Dilute hydrochrloric acid or sodium hydroxide were used to adjust the pH to that desired. Stirring was continued for 15 min and the final pH reading was made. The solid was filtered off, washed with water, and washings and filtrate combined. The unextracted cadmium(II) and zinc(II) were determined by direct EDTA titration; mercury(II) and lead(II) by displacement titration with Mg-EDTA; and silver(I) by the Volhard method.

*General chromatographic conditions*

All columns were first equilibrated with 20–30 ml of mobile phase. Most runs used a flow-rate of 1 ml/min. All injection volumes were 20 μl, and detector wave-

length was 254 nm. Mobile phases were helium-degassed for 5 min before use. All runs were at ambient temperature.

RESULTS AND DISCUSSION

*Stationary phase characteristics*

While it is well known that silver(I) has a high affinity for sulfur-containing compounds and sulfide ion (*e.g.* $pK_{sp}$ for silver(I) sulfide is *ca.* 50) it was desirable to test the interaction of silver and several other ions with MPSG. As seen in Fig. 1 silver(I) [and mercury(II)] are completely extracted by MPSG even at pH 0. In fact, only about 75% recovery of extracted silver ion was obtained with 6 *M* nitric acid. These extraction characteristics imply possible utility for MPSG as a silver-scavanging material. Efforts are underway in our laboratory to develop methods to recover the metal ion from the MPSG without destroying the solid phase.

Another implication of the extraction data is that silver-loaded MPSG should be an extremely stable HPLC stationary phase, even in acidic eluents. Indeed, column effluents were periodically monitored for silver(I) during this work by AAS and no significant loss of silver was observed. In addition, several columns were emptied after considerable use, the packing was acid-digested, and silver ion was determined by AAS. The initial silver-loading values compared favorably with the acid-digestion results, within experimental error [*e.g.* 650 $\mu$mol/g silver(I) for fresh MPSG-Ag, *versus* 670 $\mu$mol/g after 90 h of use for an Adsorbosil-based column].

While aminopropyl silica gel (APSG) has been shown to exhibit 2:1 ligand-to-metal stoichiometry with copper(II)[10], mercaptopropyl silica gel exhibits 1:1 stoichiometry with mercury(II) and silver(I). Extraction of mercury(II) (large excess of metal ion) with one batch of MPSG yielded a metal uptake value of 400 $\mu$mol/g while elemental carbon analysis gave a ligand surface coverage value of 383 $\mu$mol/g. Another batch of MPSG had a silver-uptake value of about 350 $\mu$mol/g and an elemental
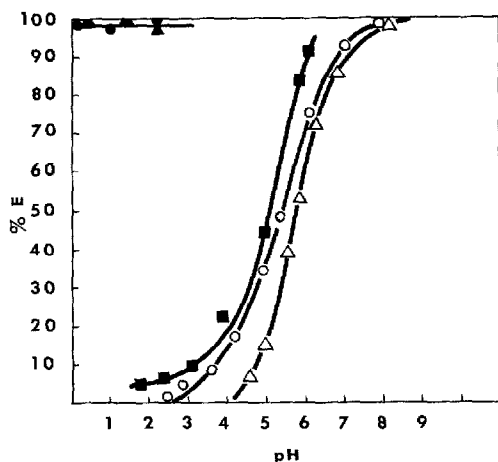


Fig. 1. Percent metal ion extracted (%E) by MPSG as a function of pH. Metal-uptake capacity was about 400 $\mu$mol/g. ● = mercury(II); ▲ = silver(I); ■ = lead(II); ○ = cadmium(II); △ = zinc(II).

TABLE II

RETENTION ON MPSG AND MPSG-Ag IN METHANOL–WATER MIXTURES

Both phases were from the same batch of Adsorbosil-based material, 680 μmol/g.

| Compound | Retention time (min) | | | |
|---|---|---|---|---|
| | MPSG | | MPSG-Ag | |
| | 70:30 | 50:50 | 70:30 | 50:50 |
| Naphthalene | 2.9 | 3.6 | 4.4 | – |
| Acenaphthene | 3.0 | 6.2 | 6.2 | 16.3 |
| Anthracene | 3.0 | 9.3 | 9.6 | 36.5 |

sulfur analysis of 367 μmol/g. The implication of these results is that one of the two silver(I) coordination sites is available for interaction with analyte ligands.

With regard to the reactivity of these phases we have indirect evidence that the –SH groups of MPSG columns can be gradually converted to disulfides by certain mobile phases or samples. However, in the silver-loaded form, this sort of column degradation is minimized. Furthermore, protection of the –SH group by the silver ion permits selective end capping of residual silanols, while the silver content of the MPSG-Ag is not substantially altered, even in refluxing toluene-trimethylchlorosilane. One batch of MPSG-Ag had silver contents of 136 and 133 μmol/g before and after end capping, respectively.

*Chromatographic characterization*

As silver ion is expected to interact less strongly with unsaturated compounds than with nitrogen or sulfur bases, the PAHs were chosen as initial test probes for the MPSG-Ag phases. We first compared a MPSG column with a MPSG-Ag column to see what effect the silver ion had on retention of the PAHs. It is clear from Table II that there is significantly less interaction of the PAHs with the mercaptopropyl group itself than with the silver-loaded phase. Indeed, at high methanol content there is practically no interaction of the smaller PAHs, whereas interaction with MPSG-Ag is extensive.

It would appear that, for aromatic compounds at low water content, essentially a single solute–stationary phase mechanisms obtains for MPSG-Ag columns, namely the interaction with the silver ion.

A second portion of the same batch of Adsorbosil-based MPSG was loaded to a lower silver content, 400 μmol/g as compared to 680 μmol/g for the first batch, and several PAHs run in methanol–water (70:30) and compared to the same system on the more highly-loaded column. Not unexpectedly, shorter retention times were observed with lower silver loading. For example, retention times for anthracene were 9.6 min on the more heavily loaded and 6.2 min on the less heavily loaded column.

MPSG-Ag was used for the separation of mixtures of several PAHs in both normal- and reversed-phase eluents. In Fig. 2 is shown the chromatogram for five compounds in isooctane–isopropanol (70:30) on Adsorbosil-based MPSG-Ag. Good resolution is achieved in about 8 min.
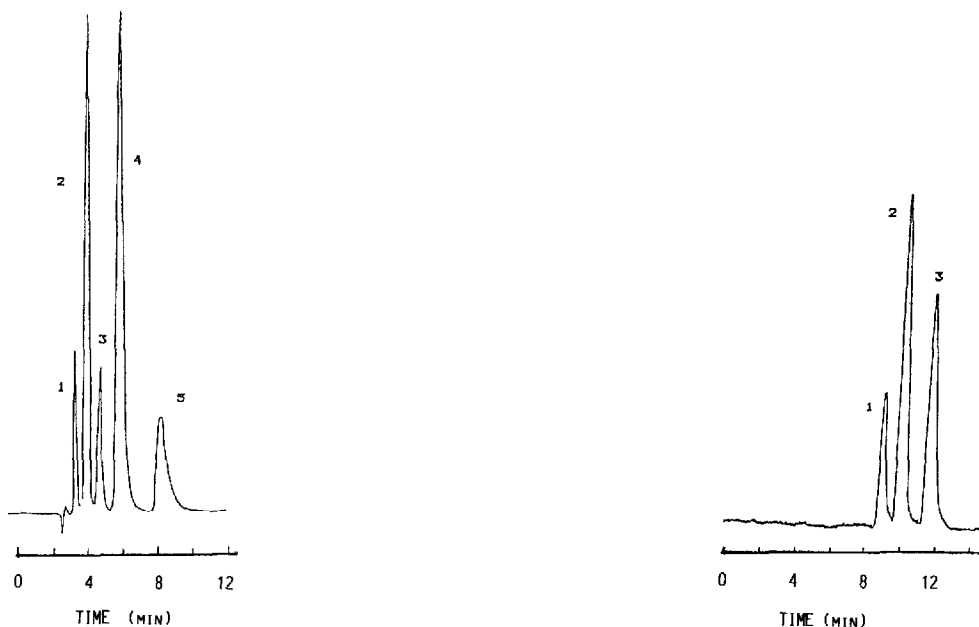
Fig. 2. Separation of five PAHs on 10 μm, Adsorbosil-based MPSG-Ag, uptake capacity 650 μmol/g, in isooctane–isopropanol (70:30, v/v). 1 = acenapthene; 2 = anthracene; 3 = pyrene; 4 = chrysene; 5 = perylene.

Fig. 3. Separation of three PAHs on 5 μm, LiChrosorb Si 100-based MPSG-Ag, uptake capacity 440 μmol/g, in 100% methanol. 1 = benzo[b]fluoranthene; 2 = benzo[k]fluoranthene; 3 = benzo[a]pyrene.

Some work was also done with 5-μm diameter phases. In Fig. 3 is shown the separation of three 5-ring compounds in 100% methanol on 5-μm LiChrosorb Si 100 based MPSG-Ag. Extensive interaction of these aromatic compounds evidently occurs, as even in this strong mobile phase significant retention is observed.

A finding which suggests that further investigation is required, however, is the significant drop in efficiency on going from 3-ring compounds to larger ones, as exhibited by broader peaks with noticeable tailing. For example, the number of theoretical plates for the Adsorbosil-based 70-Å MPSG-Ag column was 3200 for acenapthene, 2700 for naphthalene, but dropped to 1000 for chrysene (four rings) and to 500 for perylene (five rings) in isooctane–isopropanol (92:8). Similar effects were found for other MPSG-Ag columns and other mobile phases. Anthracene exhibited a plate count of 2500 on a LiChrosorb Si 100 based MPSG-Ag column in methanol water (75:25), while the number of plates for pyrene (four rings) was just 1300.

It is possible that these effects are due to increasingly poor mass transfer in the relatively small-pore structure of the 70- and 100-Å pore materials. Sander and Wise[11] showed that larger ring compounds are more efficiently separated on larger pore silicas, and so we performed some preliminary studies on 300-Å Hypersil silica. A trade off is involved, however, as the larger pore materials have lower surface areas. Indeed, the capacity of the Hypersil-based MPSG was only about 175 μmol/g,

although this coverage, 2.9 $\mu$mol of ligand per m$^2$ of original surface, is more complete than that of the smaller pore materials described above. In any case, our preliminary qualitative results do indicate that higher efficiency is obtained for the larger ring compounds on this large-pore material. More intensive investigation is required, however, before we can be more definitive about this question.

It is also possible that some of the tailing is due to interaction of the larger, more polarizable PAHs with residual silanol sites. The fact that poor peak shape and long retention times were observed in 100% hexane or isooctane but that only a fraction of a percent of isopropanol sharpended up the peaks and dramatically reduced retention lends some credence to this suggestion. The polar co-solvent apparently attenuates the effect of the silanols. We are presently studying the effect of end capping the MPSG-Ag phases to determine if better efficiencies are obtained as compared to those on the uncapped material described here.

While the PAHs can be separated quite well on reversed-phase non-polar columns, the significant interaction of these test compounds with the MPSG-Ag columns, and the stability of the phases under use, imply that these materials can be employed for more difficult separations requiring the greater selectivity that the silver(I) ion affords. Intensive investigation is underway in our laboratory with several classes of nitrogen- and sulfur-containing analytes on these new materials.

ACKNOWLEDGEMENTS

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