The Use of Hydrophilic Counterion for Superior Retention and Separation of Anionic Metal Chelates in Reversed-Phase Partition High Performance Liquid Chromatography

Emiko Kaneko, Hiroyuki Noda, Hitoshi Hoshino, and Takao Yotsuyanagi*
Department of Applied Chemistry, Graduate School of Engineering, Tohoku University, Aoba-ku, Sendai 980-77

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It has been found that hydrophilic ions, such as protonated tris(hydroxymethyl)aminomethane (H^+THMAM), are eminently suitable as counterion for reversed-phase partition high performance liquid chromatography. The anionic metal chelates with 2,2'-dihydroxyazobenzene were separated on C_{18}-bonded silica packing with an aqueous methanol mobile phase (pH 7.9) containing THMAM, and detected spectrophotometrically.

The ability of reversed-phase high performance liquid chromatography (RP-HPLC) as a highly sensitive and selective method has led to its widespread use in trace determination. There is also a large body of literature on ion-pair (IP) partition RP-HPLC. Earlier works demonstrated that the retention of charged eluites on nonpolar bonded stationary phases were augmented by the presence of large hydrophobic oppositely charged ions in the mobile phase.[1-3] Reagents, such as alkyl sulfates or alkyl sulfonates, increased the capacity factors for cations. Alkylammonium salts did likewise for anions. In early attempts, this technique was referred to as soap chromatography,[2] ion-pair chromatography,[4] and hetaeric chromatography.[5] In 1978, IPRP-HPLC was extended to charged metal chelates by Hoshino et al.,[6] and Valenty et al.,[7] independently. This technique has become extremely popular involving metal chelates and many other compounds of pharmaceutical or biochemical interest. There are also several established approaches in IPRP-HPLC to optimize separation quality by using a suitable composition of a binary mobile phase containing hydrophobic counterion. Because of the original concept of classical ion-pair liquid-liquid extraction,[6,8] most studies involving counterion have been limited to the use of hydrophobic ions. Although the effect of buffer components [9] or inorganic salts[10,11] on the retention was taken into account in a few studies, there do not appear to be any reports of RP-HPLC with hydrophilic counterion.

The object of this work was to develop a superior RP-HPLC for charged analytes with particular interest in separation by using hydrophilic counterions. Also it is urged that this technique not be viewed as a well-understood extension of a very classical idea of solvent extraction. In the discussion below, it is shown that a marked improvement in separation and sensitivity is attained when tris(hydroxymethyl)aminomethane (THMAM) was employed as the counterion and pH buffer agent for separation of anionic metal chelates with 2,2'-dihydroxyazobenzene (DHAB). In our studies on precolumn chelating agents, DHAB had been previously investigated for the determination of trace metal ions with IPRP-HPLC by using tetrabutylammonium (TBA^+) ion .[12-15] Such a series, with various hydrophilic ions, will span a large range of mobile phase composition and improve the separation quality of RP-HPLC.

The reagent, DHAB, was purchased from Dojindo Laboratories (Kumamoto, Japan) and used in the solution ca. 1.0×10^{-3} mol dm^{-3} that was prepared by dissolving it in a slightly alkaline (pH 9 - 10) aqueous solution containing 4 wt% PONPE- 20 (4-(nonyl)-phenoxypolyoxyethyleneglycol with 20 oxyethylene units, from Tokyo Kasei Kogyo Co. Ltd.). Except for vanadium(V) solution, which was prepared from ammonium metavanadate, the standard solutions of metal ions were prepared from the chlorides or the nitrates and standardized by complexometric titration with EDTA. Hydrochloric acid (ultrapure grade) and 3 mol dm^{-3} potassium hydroxide (ultrapure grade) were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). All other reagents were of guaranteed reagent grade. The mobile phase was an aqueous methanol solution (pH 7.9) containing 2×10^{-3} mol kg^{-1} THMAM or other buffer component and 1×10^{-4} mol kg^{-1} disodium EDTA. The HPLC set-up consisted of a JASCO PU-980 pump unit, and a JASCO UV-970 spectrophotometric detector with a 1 cm cell. The detection wavelength was 500 nm. A Cica-Merck LiChrospher RP-18 column (4 mm i.d. x 125 mm length) was also used.

A typical procedure is as follows: To a sample solution containing metal ions, add DHAB solution and the buffer solution. For complexation, heat the mixture in a water bath at 70°C for 20 min. After cooling to room temperature, dilute to 25 cm^3 with water. Inject the solution to HPLC with a 100 μL loop injector. The anionic chelates are separated on C_{18}-bonded silica packing with an aqueous methanol mobile phase that contains various counterions, and detected spectrophotometrically.

The counterion agents investigated extend from TBA^+ to the hydrophilic buffer components, THMAM, N-tris(hydroxymethyl)methyl- 2-aminoethanesulfonic acid (TES), N - tris - (hydroxymethyl) methyl - 3 - aminopropanesulfonic acid (TAPS), 2-hydroxy-N-tris(hydroxymethyl) methyl- 3-aminopropanesulfonic acid (TAPSO), and N-[tris(hydroxymethyl) methyl] glycine (Tricine). Although all the buffer components tested were effective to retard the anionic DHAB chelates, the best result was obtained when THMAM was employed as described below. In our previous reports[6,12-15] of kinetic differentiation mode RP-HPLC no chromogenic reagent was added in the eluent. The method was found to have a number of advantages for the determination of trace metal ions, offering a high sensitivity and selectivity. A highly efficient separation was accomplished by this technique. Labile chelates dissociated during elution and kinetically inert chelates and the reagent were eluted at very different retention

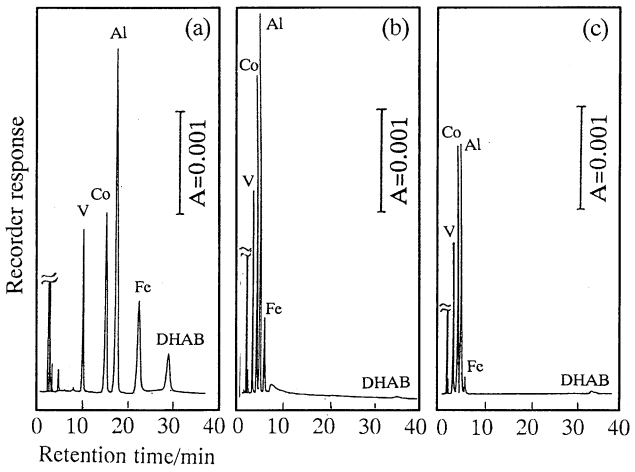

Figure 1. Effect of counterion on the chromatogram of DHAB chelates. Counterion: (a)TBA^+, (b)H^+THMAM, (c)H^+TAPSO^-; Metal ions added: Al, Be, Co, Cu, Cd, Fe, Mn, Mo, Ni, Pb, V, Zn; [Metal]: 5×10^{-7} mol dm^{-3} (for each); $[DHAB]_T$: 2×10^{-5} mol dm^{-3}; Mobile phase : (a) 60 wt% aqueous methanol containing 6×10^{-3} mol kg^{-1} TBABr , 2×10^{-3} mol kg^{-1} THMAM (pH 7.9), 1×10^{-4} mol kg^{-1} EDTA, (b) 50 wt% aqueous methanol containing 2×10^{-3} mol kg^{-1} THMAM (pH 7.9), 1×10^{-4} mol kg^{-1} EDTA, (c) 50 wt% aqueous methanol containing 2×10^{-3} mol kg^{-1} TAPSO (pH 7.9) , 1×10^{-4} mol kg^{-1} EDTA; Flow rate: 0.5 ml min^{-1}; Column: LiChrospher RP-18; Detection wavelength: 500 nm.

times. However, during our earlier study of IPRP-HPLC of DHAB chelates, TBA^+ had been long used as the counterion for the separation of anionic metal chelates, where it was difficult to separate the four detected metal chelates, vanadium(V), cobalt(III), aluminium(III), and iron (III), within 20 min (Figure 1(a)). In this study, a significant improvement in chromatographic performance assessed by peak height, separation factor and appropriate retention time, was achieved with THMAM (Figure 1(b)). The separation factors, defined as the ratio of capacity factors, were 1.86 for V and Co, 1.35 for Co and Al, 1.38 for Al and Fe with THMAM, and 1.73, 1.18, and 1.37 with TBA^+, respectively. The shorter retention time would be attributed to the weaker interaction of anionic chelates with the stationary phase in the presence of protonated buffer component, H^+THMAM. The retention of excess reagent, which was retarded as a neutral species, H_2L, was not affected by the counterions.

It was also noted that there was a considerable change in the peak height on changing the buffer and counterion agent. The use of TAPSO, which has the protonated form of H^+TAPSO^- and is also effective to ratain the chelates, resulted in decrease of the peak heights even at the same pH (Figure 1(c)). The same phenomenon was observed for TAPS and Tricine. Although the chelates were not retarded when borax buffer is used, they gave well resolved peaks by the addition of sodium sulfate in the mobile phase.

The example in this study, is an impressive illustration that the hydrophilic counterion enables a significant improvement of RP-HPLC for charged species. A matter of great importance in the proposed method is that hydrophilic counterion not only brings about rapid and sensitive determination of charged analytes, but also is an important aid for saving a considerable amount of organic solvent. The advantages of hydrophilic counterions will apply well to other charged analytes. Future work will focus on the basis underlying the chromatographic process of charged species with hydrophilic counterion.

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