CHEMISTRY LETTERS, pp. 881-884, 1994. 

# Preparation and Characteristics of Pyridyl and 4,5-Dihydroimidazolyl Groups Bonded Silica Gels as New Column Packing Materials for Separation of Metal Chelates by High Performance Liquid Chromatography

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Pyridyl and 4,5-dihydroimidazolyl groups bonded silica gel packed columns were newly prepared and applied to high performance liquid chromatographic analysis of coordinatively saturated as well as unsaturated metal chelates, *i.e.*, cobalt(III) and copper(II) acetylacetonates, respectively. The separation of those metal chelates may be caused by coordination interaction between the central metals and the immobilized neutral ligands.

Reversed phase high performance liquid chromatography (RP-HPLC) has often been applied to the separation of metal chelates, in which the mechanism of separation is supposedly based on their partition between mobile phase (polar solvent) and stationary phase (nonpolar solvent).[1] However, the mutual separation of metal chelates is not always an easy task. To improve such a situation, we have successfully proposed the utilization of adduct formation and masking effect for the mutual separation of some metal chelates in RP-HPLC, in which neutral ligands such as pyridine bases were added to the mobile phase.[2-4] On the other hand, if such ligands were immobilized on the stationary phase support, metal chelates could be separated each other with the coordination interaction between the central metals and the immobilized neutral ligands.

In the present work, we immobilized different pyridyl and 4,5-dihydroimidazolyl groups on silica gel as shown in Fig.1 and investigated the retention behavior of coordinatively saturated as well as unsaturated metal chelates [cobalt(III) and copper(II) acetylacetonates: $Co(acac)_3$ and $Cu(acac)_2$] on those columns.

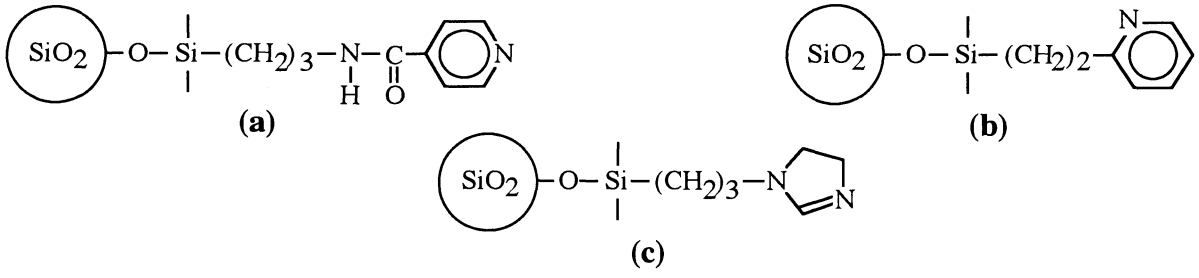


Fig. 1. Structure of modified silica gels.
(a): 3-(4-pyridinecarboxamide)propyl bonded silica gel (4-PCAPS); (b): 2-(2-pyridyl)ethyl bonded silica gel (2-PES); (c): 3-(4,5-dihydroimidazol-1-yl)propyl bonded silica gel (4,5-DHIPS).

The silica gel used for this work was Super Micro Bead Silica Gel 100A-10D (mean particle size, 9.4 $\mu$m

(spherical); mean pore diameter, 133 Å; surface area, 344 $m^2g^{-1}$; pore volume, 1.14 $mlg^{-1}$) from Fuji Silysia Chemical Ltd. The solvents used for the mobile phase were of HPLC grade and all the other chemicals used were of guaranteed grade and used without further purification.

Preparation procedure of 3-(4-pyridinecarboxamide)propyl bonded silica gel (4-PCAPS, Fig. 1(a)): Aminopropyl-bonded silica gel (APS) was prepared according to the method of Nomura *et al.*[5] The APS (10 g) and isonicotinoyl chloride hydrochloride (14.8 g, Aldrich) in pyridine (100 ml) were refluxed with stirring for 30 min. After the vessel was cooled to room temperature, the 4-PCAPS was filtered off by suction using a sintered glass funnel, and washed successively with methanol, deionized water until *p*H of the washings was almost neutral, then with methanol-deionized water (1:1,v/v), methanol and finally with dichloromethane. It was dried in vacuo at 90–100 °C for 8 h.

Preparation of 2-(2-pyridyl)ethyl bonded silica gel (2-PES, Fig. 1(b)): The native silica gel (3.3 g) and 2-(trimethoxysilyl)ethyl-2-pyridine (1 ml, Chisso) in toluene (33 ml) were refluxed with stirring for 1 h. After cooling to room temperature, the 2-PES was filtered off by suction and washed successively with toluene and dichloromethane and was dried in vacuo at 90–100 °C for 8 h.

Preparation of 3-(4,5-dihydroimidazol-1-yl)propyl bonded silica gel (4,5-DHIPS, Fig. 1(c)): The native silica gel (3.2 g) and N-[3-(triethoxysilyl)propyl]-4,5-dihydroimidazole (1.2 ml, Chisso) in toluene (32 ml) were refluxed with stirring for 1 h. The 4,5-DHIPS was treated with the same procedure as described on the 2-PES.

Endcapping of the residual silanol group: Those modified silica gels (3.3 g) and N-trimethylsilylimidazole (2 ml, Chisso) in chloroform (37 ml) were refluxed with stirring for 8 h. After cooling to room temperature, the endcapped silica gel was filtered off by suction and washed successively with chloroform and dichloromethane and was dried in vacuo at 90–100 °C for 8 h. The amounts of the neutral coordinating groups immobilized on silica gel were estimated from the nitrogen content before endcapping (Table 1).

Packing of Columns: About 2.5 g of the endcapped silica gel was slurried with 20 ml of liquid paraffin-carbon tetrachloride (1:1,v/v)[6] by stirring, then in ultrasonic bath for 5 min. The slurry was packed into 250 mm X 4.6 mm i.d. stainless-steel tube by pumping 200 ml of hexane as a packing solvent under a pressure of 29.4 MPa.

The mixed solvent of cyclohexane-ethanol was used as mobile phase to study the separation behavior of metal chelates on the modified silica gel packed columns. Cobalt(III) and copper(II) acetylacetonates were used as analytes. They were dissolved in chloroform and aliquots (15 $\mu$l) of the solution were injected into the columns.

Table 1. Elemental analysis and surface coverage of coordinating groups on modified silica gels

| | H / % | C / % | N / % | surface coverage / mmol/g |
|---|---|---|---|---|
| APS | 1.39 | 5.05 | 1.84 | 1.31 |
| 4-PCAPS | 1.65 | 13.70 | 3.46 | 1.32[a] |
| Endcapped 4-PCAPS | 1.78 | 14.26 | 3.20 | |
| 2-PES | 1.01 | 8.21 | 1.20 | 0.86 |
| Endcapped 2-PES | 1.37 | 9.39 | 1.23 | |
| 4,5-DHIPS | 1.53 | 6.91 | 2.23 | 0.80 |
| Endcapped 4,5-DHIPS | 1.62 | 7.74 | 2.20 | |

a) Estimated according to the calculation method in reference 12.

For the preliminary evaluation of the modified silica gel packed columns, we tried to separate $Co(acac)_3$ and $Cu(acac)_2$ from each other. As $Co(acac)_3$ is kinetically inert[7] and $Cu(acac)_2$ is highly stable,[8] they are resistant against dissociation in the column. Since $Co(acac)_3$ is a coordinatively saturated metal chelate, it should hardly interact with the neutral coordinating group in the stationary phase. On the other hand, although $Cu(acac)_2$ shows relatively weak interaction with a neutral ligand because of Jahn-Teller effect, the adduct formation of $Cu(acac)_2$ with pyridine bases was still reported; *i.e.*, the adduct formation constant is 2.0 for pyridine and 0.66 for 2-methylpyridine.[9] Therefore, $Cu(acac)_2$ may possibly be retained on the neutral coordinating group in the stationary phase. For the separation of the metal chelates on those modified silica gel packed columns, cyclohexane was used as a major component of the mobile phase to avoid dissociation of metal chelates in the column and deterioration of the column by dissolution of silica support due to basicity of pyridyl and 4,5-dihydroimidazolyl groups on the silica surface. Moreover, ethanol was added to the mobile phase as a competitive ligand. Since ethanol can coordinate to the central metal in a coordinatively unsaturated metal chelate, it would compete with the neutral coordinating group in the stationary phase. Furthermore, the proton of hydroxyl group in ethanol can form the hydrogen bond to the nitrogen atom of the neutral coordinating group in the stationary phase. Therefore, ethanol would desorb those metal chelates from the stationary phase due to the above two reasons.

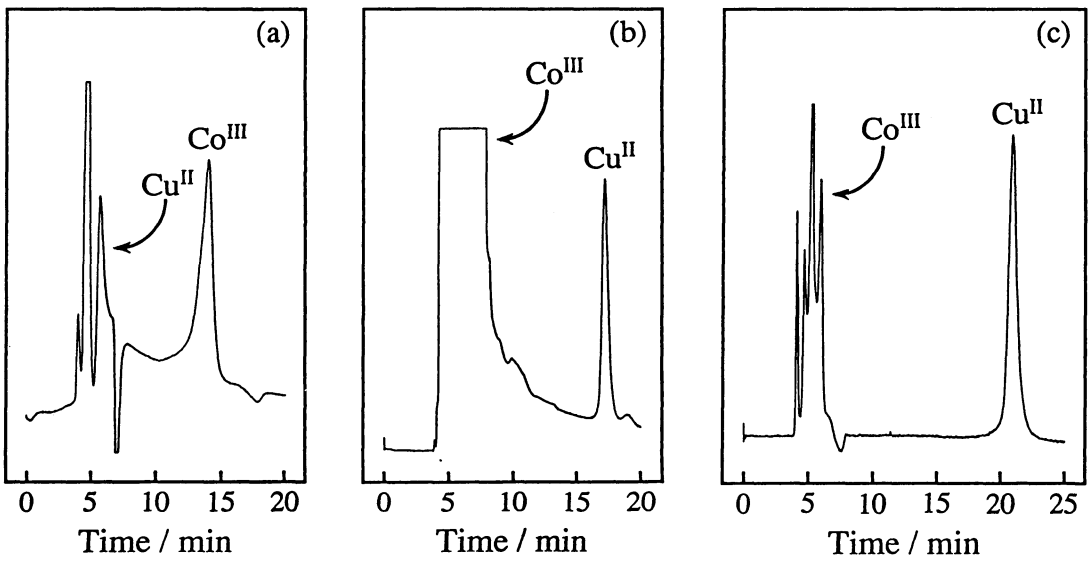


Fig. 2. Chromatograms of $Co(acac)_3$ and $Cu(acac)_2$ on the 4-PCAPS (a), the 2-PES (b) and the 4,5-DHIPS packed column (c).
HPLC conditions: flow rate, 0.8 ml $min^{-1}$; column temperature, 35 °C; detector wavelength, 254 nm for (a) and (b), 280 nm for (c); mobile phase, cyclohexane-ethanol (99.5 : 0.5, v/v) for (a), (99 : 1, v/v) for (b), (98 : 2, v/v) for (c).

Figure 2 shows the typical chromatograms of $Co(acac)_3$ and $Cu(acac)_2$ on the 4-PCAPS (a), the 2-PES (b) and the 4,5-DHIPS packed column (c). In Fig.2(b), the peak of $Co(acac)_3$ overlapped with the blank peak. When only cyclohexane was used as the mobile phase, $Cu(acac)_2$ was eluted but $Co(acac)_3$ retained in the 4-PCAPS packed column, while $Co(acac)_3$ was eluted but $Cu(acac)_2$ retained in the 2-PES and the 4,5-DHIPS packed columns. If the retention mechanism is explained by the coordination interaction,

coordinatively unsaturated metal chelates should be retained on the neutral coordinating group in the stationary phase, while coordinatively saturated ones would hardly be retained. However, the experimental result on 4-PCAPS packed column (Fig.2(a)) differs from the above consideration. The molecular structure of pyridyl amide moiety in 4-PCAPS is similar to that of isonicotinamide. Since the *p*Ka value of isonicotinamide (3.67)[10] is almost as low as that of formic acid (3.49),[10] pyridyl group in 4-PCAPS may act as an acid rather than as a base because of the electron-attracting amide group as a spacer. Thus, the pyridyl group in 4-PCAPS might hardly coordinate to $Cu(acac)_2$. Consequently, the retention of $Co(acac)_3$ and $Cu(acac)_2$ on the 4-PCAPS packed column may be ascribed to the hydrophobic interaction with the aromatic and spacer parts in the stationary phase. On the other hand, as shown in Fig. 2(b) and (c), the results on the 2-PES and 4,5-DHIPS packed columns are in accordance with the above consideration, *i.e.*, the coordination interaction between metal chelates and pyridyl and 4,5-dihydroimidazolyl groups in the stationary phases should be dominant. Because steric hindrance caused by 2-position (Fig. 1(b)) may not be necessarily negligible on the 2-PES, and the basicity of 4,5-dihydroimidazolyl group on the 4,5-DHIPS should be stronger than that of pyridyl group on the 2-PES,[10, 11] the 4,5-dihydroimidazolyl group may have higher coordinating ability than the pyridyl group. The above view corresponds to the fact that the retention time of $Cu(acac)_2$ on the 4,5-DHIPS packed column is longer than that of 2-PES as shown in Fig. 2(b) and (c).

In conclusion, the mutual separation of $Co(acac)_3$ and $Cu(acac)_2$ on the stationary phases with neutral coordinating groups such as the 2-PES and the 4,5-DHIPS packed columns was dominantly caused by the coordination interaction. We are now undergoing the synthesis of 2-(4-pyridyl)ethyl bonded silica gel (4-PES), and the application of the 2-PES, the 4,5-DHIPS and the 4-PES packed columns to the mutual separation of metalloporphyrins.

We thank Dr. A. Nomura, National Institute of Materials and Chemical Research, and Mr. K. Nobuhara, Fuji Silysia Chemical Ltd., for their useful help and discussion. This work was supported in part by a Grant-in-Aid for Scientific Research No. 03640486 from the Ministry of Education, Science and Culture.

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( Received February 4, 1994 )