Electromigration behavior of metal ions in the presence of complexing polymer

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Abstract

Electromigration behavior of metal ions in the presence of high concentrations of polyethylene glycol as a complexing agent was investigated under acidic non-buffered conditions. Simulation of the isotachophoretic steady state of the system containing the neutral polymer was performed. Both the complexation and the influence of viscosity on the electrophoretic mobility were taken into account. Good agreement between the calculated results and the experimentally obtained values was achieved. Based on the theoretical model, a new approach for the estimation of polymer–metal stability constants from experimental isotachophoretic data was proposed. Stability constants of Li^+, Na^+, Cs^+, Mg^{2+}, Sr^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Lu^{3+} and Y^{3+} ions with polyethylene glycol were determined. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Electromigration methods for the analytical separation of metal ions in aqueous solutions are still actual and attractive although other analytical tools, such as atomic absorption spectroscopy, compete with them. Analysis of metal ions [1–4] covers a relatively small part of isotachophoretic (ITP) and capillary zone electrophoretic (CZE) applications compared with the separation of small anions, pharmaceuticals, peptides, proteins, oligonucleotides and DNA, where electromigration methods are used very often nowadays. The main problems in electrophoretic separation of metal ions are similar electrophoretic mobilities and weak UV absorption.

Metal ions mostly belong to strong electrolytes and, therefore, their effective mobilities cannot be efficiently controlled by changes of pH of the background electrolyte. In this case, changing solvation by using mixed solvents [5,6] in the system can be used to influence the mobility and hence the selectivity of separation.

Utilizing complexation is another possibility for improving separation using electromigration methods. Either charged or neutral agents can be used. α-Hydroxyisobutyric acid (α-HIBA) often serves as a charged weakly complexing agent in the electromigration separation of metal ions. The metal ions from a model solution of high level liquid waste (HLLW) from nuclear power plants, some of which are used as samples in this work, were successfully isotachophoretically separated by Hirokawa et al. [7] using α-HIBA as the complexing agent. α-HIBA was used also for the complete separation of heavy metals by Everaerts et al. [2] and for the separation of lanthanides by Nukatsuka et al. [3]. All of these

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experiments were formed with pH values of the electrolyte solutions being approximately 5.

Neutral complexing agents (NCAs) have some advantages over ionic agents. There is a significant difference between strongly and weakly complexing agents. If the stability constant of the metal–NCA complex is high, a low concentration of the complexing agent is sufficient to effectively influence the mobility of the metal ion. The conductivity of the separation system is not substantially affected. The most common NCAs in this group are cyclic polyethers (e.g. 18-crown-6-ether) [8,9] and cyclodextrins.

Neutral weakly interacting ligands bring additional complexity to the separation mechanism. Because of the weak interaction of those ligands with the analyte, they have to be used at a rather high concentration to enable successful separation, which consequently alters other characteristics of the solution, namely its viscosity. Mobilities of solutes are then affected by both complexation and viscosity effects. Polyethylene glycol (PEG) can serve as an example of a weakly interacting ligand. Kaniansky et al. [10] used 40–45% (v/v) polyethylene glycol in water for the effective separation of alkali and alkaline earth metal cations. Stathakis and Cassidy [11] used PEGs of molecular masses ranging from 200 to 20 000 000 as background additives to influence the selectivity of CZE separation.

A theoretical description of migration in a system containing a complexing agent enables one to determine basic physicochemical quantities, mainly the stability constant, from experimentally obtained results. A number of methods using different approaches exist, a review of which is given by Rundlett and Armstrong [12].

Electromigration methods are considered to be a serious candidate for the preparative separation of various metal ions from HLLW after the extraction of plutonium and uranium. The metals are in a very acidic solution after dissolution of the burned fuel in nitric acid. It is therefore difficult to separate them in common electrolyte systems, as the excess of nitric acid in the sample would react with the buffer. Also, it is not possible to use weak acidic ligands (like α-HIBA) as complexing agents, although they work well in buffered systems at pH values of about 5. In this case, PEG could be a good choice as the complexing agent, because its complexing ability remains unchanged in acidic solutions.

The aim of this paper is to investigate the electromigration behavior of metal ions in the presence of high concentrations of PEG under non-buffered acidic conditions by means of ITP. As high concentrations of PEG in the solution also cause a rather big increase in its viscosity, the viscosity effect must be taken into account. When estimating complexity or stability constants of metals by ITP, we try to distinguish between the viscosity effect and complexation of metal ions with PEG.

2. Experimental

2.1. Chemicals

The chlorides or nitrates of metals and nitric acid were obtained from Lachema (Neratovice, Czech Republic) and were of analytical-reagent grade. PEG ($M_r = 4000$) of guaranteed grade was obtained from Katayama Kagaku (Tokyo, Japan). Tris(hydroxymethyl)aminomethane (Tris), of analytical-reagent grade purity, was purchased from Merck (Darmstadt, Germany). Water used in all measurements was distilled and then deionized using a MilliQ-Ultra Pure System (Molsheim, France).

2.2. Electrolyte system

The leading electrolytes consisted of 0.01 mol l^{-1} nitric acid and various % (w/w) of PEG. The terminating electrolyte contained 0.005 mol l^{-1} Tris. Table 1 gives % (w/w) of PEG added to the leading electrolyte, the concentration of PEG in the leading

Table 1
Parameters of the leading electrolytes: values of PEG [% (w/w)], concentration of PEG given as the concentration of monomer units, c_{PEG}, and mass density ρ_M of PEG solutions

% (w/w) of PEG	c_{PEG} (mol l^{-1})	ρ_M (kg m^{-3})
6.96	1.593	1007.0
13.80	3.194	1018.3
19.50	4.551	1028.9
24.13	5.689	1037.5
31.28	7.464	1050.2
37.79	9.105	1058.0

electrolyte, c_{PEG}, expressed as the concentration of –CH$_2$–CH$_2$–O– monomer units, and the mass density, ρ_M, of the leading solutions.

2.3. Samples

Samples of metal ions (Li$^+$, Na$^+$, Cs$^+$, Mg^{2+}, Sr^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Lu^{3+} and Y^{3+}) were prepared from stock solutions of chlorides or nitrates at a concentration of $2 \cdot 10^{-4}$ mol l^{-1} and their pH values were adjusted to 3 by the addition of nitric acid. The samples were introduced into the ITP instrument using a Hamilton syringe in a volume of 6 µl.

2.4. Instrumentation

Laboratory-made ITP equipment was used for all experiments. The detector was a high frequency contactless conductivity detection (HFCCD) system, as developed by Gaš et al. [13]. Its properties, including reproducibility and stability of the signal, were demonstrated by Hirokawa et al. [14]. After calibration, the detector is able to measure directly the resistivity of solutions. The detector cell was thermostated at 25°C by a built-in Peltier device. The separation column was a PTFE capillary (25 cm× 0.32 mm I.D.).

Specific conductivities of the calibration solutions and polyethylene glycol solutions were measured on a CDM210 low frequency conductivity meter from Radiometer (Copenhagen, Denmark).

3. Results and discussion

3.1. Influence of polyethylene glycol on conductivity

Ionic mobilities are strongly dependent on the viscosity of the environment. A distinction must be made between the macroscopic and microscopic viscosity of the solution. The macroscopic viscosity characterizes the bulk liquid and is experimentally measurable by means of a viscosimeter. Stokes law describes the velocity of a spherical particle moving in the environment of particles of similar size. The Walden rule, stating that the product of ionic mobili-

ty and macroscopic viscosity is constant [15], is valid with good precision in this case.

The microscopic viscosity should be taken into account if large molecules form obstacles to the movement of small ionic species. Here, the Walden rule is violated. Striking examples are hydrophilic gels that exhibit infinite macroscopic viscosity but small ions can still penetrate them. The small ions have to go around the obstacles and the increased friction force can be regarded as a consequence of the lengthening of the pathway. This obstruction theory results in the relationship between specific conductivity of the electrolyte with non-electrolyte (e.g. NCA) added and the volume fraction of non-electrolyte, φ [16]:

$$\frac{\kappa'}{\kappa} = 1 - 1.5\varphi, \tag{1}$$

where κ' and κ are the specific conductivities of the electrolyte with and without non-electrolyte, respectively.

Fig. 1 gives the experimental dependences of the ratio of conductivities κ'/κ of several electrolytes on the concentration of PEG. These electrolytes are supposed to interact with PEG to different degrees. It is obvious that the dependence of the relative conductivity on PEG concentration is almost linear. This behavior would correspond with the obstruction theory, which also predicts the linear dependence [see Eq. (1)]. However, φ in Eq. (1) is the volume fraction of the non-electrolyte instead of the molar concentration on the x-axis in Fig. 1. The concentration of polymer (expressed as the molar concentration of monomer units) is almost linearly related to % (w/w) of PEG, as can be deduced from Table 1. Furthermore, even high concentrations of PEG in the electrolyte causes a change of only a few percent in the mass density of the solution. Therefore, the concentration of PEG is almost linearly related to its volume fraction in the solution.

It seems reasonable to relate the behavior of ions interacting with PEG to a reference ion, which undergoes only the obstruction of its movement. To distinguish between the viscosity effect and complexation, it is, unfortunately, difficult to find such reference ions that do not interact with the oxygen of the PEG molecule. However, we assume that no anion interacts with PEG in this way, and that only a
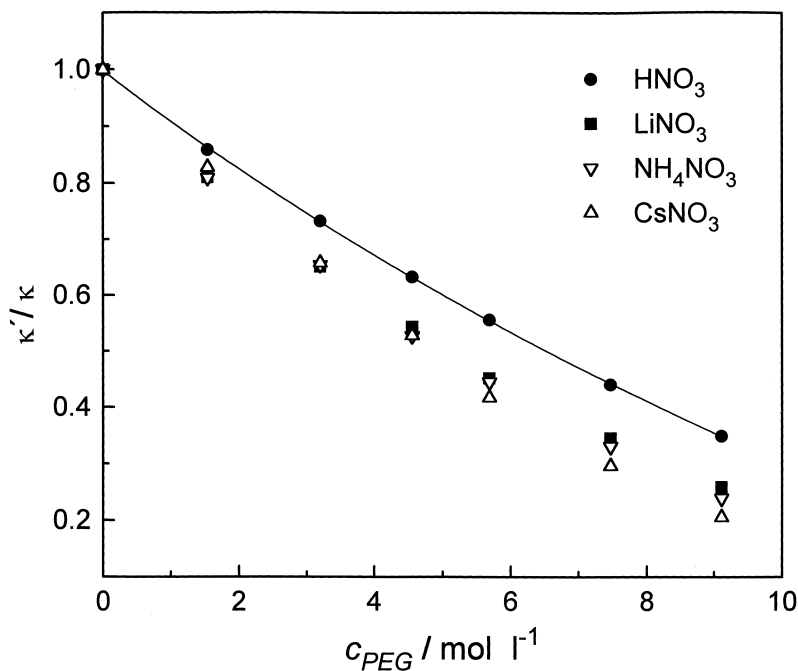
Fig. 1. Experimental dependence of the relative conductivities κ'/κ of electrolytes on the concentration of polyethylene glycol, c_{PEG}. These electrolytes (HNO_3, $LiNO_3$, NH_4NO_3 and $CsNO_3$, all at a concentration 0.005 mol l^{-1}) are supposed to interact with polyethyleneglycol to different degrees. The solid line is the exponential fit of HNO_3 data given by the function $f(c_{PEG}) = -0.617 + 1.615 \exp(-0.0565 c_{PEG})$.

hydrogen bond can possibly be formed between anions containing oxygen and the terminating OH-groups of PEG. We postulate here that the curve for HNO_3 (see Fig. 1) lying above all of the others will be considered as the reference, reflecting only the viscosity effect.

The experimentally obtained values κ'/κ for HNO_3 can be better fitted by an exponential function $f(c_{PEG})$, viz. Fig. 1:

$$f(c_{PEG}) = -0.617 + 1.615 \exp(-0.0565 c_{PEG}), \quad (2)$$

If the viscosity effect is supposed to influence the mobility of all ions in the same manner, Eq. (2) can be applied for mobilities and rewritten as follows:

$$\frac{\mu_i'}{\mu_i} = f(c_{PEG}) \quad (3)$$

where μ_i' and μ_i are the mobilities of the ith ion in solutions with and without PEG, respectively. This relation is used in further considerations and calculations.

3.2. Simulations of ITP steady state in the presence of polymer

Separation of cations in a non-buffered electrolyte system with a strong monovalent acid as the leading electrolyte is considered. The hydroxonium cation as well as the counter-ion are not supposed to interact with the polymer in the sense of a complexing reaction. The concentration of polymer is formally considered as the molar concentration of monomer units. All parameters of the leading zone are known and are regarded as input parameters. They are denoted by subscript 1. The second zone, parameters of which have the subscript 2, is the zone containing the metal ion of interest. The metal ion is allowed to interact with polymer. Letters M, P, PM, A and H stand for metal ion, polymer, polymer–metal complex, monovalent counter-ion and hydroxonium ion, respectively. Primed symbols and those without the prime denote conductivities and mobilities, κ and μ, in the presence and absence of PEG, respectively. The mobility of polymer as the neutral agent is equal

to zero. We assume in this work that the mobility of the polymer–metal complex is also zero as the polymer molecule is supposed to be much larger than the metal ion.

To calculate four unknown parameters of the sample zone (i.e., $[M]_2$, $[P]_2$, $[PM]_2$, $[A]_2$), a set of four equations describing the ITP steady state was used:

(1) The condition of electroneutrality,

$$z[M]_2 + z[PM]_2 = [A]_2 \qquad (4)$$

where z is the charge number of the metal ion,

(2) the mass balance of polymer in the first and second zone

$$[PM]_2 + [P]_2 = [P]_1 \qquad (5)$$

(3) definition of the apparent stability constant

$$K = \frac{[PM]_2}{[M]_2 \cdot [P]_2} \qquad (6)$$

(4) the isotachophoretic condition in the form

$$\frac{\kappa'_1}{\kappa'_2} = \frac{\mu'_{H,1}}{\mu'_{eff,M,2}} \qquad (7)$$

where $\mu'_{eff,M,2}$ is the effective mobility of the metal ion given by:

$$\mu'_{eff,M,2} = \mu'_{M,2} \frac{[M]_2}{[M]_2 + [PM]_2} \qquad (8)$$

When substituting κ'_1, κ'_2 and $\mu'_{eff,M,2}$ in Eq. (7), the equation can be written as:

$$\frac{\mu'_{H,1}[H]_1 + \mu'_{A,1}[A]_1}{z\mu'_{M,2}[M]_2 + \mu'_{A,2}[A]_2} = \frac{\mu'_{H,1}}{\mu'_{M,2} \dfrac{[M]_2}{[M]_2 + [PM]_2}} \qquad (9)$$

The solution of the above set of the nonlinear equations, Eqs. (4)–(6), (9) was performed by the Newton iteration method.

To understand the contribution of viscosity effect due to the presence of polymer, calculations were performed for two cases: (i) only complexation was considered, then primed quantities were not used in Eq. (7), (ii) the influence of viscosity on ionic mobilities was put to use according to Eq. (3). It should be mentioned that calculated concentration values of all species are the same in both cases. However, the specific resistivities are different. Calculated dependences of specific resistivity, ρ_2, of sample zone on concentration of polymer in the leading zone for various stability constants are depicted in Fig. 2.

There is an evident difference between the resistivities calculated for the lowest K value, $K = 0.1$ l mol^{-1} (compare lines a and b in Fig. 2). While the dependence is almost linear in the simplified approach (i), a significant curvature of this dependence can be observed in the extended approach (ii). Conversely, the dependences for higher values of K remain almost unchanged in both cases (i) and (ii). This corresponds to the fact that, for higher K values, the mobility of the ion is substantially changed at low concentrations of polymer, where the increase in the viscosity is negligible.

Examples of the experimentally measured specific resistivities of some metal ions plotted against PEG concentration are shown in Fig. 3. The concentration has to be very high to significantly influence the sample zone resistivity, which implies that the stability constant is low [compare with the dependence (b) in Fig. 2 for $K = 0.1$ l mol^{-1}]. The curvature of the dependences clearly indicates that the presence of PEG as a polymeric non-electrolyte influences ionic mobilities in the real system and that the viscosity effect plays a significant role.

3.3. Determination of stability constants of metal ions with PEG

The proposed theoretical model of ITP migration provides, in principle, a chance to determinate stability constants of complexes of metals with PEG. However, there is a serious difficulty in determining the activities of all individual components. Although the activity of ions could be calculated using the Debye-Hückel theory with sufficient accuracy, as they are in low concentrations (mostly much less than 0.01 mol l^{-1}), the polymer is in high concentrations (tens of %, w/w). Under those conditions, it is very difficult to find a reliable method for calculation or measurement of the activity coefficients of the polymer. Having those restrictions in mind, we propose rather to consider the apparent stability constants in terms of concentrations instead
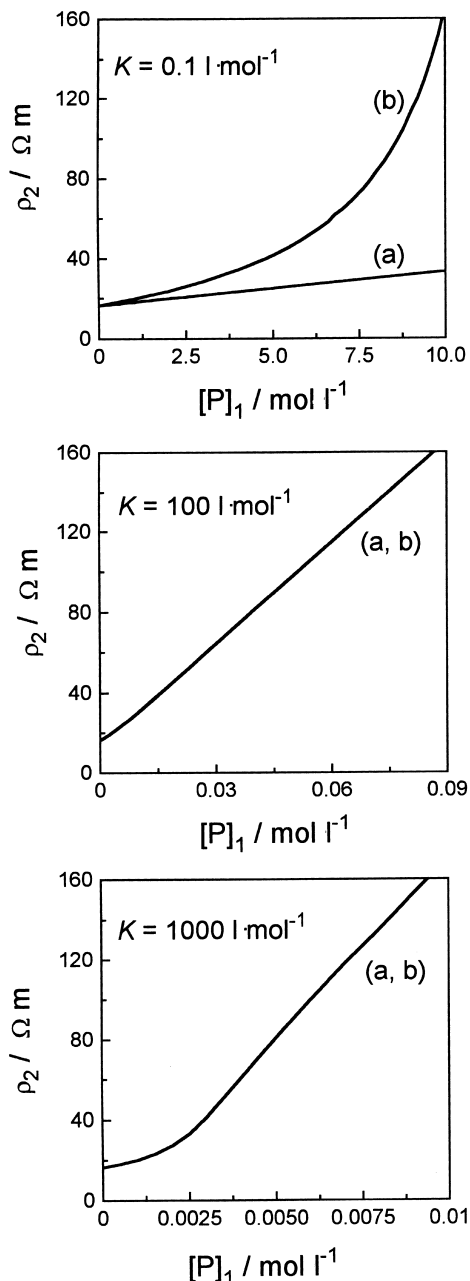
Fig. 2. Calculated dependence of the specific resistivity of the sample zone, ρ_2, on the concentration of polymer in the leading electrolyte, $[P]_1$, for various stability constants, K. Dependences: (a) the viscosity effect is omitted, (b) the viscosity effect is considered (see text). Input parameters: $[H]_1 = [A]_1 = 0.01$ mol l^{-1}; $\mu_H = 363 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; $\mu_A = 74.1 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; $\mu_M = 51.9 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; all ions are considered as being monovalent.

of activities, viz. Eq. (6). The approach for estimation of those stability constants is based on assumptions, which have been mentioned before and can be summarized as follows:

1. The addition of polymer as a non-electrolyte influences the mobilities of hydroxonium ions and metal ions due to the viscosity effect in the same way [see Eq. (3)].
2. The mobilities of polymer and polymer–metal complex are zero.
3. The mobility is not influenced by the ionic strength, i.e., only limiting ionic mobilities are considered.
4. The polymer concentrations in the first and second zone are the same, $[P]_1 = [P]_2$. This approximation is fulfilled quite well in the case of PEG, as its concentration is 100–1000 times higher than the concentration of the metal ion, so that only a small fraction of PEG is supposed to be in a complexed state.

For characterization of ITP zones, the Re value, defined as the ratio of specific conductivities of the leading zone to the sample zone, $\mathrm{Re} = \kappa_1' / \kappa_2'$, is often used. The main advantage of this relative characteristic is given by the ITP condition stating that the Re is directly equal to the ratio of the effective mobilities of the leading and sample constituents (viz. Eq. (7)). Combining Eqs. (3), (6)–(8), gives

$$\mathrm{Re} = \frac{\mu_H f([P]_1)}{\mu_M f([P]_2)} (1 + K[P]_2), \qquad (10)$$

and using assumption (iv), $[P]_1 = [P]_2$, then

$$\mathrm{Re} = \frac{\mu_H}{\mu_M} (1 + K[P]_1). \qquad (11)$$

This is a very simple equation, indicating that, unlike the specific resistivity of the sample zone, the dependence of Re on the concentration of the polymer in the leading electrolyte is linear. This fact is demonstrated in Fig. 4, which shows the experimentally obtained dependences of Re on the PEG concentration in the leading electrolyte for some metals. The linear fit can be used to determine the stability constant K and also the mobility of free metal ion μ_M, if the mobility of the hydroxonium ion, μ_H, is considered as the input value.
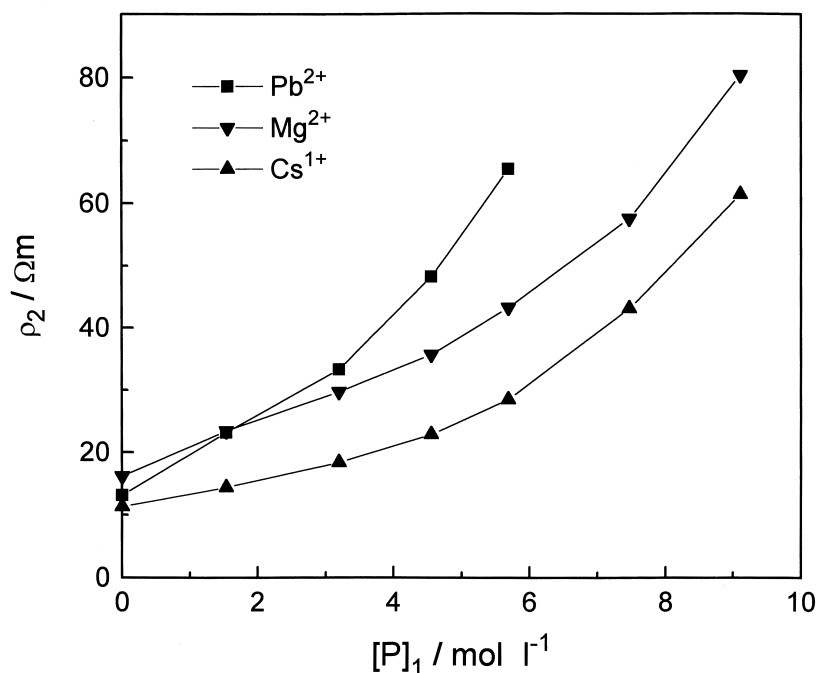
Fig. 3. Examples of experimentally obtained specific resistivities of some metal ions plotted against the concentration of PEG in the leading electrolyte.

Resulting values of ionic mobilities of metals, stability constants of PEG–metal complexes, their standard deviations and correlation coefficients of the linear fit are summarized in Table 2. Limiting mobilities, μ^0 [17], given in Table 2 correspond with the evaluated mobilities quite well.

Table 2 shows that the experimentally obtained stability constants of PEG with metal ions are very low, about 0.01–0.1 1 mol^{-1}. (For example, good complexing agents, like cyclodextrins or crown-ethers exhibit K values in the range 10–100 1 mol^{-1} [18].) The smallest value of the stability constant of the PEG–metal complex was found for Li^+ and Na^+ ions, which is in good agreement with the results of Ito and Hirokawa [19] and Kaniansky et al. [10]. They studied the effect of the addition of PEG to the electrolyte system on the mobility of alkali and alkaline metal cations and used Li^+ ion as a reference constituent, supposing that it does not interact with PEG. Although the stability constants are not given in the above-mentioned papers, the authors give the order of retardation of alkali and alkaline metal ions. They determined the following order:

$Cs^+ > Na^+ \approx Li^+$ and $Sr^{2+} > Mg^{2+}$, which is in agreement with the values of stability constants determined in our work.

4. Conclusion

The mathematical model presented in this paper describes the ITP migration of metal ions in electrolyte systems containing high concentrations of neutral weakly complexing polymer. As high concentrations of the polymer cause a significant increase in the viscosity of the system, the ionic mobilities are also influenced by the viscosity effect. The model takes into account both factors, i.e., complexation and viscosity effects.

It was shown that the isotachophoretic Re value eliminates the viscosity effect under given presumptions. The most important of them is the choice of the leading ion, that does not interact with polymer in the sense of complexation. In this way, Re can be conveniently utilized for calculation of the binding parameters of the metal ion of interest. In this work,
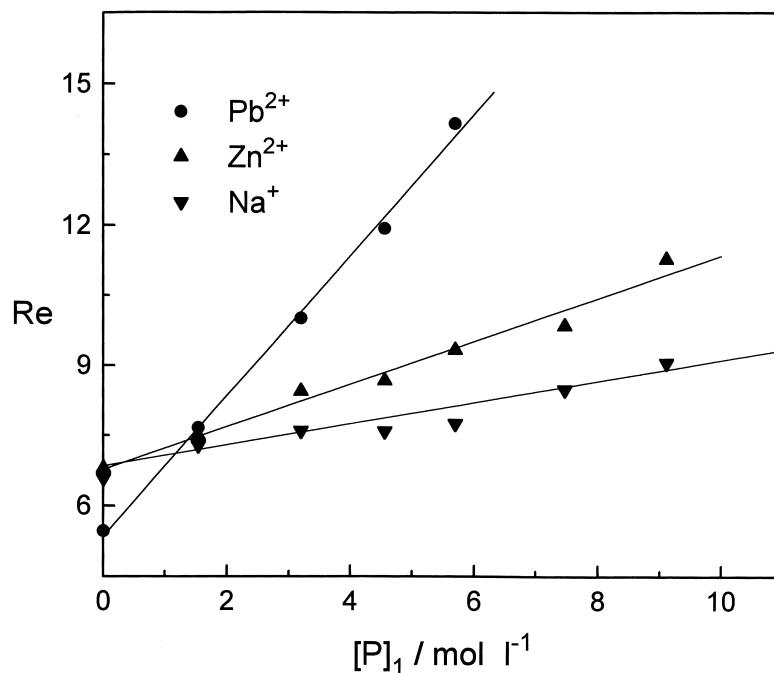
Fig. 4. Example of experimentally obtained Re value plotted against the concentration of PEG in the leading electrolyte. Thin solid lines are linear fits according to Eq. (11). Regression coefficients of the linear fit are given in Table 2.

hydroxonium ion was considered as the leading and reference ion. Nevertheless, such a choice is not critical for the validity of the model, so, instead of hydrogen, another ion that does not interact with the polymer could be regarded as the reference ion. In addition, as the concentration of the polymer is very high and almost the same in the leading and sample zones, the model can be utilized under conditions of

Table 2
Overview of the obtained stability constants K of PEG–metal complexes, mobilities μ_M, their estimations of standard deviation SD K, sd μ_M, and correlation coefficients of the linear fit R[a]

Metal ion	K (l mol^{-1})	SD K (l mol^{-1})	μ_M (10^{-9} m^2 V^{-1} s^{-1})	SD μ_M (10^{-9} m^2 V^{-1} s^{-1})	μ^0 (10^{-9} m^2 V^{-1} s^{-1})	R
Li$^+$	0.037	0.009	40.2	1.8	40.1	0.893
Na$^+$	0.037	0.004	54.4	1.2	51.9	0.918
Cs$^+$	0.103	0.017	87.0	6.8	80.1	0.983
Mg^{2+}	0.070	0.005	53.3	1.4	55.0	0.989
Sr^{2+}	0.106	0.019	63.8	5.6	61.6	0.946
Mn^{2+}	0.071	0.007	53.0	1.8	55.4	0.981
Co^{2+}	0.072	0.006	54.2	1.6	57.0	0.987
Ni^{2+}	0.070	0.007	53.2	1.8	53.9	0.980
Zn^{2+}	0.067	0.005	53.4	1.3	54.7	0.988
Pb^{2+}	0.280	0.014	67.5	2.4	72.0	0.998
Lu^{3+}	0.116	0.019	64.4	5.6	67.0	0.955
Y^{3+}	0.102	0.013	62.4	3.9	64.7	0.970

[a] Limiting mobilities μ^0 are taken from Ref. [17]. Temperature 25°C.

zone electrophoresis. In this case, an arbitrary ion that does not interact with the polymer could be considered as a reference ion.

Furthermore, we investigated experimentally the isotachophoretic behavior of 12 metal ions and their interaction with PEG. Utilizing the theoretical model, we were able to determine the PEG–metal stability constants from the experimental data obtained.

In future work, this model should be extended to improve some aspects of it. The key problem is the viscosity effect, which must be understood in a more complex way. Moreover, the influence of ionic strength on physicochemical quantities should be considered.

References

[1] T. Hirokawa, M. Ueda, A. Ijyuin, S. Yoshida, F. Nishiyama, Y. Kiso, J. Chromatogr. 633 (1993) 261.

[2] F.M. Everaerts, Th.P.E.M. Verheggen, J.C. Reijenga, G.V.A. Aben, P. Gebauer, P. Boček, J. Chromatogr. 320 (1985) 263.

[3] I. Nukatsuka, M. Taga, H. Yoshida, J. Chromatogr. 205 (1981) 95.

[4] M. Macka, P.R. Haddad, Electrophoresis 18 (1997) 2482.

[5] H. Salimi-Moosavi, R.M. Cassidy, J. Chromatogr. A 749 (1996) 279.

[6] E. Kenndler, K. Sarmini, J. Chromatogr. A 811 (1998) 201.

[7] T. Hirokawa, J.-Y. Hu, S. Eguchi, F. Nishiyama, Y. Kiso, J. Chromatogr. 538 (1991) 413.

[8] E. Šimuničová, D. Kaniansky, K. Kokšíková, J. Chromatogr. A 665 (1994) 203.

[9] Y.C. Shi, J.S. Fritz, J. Chromatogr. A 671 (1994) 429.

[10] D. Kaniansky, I. Zelenský, I. Valášková, J. Marák, V. Zelenská, J. Chromatogr. 502 (1990) 143.

[11] C. Stathakis, R.M. Cassidy, Analyst 121 (1996) 839.

[12] K.L. Rundlett, D.W. Armstrong, Electrophoresis 18 (1997) 2194.

[13] B. Gaš, J. Zuska, J. Vacík, J. Chromatogr. 470 (1989) 69.

[14] T. Hirokawa, Q. Mao, N. Ikuta, B. Gaš, Chromatographia 19 (1998) 9.

[15] P. Walden, H. Ulich, G. Busch, Z. Phys. Chem. 123 (1926) 429.

[16] T. Erdey-Grúz, in: Transport Phenomena in Aqueous Solutions, Akadémiai Kiadó, Budapest, 1974, p. 352.

[17] T. Hirahara, Thesis, 1997.

[18] A.E. Martell, R.M. Smith, in: Critical Stability Constants, Plenum Press, NewYork, 1977, p. 266.

[19] K. Ito, T. Hirokawa, J. Chromatogr. A 742 (1996) 281.