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# DEVELOPMENTS IN THE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF METALLO-ORGANIC COMPOUNDS

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## SUMMARY

A number of developments in the high-performance liquid chromatography of metallo-organic complexes are described. Micellar chromatography of cobalt(III)–iminodiacetate isomer anions has been carried out with hexadecyltrimethylammonium bromide surfactant, with full resolution of *cis* and facial *trans* isomers. A dynamic ion-exchange micellar mechanism is proposed. Neutral N,N′-ethylenebis(acetylacetoneimine) chelates of copper(II) and nickel(II) are separated using sodium dodecyl sulfate in methanol–water as the mobile phase, which constrains the copper complex to be eluted before the nickel chelate. The separation of thermally labile isomers of aluminium trifluoroacetylacetonates is described, isomerization being precluded at 0°C. Direct-current argon plasma emission spectral detection is used to confirm elution of cobalt, chromium and aluminum complexes.

## INTRODUCTION

Although high-performance liquid chromatography (HPLC) has been used for analytical separation of organic compounds of a very wide range of functionality, its role for inorganic compounds has developed rather slowly by comparison. Most separations of neutral metal complexes and organometallic compounds have involved conventional normal-phase chromatography with organic solvents or mixed aqueous–organic mobile phases and reversed-phase substrates; ionic species have been chromatographed by ion-exchange or ion-pairing approaches. This topic has been reviewed by Willeford and Veening[1]. Recent developments in HPLC methodology including sub-ambient temperature application and micellar mobile phases provide expanded versatility and represent fruitful approaches for the chromatography of metallic compounds.

The technique of micellar HPLC, based on the use of aqueous solutions of

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surfactants under specific conditions was proposed by Armstrong and Fendler[2] for the size-exclusion separation of transfer RNAs. Subsequent applications have included TLC of polycyclic aromatic hydrocarbons (PAHs)[3], chlorinated pesticides[4], vitamins[5] and other compounds. HPLC methods for phenols and PAHs have been described[6,7] as has micelle-stabilized room-temperature phosphorescence detection[8]; applications for metal complexes have not been reported, however. The use of surfactant and micellar solutions in inorganic analytical applications has been limited to ion-pair chromatography and micelle-enhanced spectrophotometry, wherein solubility and solution stability of metal chelates has been augmented. The ability of micellar solutions to solubilize hydrophobic compounds of limited aqueous solubility enables reversed-phase HPLC methods to be extended to metallic compounds normally amenable mainly to normal-phase and ion methods. The micellar system offers a variety of environments, including a hydrophobic, organic core and a polar charged surface to impart specific solubilization to hydrophobic, ionic and amphiphilic species.

The HPLC of metallo-organic compounds is dependent on a range of solution characteristics and inter-phase parameters. Of particular importance in elution with desirable peak characteristics are the absence of irreversible adsorption, the absence of solvolysis and particularly hydrolysis or hydrogen-bonding surface effects, and the effective shielding of metal atoms by bulky organic complexing groups. It appears that the action of micelles in the mobile phase will have a favorable effect in reducing the former effects. Another parameter which has been shown to have a desirable effect in inorganic HPLC is the utilization of sub-ambient temperatures. The use of lowered temperatures in reducing on-column decomposition and also in minimizing complex isomerization rates has been demonstrated and reviewed recently[9,10]. The effect of temperature change on chelate reaction is described here. In addition to molecular spectral detection, atomic plasma emission spectral detection is used to follow the presence of specific elements throughout the chromatographic elution. Examples from a number of metal complex classes are chosen to illustrate these various effects.

## EXPERIMENTAL

The liquid chromatographic system used was an IBM Instruments (Danbury, CT, U.S.A.) microprocessor-controlled Model LC/9533 ternary gradient instrument equipped with a Rheodyne Model 7125 sample injection valve with a 10-$\mu$l loop and an IBM Instruments Model 9522 ultraviolet detector (254 nm, 10-$\mu$l flow cell). The columns used included IBM Instruments 5-$\mu$m methyl- and phenyl-bonded, 250 × 4.6 mm columns, a Phenomenex (Palos Verdes Estates, CA, U.S.A.) 5-$\mu$m nitrile-bonded column (250 × 5 mm) and a 150 × 4.6 mm column packed with Spherisorb (Phase Separations, Norwalk, CT, U.S.A.) nitrile phase.

A three-electrode Spectraspan IV direct-current argon plasma emission spectrometer (Beckman Instruments, Irvine, CA, U.S.A.) was used as an element-specific detector to verify eluted peak metal content.

Reagents used were sodium dodecyl sulfate (SDS), electrophoresis grade (Bio-Rad Labs., Richmond, CA, U.S.A.), hexadecyltrimethylammonium bromide (CTAB), reagent grade, recrystallized from acetone and water (Fisher Scientific, Pitts-

burgh, PA, U.S.A.) and distilled water purified by a Norganics Water System (Millipore, Bedford, MA, U.S.A.). The HPLC-grade methanol (Fisher Scientific) was used as received.

The metallo-organic compounds were prepared as follows. The geometrical isomers of the cobalt(III)–iminodiacetate chelate [$Co(IDA)_2$] were prepared according to the method of Weyh[11]. The tetradentate N,N′-ethylenebis(acetylacetoneimine) chelates of copper(II) and nickel(II) [$Cu(enAA_2)$ and $Ni(enAA_2)$] were made according to the method of Clark *et al.*[12]. The cobalt(III)–, chromium(III)–, and aluminium(III)–trifluoroacetylacetonates [$Co(TFA)_3$, $Cr(TFA)_3$ and $Al(TFA)_3$] were prepared according to Fay and Piper[13].

The mobile phases were prepared by dissolving the appropriate weight of the surfactant in water; all surfactant and sample solutions were filtered through a 0.45-$\mu$m membrane filter (Millipore). Mobile phases were degassed using an ultrasonic bath prior to use and helium was passed continuously through the solution. Sub-ambient conditions were generated as needed by a simple liquid cold bath surrounding the column.

## RESULTS AND DISCUSSION

One of the most widely studied classes of metal complexing agents used in analytical chemistry is that of the aminopolycarboxylic acids, sometimes called "complexones", typified by ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA). Such chelating agents are also used in industrial processes, in detergent formulations and as heavy-metal binding agents in preservative applications. Their quantitation as individual complexes in environmental aquatic systems is thus of increasing importance, as they may play roles in solubilization and reaction in such areas. Iminodiacetic acid (IDA) [$HN(CH_2COOH)_2$] typifies such compounds; the line structures of the geometrical isomers possible for an IDA complex with a hexacoordinate metal ion such as Co(III) are illustrated in Fig. 1, ligand atoms only being shown. Such complexes exhibit a 1:2 metal ion-to-ligand ratio, which gives a net charge of $-1$ on the chelate. The *cis* isomer is a relatively polar form, from con-

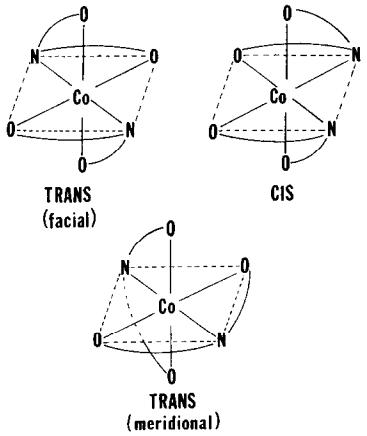


Fig. 1. Ligand atom diagrams of the geometrical isomers of the cobalt(III)–iminodiacetate system.

sideration of the positioning of nitrogens on the same edge; the *trans* isomers are considered as relatively less polar, because of the higher symmetry of ligand orientation. The meridional form of the *trans* isomer is not favored due to strain in ligand placement, as has been shown by NMR spectroscopy[14]. A preparative separation of $Co(IDA)_2$ isomers has been reported on an ion-exchange column[11] although the mechanism of separation was not clear.

In Fig. 2 an isocratic separation is shown of the *cis* and facial *trans* isomers on a methyl-bonded column using a mobile phase of 0.1 *M* CTAB. The isomers are eluted with good peak shape and chromatographic efficiency (*trans*, 38,500 plates per meter; *cis*, 45,000 plates per meter) and with a resolution factor of $R_s = 4.85$. Peak identification was by means of retention comparison with authentic samples of individual isomers; integrity of elution was also confirmed by monitoring the effluent with the d.c. argon plasma emission spectrometer, cobalt detection being performed at 350.23 nm. It is possible that the small peak at the tail of the *trans* facial isomer could be the trans meridonal isomer.

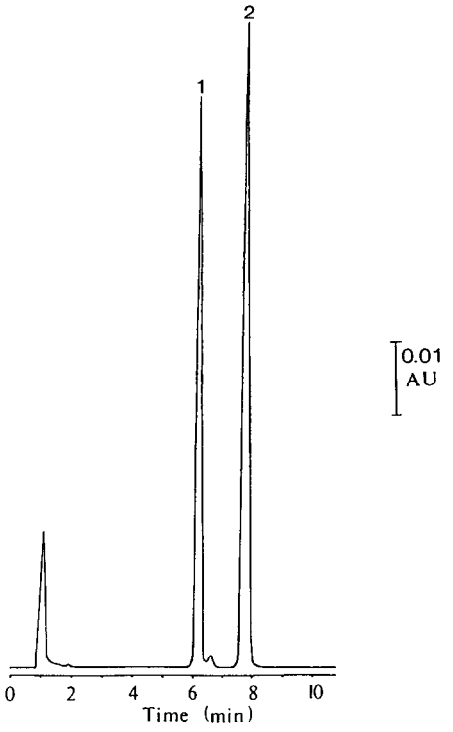


Fig. 2. Separation of *trans*- (1) and *cis*- (2) Co(III)–iminodiacetate isomers. Column 25 cm × 4.6 mm I.D., 5-$\mu$m methyl-bonded phase. Mobile phase 0.1 *M* CTAB, flow-rate 1.5 ml/min.

The elution characteristics of this charged chelated anion can be explained in terms of a simple model which incorporates a dynamic ion-exchange process, micellization and solubilization[15]. Micelles are not static species, but exist above the critical micellar concentration in equilibrium with surfactant monomers. In the chro-

matographic column, monomer surfactants can be adsorbed on the surface of the hydrocarbon-bonded reversed phase to form a dynamic ion-exchange surface (Fig. 3). Several investigations have considered the adsorption of surfactants on reversed-phase surfaces in relation to ion-pairing chromatography[15,16].

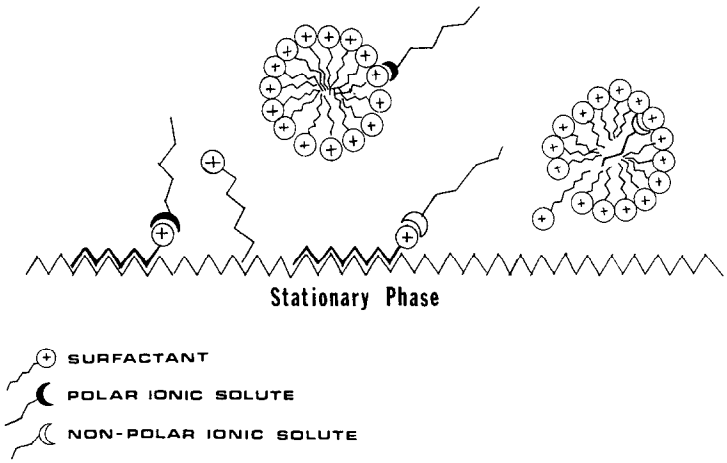


Fig. 3. Model for micellar action for ionic solutes on hydrocarbon reversed phase.

As an ionic solute passes through the column it distributes itself and interacts with the oppositely charged surfactant molecules on the surface of the stationary phase in an ion-pairing and/or ion-exchange mode. While this electrostatic interaction explains the retention of the negatively charged $Co(IDA)_2$ isomers, it does not necessarily account completely for the separation observed. This must be explained in terms of their difference in relative polarities. As illustrated in Fig. 3 for a generalized pair of ionic solutes, the relatively less polar *trans* facial isomer (designated non-polar ionic solute) distributes itself into the micellar core in the mobile phase, while the relatively polar *cis* isomer (designated polar ionic solute) remains excluded from the micelle. The overall distribution between mobile and stationary phases thus favors the *trans* facial form, which is thus eluted first. This argument gives the extreme case conditions and the actual interactions reflect intermediate behavior for both isomers.

The added dimension to inorganic complex HPLC introduced by these surface–micellar interactions suggests useful separations of charged complex ions with respect to both ligand and complexed metal.

*Micellar neutral metal chelate separations*

The major mode of separation to be affected for neutral metal complexes will be the differential solubilization and thus interphase distribution introduced by the micellar component of the mobile phase. A very useful probe system, otherwise well characterized, which is suitable to investigate this effect is that of the divalent transition metal tetradentate beta-ketoamine complexes. These are typified by the N,N′-ethylenebis(acetylacetoneimine) chelates of copper(II) and nickel(II). These complexes with both non-fluorinated and fluorinated ligands have been widely stud-

ied by both gas chromatography[17] and HPLC[18,19]. A noteworthy feature of the latter separations has been that both normal-phase adsorption chromatography on silica with dichloromethane and mixed dichloromethane–acetonitrile phases[18] *and* reversed-phase distribution on ODS with water–methanol[19] phases *both* show an elution order with complete resolution of the nickel complex followed by the copper complex. Normal-phase chromatography on a nitrile phase with a dichloromethane–tetrahydrofuran gradient also showed the same elution order[20]. Sequencing mechanisms in these various cases all combine to produce the same result.

In Fig. 4 the separation is shown of the two chelates on a 25 cm nitrile-bonded phase employing a mobile phase of water–methanol (95:5) which was 0.25 *M* in SDS. The copper chelate [Cu(enAA$_2$)] is eluted before and is completely separated from its nickel analogue [Ni(enAA$_2$)]. Dorsey *et al.*[21] have reported that the addition of low concentrations of an organic modifier such as methanol to the micellar mobile phase improves chromatographic efficiency by wetting the surface of the stationary phase thus reducing problems due to slow mass transfer.

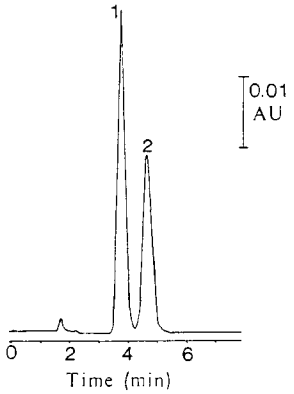


Fig. 4. Separation of Cu(enAA$_2$) (1) and Ni(enAA$_2$) (2). Column 25 cm × 5 mm I.D., 5-$\mu$m nitrile-bonded phase. Mobile phase, 0.25 *M* SDS in water–methanol (95:5), flow-rate 1.5 ml/min.

The reversal of elution order from previously observed separations by various contrasting modes can be best explained by reference to the relative solubilities of the two chelates in the surfactant system. In Table I the solubilities of Cu(enAA$_2$) and Ni(enAA$_2$) in water and in 0.1 *M* SDS are compared, the values being obtained by placing saturated solutions of the chelates in an ultrasonic bath at elevated temperatures overnight. While the solubility order remains the same, both are greatly enhanced in the surfactant system. The overall distribution of the copper chelate from the hydrocarbon substrate into the surfactant phase clearly exceeds that of the nickel complex under these conditions.

The ability to modify elution order with respect to both normal- and reversed-phase sequences has important implications in inorganic-complex HPLC and merits further investigation. Further, the ability of aqueous solutions of surfactants to solubilize hydrophobic chelates allows the application of reversed-phase chromato-

TABLE I

COMPARISON OF THE RELATIVE SOLUBILITIES OF TETRADENTATE COPPER AND NICKEL BETA-KETOAMINE COMPLEXES IN WATER AND MICELLAR MOBILE PHASE

| *Solvent* | *Solubility (mg/100 ml)* | |
|---|---|---|
| | *Cu(enAA$_2$)* | *Ni(enAA$_2$)* |
| Water | 42 | 8 |
| 0.1 *M* SDS | 840 | 310 |

graphy to species normally amenable only to normal-phase chromatography without the need for prior extraction.

*Sub-ambient temperature separation of thermally labile chelates*

The effect of temperature on HPLC separations has been less studied than modification of mobile and stationary phases; nevertheless it provides a parameter highly relevant to systems subject to on-column reaction with either phase or to kinetically controlled degradation or modification. Among the effects which are highly temperature dependent are exchange reactions of ligand groups on complexes such as octahedral trivalent metal beta-diketonates. The general HPLC of these complexes has been recently reviewed[1] and Henderson and Novak[9] have described the successful elution of a species such as Fe(III)–tris-1,1,1-trifluoroacetylacetonate, which is unstable chromatographically at room temperature, by operating at −30°C.

One of the most fully studied of HPLC chelate separations has been that of isomeric forms of octahedral beta-diketonates where the ligands are asymmetrical; 1,1,1-trifluoroacetylacetonates are typical examples. The geometrical isomers are the *trans* or meridional (mer) form and the *cis* or facial (fac) form which differ in the relative orientation of the methyl and trifluoromethyl groups on one ligand. Structural isomers of this type can interconvert at differing rates which depend on the metal complexes, the solution conditions and the temperature. There have been a number of separations reported of isomers of chelates of metals such as chromium for which isomerization rates are negligible under normal chromatographic conditions[22]. With an acetonitrile–dichloromethane (6:94) mobile phase on silica, isomer resolution for a range of chelates was obtained, meridonal chelates being eluted before facial and chromium complexes before analogous cobalt complexes. By contrast, chelates of "labile" metals such as aluminum for which isomerization is rapid under ambient temperature conditions present a greater challenge.

Among many stationary phases evaluated for separations of this kind, the greatest versatility and degree of resolution of isomer pairs was found for a phenyl-bonded phase column and low-polarity solvent mixtures such as hexane with low percentages of halocarbons. In Fig. 5 dual-detection isomer separations are shown for cobalt and chromium 1,1,1-trifluoroacetylacetonates (TFA). UV detection is at 254 nm while d.c. argon plasma detection is at 534.5 nm for cobalt and at 425.4 nm for chromium. Resolution and peak shape are excellent and metal specific detection confirms that no on-column isomerization or degradation has occurred.

A chromatogram at ambient temperature (20°C) of a mixture of aluminum,

cobalt and chromium TFA chelates gives the separation shown in Fig. 6. The identification of all eluent peaks was confirmed by separate individual injection and d.c. plasma specific metal detection. The cobalt and chromium complexes are all eluted and resolved as before. However, only a single peak is seen for $Al(TFA)_3$, which is eluted prior to the meridional chromium and cobalt isomers. A distinct peak tail is also evident from the aluminum chelate peak which persists almost as far as the facial chromium isomer. Separate plasma emission monitoring shows that aluminium is present throughout this peak tail and it is attributed to on-column isomerization of

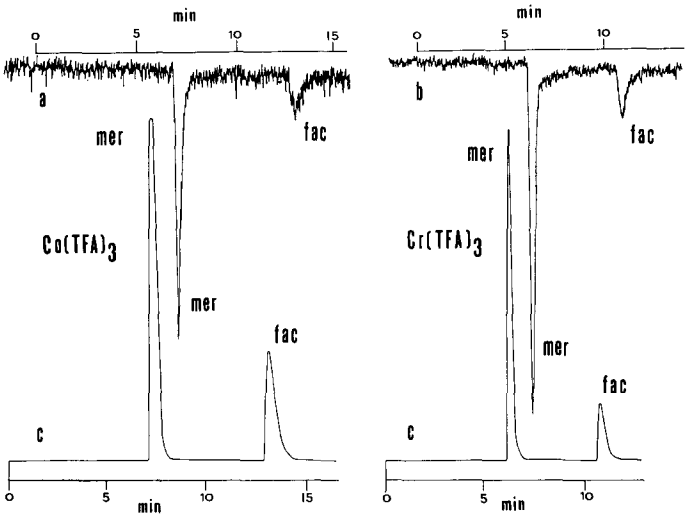


Fig. 5. Dual-detector chromatograms of cobalt(III)- and chromium(III)-trifluoroacetylacetonate isomers. Upper tracings, d.c. argon plasma emission detection of cobalt at 534.5 nm (a) and chromium at 425.4 nm (b). Lower tracings, (c), UV detection at 254 nm.

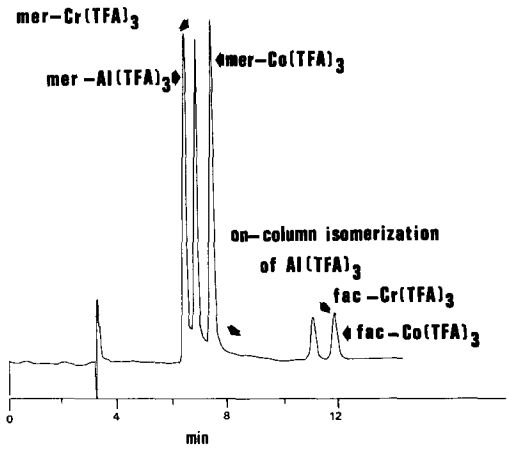


Fig. 6. Separation of isomers of aluminum-, cobalt- and chromium-1,1,1-trifluoroacetylacetonates at 20°C. Column 25 cm × 4.6 mm I.D., 5-$\mu$m phenyl-bonded phase. Mobile phase, chloroform–hexane (10:90).

meridional to facial aluminum chelate isomers. It may be understood by considering that a given chelate molecule spends a portion of its time in passage through the column in the facial isomer form, the retention time of which would probably be similar to that of the facial cobalt and chromium isomers. The overall aggregate retention would thus be between the limiting meridional and facial elution retentions. The reduction of column temperature to 0°C produces the chromatogram shown in Fig. 7. The general form of the chromatogram remains almost unchanged except that the tail from the initial alumnium complex peak is now absent; further the relative magnitude of the fourth peak is almost doubled. Independent specific element plasma monitoring showed that this peak now contains both aluminum and chromium chelates. Peak shape and resolution of the aluminum chelate pair indicates that at 0°C on-column isomerization is absent and the facial isomer peak corresponds to the proportion of that complex present in the injected solution chromatographed unchanged. This example illustrates that even a relatively small reduction in column temperature may have a substantial effect on column reactions for metal complex species.

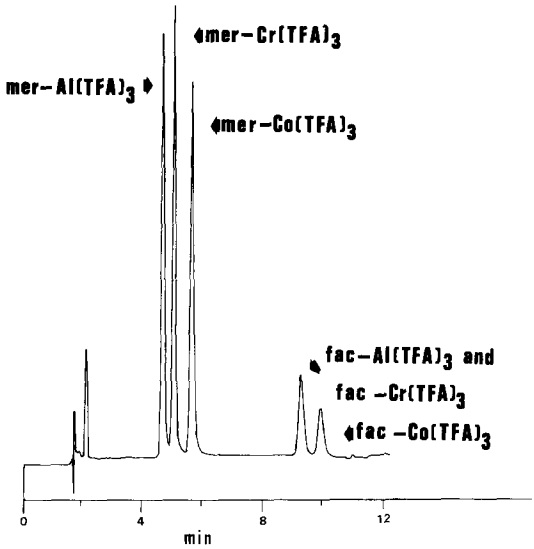


Fig. 7. Chromatogram as in Fig. 6, but at 0°C and with mobile phase chloroform–hexane (6:94).

## CONCLUSIONS

The metallo-organic complex systems investigated here show the advantages which a range of HPLC parameters can impart to separation problems. Resolution sequences which remain the same in simple normal- and reversed-phase modes may be modified by the action of micellar surfactants; these also may be employed to resolve both neutral and charged complexes in the same chromatogram. Column temperature variation also promises to be a major factor in the modification of metal chelate elution behavior. An interesting approach may be the combination of tem-

perature control and micellar mobile phase composition, and efforts in this direction are presently underway.

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