



ELSEVIER

Journal of Chromatography A, 825 (1998) 189–194

JOURNAL OF  
CHROMATOGRAPHY A

# Thin-layer chromatography of mixed-ligand zinc complexes coupled with atomic absorption spectrometric analysis of zinc<sup>1</sup>

Andrej Oriňák<sup>a,\*</sup>, Renáta Oriňáková<sup>a</sup>, L'udmila Turčániová<sup>b</sup>

<sup>a</sup>*Department of Physical and Analytical Chemistry, Faculty of Science, University of P.J. Šafárik, Moyzesova 11, 041 54 Košice, Slovak Republic*

<sup>b</sup>*Institute of Geotechnics, Slovak Academy of Science, Watsonova 45, 041 01 Košice, Slovak Republic*

Received 1 April 1998; received in revised form 6 August 1998; accepted 11 August 1998

## Abstract

Off-line coupling of two analytical methods: thin-layer chromatography (TLC) and atomic absorption spectrometry (AAS) was investigated for preliminary stability studies of zinc carboxylato complexes bound to nicotinic acid and their chromatographic separation on a Silufol layer. Zinc content, determined by an AAS method in fractions scrapped off the layers after zinc carboxylato complex (ZC) chromatographic analyses responds to ZC dissociation during chromatographic process. It was found that solute chromatographic stability is influenced substantially by the concentration of organic modifier in the eluent. To reveal relations between zinc distribution, corresponding solute retention and complex stability was the main aim of the presented paper. © 1998 Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Atomic absorption spectrometry; Zinc complexes

## 1. Introduction

Hyphenation techniques have been recently very often emphasized in chromatographic analysis. These are on-line chromatographic methods like high-performance liquid chromatography (HPLC) hyphenated with mass spectrometry (MS) [1], HPLC coupled with atomic absorption spectrometry (AAS) in analysis of metal complexes [2]. Use of HPLC–Fourier transform infrared spectrometry [3] and

especially HPLC with nuclear magnetic resonance (NMR) is especially common [4].

Perhaps one of the more significant advances in thin-layer chromatography (TLC) is the coupling of TLC with MS, made possible by the development of small, high-performance mass spectrometers [5]. Secondary ion mass spectrometry (SIMS) with a laboratory-designed apparatus was described by Busch [6]. De Brabander et al. [7] presented coupling of TLC with ion-trap detection (ITD 800). A new technique developed by Merschel et al. [8] described trace analysis in TLC hyphenated with time-of-flight (TOF) SIMS. Enzymatic and biological in-situ detection in high-performance TLC is discussed in Ref. [9]. Information on coupling TLC

\*Corresponding author.

<sup>1</sup>Presented at the 5th International Symposium on Hyphenated Techniques in Chromatography and Hyphenated Chromatographic Analyzers, Bruges, February 11–13, 1998.

with AAS for metal complexes analyses has been not found in the literature.

Reports on zinc carboxylate complexes binding different ligands were very frequent in the end of 1980s. Structures of selected metal carboxylate complexes like: zinc propanoate [10], crotonate [11], 3,3-dimethylacrylate [12], and lead crotonate [13], together with two basic zinc carboxylates, the hydroxy-centered crotonate [13] and the oxo-centered pivalate [14], were studied. Preparation and characterization of linear mixed-metal trinuclear carboxylate complexes was also well documented [15]. The preparation and structural characterization of mixed air-stable binuclear zinc(II) carboxylate complexes together with 13 air-stable linear complexes of general formula  $[\text{Zn}_2\text{M}(\text{-carboxylate})_6(\text{base})_2]$ , where  $\text{M}^{\text{II}} = \text{Zn, Co, Ni, Cd, Mg, or Ca}$  were reported by Clegg et al. [16]. The main feature of interest is the flexibility of the tetracarboxylate framework which can bridge across a wide range of metal-metal distances. An ultrasonic absorption study of the complex formation of zinc chloracetate and *n*-butyrate in aqueous solution was published by Ishihawa et al. [17].

In our laboratory we synthesized zinc carboxylate complexes of formula  $\text{Zn}(\text{R-COO})_2\text{L}_n$ , where  $\text{R} = \text{H, CH}_3, \text{CH}_3\text{-CH}_2\text{-}, \text{CH}_3\text{-(CH}_2\text{)-}, (\text{CH}_3)_2\text{-CH-};$  and  $\text{L} = \text{thiourea, caffeine, nicotinic acid (nica), phenazone}; n = 1 \text{ or } 2$ . Our interest centered on the chromatographic analysis of zinc carboxylate mixed-ligand complexes and determination of their stability using chromatographic data. After preliminary HPLC analysis it was observed that complexes are unstable and decompose during the chromatographic process forming adsorption complexes with silica gel or interact strongly with mobile phase [18,19]. The problem of low chromatographic stability is of special concern for zinc(II) complexes, because the majority of these, as well as being of low kinetic inertness, are thermodynamically unstable. For better understanding of the chromatographic behaviour of zinc complexes and their stability, AAS was off-line coupled with a TLC method [20,21].

The complexes studied showed different chromatographic behaviour in selected chromatographic systems [18,22] and promising antibacterial activity [23,24].

## 2. Experimental

### 2.1. Complexes, chemicals, solvents and reagents

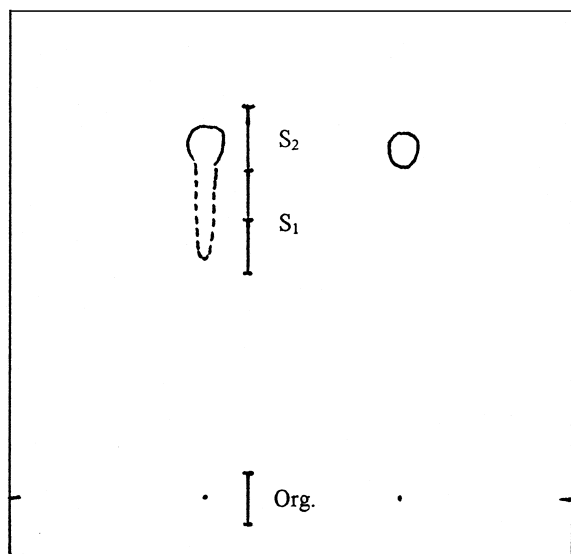
Zinc carboxylate mixed-ligand complexes were synthesized by Department of Inorganic Chemistry of P.J. Safarik University, Kosice, Slovak Republic. Stock solutions of ZC-bound nicotinic acid were prepared in concentrations of 0.05 M in redistilled water (conductivity 0.4  $\mu\text{S}$ ). Methanol (MeOH) was purchased from Merck (Darmstadt, Germany), hydrofluoric acid (HF) at 38% concentration and polystyrene tubes were obtained from Medika (Bratislava, Slovak Republic). The spraying solution for zinc detection was prepared by dissolving 4-(2-pyridylazo)resorcinol (PAR), (Merck), in a water-methanol (1:1, v/v) mixture to give a 0.04% solution. To detect the organic bases bound in ZC, potassium permanganate and bromocresol green (0.004% concentration) reagents were used as aqueous solutions.

### 2.2. Chromatography

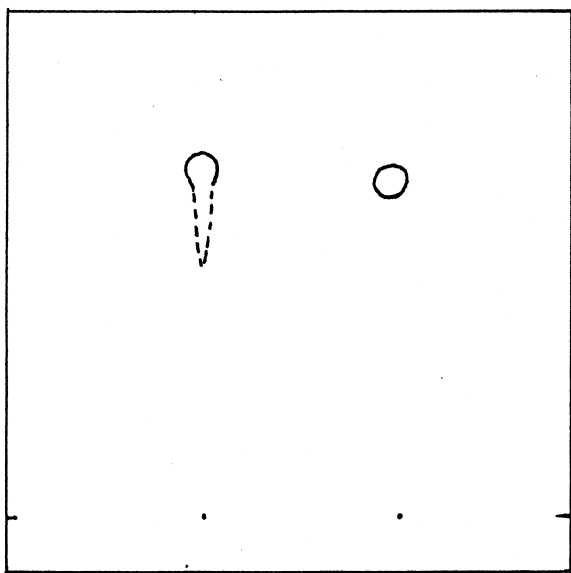
TLC was performed by ascending technique on precoated silica gel plates (9×9 cm) Silufol UV<sub>254</sub> (Votice, Czech Republic). Silica gel layers were cleaned by eluting with a methanol-chloroform (50:50, v/v) solution, then dried and stored in a desiccator. Freshly prepared aqueous solutions of ZC (5  $\mu\text{l}$ , 40 mg/3 ml) have been applied by a microliter syringe (Hamilton, USA). The layer was dried and put into a 10×15×4 cm glass chromatographic chamber where the development was performed until the front of the mobile phase moved to 5 mm from the top of the layer. No temperature control was available. After development, the chromatographic zones were first revealed under a UVMin lamp (Desaga, Germany) and then sprayed with the detection reagents.

### 2.3. Zinc carboxylate complex chromatographic zone transfer

After layer developing were detected solute positions and spots (1×1 cm) were scrapped from: (1) start, (2) part of zone spread (if formed), spot 1; (3)



1a



1b

Fig. 1. TLC chromatograms of: (a)  $\text{Zn}(\text{HCOO})_2(\text{nica})_2$ ; 60% MeOH–water eluent; silufol thin layer, detection: UV, reagent. Org=Start; S<sub>1</sub>=spot 1; S<sub>2</sub>=spot 2; scrapped positions. (b)  $\text{Zn}(\text{CH}_3\text{COO})_2(\text{nica})_2$ ; 60% MeOH–water eluent; Silufol thin-layer, detection: UV, reagent.

zone due to the molecular species of complex ZC, spot 2 (Fig. 1). Each fraction was collected with a special fractionation device described in Ref. [25]. The collected fraction was carefully weighed, quantitatively transferred into polystyrene tube and then completely dissolved by addition of 1 ml 38% HF. The resulting solutions were diluted with redistilled water as necessary and the zinc content was determined by the AAS method against a HF blank.

#### 2.4. Atomic absorption spectrometry

The flame AAS performed with using a Pye Unicam 9100X spectrometer (Germany) was operated at air–C<sub>2</sub>H<sub>2</sub> flame (fuel pressure 121.59kPa, oxidant pressure 222.92 kPa, optimum burner height 8 mm, zinc hollow-cathode lamp at 10 mA). The absorbance was monitored at 213.9 nm with a spectral band width of 0.5 mm. Because the layers used contain a zinc salt (presumably, ZnSiO<sub>3</sub>) as the fluorescent indicator, the background signal was also recorded and subtracted to plot the calibration curve.

### 3. Results and discussion

Off-line coupling TLC with AAS was used in the study of retention behaviour and stability of nicotinic acid–zinc complexes and results are given in Figs. 2 and 3. In the case of the  $\text{Zn}(\text{formate})_2(\text{nica})_2$  complex (Fig. 2) the retention of solute in the spot 1 increases linearly (about 0.3 units of  $R_F$ ) with increase of methanol (MeOH) in the eluent. Zinc content determined is the highest at 40% of MeOH in eluent (Fig. 2a). The entirely different is retention of solute represented by spot 2, where retention first increases until MeOH content is 60% (and maximum zinc content determined), and then decreases strongly with permanent linear organic modifier increasing in eluent (Fig. 2b). The solute retention decrease corresponds well to formation of adsorption complexes, that originates in the first step before the complex decomposes [26]. Those parts of the complex that are strong adsorbed on thin-layer can affect retention of other parts by modification of layer surface area. Spreading of the zone increases with percentage methanol increase in the eluent.  $R_F$  solute

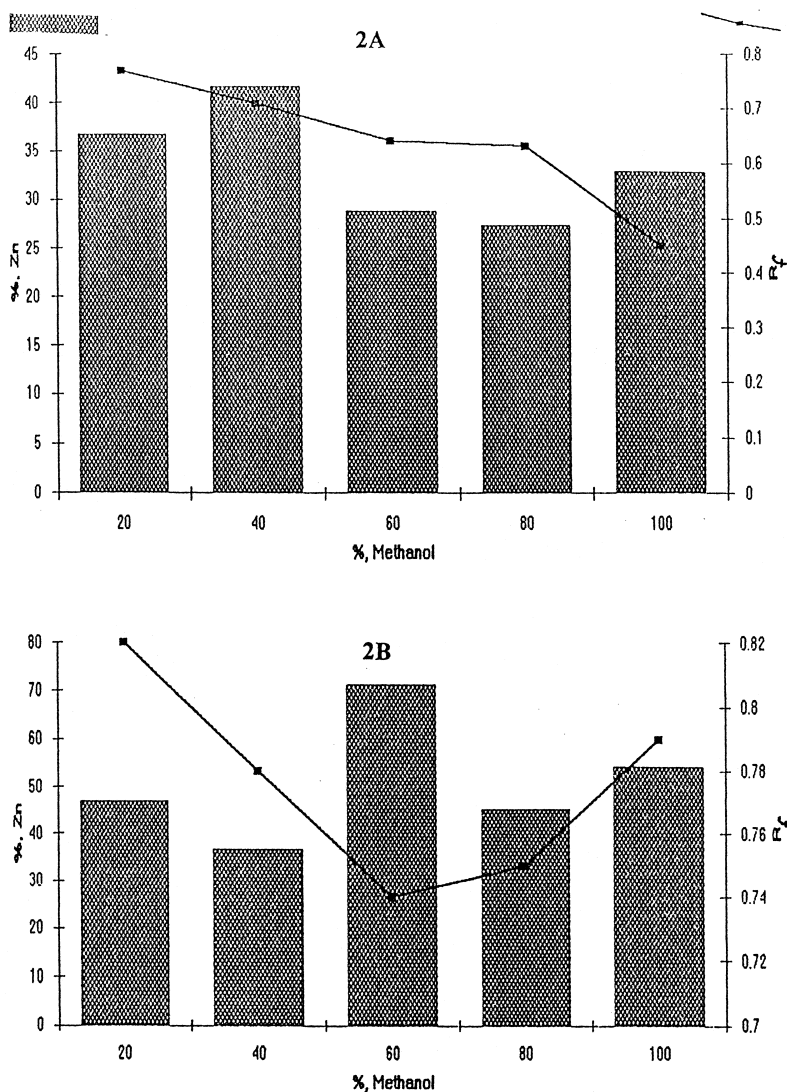


Fig. 2. Relation between zinc distribution zinc carboxylate complex stability and solute retention for  $Zn(HCOO)_2$  nicotinic acid  $_2$ ; in spot 1 (Fig. 1a); in spot 2 (Fig. 1b). Thin layer: silica gel, water–methanol binary eluents; detection: UV, detection reagents.

value changes from 0.78 (20% MeOH) to 0.45 (100% MeOH). Conversely, in the case of zinc acetate complex, the chromatographic zone (spot 1) is more spread and  $R_f$  solute values range from 0.75 (20% MeOH) to 0.15 (100% MeOH).

Retention of solute  $Zn(acetate)_2(nica)_2$  showed strongest increase of solute retention represented by spot 1, with maximum zinc content at 60% of MeOH in the eluent at its linear increase (Fig. 3a). Retention of solute is comparable with retention of nicotinic

acid standard chromatographed under the same conditions as ZC. The solute retention in the spot 2 slightly increases with maximum zinc content at 60% of MeOH in the eluent used. This fact confirms decomposition of complex with following effect of adsorbed zinc on the next retention ability of nicotinic acid (Fig. 3b). Of interest is the small effect of organic modifier volume fraction in the eluent on ligand retention (Fig. 3b). While the formate zinc complex bound nicotinic acid is presented in molecu-

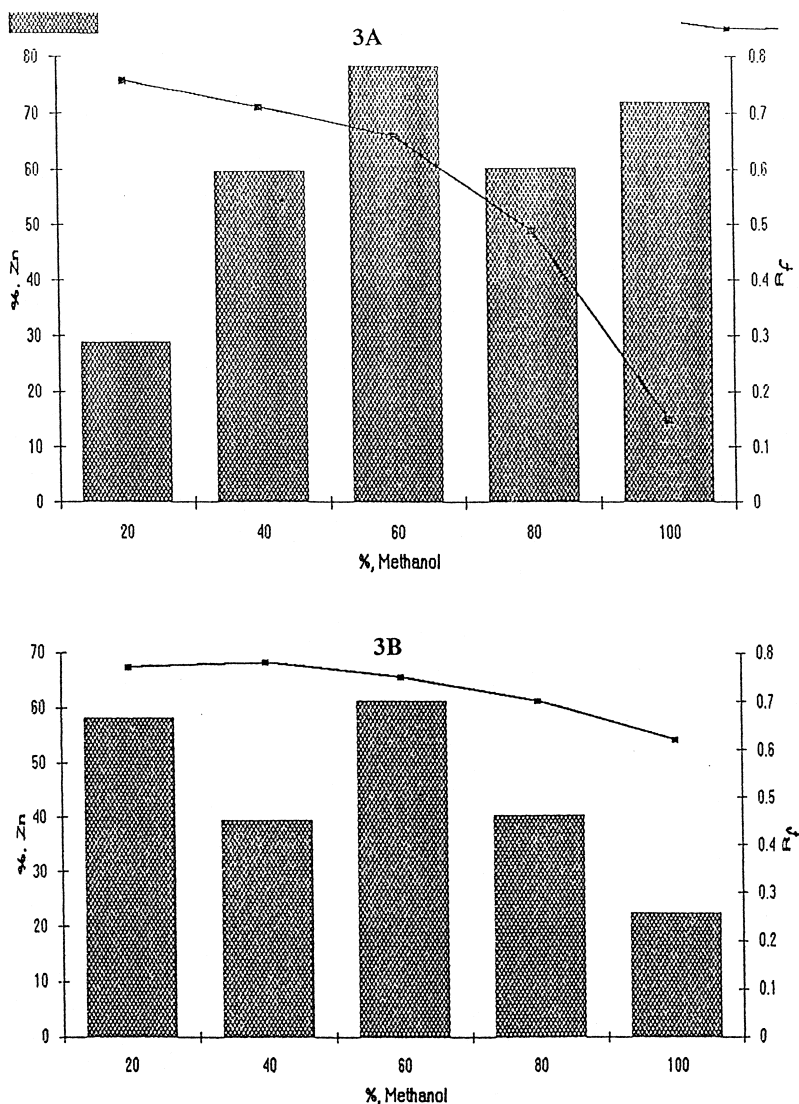


Fig. 3. Relation between zinc distribution zinc carboxylate complex stability and solute retention for  $\text{Zn}(\text{CH}_3\text{COO})_2$  nicotinic acid<sub>2</sub>; in spot 1 (Fig. 2a); in spot 2 (Fig. 2b). Thin layer: silica gel, water–methanol binary eluents; detection: UV, detection reagents.

lar form in spot 2 (Fig. 2b), strongly retained; spot 1 (Fig. 2a) represents free ligand–nicotinic acid–carboxylate complex after initial complex decomposition.

From results published previously [19] the ZC-bound nicotinic acids were found of the lowest stability following with those ZC-bound thiourea, caffeine or phenazone molecules.

Stability of the same ZC in the dioxane–water binary eluents will be tested in future studies.

#### 4. Conclusions

In this investigation, the use of a novel TLC–AAS off-line method was tested in stability examinations of mixed-ligand zinc carboxylate complex bound nicotinic acids, as a function of the type of chromatographic system, molecular structure of ZC and spot position on the thin-layer. From studies given above it is concluded that (1) different eluent combinations examined affect the stability behaviour

and ZC dissociation; (2) the increased overall polarity of chromatographic system leads to a lower stability of the complexes studied; (3) zinc formate complex bound two molecules of nicotinic acid is highly stable; the zinc acetato–nicotinic acid complex strongly decomposes; (4) the off-line TLC–AAS method is very suitable for monitoring of zinc distribution moving up the layer with zinc complex during the separation process; (5) the method can be used with layers containing fluorescent indicator.

### Acknowledgements

The authors wish to thank Mgr. Lívía Laczová for compounds she synthesized and Dr. Katarína Gyoryová, for the same preparations she provided. This work was supported by grant IMG-SQ-1001/96 supplied by European Communities Phare Programme and by grant No. 2/6104/99 MŠ SR and SAV.

### References

- [1] G.D. Allen, R. Griffiths, R.W. Abbott, S. Bartlett, T.A. Brown, V.A. Lewis, M. Nash, G. Rhodes, J.A. Rontree, *LC·GC Int.* 8 (1995) 699–703.
- [2] D. Ishii, T. Takeuchi, *J. Chromatogr. Sci.* 27 (1989) 71–76.
- [3] C. Fujimoto, T. Morita, K. Jinno, S. Ochiai, *Chromatographia* 23 (1987) 512–516.
- [4] M. Spraul, M. Hoffmann, U. Braumann, U.G. Sidelmann, 4th International Symposium on Hyphenated Techniques in Chromatography and Hyphenated Chromatographic Analysers HTC'4, Bruges, 1996, Book of Abstracts, B29.
- [5] J.C. Touchstone, *LC·GC Int.* 6 (1993) 406–410.
- [6] K.L. Busch, *Trends Anal. Chem.* 6 (1987) 95–100.
- [7] H.F. De Brabander, F. Smets, G. Pottie, J. Planar. *Chromatogr.* 4 (1991) 52–57.
- [8] L. Merschel, W. Sichtermann, N. Buschmann, A. Benninghoven, 4th International Symposium on Hyphenated Techniques in Chromatography and Hyphenated Chromatographic Analysers HTC'4, Bruges, 1996, Book of Abstracts, B20.
- [9] T.T. Chang, J.O. Lay, R.J. Francel, *Anal.Chem.* 56 (1984) 111–113.
- [10] W. Clegg, I.R. Little, B.P. Straughan, *Acta Crystallogr. C* 42 (1986) 1701.
- [11] W. Clegg, I.R. Little, B.P. Straughan, *Acta Crystallogr. C* 43 (1987) 456.
- [12] W. Clegg, I.R. Little, B.P. Straughan, *Acta Crystallogr. C* 42 (1986) 919.
- [13] W. Clegg, I.R. Little, B.P. Straughan, unpublished work.
- [14] W. Clegg, I.R. Little, B.P. Straughan, *Acta Crystallogr. C* 42 (1986) 1319.
- [15] W. Clegg, P.A. Hunt, B.P. Straughan, M.A. Mendiola, *J. Chem. Soc. Dalton Trans.* (1989) 1127.
- [16] W. Clegg, I.R. Little, B.P. Straughan, *J. Chem. Soc. Dalton Trans.*, (1986) 1283.
- [17] A. Ishikawa, K. Tamura, *J.Phys.Chem.* 97 (1993) 4820.
- [18] A. Oriňák, K. Gyoryová, E. Matisová, L. Šlesárová, J. Skoršepa, I. Rosival, M. Ganajová, *J. Chromatogr.* 660 (1994) 67.
- [19] A. Oriňák, E. Matisová, L. Šlesárová, K. Gyoryová, *Anal. Chim. Acta* 291 (1994) 169.
- [20] A. Oriňák, A.R. Timerbaev, R. Stasková, J. Vojtek, K. Ubik, *Chromatographia* 43 (1996) 537.
- [21] A. Oriňák, R. Oriňáková, L. Šlesárová, V. Gazdová, *J. Planar Chromatogr., Modern Thin Layer Chromatogr.* 10 (1997) 44.
- [22] A. Oriňák, K. Gyoryova, S. Lancaricova, *J. Planar Chromatogr.* 6 (1993) 153–156.
- [23] A. Oriňák, M. Molokacova, E. Sinajova, unpublished results, 1995.
- [24] O.P. Semenova, A. Orinak, E. Matisova, A.R. Timerbaev, *J. Cap. Electrophoresis* 2 (1995) 203–207.
- [25] D. Dekker, *J. Chromatogr.* 168 (1979) 508–510.
- [26] V.V. Salov, A.R. Timerbaev, O.M. Petrukhin, *J. Planar Chromatogr.* 3 (1990) 73–76.