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Measurement of Ion Association Constants from Decrease of Peak Areas by Capillary Electrophoresis Technique with Indirect Spectrophotometric Detection

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The new method to determine association constants using capillary electrophoresis technique with indirect spectrophotometric detection was investigated. An association constant was calculated from decrease of corrected peak area. The equations for correction of peak areas and calculation of association constants were examined using zone mathematical model of electrophoretic ion motion. This method was used to measure association constant of calcium - ions with sulfate - ions: $\lg K_{ass}(\text{CaSO}_4) = 2,39 \pm 0,09$.

Keywords: ion association, capillary electrophoresis.

Introduction

The capillary electrophoresis technique (CE) is used for measurement of ion association constant from electrophoretic mobility changes [1-10]. However if association constant has low value it is difficult to divide contribution in electrophoretic mobility changes ion association and electrostatic interactions because of the estimation of electrostatic interactions in solution containing of several ions is a problem. But then we observed that on real electropherograms for case of indirect spectrophotometric detection the decreases of peak areas were occurred for system in which the ion association takes place. This decrease of peak area will be bound to fraction

of nonassociated ions. In present study the new method to determine the association constants using capillary electrophoresis technique with indirect spectrophotometric detection from decrease of corrected peak area was investigated.

Experimental

All experiments were carried out on a capillary electrophoresis system Agilent ^{3D}CE G1600A (Agilent Technologies, USA) equipped spectrophotometric diode-array detection system (DAD). Fused-silica capillary of 64,5cm total length (effective length 56 cm) and 50 μm inner diameter was used. The measuring wavelength of the DAD system was 220 nm with reference 400

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nm. Analytical data signals processing and system control were handled by HP ChemStation software (Rev.A.10.02). The temperature held at 25 °C. Samples were injected in the hydrodynamic mode by applying an external pressure 50 mbar for 4 s. To measure electroosmotic flow velocity a neutral marker was injected to outlet by hydrodynamic pressure 50 mbar for 2 s. The capillary was washed daily for 10 min with 0,1 M NaOH, two times for 10 min with double distilled water, for 10 min with run buffer and for 20 min with keeping under voltage. Between injections capillary was washed too in 10 min with run buffer.

All chemicals used were of analytical grade or better. Sodium chloride and sodium sulfate were dried at 300° C. Solutions were prepared using double distilled water with conductivity less $1 \cdot 10^{-6}$ Ohm⁻¹cm⁻¹. As background electrolyte 5 mM calcium nitrate was employed. To measure electroosmotic flow velocity used 0,1 mM benzyl alcohol solution. Chloride –ion was employed as internal standard.

The equations for correction peak areas and calculation association constants

Indirect detection in capillary electrophoresis technique is based on decrease of concentration of background electrolyte ions in zone of separated ions. This decrease is frequently recorded by spectrophotometric method and depended on what fraction of ions is ionized. To compare peak area of nonassociating ions with peak area of associating ions we may calculate association constant with background electrolyte ions. In addition the peak areas in capillary electrophoresis with indirect detection depend on concentration of ions but also on their electrophoretic mobility, velocity of ion motion past detector, nature and concentration of background electrolyte [11-13].

In cases where indirect spectrophotometric detection is used on the ground of equations relating optical density change with ion concentration [13]

we suggested the equation for correction peak areas relative to peak area of internal standard:

$$S_i'' = \left(\frac{1 + \mu_M / \mu_{st} \cdot \frac{z_{st}}{z_i}}{1 + \mu_M / \mu_i} \right) \cdot \frac{S_i'}{S_{st}'}, \quad (1)$$

where μ_M , μ_{st} and μ_i are electrophoretic mobility of background electrolyte cation, internal standard ion and studied ion; z is charge of ions. S_i' calculated from

$$S_i' = \frac{S_i}{t_i}, \quad (2)$$

where S_i is peak area found from electropherogram, t is migration time or using equation

$$S_i' = \sum_j \frac{\Delta A_j}{t_j}, \quad (3)$$

where ΔA_j is optical density change в j point, j corresponds to reading number in data set of electropherogram. Corrected area depends on only ion concentrations and association.

To calculate electrophoretic mobility of anions μ_{ep} from experimental data we used follow equation:

$$\mu_{ep,i} = \mu_{tot,i} + \mu_{EOF} - \mu_p = \frac{l}{U} \left(\frac{l_{eff}}{t_i} + \frac{l - l_{eff}}{t_{mark}} \right), \quad (4)$$

where $\mu_{tot,i}$ is total electrophoretic mobility of ion, μ_{EOF} and μ_p are contribution into electrophoretic mobility electroosmotic flow velocity and hydrodynamic pressure flow, l and l_{eff} are total and effective capillary length (distance from inlet to detector), U is voltage, t_{mark} is migration time of neutral compound.

Relation between concentration of studied ion C_i and concentration of internal standard ion C_{st} is follow:

$$C_i = k_i \cdot S_i'' \cdot C_{st}. \quad (5)$$

If ions doesn't absorb in range spectrophotometric detection and doesn't associate to ions of background electrolyte the correcting coefficient k_i must be equal 1.

If studied ion produced with background electrolyte cation the neutral ion pair then the decrease of peak area prorated fraction of associated ions. And association constant K_{ass} calculated from equation:

$$K_{ass} = \frac{k_i - 1}{[M^{\pm+}]} \cdot \frac{1}{\gamma_{\pm}^2}, \quad (6)$$

where k_i deduced from eq. (5), $[M^{\pm+}]$ is concentration of background electrolyte cation, γ_{\pm} is mean ionic activity coefficient. Because of all chemical equilibria influence on peak area decrease a background electrolyte will not contain electroosmotic flow modifier (salts of quaternary ammonium bases) which as is known associated to anions [8]. Because of the hydrodynamic pressure is used for suppression electroosmotic flow [14].

Zone mathematical model of electrophoretic ion motion

To examine deduced equations we used zone mathematical model of electrophoretic ion motion. As model system the section of capillary consist of 4000 zones was taken. Each zone is theoretical step of separation. Velocity of ion in i zone describes by equation

$$v_i = \mu_i \frac{U \cdot C_{BGE}^0 \cdot \mu_{BGE}^0}{l \cdot \sum_j C_{j,i} \cdot \mu_{j,i}}, \quad (7)$$

where C_{BGE}^0 and C_i are initial concentrations of background electrolyte and concentration of studied ions in i zone respectively, $\sum_j C_{j,i} \cdot \mu_{j,i}$ is sum of products ion concentrations and its electrophoretic mobility for all ions being in i zone.

On the basis of eq. (7) shift of each anions in each zone Δx_i in equal time interval τ was calculated:

$$\Delta x_i = v_i \cdot \tau. \quad (8)$$

Time interval τ was taken so that ions from i zone haven't time to move more than one zone. Cation

concentrations in each zone calculated from principle of electroneutrality. Then the change of anion concentration in i zone ΔC_i^t and their concentration in time τ $C_i^{t+\tau}$ equal:

$$\Delta C_i^t = \Delta C_i^t \cdot \frac{\Delta x_i}{l_{zone}}, \quad (9)$$

$$C_i^{t+\tau} = C_i^t + \Delta C_{i-1}^t + \Delta C_i^t, \quad (10)$$

where l_{zone} is zone length, C_i^t is anion concentration in i zone in point of time t .

Ion association was taken into account by follow:

$$\mu_{ep}(A^-) = \frac{1}{1 + K_{ass}(MA) \cdot [M^+]} \cdot \mu_{ep}^0(A^-), \quad (11)$$

$$C_{i,nonass} = \frac{1}{1 + K_{ass}(MA) \cdot [M^+]} \cdot C_{i,tot}, \quad (12)$$

where μ_{ep} and μ_{ep}^0 are electrophoretic mobilities of studied ion with accounting and without accounting association, $1/(1+K_{acc} \cdot [M^+])$ is mole fraction of ion form, $C_{i,nonass}$ и $C_{i,tot}$ are concentration of nonassociated anions and total concentration of the studied anions in i zone.

It is assumed that shape of the studied ion range in initial point of time is trapeziform. The mathematical model allows to calculate concentration of studied ion in each of 4000 zones at any point of time. The peak area was calculated from model electropherogram - concentration background electrolyte anion in detector area versus time. Calculations were performed by programme on basis of Visual Basic for Applications. Adequacy of mathematical model is proved that regulating Kohlrausch function is constant all over capillary [15]. Asymmetry of peaks giving by model was proved experimentally too.

Results and discussion

Due to electromigration dispersion the peaks in capillary electrophoresis are asymmetrical and each peak has proper asymmetry coefficient (see

Fig.). Since the corrected peak areas using eq. (1) for ions with different electrophoretic mobility and equal concentration may be distinguished. Because of the eq. (1) was examined with using zone mathematical model of electrophoretic ion motion. As can be seen in table 1 for conventional anions with electrophoretic mobility from $40 \cdot 10^{-9}$ to $80 \cdot 10^{-9} \text{ m}^2 \cdot \text{s}^{-1} \cdot \text{V}^{-1}$ the calculated corrected peak areas agreed to the extent of 2,3 %. If S' calculated from eq. (3) better results were turned but recalculation of real electropherograms is too hungus. Deviation in 2,3 % can be induced both asymmetry of peaks and discreteness of mathematical model.

Zone mathematical model of electrophoretic ion motion was used for examining effect of ion association on the decreases of peaks areas. Deviation of calculated association constant (eq. 6) from introduced in model using eqs. (11) and (12) for magnitude of association constant 1 - 300 were below 1,5 %.

Experimentally the separation of chloride – and sulfate-ions was studied (see Fig.) using calcium nitrate as background electrolyte.

According to eq.6 association constant of calcium with sulfate ions were calculated, mean ionic activity coefficient was calculated from second Debye-Huckel approach, μ_M - with use of Debye-Huckel – Onsager equation. As μ_M is present both in numerator and in denominator S'' value wasn't changed strongly with variation of μ_M . $\lg K_{\text{ass}}(\text{CaSO}_4)$ were $2,39 \pm 0,09$ ($P = 0,95$, 5 parallel measurement). This is agreed to results yielding with using other techniques in particular capillary electrophoresis technique from changes of electrophoretic mobility [3] (see Table 2).

Thus the new method based on decrease of corrected peak area to measure association constants using capillary electrophoresis technique with indirect spectrophotometric detection was investigated. To examine the equations for peak area correction and calculation of association constants was used zone mathematical model of electrophoretic ion motion. Using this method experimentally association constant of CaSO_4 was measured. The calculated value of association constant was agreed to value yielding other techniques.

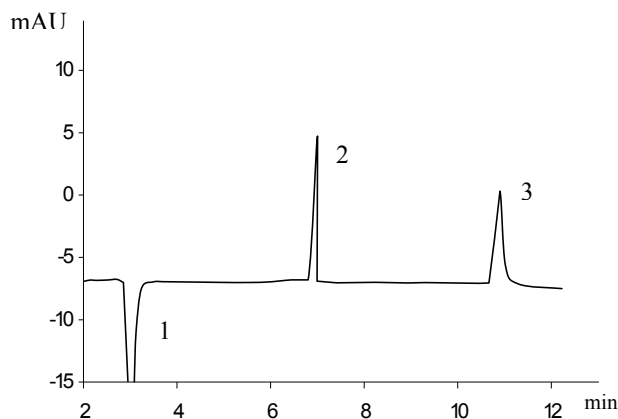


Fig. Electropherogram of chloride – and sulfate-ions. Background electrolyte: 5 mM $\text{Ca}(\text{NO}_3)_2$, $U = -15 \text{ kV}$. Peaks: 1 – EOF marker (benzyl alcohol), 2 – Cl^- , 3 – SO_4^{2-} , equivalent concentration of ions – 0.5 mM

Table 1. Calculated using eq. (1) peak areas for anions with differ electrophoretic mobility, internal standard – anion with $\mu = 60 \cdot 10^{-9} \text{ m}^2 \cdot \text{s}^{-1} \cdot \text{V}^{-1}$. S were calculated from model electropherograms. Equivalent concentration of ions – 0.5 mM

$\mu \cdot 10^9, \text{ m}^2 \cdot \text{s}^{-1} \cdot \text{V}^{-1}$	S	$S' \cdot 10^3$, Eq. (2)	S''	$S' \cdot 10^3$, Eq. (3)	S''
40	0,028725	1,143	1,023	1,121	1,010
50	0,011406	0,740	1,008	0,731	1,003
60	0,006354	0,569	1,000	0,566	1,000
70	0,004168	0,475	0,993	0,474	0,998
80	0,003006	0,415	0,989	0,415	0,996

Table 2. The association constant of CaSO_4 measured different techniques, 25° C

Technique	$\lg K_{\text{ass}} (\text{CaSO}_4)$	Reference
CE from changes of S''	$2,39 \pm 0,09$	This study
CE from changes of μ	$2,551 \pm 0,013$	[3]
Solubility	2,31	[16]
	2,27	[17]
Electromotive force	2,27	[17]
	2,57	

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