ELSEVIER

Journal of Chromatography A, 786 (1997) 321–331

JOURNAL OF CHROMATOGRAPHY A

Evaluation of capillary zone electrophoresis equilibrium data using the CELET program[1]

Josef Havel*, Pavel Janoš[2]

Department of Analytical Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, CZ-61137 Brno, Czech Republic

Received 19 April 1997; received in revised form 29 April 1997; accepted 22 May 1997

Abstract

General least squares CELET program in Fortran 77 for personal computers applying 'pit-mapping', Davidon–Fletcher–Powell, simplex, and/or Monte Carlo minimizing routines of MINUIT package has been developed to evaluate acidobasic and/or complex equilibrium data from effective mobility changes by capillary zone electrophoresis. The program is able to treat the data of effective mobilities as a function of pH, ligand$_1$, ligand$_2$ or metal ion concentration in up to quaternary system where formation of $A_pB_qC_rD_s$ species can take place. The distribution of species is also calculated. Complete statistical analysis of residuals is performed and the other fitness criteria evaluated. Modeling of mobility changes in capillary zone electrophoresis in up to eight simultaneous reactions is also possible. Simulated (theoretical) data can be loaded with random errors of a chosen level. The program was applied to evaluate data of various systems, especially those of metal ion–sulphate equilibria, Cu(II) and Ce(III)–lactate complexation, Pd(II)–chloride equilibria, etc. © 1997 Elsevier Science B.V.

Keywords: Electrophoretic mobility; Equilibrium analysis; Metal complexes

1. Introduction

Complex equilibria are of high importance in all branches of chemistry and in the last half of this century much attention has been paid to equilibria studies. Nowadays, mostly computers are used to compute equilibrium data [1], while potentiometry, spectrophotometry, and extraction methods are the most popular. Electrophoresis has been used for equilibrium studies almost half a century now and the principals are described in a monograph [2] and reviewed by Robinson and Stokes [3] and Mukerjee [4]. Perhaps for the first time basic quantitative relations were given by Tiselius [5]. One of the first applications of electrophoresis for equilibria analysis was the so called 'moving boundary' method; it was applied to the determination of ionization constants of amino acids [6], determination of stability constants of cadmium iodide [7], conalbumin, lysozome [8], etc. It seems that these results and formulations, which are valid for capillary electrophoresis as well, are rather forgotten.

Let us assume for simplicity just two components A and B. There are two basic situations: (1) if the equilibrium in the system is attained slowly in

*Corresponding author. Fax: +420 5 41211214.

[1]Presented at the 10th International Symposium on Capillary Electrophoresis and Isotachophoresis, Prague, 17–20 September 1996.

[2]Present address: Research Institute of Inorganic Chemistry, Revoluční 84, CZ-400 01 Ústí nad Labem, Czech Republic.

PII S0021-9673(97)00584-0

comparison with the duration of the experiment (*inert* systems), and (2) if the time required for equilibrium is negligible compared with the duration of an electrophoretic experiment, i.e. with the time of separation (*labile* systems). In the case of an inert system where BA_n species are formed, $N+2$ boundaries are created during separation process, and thus $N+2$ distinct peaks are observed. In the case of labile systems, a solution containing B, A, and BA_n complexes behaves as if it contained only two species, B and A, moving with apparent mobilities. In this work we will limit only to the case of labile systems. Furthermore, it will be assumed that activity coefficients and ionic mobilities do not vary significantly throughout the experiment, and that the temperature is maintained constant.

Capillary zone electrophoresis (CZE) is a relatively new separation method, and in 1994 it was also applied to equilibria studies. Importance of complex equilibria in CZE and their use for the separation of rare earth metal ions was studied by Vogt and Conradi [9], and applicability of CZE for studying metal complexation in solution was studied by Erim et al. [10]. Lee and Lin [11] determined metal cations by CZE and studied the effect of complexing agents. Theoretical estimation of CZE behaviour of metal complexes was also studied [12]. Reports of the determination of pK values [13] and dissociation constants by CZE [14], and of the spectrophotometric determination of ionization constants by CZE [15], have been published. Experimental approaches to the determination of pK_a values by CZE were investigated by Gluck and Cleveland [16]. Various expressions for the estimation of association constants by graphical methods from CZE were examined by Rundlett and co-workers [17], and automated pK_a determination at low solute concentrations by CZE were proposed by Cleveland et al. [18]. However, a completely general approach to the investigation of chemical equilibria with the aid of CZE is still missing, and in spite of the above mentioned references we feel there is still a gap in this field. Furthermore, sometimes old things are newly 're-discovered' and/or old-fashioned relations published, and graphical-based approaches which have been already overcome in the past again proposed, described and studied.

The problem of studying equilibrium is more complicated; the task is not only to calculate the acidobasic and or complex forming equilibrium constants. Generally, also the number and composition of the species formed is either not known, only quite clear, and/or certain and, thus, must be determined by equilibrium analysis [19]. In such a case the search for a so-called 'chemical model' to explain mobility changes in CZE must be performed. Chemical model determination determines: (i) the number of species; (ii) their chemical composition; and (iii) equilibria/stability constants.

Equilibrium analysis suffers from an almost sole use of a '*trial and error*' approach [1]. The chemical model is investigated so that one species after another one is 'tried' until the '*best*' model is obtained. A general solution has already been proposed using the simultaneous regression estimation of stoichiometric indices and equilibrium constants (ESI method [20,21]). The ESI approach for equilibrium evaluation has already been quite successfully applied in potentiometry [22], kinetics [23] and spectrophotometry [24].

Thus, the aim of this work was to develop a modern and quite general approach to evaluate equilibria from data of effective mobilities measured by CZE, and to create a user-friendly program, which could run on a personal computer, for the calculation of species mobilities and stability constants in CZE, the program afforded furthermore with up-to-date statistical evaluation of parameters and quality of fitness.

2. Experimental

2.1. Instrumentation

CZE measurements were done on SpectraPHORESIS 2000 (Thermo Bioanalysis Corp., Riviera Beach, FL, USA) using untreated fused-silica capillary, inner diameter 75 μm (Avery Dennison, MA, USA), total capillary length 66 cm, length up to detector window was 58.5 cm. Voltage used, 20 kV; temperature, 30°C. Direct as well as indirect photometric detection, typically at 215 nm, was used throughout the work.

Before use the capillary was first washed for 5 min with 1 *M* NaOH at 60°C, then for another 5 min with

0.1 *M* NaOH at 60°C, 10 min with water at 30°C and, finally, 10 min with background electrolyte (BGE) at 30°C. Between the measurements the capillary was washed with water and corresponding BGE solution. The samples were injected mostly with hydrodynamic injection using a vacuum (1.5 p.s.i., relative to ambient pressure; 1 p.s.i.=6894.96 Pa) for 1 s if not otherwise mentioned.

2.2. Chemicals

Stock solutions of copper sulfate and copper perchlorate were 0.1 *M*. Copper perchlorate was made from copper chloride adding a slight excess of 70% perchloric acid and digesting several times to dryness. The final preparation was then dissolved in water. Metal ion stock solutions (0.1 *M*) were prepared from corresponding nitrates, sulphates or chlorides, and were diluted before use with distilled water. Solutions were standardized using common alkalimetric or complexometric techniques [25].

Lactic acid (CIBA, Basel, Switzerland) was purified by passing through a column with a Dowex 50W-X8 cation-exchanger in the H^+ form. Concentration of the stock solution was checked by conventional titrimetric procedure. The BGEs were prepared by mixing the stock solution of lactic acid with NaOH and adjusting the pH to 6.00±0.05 with diluted perchloric acid. The other chemicals were from Lachema a.s. (Brno, Czech Republic), purity for analysis. Double distilled water from quartz still (Heraeus, Germany) was used.

3. Theoretical

3.1. Calculations

Effective mobility of the component was calculated according to

$$\mu_{\text{eff}} = \frac{l\,L}{V}\left(\frac{1}{t_{\text{m}}} - \frac{1}{t}\right) \tag{1}$$

where L is the total length of the capillary, l the length to the detector, V is voltage applied, t_{m} migration time of the compound and t_0 migration time corresponding to electroosmotic flow (EOF). The value of EOF was determined either from the position of water peak (water dip) observed during indirect detection or using acetone or mesityloxide as a neutral marker; both methods yield results in a good agreement.

3.2. Basic principals of the CELET program

For effective mobility μ_{eff} of a component B under the presence of complexing ligands C and D (where A is supposed to be mostly H^+), and assuming that a series of complexes can be formed according to the reactions

$$p\text{A} + q\text{B} + r\text{C} + s\text{D} \underset{\beta pqrs}{\Leftrightarrow} \text{A}_p\text{B}_q\text{C}_r\text{D}_s \tag{A}$$

a general relation is valid (the charges are omitted for the sake of clarity):

$$\mu_{\text{eff}} = \frac{\sum_{i=0}^{N} \mu_i \beta_i [\text{A}]^{p_i}[\text{B}]^{q_i}[\text{C}]^{r_i}[\text{D}]^{s_i}}{\sum_{i=0}^{N} q_i \beta_i [\text{A}]^{p_i}[\text{B}]^{q_i}[\text{C}]^{r_i}[\text{D}]^{s_i}} = \frac{\sum_{i=0}^{N} \mu_i \beta_i [\text{A}]^{p_i}[\text{B}]^{q_i}[\text{C}]^{r_i}[\text{D}]^{s_i}}{B_{tot}}$$

$$= \frac{\mu_B[\text{B}] + \mu_1\beta_1[\text{A}]^{p_1}[\text{B}]^{q_1}[\text{C}]^{r_1}[\text{D}]^{s_1} + \cdots + \mu_N\beta_N[\text{A}]^{p_N}[\text{B}]^{q_N}[\text{C}]^{r_N}[\text{D}]^{s_N}}{B_{tot}} \tag{2}$$

where μ_i are mobilities of individual species, β_i are total stability constants (β_0 is supposed to be equal to 1), p, q, r, and s are stoichiometric indices and B_{tot} is analytical concentration of the component B. The summation is taken over all N reactions in the system. The total concentration of the component B is given by relation

$$B_{\text{tot}} = \sum_{i=0}^{N} q_i \beta_i [\text{A}]^{p_i}[\text{B}]^{q_i}[\text{C}]^{r_i}[\text{D}]^{s_i} \tag{3}$$

and similar equations like Eq. (3) are valid for total concentrations C_{tot} and D_{tot} of the components C and D.

Analogous relations to that of Eq. (2) can be written and used in the case of effective mobility of the other component, e.g. C or D, are determined experimentally.

Evaluation of equilibria constants with the CELET program are based on the minimization of the sum of squares of residuals, i.e. the differences of experimental effective mobilities $\mu_{\text{eff,exp}}$ and mobilities calculated $\mu_{\text{eff,calcd}}$. Parameters, i.e. equilibria con-

stants and specific mobilities of the individual species, are obtained from the position of the minimum of U given by the relation

$$U = \sum_{i=1}^{N_p} (\mu_{\text{eff,exp}} - \mu_{\text{eff,calcd}})^2 = \text{minimum} \quad (4)$$

where N_p is the total number of experimental points.

The task of capillary electrophoresis equilibrium analysis is to find such values of effective mobilities μ_i, the formation constants β_i and stoichiometric indices, p_i, q_i, r_i, and s_i, of $A_pB_qC_rD_s$ species, which would yield the minimum of the sum of squares of residuals, U, given by Eq. (4). Naturally, it is not trivial to find out the value of N, i.e. the number of equilibrium reactions. Usually, this number is obtained just at the end of equilibrium model search when the 'best' fit has been reached.

Once the model is found and equilibria constants and ionic mobilities are obtained, the distribution of individual complexes is calculated by the CELET program, and the tables of the distribution diagrams are printed. The distribution of individual species can be conveniently expressed by the fraction of $A_pB_qC_rD_s$ species versus total concentration of the component.

These distribution functions α_i, β_i, γ_i are defined by

$$\alpha_i = \frac{q_i[A_{p_i}B_{q_i}C_{r_i}D_{s_i}]}{B_{tot}} \quad (5)$$

$$\beta_i = \frac{r_i[A_{p_i}B_{q_i}C_{r_i}D_{s_i}]}{C_{tot}} \quad (6)$$

$$\gamma_i = \frac{q_i[A_{p_i}B_{q_i}C_{r_i}D_{s_i}]}{D_{tot}} \quad (7)$$

3.3. Statistical evaluation of fitness test

Besides the sum of residual squares, U, defined by Eq. (4), residual mean, mean residual, variance, standard deviation $s(\mu_{\text{eff}})$ of the measured quantity, and Hamilton R factor (%) are calculated. The definition of the variables and their application for the evaluation of the fit are described elsewhere [1,26,27].

The above-mentioned relation (Eq. (2)) and/or analogous ones for the other components in the system are quite general and they include any kind of: (i) acid–base equilibria; (ii) metal–ligand complexation with one or two ligands simultaneously; (iii) host–guest interactions; (iv) metal ion–hydrolytic equilibria, including polynuclear species, etc.; (v) concurrence reactions of a ligand D with respect to the complexation of B with complexing agent C, etc.

The formation of mono- and/or polynuclear species or any combination of (i)–(v) chemical equilibria is possible to evaluate. The only precondition is that interferences, like sorption of the species on the capillary wall, etc., are eliminated or can be neglected. Several illustrative cases will be considered below and the possibilities of the use of the CELET program demonstrated.

3.4. Some simplifications

3.4.1. Stepwise complex formation of ML_n complexes

In this case Eq. (2) can be simplified. For example, for effective mobility μ_{eff} of a metal cation M in the presence of complexing ligand L under the formation of a series of mononuclear ML_n complexes, the following relation is valid (the charges are omitted for the sake of clarity)

$$\mu_{\text{eff}} = \frac{\sum_{i=0}^{N} \mu_i \beta_i [\text{M}][\text{L}]^i}{\sum_{i=0}^{N} \beta_i [\text{M}][\text{L}]^i} = \frac{\mu_{\text{M}}[\text{M}] + \mu_1\beta_1[\text{M}][\text{L}] + \mu_2\beta_2[\text{M}][\text{L}]^2 + \ldots \mu_N\beta_N[\text{M}][\text{L}]^N}{M_{tot}} \quad (8)$$

where μ_i are mobilities of the individual species and β_i total stability constants (β_0 is supposed to be equal to 1) and M_{tot} is total (analytical) concentration of the metal ion. Furthermore, e.g., in case of the equilibrium of a divalent metal ion M with a ligand L, for example with sulfate, if it is possible to neglect the formation of the higher (anionic) complexes, Eq. (8) can be written in an even more simple way [28].

$$\frac{1}{\mu_{\text{eff}}} = \frac{1}{\mu_{\text{M}}}(1 + \beta_1[\text{L}]) \quad (9)$$

Thus, reciprocal effective mobilities are linearly dependent on ligand (sulfate) concentration in the

basic electrolyte. The value of μ_M is ionic mobility of free cation M^{2+} and [L] is free ligand concentration. Using sufficient excess of the ligand L, free concentration [L] can be approximated with total analytical concentration c_L. Such a relation can be very well used for a graphical solution, as demonstrated recently [28]. However, we would like to stress here that if the condition $c_L \gg c_M$ is not fulfilled, then the approximation $c_L \sim c_M$ is not valid and the results applying Eq. (9) are only approximate, as has recently been shown for the case of metal ion-sulfate equilibria [28].

3.4.2. Simple acidobasic equilibrium

In the case of a general acidobasic equilibrium

$$H_nL \Leftrightarrow H_{n-1}L^- + H^+ \qquad (B)$$

the effective mobility of an anion L co-existing in various protonated forms can be expressed as follows:

$$\mu_{eff} = \frac{\sum_{i=0}^{n} \mu_i [H_{n-i}L^{i-}]}{\sum_{i=0}^{n} [H_{n-i}L^{i-}]} = \frac{\mu_0[H_nL] + \mu_1[H_{n-1}L^-] + \mu_2[H_{n-2}L^{2-}] + \cdots + \mu_n[L^{n-}]}{L_{tot}} \qquad (10)$$

μ_i are mobilities of the individual species, again (the mobility μ_0 is considered equal to zero). For a dibasic acid H_2L, Eq. (10) can be further simplified and rewritten using dissociation constants K_{a1} and K_{a2}:

$$\mu_{eff} = \frac{\mu_{HL} K_{a1}[H^+] + \mu_{L^{2-}} K_{a2}}{K_{a1}K_{a2} + K_{a1}[H^+] + [H^+]^2} \qquad (11)$$

4. Results and discussion

The program CELET was constructed on the basis of Eqs. (2)–(7). From the many minimizing methods available and used in equilibria analysis reviewed elsewhere [1,27] a '*pit-mapping*' approach in the form of ABLET [26], described in detail elsewhere [27], has been used. The ABLET has been modified and adapted to be used on PCs and enlarged up to 16 parameters to be estimated simultaneously.

As the ABLET family of programs has been sufficiently described [1,26,27], and manuals for several programs of this family are available [27], the input data and program structure will not be given in detail here. The MAIN part of the program reads some items of input and has the organizational role mainly. The input block DATA reads most of the data, i.e., (μ_{eff}, pH) or (μ_{eff}, L_{tot}), pairs, the residual squares sum block UBBE calculates the sum of squares of residuals according to Eq. (4). Subroutines LETAG, MULLE, PINUS, INVER, WEIGHT, READR, READI, STATS, PLOTT, SIMUL, NORAND, and RANDOL are related to ABLET. Subroutine SKRIK is used to print the results and distribution tables for individual species, and it enables also semi-graphical line printer output of the experimental and calculated curves. This part of the program is, in structure, similar to POLET [22] or to the other programs of the ABLET family described in detail elsewhere [27]. ABLET is derived from LETAGROP and it uses a similar strategy. A minimization process can either be controlled by the operator or governed automatically by the program itself. A combined strategy can also be used.

Sometimes problems with convergence during the minimization process occur. For this reason the MINUIT minimizing package [29] has been modified accordingly for PC and implemented. Thus, besides the '*pit-mapping*' method, the user can switch off to the other minimizing strategies, if the need arises. Contour maps of U around the minimum can also be calculated and vizualized. It will be demonstrated below that these maps are a good indication that the minimum defined by Eq. (4) has really been reached.

In any case of input model, a starting guess for the parameters must be given as input. The parameters to be determined are β_1, β_2, ... β_n, and μ_i, and an initial guess of these parameters must be given as input in this order. It is up to the user which of them and in which order they should be calculated. Parameters not requested to be changed are automatically kept constant during the minimization process. Input data for the program should be prepared in a separate file, in the way described in the manual [27]. The data are format free and reached by applying subroutines READR and READI, while the separator is a comma or free space.

The program CELET has been validated on sever-

al simulated and experimental data sets. Below, demonstration examples of the use of CELET program are given and discussed.

4.1. Case (1): dissociation constants of a weak acid and shape of the minimum $U = f(pK_i, \mu_{eff,i})$

The program has already been applied to several experimental and simulated data [30,31]. In this case it was assumed that a weak acid H_2L dissociates with two near values of dissociation constants, $pK_1 = 3.40$ and $pK_2 = 4.15$, while individual mobility values, μ_{eff}, were 0.00, 50.00, and 100.00 for H_2L, HL^-, and L^{2-}, respectively. Data (pH, μ_{eff}) were simulated with the CELET program and μ_{eff} values were loaded with random errors possessing the value of 'instrumental error' with standard deviation $s_{inst} = \pm 2.50$ (corresponding approximately to the average accuracy of effective mobility measurement in CZE). The data are given in Table 1.

The experimental data together with residuals ($\mu_{eff,exp} - \mu_{eff,calc}$) are demonstrated in Fig. 1. Using the CELET program, pK_1, pK_2 and individual mobilities of H_2L, HL^-, and L^{2-} species were calculated. Even when starting from quite wrong initial estimates for parameters to be calculated, the program converges and the minimum of U is obtained. The results are given in Table 2.

It is evident that the value of σ is close to s_{inst} and values of parameters near the correct ones, even if the case is rather difficult for quite similar pK values. Demonstration of the shape of the minimum of U as a function of the calculated ionic mobility of L^{2-} species and $\log \beta = pK_1 + pK_2$ is given in Fig. 2. It is evident that the minimum is quite well expressed and obtained for values of parameters near the correct ones.

4.2. Case (2): stability constants of alkali and alkaline earth cations with sulfate

When studying separation conditions for alkali and alkaline earth cations, where copper sulfate was used for indirect detection, the migration behaviour of cations to be separated was found to be influenced by the level of sulfate concentration [28], which is naturally due to the formation of sulfate complexes.

Table 1
Simulated data of effective mobilities concerning dissociation of a weak acid H_2L ($pK_1 = 3.40$, $pK_2 = 4.15$, $\mu_{eff} = 0.00$, 50.00, and 100.00 for H_2L, HL^-, and L^{2-}, respectively)

pH	$\mu_{eff,exp}$	$\mu_{eff,calc}$	Residual $\mu_{eff,exp} - \mu_{eff,calc}$
1.000000	−2.112753	0.1955	−2.3083
2.000000	1.916351	1.9149	0.0015
3.000000	21.241950	15.9455	5.2964
3.100000	19.296310	19.1714	0.1249
3.200000	20.656450	22.8532	−2.1967
3.300000	27.565750	26.9838	0.5819
3.400000	31.874280	31.5325	0.3418
3.500000	37.219100	36.4425	0.7766
3.600000	39.495300	41.6326	−2.1373
3.700000	44.275080	47.0011	−2.7260
3.800000	51.456450	52.4327	−0.9762
3.900000	59.509420	57.8072	1.7022
4.000000	63.906960	63.0090	0.8979
4.100000	70.000170	67.9354	2.0648
4.200000	68.521030	72.5038	−3.9828
4.300000	74.942150	76.6562	−1.7141
4.400000	81.212610	80.3604	0.8522
4.500000	84.955950	83.6085	1.3475
4.600000	87.648980	86.4127	1.2363
4.700000	90.462000	88.8007	1.6613
4.800000	92.086640	90.8102	1.2765
4.900000	93.644870	92.4839	1.1610
5.000000	96.157840	93.8659	2.2919
5.100000	94.582440	94.9989	−0.4164
5.200000	95.039580	95.9221	−0.8825
5.300000	97.995520	96.6706	1.3249
5.400000	97.401780	97.2752	0.1266
5.500000	90.179940	97.7618	−7.5818
5.600000	95.417830	98.1525	−2.7346
5.700000	100.496500	98.4654	2.0311
5.800000	98.716690	98.7157	0.0010
5.900000	99.773910	98.9156	0.8583
6.000000	96.510330	99.0751	−2.5648
6.100000	99.236660	99.2022	0.0344
6.200000	98.068530	99.3035	−1.2349
6.300000	100.780300	99.3841	1.3962
6.400000	98.897670	99.4482	−0.5505
6.500000	101.501200	99.4992	2.0020
6.600000	98.928540	99.5398	−0.6112
6.700000	98.277370	99.5720	−1.2946
6.800000	102.486800	99.5976	2.8892
6.900000	98.836130	99.6180	−0.7819
7.000000	100.976900	99.6342	1.3427
8.000000	99.943470	99.6905	0.2529
9.000000	100.114900	99.6962	0.4187

That is why the stability constants of 12 metal cations with sulfate were determined by CZE [28]. In this study copper perchlorate was replaced continuously with sulfate. A series of electrolyte solutions

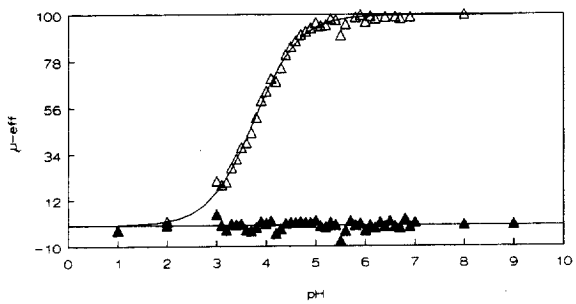

Fig. 1. Effective mobility as a function of pH for a weak acid dissociating in two steps ($pK_1 = 3.40$, $pK_2 = 4.15$): (△) experimental data $\mu_{eff,exp}$; solid curve, calculated values $\mu_{eff,calc}$; (▲) the residuals ($\mu_{eff,exp} - \mu_{eff,calc}$).

were made so that copper concentration was maintained constant and only the concentration of sulfate varied. The value of pH was kept practically constant in the range 4.85–4.90. Measured dependency of the effective mobility of Ca(II) on total sulfate concentration is given below as just one example of this extensive study (the data in the graphical form only were published in literature [28]). The data ($sulfate_{tot}$, μ_{eff} (Ca^{2+})): (0.00, 0.034114), (0.001, 0.032025), (0.002, 0.029659), (0.003, 0.027314), and (0.004, 0.025475). Because Cu(II) also forms sulfate complexes, it was necessary to correct for the parallel complexation reaction of sulfate with Cu(II) in the background electrolyte, which is quite easy with the CELET program. In this way, also the constant for the $CuSO_4$ complex can be estimated. Stability constants for $CaSO_4$ and $CuSO_4$ obtained were log $\beta = 2.15 \pm 0.01$ and 2.31 ± 0.02, respectively, while the individual mobility of Ca^{2+} calculated was 0.03423 ± 0.00007 cm^2 V^{-1} min^{-1}. For the experimental details and some other conditions see Ref. [28].

4.3. Case (3): lactate complexes of copper(II) and cerium(III)

4.3.1. Copper(II)–lactate

Copper(II) forms several complexes with lactic acid, $Cu(Lac)_n^{(2-n)+}$, where $n = 1–4$. At moderate lactate concentrations only the formation of 1:1 and 1:2 species can be assumed. There is a great discrepancy in the values of stability constants in literature, log $\beta_1 = 2.06$ (0.1 *M* $NaClO_4$) [32], while a value equal to 2.63 is reported in 2 *M* $NaClO_4$ [33]. The average value for the lowest ionic strength, 0.1, found in literature (2.06 in 0.1 *M* $NaClO_4$ [32], 2.36 in 0.1 *M* KCl [34], and 2.55 [35]) is 2.32, while 2.49 was found for 1 *M* $NaClO_4$ [36]. The average value for log β_2 is difficult to estimate, as there is even greater discrepancy. The values range from 4.11 (2 *M* $NaClO_4$ [37]) to 2.73 in 0.1 *M* $NaClO_4$ [34]. The average for the ionic strength $I = 0.1$ is about 3.05.

Data ($Lact_{tot}$, μ_{eff}): 0, 48.90; 0.001, 41.98; 0.002, 37.04; 0.005, 28.15; 0.008, 23.79; 0.01, 22.05; 0.012, 20.04; 0.015, 18.16; measured for $Cu_{tot} = 1$ mM were then evaluated with the CELET program. The results obtained for various models are given in Table 3, where the chemical model is briefly given.

It follows from Table 3, that already a rather good fit was obtained assuming only $Cu(Lac)^+$ complex formation (Run 1), worse fit was obtained assuming a 1:2 complex (Run 2) and a better fit was obtained assuming simultaneous formation of $Cu(Lac)^+$ and $Cu(Lac)_2$ complexes (Run 3). However, even in this case the statistical distribution of residuals was not satisfactory. Quite a good fit with excellent statistical analysis of residuals was obtained calculating also the effective mobility of neutral $Cu(Lac)_2$ species.

Table 2
Results of computation of data on dissociation of a weak acid from data in Table 1

Parameters	Correct	Calculated	Fitness parameters	
pK_1	4.15	4.098±0.003	*U*	194.023
pK_2	7.55	7.503±0.005	σ	2.076
Mobilities				
L^{2-}	100	99.697±0.055	χ^2	11.36 (12.60)
LH^-	50	49.734±0.472	Hamilton R factor	0.02532
LH_2	0	fixed		

σ, standard deviation of μ_{eff}.

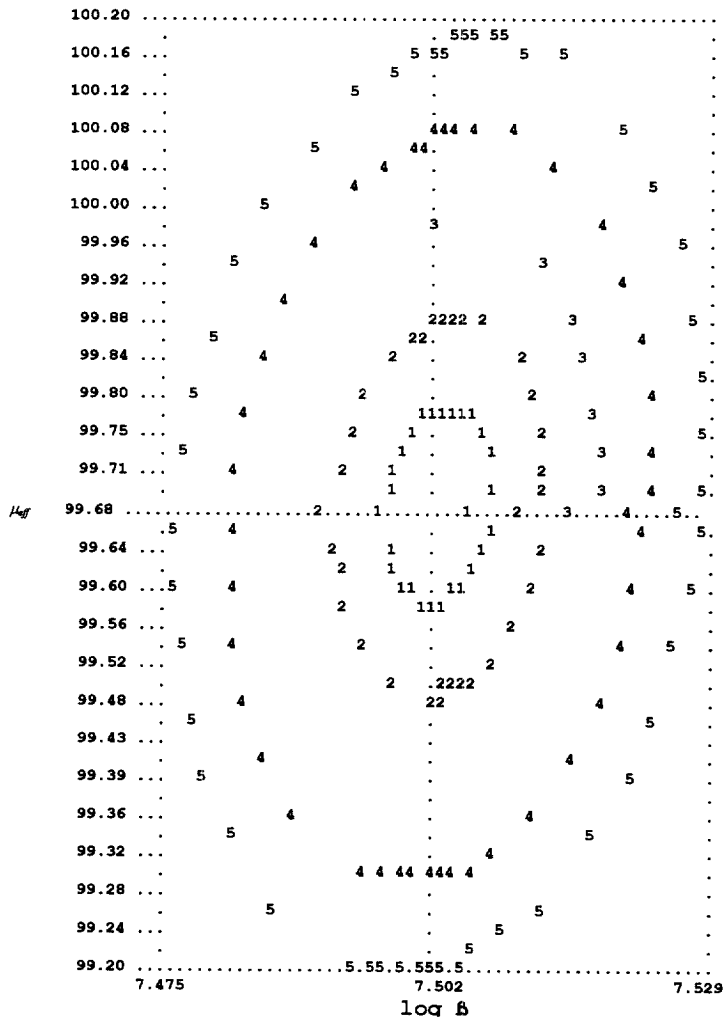

Fig. 2. Contours map for $U=f(\mu_{eff}$ of L^{2-}; $-\log\beta=pK_1+pK_2)$ around the minimum concerning data of case (1). Contour n: $U_n=U_{min}+0.25\times n^2$ where U_{min} is the value of U in minimum ($U_{min}=194.023$).

However, the mobility of $Cu(Lac)_2$ species came out negative. This is an indication that another complex with a negative charge, and thus negative mobility, is also formed. This suggestion was proved in the last run (Run 6) assuming the formation of a 1:3 complex, and the species mobility became negative for the 1:3 species, as it should be, and a reasonable value of log β_3 was obtained in a good agreement with the literature.

The distribution diagrams calculated using the final values of the equilibrium constants (as given in Table 3, Run 6) are shown in Fig. 3.

4.3.2. Ce(III)–lactate system

Cerium(III) forms $Ce(Lac)_n^{(3-n)}$ complexes, with $n=1-3$. Similarly, as in case of Cu(II), the changes of effective mobility of Ce(III) were followed as a function of lactate concentration. The data ($Lact_{tot}$, μ_{eff}: 0.00, 58.0; 0.001, 52.97; 0.002, 48.28; 0.005, 38.76; 0.008, 33.9; 0.01, 31.29; 0.012, 28.9; 0.015, 26.67; mol l^{-1} and $cm^2\ V^{-1}\ min^{-1}$) measured for $Ce_{tot}=1$ mM at pH 6 were then evaluated with the CELET program. The results obtained for various models are given in Table 4. It can be seen from Table 4 that assumption of just one complex 1:1 was

Table 3
Results of Cu(II) mobility changes due to the increasing lactate concentration evaluation with the CELET program

Run	Model	log β_i	Individual effective mobilities	Fitness criteria	
				U	$\sigma(\mu_{eff})$
1	HLac	4.49 (fixed)			
	Cu^{2+}		48.78±0.25	0.2759	0.27591
	$Cu(Lac)^{+}$	2.40±0.02	10.03±0.62		
2	HLac	4.49 (fixed)			
	Cu^{2+}		46.77±1.06	18.65	1.63230
	$Cu(Lac)_2$	5.33±0.11			
3	HLac	4.49 (fixed)			
	Cu^{2+}		48.86±0.12	0.264098	0.19424
	$Cu(Lac)^{+}$	2.46±0.005	12.90±0.13		
	$Cu(Lac)_2$	3.34±0.036			
4	HLac	4.49 (fixed)			
	Cu^{2+}		48.835±0.105	0.192378	0.16578
	$Cu(Lac)^{+}$	2.475±0.005	13.952±0.105		
		2.48 [40]			
	$Cu(Lac)_2$	3.364±0.021	− 8.36±1.37		
		3.05 [40]			
5	HLac	4.49 (fixed)			
	Cu^{2+}		48.835±0.105	0.192378	0.16578
	$Cu(Lac)^{+}$	2.475±0.005	13.952±0.105		
		2.48 [40]			
	$Cu(Lac)_2$	3.364±0.021	− 8.36±1.37		
		3.05 [40]			
6	HLac	4.49 (fixed)			
	Cu^{2+}		48.895±0.086	0.1299	0.13622
	$Cu(Lac)^{+}$	2.485±0.004	14.014±0.087		
		2.48 [40]			
	$Cu(Lac)_2$	3.333±0.027	0.0 (fixed)		
		3.05 [40]			
	$Cu(Lac)_3^{-}$	4.513±0.056	−35.086±1.525		
		4.01 [40]			

U, the sum of squares of residuals as given by Eq. (4), $\sigma(\mu_{eff})$, standard deviation.

not sufficient to explain the mobility changes. Satisfactory agreement with the data was obtained assuming the simultaneous formation of 1:1 and 1:2 complexes. Under the excess of the lactate used, the formation of a 1:3 complex was not proved as log β_3 turned out not to be real and with a high standard deviation. The equilibrium constants of $Ce(Lac)_n^{(3-n)}$ computed are in a fair agreement with literature, the distribution diagram is given in Fig. 4.

4.4. Case (4): palladium(II) chlorides

Recently, separation of platinum group metals (PGM) in chloride medium has been studied [38] and a CZE method for simultaneous determination of all PGM was worked out [39]. In this study stability constants of Pd(II) chlorides were determined at 25 and 55°C. As experimental data has not been published, and no details of calculation given, the use of CELET and computational problems will be discussed here. Effective mobility of Pd(II) as a function of chloride concentration was measured at pH 3.20 detecting Pd at 214 nm. The data (Cl_{tot}, μ_{eff}) obtained were: 0.001, −1.40; 0.005, −11.60; 0.010, −16.30; 0.020, −20.60; 0.030, −22.90; 0.040, −24.1; 0.050, −26.00; and 0.060 mol l^{-1}, −28.20 $m^2 V^{-1} s^{-1}$. The results of calculation are given in Table 5, the distribution diagram in Fig. 4.

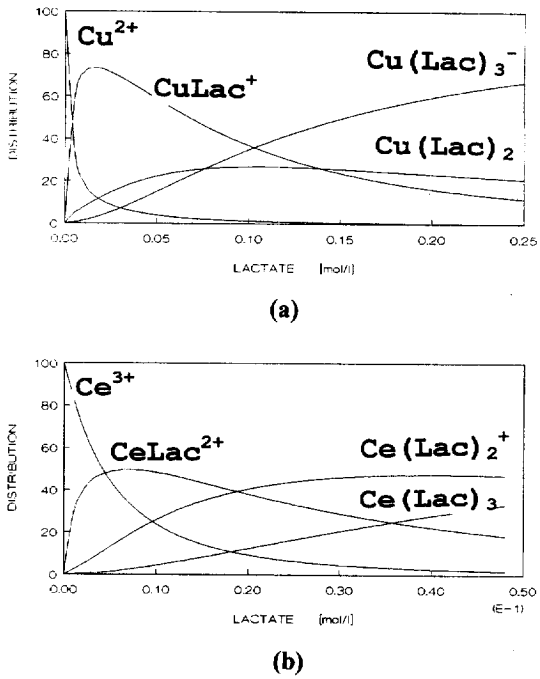

Fig. 3. (a) Distribution diagram of complex species formed in the Cu(II)–lactate system. (b) Distribution diagram of complex species formed in the Ce(III)–lactate system.

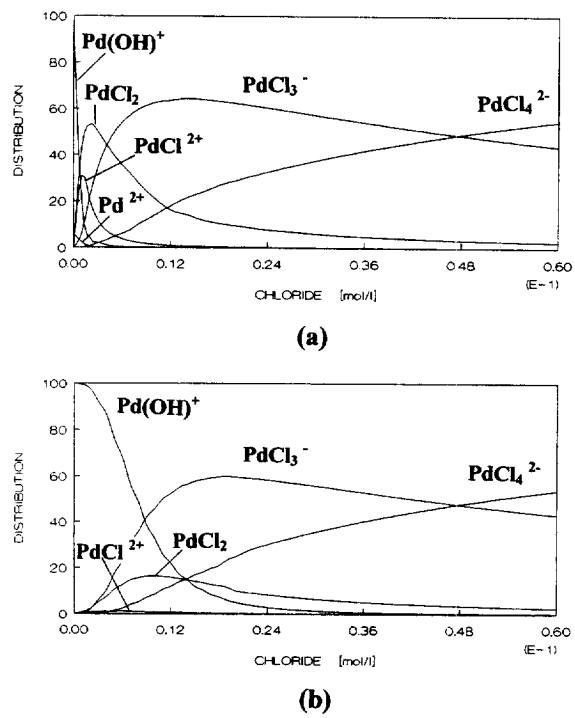

Fig. 4. Distribution of palladium chloride complexes as a function of chloride concentration: (a) pH 3.20; (b) pH 6.00.

5. Conclusions

The computer program CELET can be used with advantage to evaluate acidobasic and complex equilibria constants from effective mobility data, and also for the search of chemical models, and no approximations are needed. The computation can be done in up to quaternary systems. Also the simulation of mobility changes is possible, while up to eight simultaneous equilibria can be taken into account.

Acknowledgments

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

Table 4
Evaluation of Ce(III) mobility changes due to the increasing lactate concentration with the CELET program

Run	Model	log β_i	Individual effective mobilities	Fitness criteria	
				U	$\sigma(\mu_{eff})$
1	HLac	4.49 (fixed)			
	Ce^{3+}		58.19±0.13	0.4163	0.24388
	$Ce(Lac)^{2+}$	2.196±0.004	12.74±0.18		
2	HLac	4.49 (fixed)			
	Ce^{3+}		58.12±0.15	0.4140	0.24321
	$Ce(Lac)^{2+}$	2.384±0.012	27.19±0.21		
	$Ce(Lac)_2^+$	4.119±0.012	13.95±0.41		

Table 5
Pd(II)–chloride system

Species	log β	$s(\log \beta)$	log β (Ref. [39])	μ_{eff}	$\sigma(\mu_{eff})$
$PdCl^+$	3.82	0.80	4.47	2.52	8.93
$PdCl_2$	7.75	0.03	7.76	0[a]	−1[a]
$PdCl_3^-$	10.26	0.02	10.20	−18.02	0.29
$PdCl_4^{2-}$	11.49	0.02	11.50	−38.01	0.52
$Pd(OH)^+$	−1.46	0.37	−2.00	0.17	0.92

[a]Not calculated.

References

[1] M. Meloun, J. Havel, E. Högfeldt, Computation of Solution Equilibria – A Guide to Methods in Potentiometry, Extraction, and Spectrophotometry, Ellis Horwood, Chichester, 1988.

[2] F.J.C. Rossotti, H. Rossotti, The Determination of Stability Constants, McGraw-Hill, New York, 1961, pp. 316-318.

[3] R.A. Robinson, R.H. Stokes, Electrolyte Solutions, 2nd ed., Butterworth, London, 1959.

[4] P. Mukerjee, J. Phys. Chem. 62 (1958) 1397.

[5] A. Tiselius, Dissertation, University of Uppsala, Sweden, 1930.

[6] H. Svensson, A. Benjaminsson, I. Brattsten, Acta Chem. Scand. 3 (1949) 307.

[7] R.A. Alberty, E.L. King, J. Am. Chem. Soc. 73 (1951) 517.

[8] S. Ehrenpreis, R.C. Warner, Arch. Biochem. Biophys. 61 (1956) 38.

[9] C. Vogt, S. Conradi, Anal. Chim. Acta 294 (1994) 145.

[10] F.B. Erim, H.F.M. Boelens, J.C. Kraak, Anal. Chim. Acta 294 (1994) 155.

[11] Y.-H. Lee, T.-I. Lin, J. Chromatogr. A 675 (1994) 227–236.

[12] A.R. Timerbaev, V.P. Semenova, J. Chromatogr. A 690 (1995) 141.

[13] J.L. Beckers, F.M. Everaerts, M.T. Ackermans, J. Chromatogr. 537 (1991) 407.

[14] S.J. Gluck, J.A. Cleveland Jr., J. Chromatogr. A 680 (1994) 43.

[15] J.A. Cleveland Jr., C.L. Martin, S.J. Gluck, J. Chromatogr. A 679 (1994) 167.

[16] S.J. Gluck, J.A. Cleveland Jr., J. Chromatogr. A 680 (1994) 49.

[17] L. Kimber, D. Rundlett, W. Armstrong, J. Chromatogr. A 721 (1996) 173.

[18] J.A. Cleveland, M.H. Benko Jr., S.J. Gluck, Y.M. Walbroehl, J. Chromatogr. A 652 (1993) 301.

[19] J. Havel, Computer applications in chemistry, VIII, Problem of chemical model determination in equilibrium study, Scripta Fac. Sci. Nat. Univ. Purk. Brun; Chemia 17 (1987) 305–320.

[20] J. Havel, M. Vrchlabský, Euroanalysis V, Cracow, 26–31 August 1984, Paper XVIII-20, 465.

[21] J. Havel, M. Meloun, Talanta 33 (1986) 435.

[22] J. Havel, M. Meloun, Talanta 33 (1986) 525.

[23] J. Havel, J.L. González, React. Kinet. Catal. Lett. 39 (1989) 141.

[24] M. Meloun, M. Javůrek, J. Havel, Talanta 33 (1986) 513.

[25] A.A. Flashka, A.J. Barnard Jr., in: C.L. Wilson, D.W. Wilson (Eds.), Comprehensive Analytical Chemistry, vol. IB, Classical Analysis, Elsevier, Amsterdam, 1960, pp. 288–375.

[26] M. Meloun, J. Čermák, Talanta 31 (1984) 947.

[27] J. Havel, M. Meloun, in: D.J. Leggett (Ed.), Computational Methods For The Calculation Of Formation Constants Plenum Press, New York, 1985.

[28] J. Havel, P. Janoš, P. Jandik, J. Chromatogr. A 745 (1996) 127.

[29] F. James, M. Roos, Comp. Phys. Commun. 10 (1975) 343.

[30] G. Vargas, A. Revilla, L. Galindo, J. Havel, Computation of Acidity Constants from Capillary Zone Electrophoresis Data, Chemometrics IV, Brno, September 1996.

[31] J. Havel, P. Janoš, CELET Computer Program for the Evaluation of Equilibrium Constants from Capillary Zone Electrophoresis Data. Chemometrics IV, Brno, September, 1996.

[32] S. Ramamoorthy, M. Santappa, J. Inorg. Nucl. Chem. 30 (1968) 2393.

[33] J. Savic, M. Savic, I. Filipovic, Croat. Chim. Acta 44 (1972) 305.

[34] R. Ghosh, V.S.K. Nair, J. Inorg. Nucl. Chem. 32 (1970) 3025.

[35] V.P. Devendran, M. Santappa, Curr. Sci. 34 (1965) 145.

[36] H. Thun, W. Guns, F. Verbeck, Anal. Chim. Acta 37 (1967) 332.

[37] I. Filipovic, I. Piljac, A. Medved, S. Savic, A. Bujak, B. Bach-Dragutinivic, B. Mayer, Croat. Chim. Acta 40 (1968) 131.

[38] A.V. Pirogov, J. Havel, Presented at the 10th International Symposium on Capillary Electrophoresis and Isotachophoresis, Prague, 17–20 September, 1996, Book of Abstracts, P82, p. 116.

[39] A.V. Pirogov, J. Havel, J. Chromatogr. A 772 (1997) 347–355.

[40] H. Deelstra, F. Verbeck, Anal. Chim. Acta 31 (1964) 251.