ELSEVIER

Journal of Chromatography A, 834 (1999) 321–327

JOURNAL OF CHROMATOGRAPHY A

Determination of platinum(II,IV) and palladium(II) as thiocyanate complexes by capillary zone electrophoresis Analysis of carboplatin and similar drugs

Josef Hamáček, Josef Havel*

Department of Analytical Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Abstract

The thiocyanate complexes of Pd(II), Pt(II) and Pt(IV) were studied by capillary zone electrophoresis. Pd(II) can be detected in the form of the thiocyanate complex at 305 nm with higher sensitivity than in the form of its chloro complex (absorption maximum 214 nm). A detection limit equal to 5 ppb for Pd has been finally achieved. The possibility of simultaneous determination of Pd(II) and Pt(IV) in the form of thiocyanate complexes has also been demonstrated. When the method optimized for the determination of Pt(II) was applied to the drugs Cykloplatin and Ribocarbo (containing carboplatin) and Platidiam (containing cisplatin), good agreement of the platinum content with the declared value was obtained. Samples of vehicle exhaust particulates (National Institute for Environmental Studies, Japan, No. 8 reference material) were also analyzed.

Keywords: Palladium; Platinum; Metal complexes; Thiocyanates; Carboplatin; Cisplatin; Metal cations

1. Introduction

There is an increasing interest in the use of metal-containing compounds in medicine. Their chemical properties are often utilized in biological systems for diagnosis and therapy. The interest in the interaction of organic complexes of platinum group metals (PGMs) [1,2] with biological molecules began about 25 years ago with connection to the discovery of their anticancer activity. For the determination of these metals in different materials a number of instrumental techniques [3], often with low limits of detection (LODs), are applied. Atomic absorption spectrometry (AAS) and inductively coupled plasma (ICP)-MS [4] and adsorptive voltammetry [5] are usually used for the determination of total metal. Electrokinetic capillary chromatography [6] with MS detection or postcolumn derivatization and various types of HPLC [7–9] are used for the determination of PGM species. A number of reports describe methods for the separation and determination of intact drugs, especially cisplatin [10–12] and carboplatin [12], by HPLC procedures in biological samples like serum and urine. Capillary zone electrophoresis (CZE) is a more and more used technique due to its high separation efficiency [13] and short analysis time and has been applied to the determination of some inorganic and organic metal complexes. Buchberger and coworkers [14,15] made the separation of transient metal ions including Pd and Pt in the form of cyano complexes by CZE. Aguilar et al. [16] has used cyano complexes to determine PGMs in leaching solutions of automobile converters. Mojski et al. made the CZE separation of PGM in the form of bromo complexes [17]. Recently, Khramov and Havel used thiourea for the HPLC and CZE sepa-

*Corresponding author. e-mail: havel@chemi.muni.cz

0021-9673/99/$ – see front matter
PII: S0021-9673(98)00848-6

ration of PGM and developed a method for the determination of Pt, Rh, and Pd [18]. Alloys of PGMs are usually dissolved in a mixture of HCl–HNO_3 and CZE was applied for their determination in the form of chloro complexes [19–21]. The background electrolyte based on the mixture of HCl–KCl solution was used in these cases. In diluted aqueous solutions PGM can undergo hydrolysis and form a great number of species [22–24]. In this sense, speciation of iridium has been recently studied by capillary electrophoresis [25].

In this paper, determination of Pt and Pd in model and real samples (the anticancer drugs, National Institute for Environmental Studies, Japan [NIES] material) in the form of thiocyanate complexes was studied because the higher stability and molar absorptivity coefficients of these compared with chloro complexes are promising.

2. Experimental

2.1. Instrumentation

Spectraphoresis 2000 (Thermo Bioanalysis, CA, USA), instrumentation was used with an uncoated fused-silica capillary, length 68 cm, effective length 60.5 cm×75 μm I.D. The reversed polarity mode of the CZE system (anodic pole at the side of detection) was applied. Spectrophotometric measurements were done on UV–Vis spectrometer UV2 (Unicam, UK).

A mineralization device Plasma 1101 (IPC, CA, USA), producing high frequency (13.5 MHz, 1500 W) low-pressure (10–500 Pa) oxygen plasma that serves to effect efficient combustion of the organic compounds containing samples was used.

An ICP-MS ELAN 6000 (Perkin Elmer, UK) instrument was used for spectrometric measurements.

2.2. Reagents

The stock solution of 0.02381 mol l^{-1} Pd(II) was prepared by dissolving of the metal in 15 ml 5.692 mol l^{-1} HCl and diluting to 200 ml with distilled water. The stock solution of Pt(IV) was prepared by dissolving Pt metal (2.8428 g) in HNO_3–HCl (1:9), fuming the solution to dryness with hydrochloric acid and diluting to 100 ml with 0.2 mol l^{-1} HCl. The working solutions were prepared by diluting the stock solutions with double-distilled water. The solution of Pt(II) was prepared by heating 5.2 ml of Pt(IV) stock solution with 1.64 ml 0.8% hydrazinium hydrate up to 40°C and then dissolving it up to 100 ml with 0.2 *M* HCl.

Cykloplatin drug (containing carboplatin) and Platidiam drug (containing cisplatin) were obtained from Lachema (Brno, Czech Republic). Ribocarbo-L (containing carboplatin) drug was obtained from Ribosepharm (Haan, Germany).

All other chemicals were of analytical grade purity and were obtained from Lachema.

The electroosmotic flow was determined from migration time of acetone. Mesityl oxide, often applied for the purpose, cannot be used here because it forms complexes with Pd(II) and Pt(IV) $(C_6H_9OPdCl)_2$, $C_6H_{10}Cl_2OPt$ [26] and it was found that it interferes with the determination of both metals.

Between each run the capillary was flushed for 1 min with pure water, 3 min with 0.1 *M* HCl and followed by flushing with the running buffer for 3 min.

2.3. Sample preparation

The reference material NIES No. 8 (Vehicle Exhaust material), certified only for Al, As, Ca, Cd, Co, Cr, Cu, K, Mg, Na, Ni, Pb, Sb, Sr, V, and Zn, was dissolved in the following way: 1 g was placed on the glass dish and inserted into the combustion chamber of a Plasma 1101 mineralization apparatus. After the combustion (5 h), 0.5 ml concentrated $HClO_4$ were added, the mixture was dried under infralamp and then dissolved in 1 ml of water. This solution was filtered through a 0.45 μm filter applying a syringe and the filtrate was then analyzed by CZE.

A 100-fold dilution of the drug solutions by double distilled water was applied.

For the formation of thiocyanate complexes two different derivatization procedures for the preparation of the sample were tried:

2.3.1. Procedure 1

To the sample solution (0.1 ml) 0.5 ml of 1 mol

l^{-1} KSCN was added, the mixture was heated for 7–10 min in a boiling water-bath, after cooling down the solution was diluted by water to 10 ml and analyzed by CZE.

2.3.2. Procedure 2

To the sample solution (0.1 ml) 0.1 ml of concentrated $HClO_4$ was added, the mixture was fumed to dryness and after adding 0.5 ml of 1 mol l^{-1} KSCN and waiting for 10 min the solution was diluted to 10 ml and analyzed by CZE.

3. Results and discussion

3.1. Spectrophotometric study

The thiocyanate complexes of Pt metals show different absorption spectra in comparison to chloro complexes. The values of absorption maxima of thiocyanates are: 308, 242 and 288 nm for Pd(II), Pt(II) and Pt(IV), respectively, while absorption maxima equal to 223, 216 and 262 nm were observed for chloro complexes [27]. However, for the determination by CZE, scanning at several wavelengths is chosen; 305, 245 and 290 nm for Pd(II), Pt(II) and Pt(IV)), respectively, owing to the real possibilities of the detector. During the simultaneous determination of Pd(II) and Pt(IV), absorbance was measured at 295 nm, between their absorption maxima.

First, the formation of $Pd(SCN)_4^{2-}$, $Pt(SCN)_4^{2-}$ and $Pt(SCN)_6^{2-}$ from the chloride solutions was studied. The thiocyanate complex of Pd(II) is formed rather fast and it is then stable even after heating or standing for several days. Absorbance of the Pd(II)–thiocyanate complex is twotimes higher than that of Pd(II)–chloride. In the case of Pt(II,IV), the transfer of chloro to thiocyanate complexes is much slower. A constant absorbance value was attained only after 1–2 days of standing. However, after heating the solution in a boiling water-bath, this period can be shortened to 5–10 min.

3.2. Optimization of CZE conditions

After optimization of all conditions the following values were found to be optimal: 0.1 mol l^{-1} KSCN adjusted by adding $HClO_4$ (perchlorate does not form complexes with PGM) to pH 3 as background electrolyte (BGE), temperature 50°C, separation voltage −10 kV, hydrodynamic injection 4 s. In the sample solution the concentration of thiocyanates was maintained at 50 mmol l^{-1}. Pd(II) under the presence of an excess of thiocyanate and at pH 3 is present completely in the form of $Pd(SCN)_4^{2-}$ (Fig. 1). In case of Pt(II,IV), the presence of $Pt(SCN)_4^{2-}$ and $Pt(SCN)_6^{2-}$ is expected under the conditions described above, analogically to chloro complexes [19]. It should be noted that for sample solutions with a low ionic strength it is better to use electrokinetic injection, the signal is then 3–4 times higher than with hydrodynamic injection for the same time.

3.3. CZE separation

The standard solutions of chloro complexes of PGM were used as the samples in following measurements. Results of simultaneous determination of Pd(II) and Pt(IV) under the conditions described above are shown in Fig. 2. For both variants of the sample preparation the same results were obtained for Pd(II). In the case of Pt(IV), the higher peak was obtained using Procedure 2. Simultaneous determination of Pt(II) with Pd(II) and Pt(IV) at 295 nm (for Pt(II)–thiocyanate 245 nm need to be used) is not suitable owing to low absorbance of $Pt(SCN)_4^{2-}$, but separation appears. In addition, using Procedure 2 Pt(II) will be oxidized to Pt(IV). For the individual determination of Pd(II) and Pt(II), Procedure 1 is recommended concerning their easier conversion to thiocyanate complexes. Procedure 2 is necessary to apply for Pt(IV) determination to improve the conversion of Pt(IV) to thiocyanate complex and to prevent possible reduction of Pt(IV) to Pt(II).

The calibration curves for the individual determination of Pd(II), Pt(II) and Pt(IV) were evaluated for the peak height and they show a good linearity for concentrations in the range of at least two orders of magnitude. The equations for calibration dependencies were: $y=0.161x-0.221$ ($R^2=0.998$) for Pd(II), $y=0.236x-0.84$ ($R^2=0.999$) for Pt(II) and $y=0.495x-12.08$ ($R^2=0.998$) for Pt(IV) (R means correlation coefficient, x is concentration in mol l^{-1},

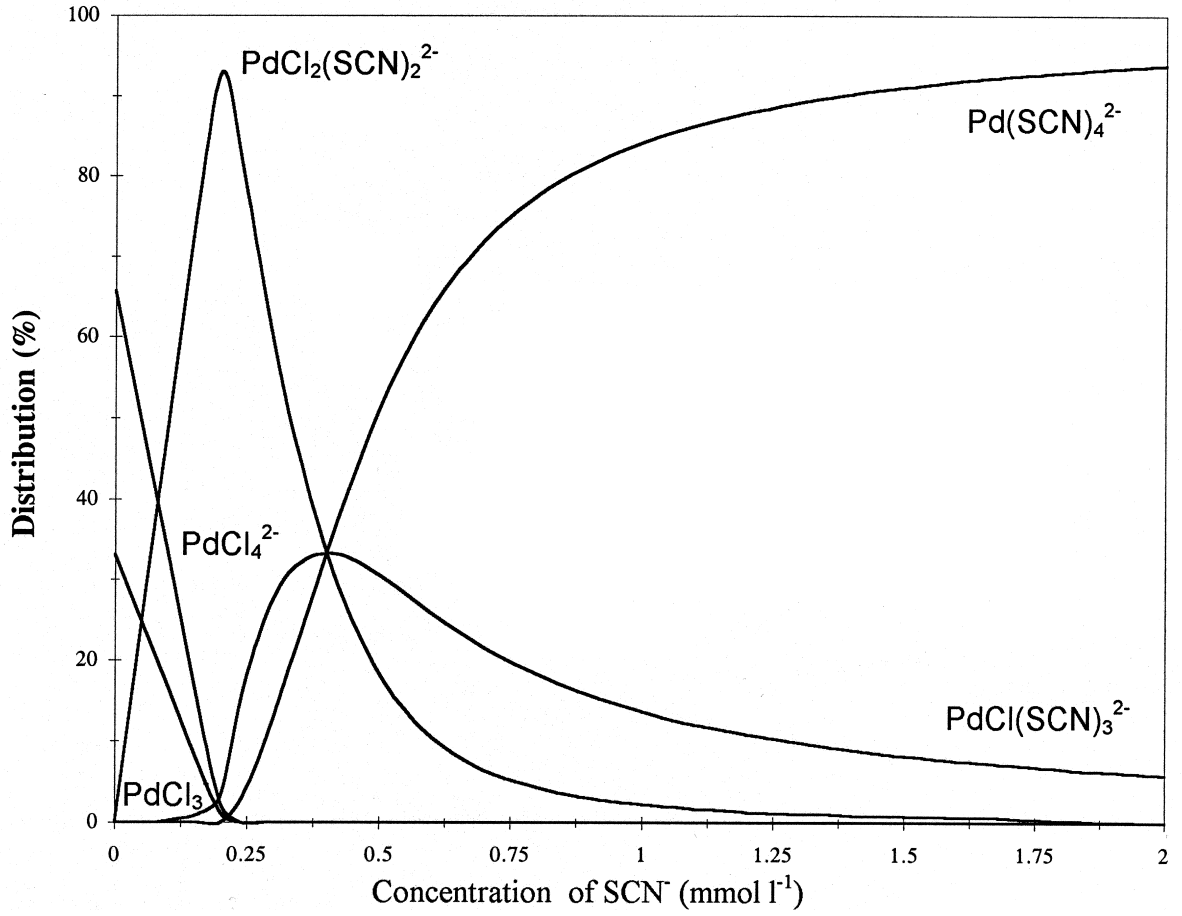
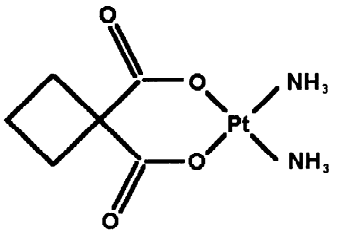


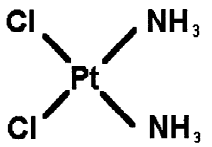
Fig. 1. Distribution diagram of Pd(II) species in chloride-thiocyanate system, concentration of Pd(II) $1.0 \cdot 10^{-4}$ mol l^{-1}, pH 3, $c_{Cl^-} = 0.1$ mol l^{-1}. Diagram calculated using stability constants as published in literature [30].

y is absorbance). However, the electrolyte used is unbuffered and this usually leads to imprecision.

Some characteristics of thiocyanate complexes are given in Table 1, where the LODs were determined for the conditions as described above. Using lower concentrations of KSCN (in diluted samples, $c_{KSCN} = 10^{-3}$ mol l^{-1}, electrokinetic injection 8.0 s), LOD as low as 5 ppb for Pd(II) was achieved.



I



II

3.4. Applications

The method developed was used to analyze Cykloplatin and Ribocarbo containing carboplatin (I) and Platidiam containing cisplatin (II).

Pt in these pharmaceutical products is better determined in the form of $Pt(SCN)_4^{2-}$ using Procedure 1. For the calibration a standard solution of $Pt(Cl)_4^{2-}$ was used. A good agreement with the declared value was achieved for analysis of cisplatin

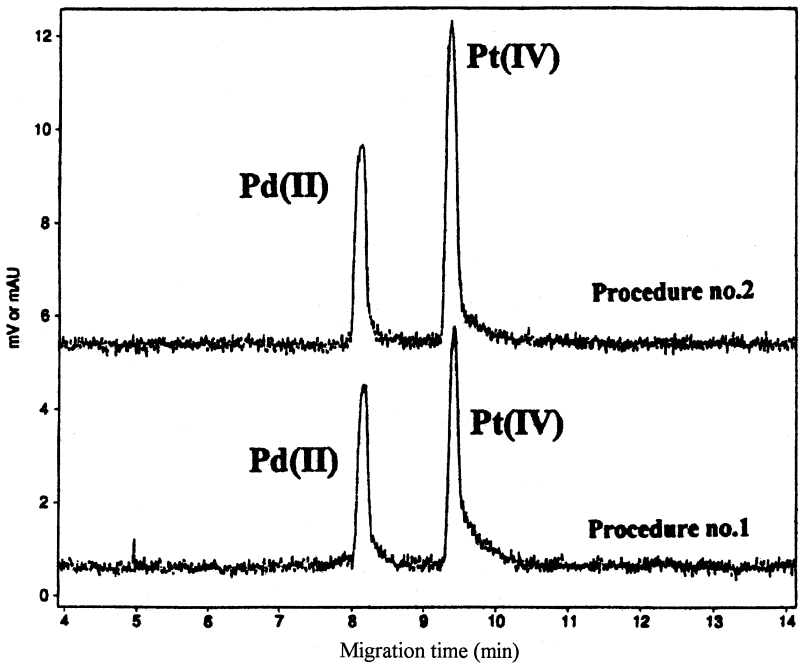


Fig. 2. Electrophoreogram of a simultaneous determination of Pd(II) and Pt(IV) at 295 nm, $c_{Pd(II)}=2.38\cdot10^{-5}$ mol l^{-1}, $c_{Pt(IV)}=7.32\cdot10^{-5}$ mol l^{-1}. Background electrolyte: 0.1 mol l^{-1} KSCN, pH 3 (adjusted by $HClO_4$), temperature 50°C, separation voltage applied −10 kV, hydrodynamic injection 4 s.

(Fig. 3). In the cases of Cykloplatin and Ribocarbo, the obtained values were lower than nominal values (Table 2), but approximately in accordance with ICP values.

The method for Pd(II) determination was also applied for NIES No. 8 standard material to determine low levels of Pd(II). The solution of NIES prepared was analyzed either by ICP-MS {120 ng Pd(II) per 1 g of material was found, while the refereed value is 180±28 ng/g [28] or 230±80 ng/g [29]}, or by CZE after the addition of thiocyanates. Owing to the complex NIES matrix, a change in migration time of Pd(II) peak with respect to that of pure Pd(II) standard was observed during CZE analysis. In spite of this, a satisfactory agreement with ICP value was obtained. The value of Pd content found by CZE was 105±34 ng/g. Our values are slightly lower than those found in the literature [28,29].

4. Conclusions

A new analytical method for the determination of Pd(II), Pt(II) and Pt(IV) was developed. This method applying thiocyanate complexes offers advantage-

Table 1
Some characteristics of thiocyanate complexes and CZE analysis

Metal	Procedure supposed	Wavelength (nm)	Migration time (min)	Mobility [10^5 $cm^2/(V\,s)$]	Limit of detection (ppm)
Pd(II)	1	305	8.3	−71.3	0.3
Pt(II)	1	245	8.5	−69.4	0.9
Pt(IV)	2	290	9.5	−60.8	2.3

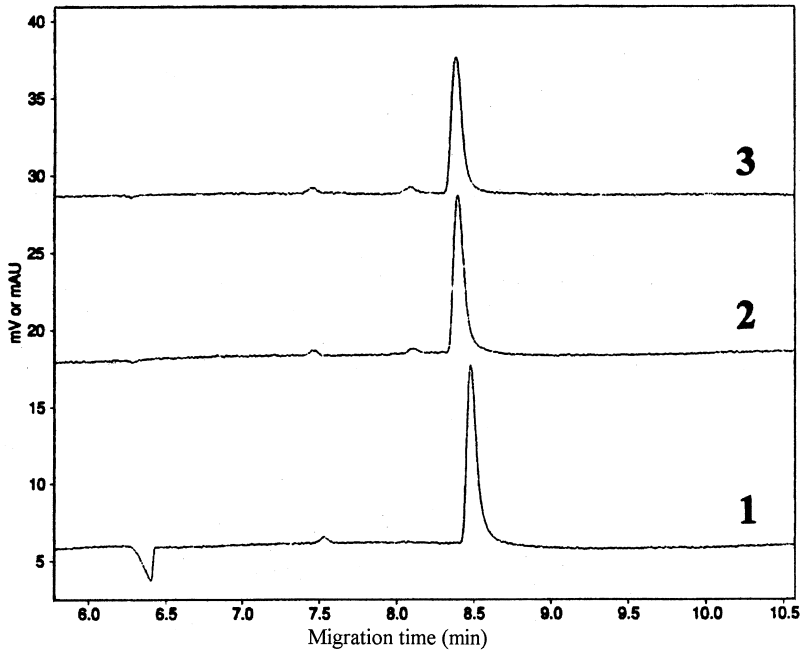


Fig. 3. Examples of electrophoreograms for determination of Pt(II) at 245 nm. Curves: (1) $Pt(Cl)_4^{2-}$, $c=7.7\cdot10^{-5}$ mol l^{-1}; (2) cisplatin, $c=6.7\cdot10^{-5}$ mol l^{-1}; (3) carboplatin, $c=6.7\cdot10^{-5}$ mol l^{-1} . The other conditions are the same as given in Fig. 2.

Table 2
Results of analysis of Pt containing drugs

	Declared (mg/g)	Found CZE (mg/g)	Relative error (%)	R.S.D. (%)	Found ICP (mg/g)	Relative error (%)	R.S.D. (%)
Carboplatin (Cykloplatin)	10.00	8.85	11.5	5.1	8.38	16.2	0.6
Carboplatin (Ribocarbo)	10.00	9.17	8.3	5.3	8.91	9.1	1.3
Cisplatin (Platidiam)	1.00	1.03	3	2.9	0.88	12	3.3

R.S.D. = relative standard deviation (obtained from five parallel analyses).

ous possibilities for the determination of these metals. In the case of Pd(II), an LOD as low as 5 ppb was reached and the method was applied to determine Pd content in NIES standard material. The method for Pt determination was applied to the determination of total Pt(II) content in several drugs. The results obtained were close to those obtained by ICP results. Conditions for the simultaneous determination of Pd(II) and Pt(IV) by CZE at 295 nm were proposed.

Acknowledgements

This work was supported by Grant Agency of the Czech Republic, grant No. 203/96/0478.

References

[1] H. Kozlowski, L.D. Pettit, in F.R. Hartley (Editor), Chemistry of the Platinum Group Metals – Recent Developments, Elsevier, Amsterdam, 1991, Ch. 15, pp. 530–542.
[2] M.J. Abrams, B.A. Murrer, Science 261 (1993) 726–730.
[3] R.R. Barefoot, J.C. Van Loon, Anal. Chim. Acta 334 (1996) 5–14.
[4] B. Michalke, S. Lustig, P. Schramel, Electrophoresis 18 (1997) 196–201.
[5] J. Messerschmidt, F. Alt, G. Tölg, Electrophoresis 16 (1995) 800–803.
[6] B.W. Wenclaviak, M. Wollmann, J. Chromatogr. A 724 (1996) 317–326.
[7] M. Macka, J. Borák, J. Chromatogr. 641 (1993) 101–113.
[8] M. Macka, J. Borák, F. Kiss, J. Chromatogr. 586 (1991) 291–295.
[9] J. Doležal, L. Sommer, Collect. Czech. Chem. Commun. 62 (1997) 1029.

[10] R. Kizu, T. Yamamoto, T. Yokoyama, M. Tanaka, M. Miyazaki, Chem. Pharm. Bull. 43 (1995) 108.
[11] A. Anderson, H. Ehrsson, J. Chromatogr. B 652 (1994) 203.
[12] E. Brandsteterova, F. Kiss, V. Chovancova, V. Reichelova, Neoplasma 38 (1991) 415.
[13] C. Vogt, G. Werner, J. Chromatogr. A 686 (1994) 325–332.
[14] W. Buchberger, O.P. Semenova, A. Timerbaev, J. High Resolut. Chromatogr. 16 (1993) 153–156.
[15] W. Buchberger, P.R. Haddad, J. Chromatogr. A 687 (1994) 343–349.
[16] M. Aguilar, A. Farran, V. Martí, J. Chromatogr. A 778 (1997) 397–402.
[17] M. Mojski, I. Gluch, N. Obarski, Chem. Anal. 42 (1997) 825–835.
[18] A.N. Khramov, J. Havel, Scripta Fac. Sci. Nat. Univ. Masaryk. Brun. 27(Chemistry) (1997) 63–73.
[19] A.V. Pirogov, J. Havel, J. Chromatogr. A 772 (1997) 347–355.
[20] B. Baraj, A. Sastre, M. Martínez, K. Spahiu, Anal. Chim. Acta 319 (1996) 191–197.
[21] H.-W. Zhang, Li Jia, Zhi-De Hu, J. Chromatogr. A 704 (1995) 242–246.
[22] D. Nachtigall, S. Artelt, G. Wünsch, J. Chromatogr. A 775 (1997) 197–210.
[23] L.I. Elding, Acta Chem. Scand. 24 (1970) 1331.
[24] M. Lederer, E. Leipzigpagani, Anal. Chim. Acta 350 (1997) 203–208.
[25] J.M. Sanchez, V. Salvado, J. Havel, J. Chromatogr. A (1998) in press.
[26] S. Khopkar, Anal. Chem. 38 (1966) 360.
[27] J. Doležal, Thesis, Masaryk University, Brno, 1995.
[28] T. Paukert, I. Rubeška, Anal. Chim. Acta 278 (1993) 125.
[29] R. Vlašánková, V. Otruba, J. Bendl, M. Fišera, V. Kanický, Talanta (1998) in press.
[30] S. Kotrlý, L. Šůcha, Handbook of Chemical Equilibria in Analytical Chemistry, Ellis Horwood, Chichester, 1985.