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USE OF COMPLEX FORMATION EQUILIBRIA IN THE ANALYTICAL ISOTACHOPHORESIS OF STRONG ELECTROLYTE IONS*

SEPARATION OF HALIDES AND SULPHATES

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SUMMARY

The possibility of effecting isotachophoretic separations by means of complex formation equilibria between a counter ion and the components being separated was investigated. This procedure was shown to be of potential significance mainly for the separation of ionic species of strong electrolytes, and the separation of sulphates and halides was examined. The use of Cd(II) as a counter ion and NO_3^- as a leading ion is suggested. The migration of sulphate and nitrate zones is considered theoretically, and data calculated for actual operating conditions are verified experimentally.

INTRODUCTION

An isotachophoretic separation is based on the differences in the effective mobilities of ionic species. Separations have been described in which effective mobilities were altered, with the aim of obtaining the required separations, by changing the solvation¹ and by selecting an appropriate pH of the leading electrolyte^{2,3}. In isotachophoresis, sufficient attention has not been devoted to the possibility of influencing the effective mobilities of the ionic species being separated by means of complex formation equilibria. Unlike the effect of pH on the effective mobilities of ionic species, complex formation may be applicable even with anions of strong acids or with a number of cations where the degree of dissociation is not affected substantially by pH.

The significance of this possibility is evident when one considers, for example, the separation of halides and sulphates, which is an important analytical problem that can hardly be solved by classical isotachophoresis using water. The general

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problem can be treated by considering such a practically important model. It follows from the principle of isotachophoresis that the only ionic species in the sample that can be separated are those with mobilities lower than that of the leading ion and greater than that of the terminating ion. There is no problem in finding a suitable terminating anion in a sample containing sulphates and chlorides because, under the conditions used in practice, *i.e.*, room temperature and with concentrations of the solutions varying from 10^{-2} to 10^{-4} M, both sulphates and halides are among the most mobile anions. However, finding a suitable leading electrolyte with which sulphates and halides will form isotachophoretic zones represents a problem.

A possible solution to the last problem is to find suitable anions with mobilities greater than those of sulphate and chloride. Such leading ionic species could be, for example, chromates, hexacyanoferrates(II) and hexacyanoferrates(III), the limiting mobilities of which are greater than those of sulphate and chloride. Experiments showed⁴, however, that under the above conditions the mobilities of these ionic species are not sufficiently high, owing either to incomplete dissociation or to a considerably greater retarding effect of the ionic strength on multivalent ions in accordance with the Debye-Hückel-Onsager relationship. Also, the use of OH^- , which is the most mobile anion of all in water, is not suitable as the leading electrolyte would acquire a pH of 11–12 and an interference with the separation could be expected from carbonates present in the solution or from possible hydrolytic products in the injection port where the leading electrolyte comes into contact with the sample. In addition, this pH value lies outside the so-called⁵ "safe region" for aqueous media.

Another possibility is to utilize selective depression of the mobilities of sulphate and halide. Such effects on the mobilities of strong ions were obtained by using non-aqueous media, *e.g.*, methanol, instead of water¹ and by isotachophoresis on paper^{6,7}, the isotachophoretic migration being combined with chromatographic effects. The mobilities of strong acid anions can also be altered by using complex formation equilibria.

If we respect the fundamental requirement of isotachophoresis, *i.e.*, that any electric current in a given zone may be transferred only by the ionic species under separation and by a counter ion, then only the complex formation equilibria between the counter ion and the ionic species being separated can be utilized. Hence the use of complex formation equilibria in isotachophoresis differs from that in zone electrophoresis, where use is made of the equilibria between the ionic species being separated and suitable complex-forming ionic species present in a background electrolyte^{8,9}.

Another important requirement is that the time of existence of each ionic species involved in the complex equilibria must be small in comparison with the time of migration, *i.e.*, the equilibrium adjustments must be very rapid (*cf.*, Tiselius¹⁰). The use of complex formation equilibria in isotachophoresis therefore differs from the isotachophoresis of kinetically inert complexes^{11–13}.

The selection of a suitable central cation that would serve as a counter ion in the isotachophoretic migration of halides and sulphates, a description of the system and an experimental verification are the subjects of this paper.

THEORETICAL

When selecting an operational electrolyte system such that sulphates and

halides are affected by complex formation in such a way that they form isotachophoretic zones, the following requirements apply:

(1) The establishment of the complex formation equilibria of halides and sulphates with a counter ion must be sufficiently rapid. The composition of the system should be such that the effective mobilities of halide and sulphate may decrease to such an extent that a suitable leading component can be found and, at the same time, the decrease should not be too great so that finding a suitable terminator would not be difficult. If some of the limiting mobilities at 25° are compared (*cf.*, ref. 14) *e.g.*, 80.0, 76.4, 71.5, 67.0, 55.0, 41.4 and $30.4 \cdot 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{sec}^{-1}$ for sulphate, chloride, nitrate, chlorate, fluoride, acetate and picrate anions, respectively, and if the concentration dependence is assumed to be approximately constant, it is evident that the decrease in the mobilities of sulphate and chloride anions due to the complex formation equilibrium should be more than *ca.* 10%. From the viewpoint of the selection of the terminator, the decrease should not exceed *ca.* 70%. Thus, for example, with sulphate the effective mobility of sulphates, \bar{u}_i , should be between 30 and 90% of the ionic mobility, $u_{\text{SO}_4^{2-}}$.

(2) The counter ion must not have a disturbing effect in combination with any of the anions assumed to be present in the sample or with the terminating electrolyte. Hence there must be no risk of the formation of precipitates or of products that are only slightly dissociated.

(3) A suitable anion must be available that virtually does not form a complex with the selected counter ion and with a sufficiently high mobility that it may serve as a leading component.

(4) It is advantageous if the complexes of halides and sulphates with the selected counter ion are well known, *i.e.*, their compositions, stability constants and temperature dependences and, moreover, if the mobilities of the individual particles involved in complex formation equilibria are also known.

Under such conditions, for a given composition of the leading electrolyte used, a mathematical description of the isotachophoretic zones can be given and the resulting net mobilities of halides and sulphates can be calculated, as will be shown. Such a case is analogous to the calculation of concentrations in isotachophoretic zones where acid-base equilibria are taken into account¹⁵.

On the basis of the above considerations, the cadmium(II)-ion was selected as a suitable counter ion as it forms in aqueous media both sulphato and halogeno complexes, the compositions and stability constants of which are known. The nitrate anion, which virtually does not form complexes with the cadmium(II)-ion in *ca.* 0.01 M solutions¹⁶ and the mobility of which is sufficiently great, was selected as the leading ionic species.

Citrate and/or tartrate can be used as terminator as both of them have sufficiently low mobilities.

A mathematical description of the zones, numerical calculation of net mobilities and experimental verification of the calculated values were carried out for sulphates and nitrates. The assumed compositions of the zones are as follows: leading zone, Cd^{2+} , NO_3^- ; and sulphate zone, Cd^{2+} , CdSO_4 , SO_4^{2-} . Data on the limiting ionic mobilities of the ions involved are available (see Experimental) and the mobility of CdSO_4 may be considered to be zero.

In all instances, the limiting ionic mobility, $u_{i,0}$, of an ion *i* is corrected for

the given concentration, c , in its zone according to the Debye-Hückel-Onsager relationship as follows¹⁷:

$$u_{i,c} = u_{i,0} - \left(\frac{29.14 z_i}{\eta (\varepsilon T)^{1/2}} - \frac{9.90 \cdot 10^{-5}}{(\varepsilon T)^{3/2}} \cdot u_{i,0} \omega \right) \cdot I^{1/2} \quad (1)$$

$$\omega = z_+ z_- \cdot \frac{2q}{1 + q^{1/2}} \quad (2)$$

$$q = \frac{z_+ z_-}{z_+ + z_-} \cdot \frac{u_+ + u_-}{z_+ u_- + z_- u_+} \quad (3)$$

$$I = 0.5 (c_+ z_+^2 + c_- z_-^2) \quad (4)$$

where $u_{i,c}$ indicates the ionic mobility at concentration c and c , z and u indicate the concentration, the valency of the ion (absolute value) and the mobility, respectively. The subscripts indicate the appropriate cation or anion. ε is relative permittivity and η the viscosity.

Further, net mobilities are calculated by using definition equations^{10,18}. For the net mobility of sulphate in its zone, $\bar{u}_{\text{SO}_4, c_S}$, where the total concentration of sulphates in their zone is c_S , the following relationship holds:

$$\bar{u}_{\text{SO}_4, c_S} = \frac{[\text{SO}_4^{2-}]}{c_S} \cdot u_{\text{SO}_4^{2-}, c_S} \quad (5)$$

\bar{u}_{Cd, c_S} represents the net mobility of Cd ionic species, where

$$\bar{u}_{\text{Cd}, c_S} = \frac{[\text{Cd}^{2+}]}{c_{\text{Cd}}} \cdot u_{\text{Cd}^{2+}, c} = \frac{[\text{SO}_4^{2-}]}{c_S} \cdot u_{\text{Cd}^{2+}, c_S} \quad (6)$$

as the equivalence of the total concentrations, $c_{\text{SO}_4} = c_{\text{Cd}} = c_S$, and of the electric neutrality, $[\text{SO}_4^{2-}] = [\text{Cd}^{2+}]$, obviously holds. The value of $[\text{SO}_4^{2-}]$ can be expressed by means of the expression for the total concentration of the ionic species, i.e., $c_S = [\text{SO}_4^{2-}] + [\text{CdSO}_4]$ and the definition for the thermodynamic complex stability constant of CdSO_4 :

$$K = \frac{a_{\text{CdSO}_4}}{a_{\text{Cd}^{2+}} \cdot a_{\text{SO}_4^{2-}}} = \frac{[\text{CdSO}_4]}{[\text{Cd}^{2+}] \cdot [\text{SO}_4^{2-}] \cdot \gamma^2} \quad (7)$$

The following equation¹⁹ is used in order to determine the activity coefficient, γ :

$$-\log \gamma = 0.5 z_{\text{Cd}} \cdot z_{\text{SO}_4} \cdot \left(\frac{I_S^{1/2}}{1 + I_S^{1/2}} - 0.3 I_S \right) \quad (8)$$

For the ionic strength in the sulphate zone, $I_S = 4[\text{SO}_4^{2-}]$.

The total concentration of sulphates in their zone, c_S , is given by a moving-boundary equation, modified for isotachophoretic migration into the following form²⁰:

$$\frac{\bar{u}_{\text{SO}_4, c_S}}{\kappa_{\text{SO}_4}} = \frac{u_{\text{NO}_3^-, c_N}}{\kappa_{\text{NO}_3}} \quad (9)$$

where κ is the specific conductivity of the sulphate or nitrate zone and $u_{\text{NO}_3^-, c_N}$ is the net mobility of nitrates in their zone, their total concentration being c_N . After expressing κ explicitly, eqn. 9 can be rearranged into the following form²¹:

$$\frac{c_S}{c_N} = \frac{\bar{u}_{\text{SO}_4, c_S}}{\bar{u}_{\text{SO}_4, c_S} + \bar{u}_{\text{Cd}, c_S}} \cdot \frac{u_{\text{NO}_3^-, c_N} + u_{\text{Cd}^{2+}, c_N}}{u_{\text{NO}_3^-, c_N}} \cdot \frac{z_{\text{NO}_3}}{z_{\text{SO}_4}} \quad (10)$$

By applying eqns. 1–4 to nitrate, sulphate and cadmium ionic species in their respective zones and by combining them with the eqns. 5–8 and 10, we obtain the system of equations that describes the isotachophoretic migration of sulphates, *i.e.*, the system of equations that describes the concentrations and the mobilities in the zones of sulphates as a function of the concentration of the leading anion, NO_3^- , in the leading electrolyte, $\text{Cd}(\text{NO}_3)_2$.

Such a system of equations can be solved by iteration in such a way that for a given concentration, c_N , a zeroth approximation, $[\text{SO}_4^{2-}]_0 = 0.5 c_N$ is selected. By substituting $[\text{SO}_4^{2-}]_0$ into eqn. 8, the first approximation, y_1 , is obtained.

By substituting c_N and $[\text{SO}_4^{2-}]_0$ into a combination of eqns. 5, 6 and 10, the first approximation, $(c_S)_1$, is obtained. Using y_1 , $(c_S)_1$ in eqn. 7 yields the first approximation $[\text{SO}_4^{2-}]_1$. The whole procedure is repeated with the approximation so obtained until two subsequent approximations are in agreement, corresponding to the required accuracy; an accuracy of better than 1% is sufficient in this instance. The required mobility of sulphate, $\bar{u}_{\text{SO}_4, c_S}$, is then calculated from eqn. 5.

EXPERIMENTAL

The equipment used for isotachophoresis consisted of a block of organic glass into which were built electrode chambers, control cocks, connecting channels and a separation capillary. The separations were performed in a separation capillary of tangular cross-section, with dimensions $200 \times 1.0 \times 0.2$ mm, created by a groove in the organic block, covered with PTFE foil pressed on the block with a thermostatted plate. Detection was carried out by measuring the electric gradient by means of platinum contacts (two wires of diameter 0.05 mm, *ca.* 0.05 mm apart), penetrating into the groove a distance of *ca.* 16 cm from the injection port. A detailed description was published earlier²².

A stabilized current of up to 400 μA at the maximum of up to 16 kV served as a high-voltage source. Detection was effected with a voltmeter with a high input resistance, simultaneously insulating galvanically a high-voltage section of the measuring circuits from the section connected to a recorder. The high-voltage source and the detection device were of our own design and have been described earlier²³.

A Perkin-Elmer Model 196 recorder was used. The chemicals used were of analytical-reagent grade (Lachema, Brno, Czechoslovakia). The thermostatted metal plate was maintained at 25° by means of circulating water.

The mobilities of the individual ionic species being investigated were calculated and measured as relative mobilities, where the leading NO_3^- ion was used as a reference ionic species; hence the calculated values represented the ratio $\bar{u}_i/u_{\text{NO}_3^-}$. Experimental measurements of the relative mobilities were carried out by using a procedure described earlier²¹. An inverse ratio of the step heights, $h_{\text{NO}_3^-}/h_i$, was measured. The ionic species being investigated were injected separately; the concentrations of standards were $5\text{--}8 \cdot 10^{-4} M$ and the sample size was *ca.* 1 μl . A solution of $\text{Cd}(\text{NO}_3)_2$ was used as the leading electrolyte with concentrations selected in the range 0.002–0.010 M ; 0.010 M citric acid was used as the terminating electrolyte.

In the device used²², at a driving current of approximately 300 μA and a thermostat temperature of 25°, the temperature of the solution in which migration occurred was 35° (ref. 24). The operating parameters were as follows: Temperature, 35°; relative permittivity, $\epsilon = 82.3$; viscosity, $\eta = 0.00716 \text{ P}$; limiting ionic mobilities at the given temperature, $u_{\text{SO}_4^{2-},0}$, $u_{\text{NO}_3^-,0}$ and $u_{\text{Cd}^{2+},0}$, 96.0, 85.8 and $64.8 \cdot 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{sec}^{-1}$, respectively. The thermodynamic complex stability constant of CdSO_4 is, under the conditions given, $\log K = 2.11$ (ref. 16). The valencies of the ions are obvious: $z_{\text{SO}_4} = 2$, $z_{\text{Cd}} = 2$ and $z_{\text{NO}_3} = 1$.

RESULTS AND DISCUSSION

Using the above relationships, data were calculated for the isotachophoretic migration of sulphate and nitrate with cadmium as counter ion, and a comparison of the experimental values of the relative mobility of sulphate with the calculated values is shown in Table I. Good agreement was found.

The effect of the complex formation equilibria on the relative mobilities of halides was evaluated experimentally in a similar manner. The calculations were not performed in these instances as the ionic mobilities of the ions of the type CdX^+ are not available. It is evident that the reverse procedure, *i.e.*, experimental determination of the mobility of the ionic species with a known composition of the equilibrium

TABLE I

COMPARISON OF EXPERIMENTAL VALUES (AVERAGE FROM THREE DETERMINATIONS) OF THE RELATIVE STEP HEIGHTS, $h_{\text{NO}_3^-}/h_{\text{SO}_4}$, WITH THE CALCULATED VALUES OF THE RELATIVE MOBILITIES, $\bar{u}_{\text{SO}_4^{2+}}/u_{\text{NO}_3^-}$.

Concentration of leading electrolyte, $\text{Cd}(\text{NO}_3)_2$ (M)	$h_{\text{NO}_3^-}/h_{\text{SO}_4}$	$\bar{u}_{\text{SO}_4^{2+}}/u_{\text{NO}_3^-}$	Difference	Difference (%)
0.002	0.917 ± 0.018	0.934	-0.017	-1.8
0.004	0.872 ± 0.018	0.870	+0.002	+0.2
0.005	0.847 ± 0.018	0.840	+0.007	+0.8
0.006	0.806 ± 0.018	0.813	-0.007	-0.9
0.008	0.763 ± 0.018	0.781	-0.018	-2.3
0.010	0.741 ± 0.018	0.752	-0.011	-1.5

TABLE II

EXPERIMENTAL VALUES OF THE RELATIVE MOBILITIES OF HALIDES, $u_i/u_{NO_3^-}$
 The data represent averages from three measurements and the confidence interval is ± 0.018 .

Concentration of leading electrolyte, $Cd(NO_3)_2$ (M)	F	Cl	Br	I
0.002	0.794	0.952	0.917	0.800
0.004	0.769	0.892	0.862	0.752
0.005	0.769	0.862	0.840	0.714
0.006	0.769	0.847	0.806	0.694
0.008	0.756	0.820	0.775	0.671
0.010	0.746	0.794	0.756	0.637

system in the zone can provide data on the ionic mobilities of complexes, which can be obtained by other means only with difficulty.

The experimental values of the relative mobilities of halides are given in Table II, and confirm the possibility of affecting the mobilities of strong electrolyte anions by means of a complex-forming cationic species. If the limiting mobilities¹⁴ tabulated are compared, *e.g.*, fluoride, chloride, bromide, iodide and nitrate have limiting mobilities of 55.4, 76.4, 78.1, 76.8 and $71.5 \cdot 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{sec}^{-1}$, respectively, it can be seen that except for fluoride, the halides are more mobile than nitrate and, moreover, the difference between the mobilities of chloride and iodide is small. On using the complex formation equilibrium with cadmium, the mobilities of halides not only become smaller than that of nitrate but they also differ sufficiently from one another and their isotachophoretic separation is then easy, as shown in Fig. 1, where the separation of a mixture of chlorides, bromides and iodides is shown.

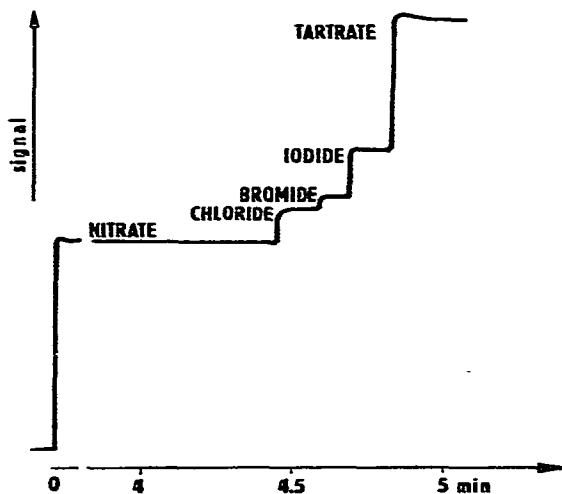


Fig. 1. Analysis of a mixture of chloride, bromide and iodide; 0.006 M $Cd(NO_3)_2$ served as the leading electrolyte. The volume injected was about $2 \mu\text{l}$ and the concentration of each component was about 0.01 M. The driving current was $400 \mu\text{A}$.

A practical analysis of 10 μl of IDA mineral water from Spa Běloves, Czechoslovakia, containing 119.0 mg of sulphate, 17.60 mg of chloride, 0.30 mg of fluoride, 6.08 mg of nitrate and 1.05 mg of arsenate in 1 l, is shown in Fig. 2.

Chloride and sulphate provide zones that can be evaluated analytically. A quantitative analysis was carried out by direct comparison with a standard mixture and the results, representing average values from three determinations, are given in Table III.

Fluoride and arsenate zones are, under the operating conditions used, below the sensitivity of the apparatus, which was about $1 \cdot 10^{-8}$ g of halides.

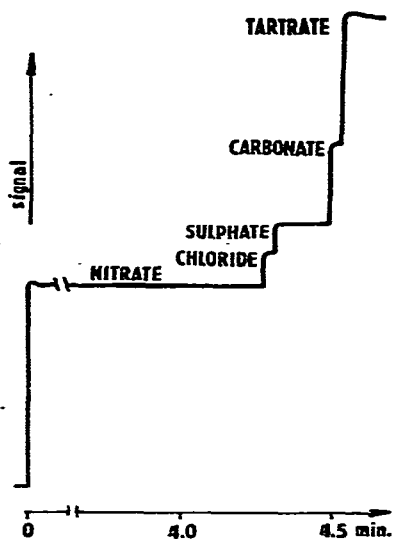


Fig. 2. Analysis of 10 μl of IDA mineral water. The leading electrolyte was 0.006 M $\text{Cd}(\text{NO}_3)_2$ and driving current was 400 μA . The carbonate zone is unstable. A; the sample is injected, with tartrate as terminator, it migrates but it vanishes during the migration and the quantitative information is lost.

TABLE III

DETERMINATION OF SULPHATE AND CHLORIDE IN IDA MINERAL WATER

Species	True concentration (mg/l)	Concentration determined (mg/l)	Standard deviation		Deviation from true value	
			mg/l	%	mg/l	%
Sulphate	119.0	117.1	2.1	1.8	-1.9	-1.6
Chloride	17.60	17.70	0.16	0.9	+0.1	+0.6

CONCLUSIONS

Complex formation equilibria are a significant factor by means of which the effective mobilities and thus also the possibility of the isotachophoretic separation of ionic species of strong electrolytes can be influenced. A complex-forming agent acts

as the counter ion; hence complex formation between the counter ion and the component being separated is involved. In the separation of the anions of strong acids, an appropriately selected cation acts as the complex-forming agent.

If the stability constants and the mobilities of all of the particles involved in the complex formation equilibrium of the component being separated with the counter ion are known, the total concentration of the ionic species being separated in its zone and its net mobility can be calculated for a given composition of the leading electrolyte. Conversely, the mobilities of complex particles can be determined from the experimental data.

The calculation was carried out for the zone of sulphate migrating isotachophoretically behind the zone of nitrate with Cd(II) as the counter ion, for concentrations of cadmium nitrate in the leading electrolyte varying over the range 0.002–0.010 M. The calculated effective mobilities of sulphate relative to nitrate were compared with the data measured experimentally and the agreement was found to be good.

The system formed by the leading nitrate anion and the cadmium counter ion appeared to be suitable for the separation of halides and sulphates. The suggested system of electrolytes was applied to the analysis of sulphates and chlorides in mineral waters. The determination took about 5 min.

REFERENCES

- 1 J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, 51 (1970) 339.
- 2 J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, 69 (1972) 165.
- 3 P. Boček, K. Lekova, M. Deml and J. Janák, *J. Chromatogr.*, 117 (1976) 97.
- 4 P. Boček and I. Miedziak, unpublished results.
- 5 R. J. Routs, *Thesis*, Technical University, Eindhoven, 1971.
- 6 V. Taglia and M. Lederer, *J. Chromatogr.*, 77 (1973) 467.
- 7 V. Taglia, *J. Chromatogr.*, 79 (1973) 380.
- 8 V. Jokl, *J. Chromatogr.*, 13 (1964) 451.
- 9 V. Jokl, *J. Chromatogr.*, 71 (1972) 523.
- 10 A. Tiselius, *Nova Acta Regiae Soc. Sci. Ups.*, 7, No. 4 (1930) 1.
- 11 E. Blasius and U. Wenzel, *J. Chromatogr.*, 49 (1970) 527.
- 12 E. Blasius, H. Augustin and U. Wenzel, *J. Chromatogr.*, 50 (1970) 319.
- 13 W. Preetz and H. L. Pfeifer, *Talanta*, 14 (1967) 143.
- 14 J. Dvořák, J. Koryta and V. Boháčková, *Electrochemistry*, Academia, Prague, 1966, p. 104 (in Czech).
- 15 F. M. Everaerts and R. J. Routs, *J. Chromatogr.*, 58 (1971) 181.
- 16 L. G. Sillén and A. E. Martell, *Stability Constants of Metal-Ion Complexes*, Special Publication No. 17, Chemical Society, London, 1964.
- 17 E. Hála and A. Reiser, *Physical Chemistry*, Vol. II, Academia, Prague, 1966, p. 274 (in Czech).
- 18 R. Conden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, 40 (1946) 33.
- 19 L. Šůcha and S. Kotrlý, *Solution Equilibria in Analytical Chemistry*, Van Nostrand Reinhold, London, SNTL, Prague, 1972, p. 32.
- 20 P. Boček, M. Deml and J. Janák, *J. Chromatogr.*, 91 (1974) 829.
- 21 H. Haglund, *Sci. Tools*, 17 (1970) 2.
- 22 P. Boček, M. Deml and J. Janák, *J. Chromatogr.*, 106 (1975) 283.
- 23 M. Deml, P. Boček and J. Janák, *J. Chromatogr.*, 109 (1975) 49.
- 24 Z. Ryšlavý, P. Boček, M. Deml and J. Janák, *Collect. Czech. Chem. Commun.*, in press.