Liquid chromatographic separation of metal–tetraphenylporphine complexes based on coordination interaction with immobilized neutral ligand in stationary phase

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Abstract

Proposed is a novel liquid chromatographic system for metal chelates based on the coordination interaction between the central metal in a complex and immobilized neutral ligand in the stationary phase. For this purpose, a 2-(4-pyridyl)ethyl bonded silica gel (4-PES) column was newly prepared, and cobalt(II)-, nickel(II)-, copper(II)-, and zinc(II)–tetraphenylporphine (TPP) complexes were separated on the 4-PES column as well as on previously prepared 4,5-dihydroimidazolyl bonded silica gel, 2-(2-pyridyl)ethyl bonded silica gel and a commercial aminopropyl bonded silica gel, packed columns in the normal phase mode. Among them, the strongest retention for the metal–TPP complexes which are considered to be coordinatively unsaturated, was observed on the 4-PES column, indicating that the separation of the metal–TPPs on these columns may mainly be based on the proposed mechanism. © 1997 Elsevier Science B.V.

Keywords: Coordination interaction; Metal complexes; Tetraphenylporphine

1. Introduction

Recently, the mutual separation of metal chelate compounds by high-performance liquid chromatography (HPLC) has become an important subject in the analytical chemistry of trace metals [1–3]. Although a lot of studies have been reported on this subject, it is not always easy to obtain adequate chromatographic separation of these compounds. One of the reasons may be that the central metal of a metal chelate is often surrounded by relatively large molecules, i.e., organic ligands, and hence the metal chelates often exhibit very similar chromatographic behavior.

In the solvent extraction of metal chelates, the so-called synergistic effect has been utilized to improve the extractability of an analyte and sometimes to enhance the extraction rate [4,5]. This effect is due to an adduct formation, i.e., the substitution of neutral ligands such as pyridine bases for residual water molecules coordinating to the central metal in an coordinatively unsaturated metal chelate. We have intended to apply the adduct formation to the HPLC separation of metal chelates. That is, we showed that the synergistic and/or the masking effect of neutral ligands such as pyridine bases, which are added to the mobile phase, can be used to control the degree

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of separation of some metal chelates by reversed-phase HPLC [6,7]. Moreover, we reported the preparation of new silica gel packing materials on which 4,5-dihydroimidazolyl and different pyridyl groups were covalently immobilized. These packing materials were preliminarily applied to the mutual separation of some metal acethylacetonates where the coordination interaction between the central metals and the immobilized neutral ligands might play an important role [8]. Two of these column packings, that is, the 4,5-dihydroimidazolyl bonded silica gel (4,5-DHIPS) and 2-(2-pyridyl)ethyl bonded silica gel (2-PES), are shown in Fig. 1a and b, respectively. Although such a coordination interaction has been utilized for the separation of some compounds in gas chromatography (GC) and HPLC where the metal chelates are used as the stationary phase [9–17], the present idea, that is, the separation of metal chelates based on the coordination interaction between the central metals and the immobilized neutral ligands in the stationary phase, has never been proposed.

In this study, in order to realize this novel concept for the separation of metal chelates, the 2-(4-pyridyl)ethyl bonded silica gel (4-PES, Fig. 1(c)), a stereoisomer with 2-PES, was newly prepared. More-

over, to evaluate the separation mechanism of these columns, some metal-tetraphenylporphine (TPP) complexes, i.e., Co^{II}-, Ni^{II}-, Cu^{II}-, and Zn^{II}–TPPs, were separated on the 4-PES column as well as 2-PES, 4,5-DHIPS, and a commercial aminopropyl-bonded silica gel (APS) packed columns in the normal phase mode. Although the N-atoms of 2-PES and 4-PES may have almost the same basicity, we expect that 4-PES will show a much greater coordination ability to coordinatively unsaturated metal chelates than 2-PES because of the steric hindrance of the ethyl group at the 2-position to the N-atom in 2-PES. Thus, a comparison between these two columns may provide important information on the separation mechanism of the metal chelates. Moreover, these metal–TPP complexes are stable and often exhibit similar chromatographic behavior to one another because the central metal ion is covered by a bulky porphyrin group, and hence they may be suitable for the evaluation of these columns.

2. Experimental

2.1. Apparatus

The HPLC system consisted of a Shimadzu (Model LC-9A, Japan) pump, a Rheodyne (Model 7010) sample injection valve with a Rheodyne (Model 7012, U.S.A.) loop filler port and a Rheodyne (Model 7020) 5-μl sample loop, a Shimadzu (Model CTO-2A) column oven, a Hitachi (Model L-4200, Japan) UV–Vis detector, and a Shimadzu (Model Chromatopac C-R6A) chromatographic data processor. An Iwaki (Model V-LDX KM, Japan) shaker, a Kokusan (Model H-102, Japan) centrifuge, and a Horiba (Model F-16, Japan) pH meter were also used.

2.2. Materials and reagents

The silica gel used in this study was Super Micro Bead Silica Gel 100A-10D (mean particle size, 9.4 μm (spherical); mean pore diameter, 13.3 nm; surface area, 344 $m^2 \cdot g^{-1}$; pore volume, 1.14 $ml \cdot g^{-1}$) from Fuji Silysia Chemical, Japan, which was the same as that used in the previous paper [8]. 4-[2-(Trichlorosilyl)ethyl]pyridine (25% in toluene) and
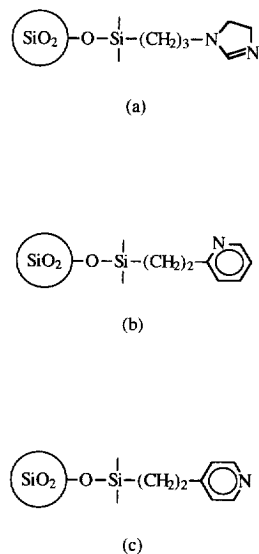

Fig. 1. Structure of coordinating group-bonded silica gels. (a): 3-(4,5-dihydroimidazol-1-yl)propyl bonded silica gel (4,5-DHIPS); (b): 2-(2-pyridyl)ethyl bonded silica gel (2-PES); (c): 2-(4-pyridyl)ethyl bonded silica gel (4-PES).

N-trimethylsilylimidazole were obtained from Chisso (Japan). The cobalt(II)-, nickel(II)-, copper(II)-, and zinc(II)–TPP complexes were purchased from Aldrich (USA). The aminopropyl-bonded silica gel used for the comparison with the stationary phases prepared in this work was Chromatorex NH-DU0010 (particle size, 10 μm (spherical); pore diameter, 100 Å; aminopropyl-group surface coverage, 0.94 mmol· g^{-1}; endcapped), which was kindly donated from Fuji Silysia Chemical. The solvents used for the mobile phase were of HPLC grade except for ethanol. All other chemicals used were of guaranteed grade and used without further purification.

2.3. Preparation of modified silica gels and their packed columns

2.3.1. Preparation of 2-(4-pyridyl)ethyl bonded silica gel (4-PES, Fig. 1(c))

The native silica gel (3.2 g), 4-[2-(trichlorosilyl)ethyl]pyridine (25% in toluene) (11 ml), and pyridine (2.8 ml) in toluene (23 ml) were refluxed with stirring for 6 h. After cooling to room temperature, the 4-PES was filtered off by suction using a sintered-glass funnel and washed successively with chloroform and a small amount of methanol, then placed in a centrifuge tube. This tube was filled with a 0.1 M NaH_2PO_4–0.1 M Na_2HPO_4 buffer solution (pH 7) and vigorously shaken. After the buffer solution and the 4-PES were separated by centrifugation at 8000 rpm for 20 min, the supernatant (pH 4–5) was discarded. This tube was filled once more with the phosphate buffer solution (pH 7), and the 4-PES was dispersed. The 4-PES was filtered off by suction using a sintered-glass funnel and washed successively with deionized water (until the pH of the washings became almost neutral), then with methanol–deionized water (1:1, v/v), methanol, and finally with dichloromethane. It was then dried in vacuo at 90–100°C for 8 h. Endcapping of the residual silanol group in the modified silica gel was treated using the same method as described in the previous paper [8]. The amount of the neutral coordinating group immobilized on the silica gel (0.8 mmol·g^{-1}) was estimated from the nitrogen content before endcapping (see below). Anal. Found: C,

8.21; H, 1.01; N, 1.20% for 4-PES, C, 9.39; H, 1.37; N, 1.23% for endcapped 4-PES.

2.3.2. Column packing

The prepared 4-PES and commercial APS were packed into a 250 mm×4.6 mm I.D. stainless steel tube by the method described previously [8].

The 2-PES and 4,5-DHIPS packed columns in this study were identical with those used previously [8]. Although the 2-PES packed column has already been prepared by R.E. Majors and M.J. Hopper, they used this column for the separation of azobenzenes and steroids but not for metal chelates [18].

2.4. HPLC conditions

The mixed solvent of toluene–ethanol was used as the mobile phase throughout the experiments. The flow-rate of the mobile phase was set at 0.8 ml· min^{-1}. The temperature of the column was maintained at 40°C throughout the measurements. Among the temperatures tested (30 to 40°C), 40°C gave the most favorable separation. This may be attributable to relatively slow exchange kinetics on the columns at lower temperature. The detection wavelength was 409 nm unless otherwise indicated. The cobalt(II)-, nickel(II)-, copper(II)- and zinc(II)–TPP complexes were dissolved in chloroform to give a final concentration of 1.0×10^{-5} M, and aliquots (with a 5-μl loop injector) of the solution were injected into the columns.

3. Results and discussion

The schematic diagram of the separation mechanism of a metal chelate which we postulate on the prepared coordinating group bonded silica gel column is shown in Fig. 2. When an analyte is a coordinatively unsaturated metal chelate, it may be retained on the stationary phase through a ligand exchange reaction between the residual water molecule and the neutral coordinating group in the stationary phase, then eluted with the addition of a competitive ligand into the mobile phase, i.e., ethanol, in this figure as an example.

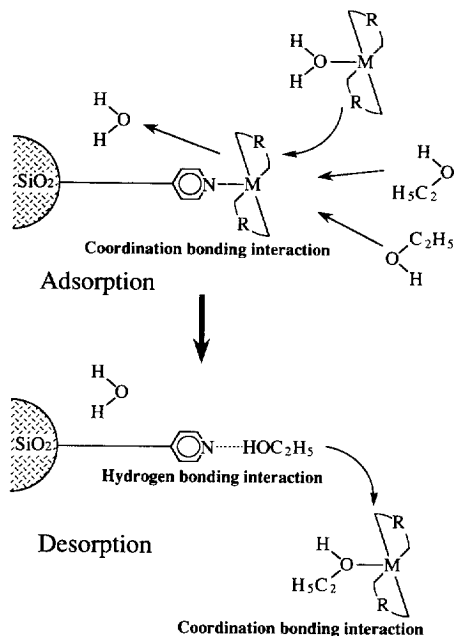Figs. 3 and 4 show the effect of the mobile phase composition on the retention of the metal–TPP

Fig. 2. Postulated separation mechanism of metal chelate compounds on coordinating group-bonded silica. R=chelating agent; M=metal.

complexes on the 4,5-DHIPS and 4-PES packed columns, respectively. Although the results are not shown, the same experiments were also done on the 2-PES and APS packed columns. The APS packed column gave almost the same results as the 4,5-DHIPS packed column. On the other hand, all the
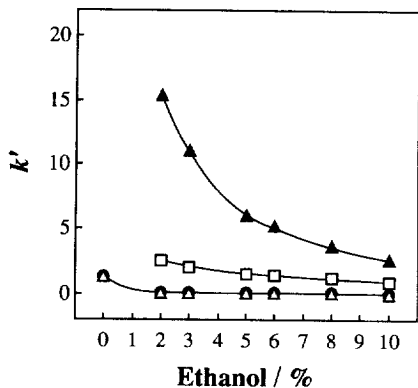

Fig. 3. Effect of ethanol content in the mobile phase on the retention of metal–TPP complexes on 4,5-DHIPS packed column. Mobile phase: toluene–ethanol (v/v). See Section 2 for further chromatographic conditions. □, Co^{II}; ●, Ni^{II}; △, Cu^{II}; ▲, Zn^{II}.
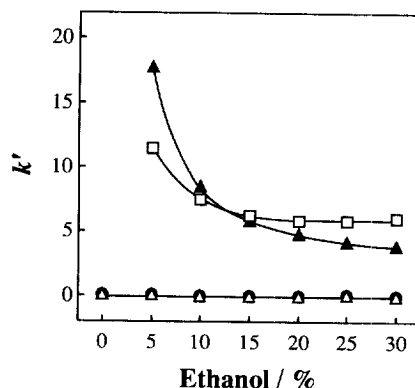

Fig. 4. Effect of ethanol content in the mobile phase on the retention of metal–TPP complexes on 4-PES packed column. Mobile phase: toluene–ethanol (v/v). Other chromatographic conditions are the same as in Fig. 3. □, Co^{II}; ●, Ni^{II}; △, Cu^{II}; ▲, Zn^{II}.

metal–TPP complexes were hardly retained on the 2-PES column, in other words, the 2-PES column showed the weakest retention ability for these complexes. As shown in Figs. 3 and 4, Co^{II}- and Zn^{II}–TPPs were strongly retained on 4,5-DHIPS and 4-PES, and the retention of the Co^{II}- and Zn^{II}–TPP complexes decreased with an increase in the ethanol content of the mobile phase. On the other hand, the Ni^{II}- and Cu^{II}–TPP complexes were hardly retained and came out in the void volume with the toluene–ethanol eluents on all the columns tested. These phenomena for the Cu^{II}- and Ni^{II}–TPPs can be explained as follows; a Cu^{II} chelate does not generally form a stable adduct because of the weak metal–ligand interaction in the axial directions of the chelate on the basis of the Jahn–Teller effect [19–22]. As for the Ni^{II}–TPP complex, its coordination reaction of a neutral ligand to the axial positions of the complex may be too slow because Ni^{II} is well known to be kinetically inert against ligand exchange in contrast to Co^{II} and Zn^{II} [23,24]. In addition to it, the complex formation constants of the Ni^{II}–TPP complex with nitrogenous bases are relatively small (see below). As is shown in the figures, the order of the retention of the metal–TPP complexes was Ni$^{II}\approx$ Cu$^{II}<$Co$^{II}<$Zn^{II} on all the stationary phases tested. This order was in accordance with that of the complex formation constants (Ni$^{II}\approx$Cu$^{II}<$Zn^{II}) of the metal–TPP complexes with nitrogenous bases

[25,26] and the retention volumes ($Co^{II} < Zn^{II}$) of nitrogen-containing compounds on the metal–TPP complex coated adsorbents in a packed-column GC [10]. Moreover, the order of retention ability for the Co^{II}- and Zn^{II}–TPP complexes was 2-PES < 4,5-DHIPS ≈ APS < 4-PES. This order was in good agreement with the retention volumes of the nitrogen-containing compounds (2-methylpyridine < ethyl-amine < 4-methylpyridine), which are analogous to the immobilized groups on the stationary phases in this study, on the Co^{II}- or Zn^{II}–TPP complex coated adsorbents in GC [10,11]. The weakest retention ability of 2-PES may be explained in terms of the steric hindrance due to the ethyl group at the 2-position. All the results mentioned above are in accordance with the proposed mechanism of Fig. 2.

Fig. 5 is a typical chromatogram of the metal–TPP complexes on the 4-PES packed column. The efficiencies of the columns tested were evaluated from the elution of the Zn^{II}–TPP complex with toluene–ethanol (90:10, v/v) as a mobile phase for the 4,5-DHIPS and APS columns and from that of the Co^{II}–TPP complex with toluene–ethanol (75:25, v/v) for the 4-PES. The theoretical plate number of the 4,5-DHIPS and 4-PES packed columns was 2700

and 2200, respectively, while that of the commercial APS packed column was 2300.

4. Conclusion

The separation system based on the coordination interaction between the central metal of the metal chelates and the stationary phase was accomplished on the stationary phases having immobilized neutral ligands (4,5-DHIPS, 2-PES, 4-PES, and APS). Among them, the strongest retention for the metal–TPP complexes which are considered to be coordinatively unsaturated, i.e., Co^{II}- and Zn^{II}–TPPs, was observed on the 4-PES column. The separation system based on such a concept may be particularly useful for the mutual separation of metals as porphyrin complexes.
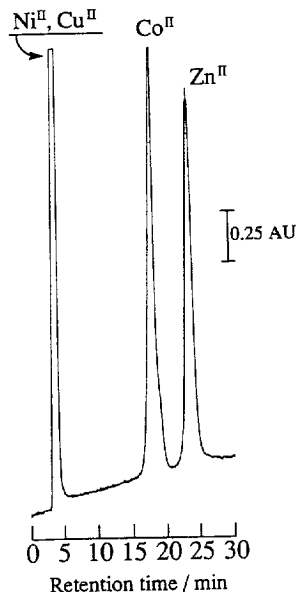
Acknowledgements

Fig. 5. Chromatogram of metal–TPP complexes on 4-PES packed column. Mobile phase: toluene–ethanol (75:25, v/v). Other chromatographic conditions are the same as in Fig. 3.

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