Journal of Chromatography, 623 (1992) 337–344
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 428

Electromigration in systems with additives in background electrolytes

I. Addition of the neutral complexing agent

Eva Dubrovčáková, Bohuslav Gaš, Jiří Vacík and Eva Smolková-Keulemansová

Faculty of Science, Charles University, Albertov 2030, 128 40 Prague 2 (Czechoslovakia)

(First received March 3rd, 1992; revised manuscript received June 12th, 1992)

ABSTRACT

A mathematical model describing the electrophoretic migration of strong electrolytes in the presence of a neutral complexing agent (*e.g.*, cyclodextrin) is formulated. The model allows computer simulation of the dynamics of the electrophoretic separation in general and the calculation of the isotachophoretic steady state. The approach for the determination of stability constants and mobilities of complex compounds from the experimental isotachophoretic data is described.

INTRODUCTION

The effective mobilities of compounds in electromigration methods, *i.e.*, isotachophoresis or zone electrophoresis, can be controlled by changes in the pH of the electrolytes used or by complex formation between a separated compound and a buffering counter ion acting as a charged complexing agent. The utilization of a neutral complexing agent (NCA) is another possibility of using complex formation for separation in electromigration methods. Tazaki *et al.* [1] and Stover [2] suggested the use of cyclic crown ethers and cyclodextrins. The ability to form inclusion complexes and the chiral character of cyclodextrins allow the separation of structurally related and isomeric compounds, including enantiomers [3,4].

Neutral complexing agents have some advantages over ionic agents. They do not substantially influence the conductivity of the separation system and their molecules are not transported by migration unless they are not bound to the separated ions. These agents can be therefore used in high concentration and hence are able to influence the mobility and provide successful separations even when the stability constant is very low.

Although separations using NCAs are often utilized in analytical practice, the theoretical description of such separations has not yet been fully formulated.

Tazaki *et al.* [5] derived a relationship for the effective mobility of a compound interacting with an NCA. They also determined the stability constants of some quaternary ammonium salts with α-cyclodextrin from isotachophoretic measurements. Ionic mobilities of these complexes were not determined from experimental data, but calculated using the equation suggested by Jokl [6]. Jelínek *et al.* [7] presented the concept of effective and non-effective inclusion and also showed [8] the influence of interaction of the counter ion on separation in isotachophoresis. In our recent studies [9] some

Correspondence to: Dr. B. Gaš, Faculty of Science, Charles University, Albertov 2030, 128 40 Prague 2, Czechoslovakia.

0021-9673/92/$05.00

general ideas about the electromigration of cyclodextrin complexes of azo compounds were considered.

The purpose of this paper is to present a model of the electromigration of compounds in the presence of a neutral complexing agent. In continuation of our previous work [10] we describe the dynamics of separations in electrophoretic methods in general. The model also allows the calculation of compound concentrations in all zones in the isotachophoretic steady state. In addition, stability constants and mobilities of complex compounds can be determined from the experimental isotachophoretic data.

MATHEMATICAL MODEL

Electromigration

Let us consider n ions of strong univalent electrolytes and a neutral complexing agent present in the solution. This agent can form a complex compound with any of the ions in a 1:1 ratio. Let us assume a negligible concentration of H^+ or OH^- ions produced by ionization of water. The ionic electrophoretic mobilities are presumed to be positive numbers. The total concentration of an ion j with a relative charge z_j (*i.e.*, the concentration of free and complex ions) is denoted by c_j and the concentration of free ion is denoted by c_j^z. The relative charge of the ion can be $+1$ or -1. Further, the total concentration of the NCA (*i.e.*, the concentration of the free neutral form and charged bound form) is c_c and the concentration of the free NCA is c_c^0. It is evident that $c_c^0 = c_c - \sum_{l=1}^{n} (c_l - c_l^z)$. The formulation of electromigration equations has the same basis as previously [10]. The continuity equations, which describe the one-dimensional mass flow of an ion j in an electric field in a capillary tube, have the form

$$\frac{\partial c_j}{\partial t} = -z_j i \cdot \frac{\partial}{\partial x}\left[\frac{u_j c_j^z + m_j(c_j - c_j^z)}{\kappa}\right] \tag{1}$$

where

$$\kappa = F \sum_{l=1}^{n} [c_l^z u_l + (c_l - c_l^z) m_l] \tag{2}$$

and $j = 1, 2, \ldots, n$, n is the number of ions, t is the time, i is the current density in a capillary tube, κ is the specific conductivity, u_j is the ionic mobility of the ion j, m_j is the ionic mobility of the ion j bound on an NCA, x is the length coordinate along a capillary tube and F is the Faraday constant.

The continuity equation describing the transport of NCA can be written in a similar way. In this instance we have to remember that the agent can migrate only when it is bound to an ion and forms a complex compound:

$$\frac{\partial c_c}{\partial t} = -i \cdot \frac{\partial}{\partial x}\left[\frac{\sum_{l=1}^{n} z_l m_l (c_l - c_l^z)}{\kappa}\right] \tag{3}$$

We assume that the ionic mobilities u_j and m_j depend neither on the viscosity nor on the ionic strength of solution and therefore can be approximated by the limiting ionic mobilities. We also do not consider the electroosmotic flow and the effect of temperature.

Reaction of an ion with a ligand is mostly reversible and fast and, consequently, the rate of the reaction is controlled only by diffusion, especially when the reaction of small molecules without steric hindrance [11] is considered. Yoshida *et al.* [12,13] have studied the dynamic aspects of the inclusion reactions between alkyl-substituted hydroxyphenylazo compounds and cyclodextrins and found that the inclusion reaction is very fast when the alkyl groups are small. They have also determined the stability constants of these complexes spectroscopically.

In our model, we assume that the kinetics of complex formation do not play a considerable role in migration and the concentrations of the compounds in the system are determined by complexation equilibria. The equations describing the equilibria are of the form

$$K_j = \frac{c_j - c_j^z}{c_j^z c_c^0} \tag{4}$$

where K_j is the stability constant of the complex between the ion j and the agent.

The electroneutrality condition

$$\sum_{l=1}^{n} c_l z_l = 0 \tag{5}$$

can replace one of the equations from the set 1, preferably that which describes the migration of the counter ion.

The terms of diffusion flow can be added to the right-hand sides of the continuity equations. Values of diffusion coefficients are available only for a small number of compounds. For this reason we used the Nernst–Einstein equation, $D = uRT/(|z|F)$, which describes the relationship between the mobility u and the diffusion coefficient D of an ion with charge z (R is the gas constant and T is absolute temperature).

The Nernst–Einstein equation cannot be used for neutral compounds. Therefore, the diffusion coefficient of NCAs was interpolated from available tables of data for structurally related compounds (*e.g.*, the value of $5 \cdot 10^{-10}$ m^2 s^{-1} was estimated for β-cyclodextrin). The final form of the continuity equations (disregarding terms describing the diffusion potential gradient resulting as a cross-effect of the diffusion of ions with different mobilities [14]) is

$$\frac{\partial c_j}{\partial t} = \frac{RT}{F} \cdot \frac{\partial^2}{\partial x^2} [u_j c_j^z + m_j(c_j - c_j^z)] - z_j i \cdot \frac{\partial}{\partial x}\left[\frac{u_j c_j^z + m_j(c_j - c_j^z)}{\kappa}\right] \quad (6)$$

and

$$\frac{\partial c_c}{\partial t} = \frac{RT}{F} \cdot \frac{\partial^2}{\partial x^2}\left[\sum_{l=1}^{n} m_l(c_l - c_l^z)\right] + D \cdot \frac{\partial^2 c_c^0}{\partial x^2} - i \cdot \frac{\partial}{\partial x}\left[\frac{\sum_{l=1}^{n} z_l m_l(c_l - c_l^z)}{\kappa}\right] \quad (7)$$

The model defined by eqns. 4–7 enables the main features of the separation process to be described.

Isotachophoretic steady state

Calculation of the isotachophoretic steady state (*i.e.*, the determination of concentrations of all compounds in isotachophoretic zones) is analogous to the calculation of the steady state in the migration of weak electrolytes describe earlier [15].

Let us consider an isotachophoretic system of two zones 1 and 2. Let the first zone contain ion 1 and the second zone contain ion 2 and there is a common counter ion 3 and the NCA (which can form a complex compound with any of the ions) in both zones 1 and 2. The notation of the concentrations and mobilities of ions below consists of two subscripts, one representing a compound and the other a zone.

Our aim is to determine the parameters of the second zone in the isotachophoretic steady state, assuming we know the physico-chemical characteristics of all the compounds (the ionic mobilities and the stability constants). Let the first zone be the zone of a leading electrolyte where the concentration of a leading ion and the total concentration of an NCA are known (and as a result of the electroneutrality condition, the total concentration of the counter ion is also known). All necessary concentrations in the zone 1 can be calculated by eqns. 10 and 12, which describe the complexation equilibria.

The following five unknown parameters of zone 2 should be determined: concentrations c_{22}^z and c_{32}^z (the concentration of the free form of the ion 2 and the concentration of the free counter ion 3, respectively), concentrations c_{22} and c_{32} (the total concentrations of the free and bound forms of the ion 2 and the counter ion 3, respectively) and concentration c_{c2}^0 (the concentration of the free complexing agent).

The following equations hold:

(1) the electroneutrality condition:

$$c_{11} = c_{31} \quad (8)$$

$$c_{22} = c_{32} \quad (9)$$

(2) the equations describing complexation equilibria:

$$K_1 = (c_{11} - c_{11}^z)/(c_{11}^z c_{c1}^0) \quad (10)$$

$$K_2 = (c_{22} - c_{22}^z)/(c_{22}^z c_{c2}^0) \quad (11)$$

$$K_3 = (c_{31} - c_{31}^z)/(c_{31}^z c_{c1}^0) \quad (12)$$

$$K_3 = (c_{32} - c_{32}^z)/(c_{32}^z c_{c2}^0) \quad (13)$$

(3) the equation describing the isotachophoretic condition (the form of the equation is written in terms of the effective mobilities):

$$\bar{u}_{11}/\kappa_1 = \bar{u}_{22}/\kappa_2 \quad (14)$$

where $\bar{u}_{11}$ is the effective mobility of compound 1 in zone 1, $\bar{u}_{22}$ is the effective mobility of compound 2 in zone 2 and κ_1 and κ_2 are the specific conductivities of zone 1 and zone 2, respectively.

The conductivities of both zones can be determined from eqn. 2.

The effective mobility $\bar{u}_{ii}$ ($i = 1, 2$) is expressed as

$$\bar{u}_{ii} = [u_i c_{ii}^z + m_i(c_{ii} - c_{ii}^z)]/c_{ii} \quad (15)$$

For the last equation one can use, *e.g.*, the mass balance of the NCA, which has the form

$$c_{c1}(\bar{u}_{11} - \bar{u}_{c1})/\kappa_1 = c_{c2}(\bar{u}_{22} - \bar{u}_{c2})/\kappa_2 \quad (16)$$

where c_{c1} and c_{c2} are the total concentrations of the NCA and $\bar{u}_{c1}$ and $\bar{u}_{c2}$ are the effective mobilities of the NCA in zones 1 and 2, respectively. These effective mobilities are determined by the following equations:

$$\bar{u}_{c1} = [m_1(c_{11} - c_{11}^z) - m_3(c_{31} - c_{31}^z)]/c_{c1} \quad (17)$$

$$\bar{u}_{c2} = [m_2(c_{22} - c_{22}^z) - m_3(c_{32} - c_{32}^z)]/c_{c2} \quad (18)$$

The five unknown concentrations c_{22}^z, c_{32}^z, c_{22}, c_{32} and c_{c2}^0 can obviously be determined from eqns. 9, 11, 13, 14 and 16.

The dependence of the resistance of the sample zone on the concentration of the NCA in the leading electrolyte can be easily obtained experimentally. It permits the determination of the stability constants and the mobilities of the free and complex compounds. For this purpose, a computational program was written which iterativelly fits the experimental isotachophoretic data by a simulated curve using the least-squares method.

NUMERIC METHODS

The partial differential eqns. 6 and 7 describing the electromigration was solved by the CTDS method of lines (continuous time, discrete space). The CTDS method consists in discretizing the spatial derivatives at a set of grid points to generate a set of ordinary differential equations with time as the independent variable. The finite-difference approximation is based on the first-order symmetrical difference approximation for either first and second derivatives.

Hamming's modification of the fourth-order predictor–corrector method [16] was used for the solution of the resulting set of ordinary differential equations. Simultaneously, the set of non-linear algebraic eqns. 4 must be solved at every time increment together with the sets of eqns. 6 and 7. This set of non-linear algebraic equations was solved by the Newton–Raphson iterative method [16].

For solution of the isotachophoretic steady state, the set of eqns. 9, 11, 13, 14 and 16 was solved by the Newton–Raphson method [16], instead of using of the RFQ method [17].

EXPERIMENTAL

The algorithm for solving the dynamics of electromigration and the isotachophoretic steady state was programmed in Pascal and runs on an IBM PC computer or compatible types. The results can be presented in graphical form.

The isotachophoretic measurements were made on a one-column isotachophoretic analyser consisting of the preseparation hydraulic part from a ZKI 01 instrument (Labeco, Spišská Nová Ves, Czechoslovakia) and a high-frequency contactless detector [18]. The leading ion was chloride (0.0111 *M*) and the counter ion ε-amino caproic acid; the pH of the leading electrolyte was 4.21. The terminating electrolyte was caproic acid. Chemicals used for the preparation of the leading and terminating electrolytes were of analytical-reagent grade and used without further purification. No additives were applied to decrease the electroosmotic flow. 4-Hydroxyphenylazobenzenesulphonic acid was synthesized in the Research Institute of Organic Syntheses (Pardubice, Czechoslovakia) and purified on a cellulose packed column. The complexing agent was β-cyclodextrin (Chinoin, Budapest, Hungary).

RESULTS AND DISCUSSION

NCAs are often used in capillary electromigration methods for the separation of structurally similar compounds. Compounds that originally had the same migration characteristics (*e.g.*, optical antipodes) can be separated assuming that their complexation with NCA is different.

Molecules of the NCA can migrate in an electric field only when they are bound on charged particles. In isotachophoresis, neutral complexing agents, especially cyclodextrins, are used as additives to the leading electrolyte. Fig. 1 simulates such an isotachophoretic migration where the sample contains only one ion interacting with cyclodextrin while the leading ion, the counter ion and the terminating ion do not form any complexes. In this case, the concentration of cyclodextrin remaining during migration in the terminating electrolyte is the same as in the leading electrolyte. Cyclodextrin as a whole does not move in the capillary, only the sample zone creates "a migrating bulge" on the concentration profile of cyclodextrin.

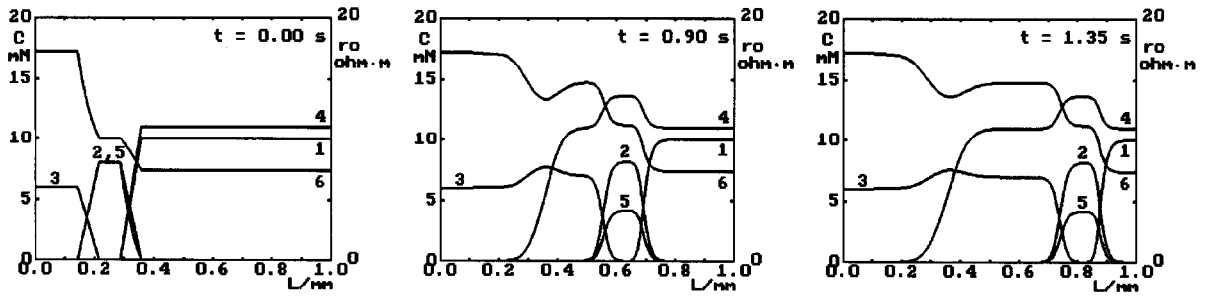

Fig. 1. Simulation of isotachophoretic migration. 1 = Leading anion; 2 = total sample; 3 = terminating anion; 4 = total cyclodextrin; 5 = free sample; 6 = specific resistance. Mobility of leading anion, $80 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; mobility of sample, $60 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; mobility of complexed sample, $30 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; mobility of terminating anion, $40 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; mobility of counter cation, $70 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; stability constant of sample, 100. L = Length coordinate of the capillary tube; C = concentration; ro = specific resistance.

When both the sample and the terminating ions interact with cyclodextrin (Fig. 2), the cyclodextrin zone moves in the zone of the terminating electrolyte in the same direction as the sample and has a higher concentration than that in the leading electrolyte.

In some instances both the counter ion and the sample can interact with a complexing agent [8]. The concentration profiles corresponding to such a situation are presented in Fig. 3. Now the cyclodextrin zone migrates in the opposite direction and has a lower concentration. The leading (Fig. 2) or trailing (Fig. 3) edge of the cyclodextrin boundary in the terminating electrolyte has interesting sharpening properties and is the subject of further studies.

Let us analyse in detail the isotachophoretic migration in a system of two migrating adjacent zones, 1 and 2. The first zone belongs to ion 1 and the second zone to ion 2 and in both zones the common counter ion 3 and NCA are present. We assume that zone 1 is the zone of the leading electrolyte with all parameters known and zone 2 is the zone of the sample. The NCA is able to interact only with ion 2. The effective mobility of the ion interacting with NCA is determined by eqn. 15. The dependence of the specific resistance ρ_2 of zone 2 ($\rho_2 = 1/\kappa_2$) on the total concentration of the NCA, c_{c2}, in zone 2 for the different values of the stability constants are presented in Fig. 4a.

The concentration c_{c2} of the agent in zone 2 is not experimentally available. However, it can be calculated using the model of the isotachophoretic steady state described above. Fig. 4b shows the dependence

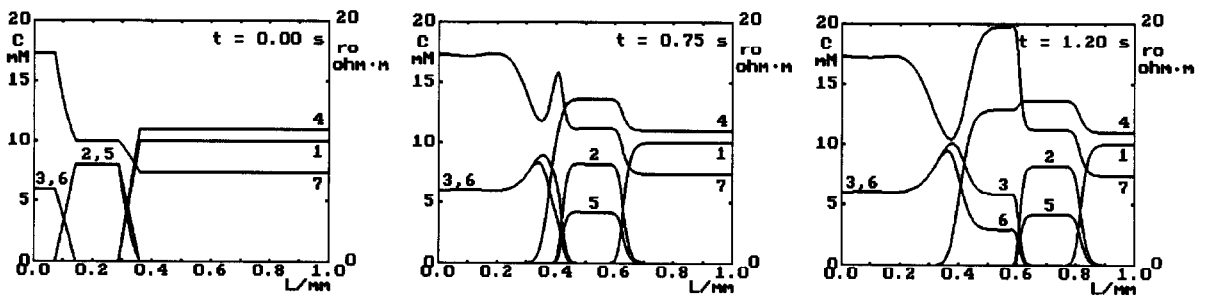

Fig. 2. Simulation of isotachophoretic migration. 1 = Leading anion; 2 = total sample; 3 = terminating anion; 4 = total cyclodextrin; 5 = free sample; 6 = free terminating anion; 7 = specific resistance. Mobility of the complexed terminating anion, $20 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; stability constant of the terminating anion, 100. Other mobilities, stability constants and symbols as in Fig. 1.

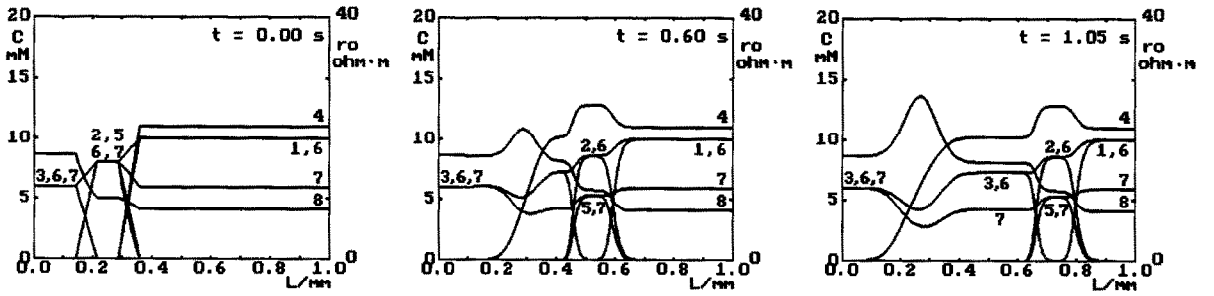

Fig. 3. Simulation of isotachophoretic migration. 1 = Leading anion; 2 = total sample; 3 = terminating anion; 4 = total cyclodextrin; 5 = free sample; 6 = total counter cation; 7 = free counter cation; 8 = specific resistance. Mobility of the complexed counter cation, $35 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; stability constant of the counter cation, 100. Other mobilities, stability constants and symbols as in Fig. 1.

of the specific resistance of zone 2 on concentration c_{c1} of the agent in the zone of the leading electrolyte for different stability constants. The existence of inflection points on the curves for higher stability constants is an interesting feature.

Let us assume that there are now two compounds A and B in the sample, both of which have the same mobilities and can interact with NCA. K_A and K_B are the stability constants of compounds A and B. The ratio $r = \bar{u}_A/\bar{u}_B$ ($\bar{u}_A$ and $\bar{u}_B$ are the effective mobilities) is the criterion of the separation ability. This ratio is also the ratio of the specific resistances of the zones, $r = \rho_B/\rho_A$, because of the validity of the isotachophoretic condition. Fig. 5a shows the graphical dependences of the ratio r on the concentration of NCA in the leading electrolyte for the same stability constants as in Fig. 4b (for curves 1 and 2, 2 and 3, ..., 6 and 7). Each curve has a maximum that corresponds to the maximum separation efficiency for the given pair of compounds and that gives the optimum concentration of NCA in the leading electrolyte. If the counter ion also interacts with the NCA, these maxima are shifted to higher concentrations (Fig. 5b).

The basic validity of the described model was verified by determination of the stability constant of the inclusion complex between 4-hydroxyazobenzenesulphonic acid and β-cyclodextrin and by the determination of the mobilities of free and complex compounds. The system with ε-aminocaproic acid as

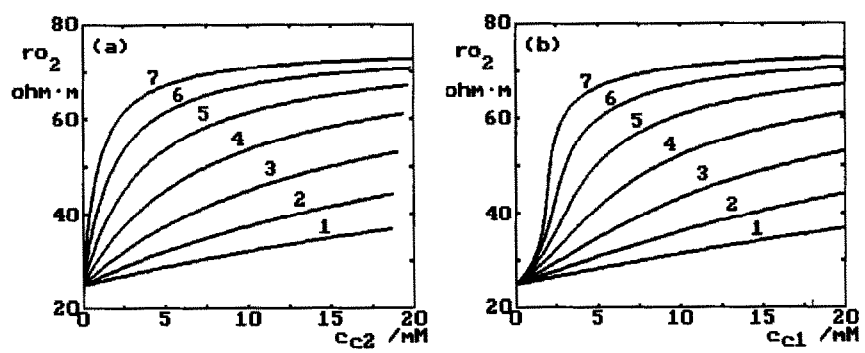

Fig. 4. Dependence of the specific resistance of the sample zone on the total concentration of complexing agent. ro_2 = Specific resistance of the sample zone. Concentration of the leading ion, 10 mM. Mobility of the leading ion, $79.1 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; mobility of the sample, $30 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; mobility of the complexed sample, $10 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; mobility of the counter ion, $30 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}. Stability constant of the sample: (1) 50; (2) 100; (3) 200; (4) 400; (5) 800; (6) 1600; (7) 3200. (a) c_{c2} = Total concentration of cyclodextrin in the sample zone; (b) c_{c1} = total concentration of cyclodextrin in the leading zone.

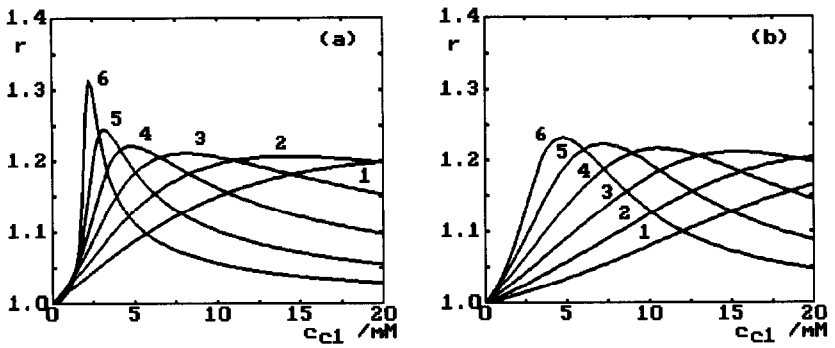

Fig. 5. Dependence of the ratio $r = \rho_B/\rho_A$ on total concentration of complexing agent in the leading zone. c_{c1} = Total concentration of cyclodextrin in the leading zone. Concentration of the leading ion, 10 m*M*. Mobility of the leading ion, $79.1 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; mobility of the samples A and B, $30 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; mobility of the complexed samples, $10 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; mobility of the counter ion, $30 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}. Curves: (1) $K_A = 50$, $K_B = 100$; (2) $K_A = 100$, $K_B = 200$; (3) $K_A = 200$, $K_B = 400$; (4) $K_A = 400$, $K_B = 800$; (5) $K_A = 800$, $K_B = 1600$; (6) $K_A = 1600$, $K_B = 3200$. (a) Only samples A and B interact with cyclodextrin; (b) the counter ion also interacts with cyclodextrin. Mobility of the complexed counter cation, $15 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; stability constant of the counter cation, 500.

the weak buffering counter ion (pH = 4.21) with chloride as the leading ion was used. The specific resistance of the leading electrolyte is not influenced by addition of β-cyclodextrin, which implies that neither the counter ion nor the leading ion interacts with β-cyclodextrin. We can expect that 4-hydroxyazobenzenesulphonic acid will be almost totally dissociated at this pH, but ε-aminocaproic acid only *ca.* half dissociated. The model was developed for strong electrolytes only and, for this reason, we assume that the mobility of the counter ion is equal to its effective mobility. The computer simulation program [10] of the isotachophoretic separation gave $17.92 \cdot 10^{-9}$ m^2 V^{-1} s^{-1} for this effective mobility value. The limiting mobility of the leading Cl^- ion was assumed to be $79.1 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}. The measured dependence of the specific resistance of the sample zone on the concentration of β-cyclodextrin in the leading electrolyte is shown in Fig. 6. The experimental points were fitted by a theoretical curve and the stability constant and the mobilities of the free and complex form of 4-hydroxyazobenzenesulphonic acid with β-cyclodextrin were iteratively determined. We obtained the following values: limiting mobility of the free anion, $21.4 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; limiting mobility of the complex anion, $10.3 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}; and stability constant, 2040. Yoshida *et al.* [13] spectroscopically determined the stability constant for this compound to be 2000, which corresponds well with our result.

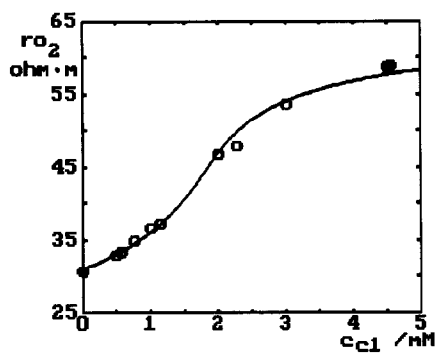

Fig. 6. Experimental data and fitting curve of the isotachophoretic measurement of 4-hydroxyphenylazobenzenesulphonic acid. Leading electrolyte, 0.0111 *M* Cl^-–ε-aminocaproic acid (pH 4.21); terminating electrolyte, 0.005 *M* caproic acid. ρ_2 = Specific resistance of the sample zone; c_{c1} = total concentration of cyclodextrin in the leading electrolyte. Circles = experimental data; full line = fitting curve.

CONCLUSION

The model presented in this paper describes all the basic features of the migration of strong monovalent electrolytes in the presence of a neutral complexing agent. It allows the determination of mobilities and stability constants and the prediction of the optimum separation conditions. In the future work this model should be extended to weak electrolytes and

their interaction with the neutral complexing agent and further it should also include the Debye–Hückel–Onsager theory of the dependence of mobilities on the ionic strength of the solution.

REFERENCES

1 M. Tazaki, M. Tagaki and K. Ueno, *Chem. Lett.*, (1982) 639.
2 F. S. Stover, *J. Chromatogr.*, 298 (1984) 203.
3 J. Snopek, I. Jelínek and E. Smolková-Keulemansová, *J. Chromatogr.*, 438 (1988) 211.
4 E. Smolková-Keulemansová, in O. Huber and J. Szejtli (Editors), *Proceedings of the 4th International Symposium on Cyclodextrins*, Kluwer, Dordrecht, 1988, p. 451.
5 M. Tazaki, T. Hayashita, Y. Fujino and M. Tagaki, *Bull. Chem. Soc. Jpn.*, 59 (1986) 3459.
6 V. Jokl, *J. Chromatogr.*, 13 (1964) 454.
7 I. Jelínek, J. Snopek, J. Dian and E. Smolková-Keulemansová, *J. Chromatogr.*, 470 (1989) 113.
8 I. Jelínek, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 557 (1991) 215.
9 E. Dubrovčáková, B. Gaš, J. Vacík and E. Smolková-Keulemansová, in Å. Sopková (Editor), *Proceedings of the 4th International Seminar on Inclusion Complexes, Stará Lesná, 17–21 June, 1991*, Slovak Chemical Society, Bratislava, 1991, p. P17.
10 B. Gaš, J. Vacík and I. Zelenský, *J. Chromatogr.*, 545 (1991) 225.
11 W. Saenger, in O. Huber and J. Szejtli (Editors), *Proceedings of the Fourth International Symposium on Cyclodextrins, Munich, April 20–22, 1988*, Kluwer, Dordrecht, 1988, p. 159.
12 N. Yoshida, A. Seiyama and M. Fujimoto, *J. Phys. Chem.*, 94 (1990) 4246.
13 N. Yoshida, A. Seiyama and M. Fujimoto, *J. Phys. Chem.*, 94 (1990) 4254.
14 T. Erdey-Gruz, *Transport Phenomena in Aqueous Solutions*, Akadémiai Kiadó, Budapest, 1974.
15 V. Kašička, J. Vacík and Z. Prusík, *J. Chromatogr.*, 320 (1985) 33.
16 A. Ralston, *A First Course in Numerical Methods*, McGraw-Hill, New York, 1965.
17 J. L. Beckers, *Thesis*, Technical University of Eindhoven, Eindhoven, 1973.
18 B. Gaš, J. Zuska and J. Vacík, *J. Chromatogr.*, 470 (1989) 69.