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Quantitative analysis in capillary zone electrophoresis with conductivity and indirect UV detection

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ABSTRACT

An interesting point in quantitative capillary zone electrophoresis, when applying conductivity detection or indirect UV detection with non-UV absorbing components, is the existence of a relationship between effective mobilities and peak area, independent of the kind of ionic species. This relationship is theoretically considered for fully ionized monovalent ions resulting in a linear relationship, passing through the origin, between temporal peak area and the product of a correction factor (dependent only on the effective mobilities of the ionic species) and migration time for an equimolar sample composition. A good correlation between theory and practice could be established by applying experimental measured data.

INTRODUCTION

Huang *et al.* [1] reported on the unique advantage of quantitative capillary zone electrophoresis (CZE) using conductivity detection that the use of an internal standard allows the accurate determination of absolute concentrations in a mixture without separate calibration of the response for each component and they found a direct relationship between peak area and migration times. Further consideration of the principle of the measured conductivity shows, however, that although a relationship exists between peak area and migration time it is nearly linear only over a small mobility range. In this paper, the relationship between peak area and effective mobility for conductivity and indirect UV detection in CZE is considered for fully ionized monovalent ions.

THEORETICAL

Assuming only the presence of fully ionized monovalent ionic constituents, the electrophoretic separation mechanism can approximately be described by Kohlrausch's regulation function:

$$\sum_i c_i/m_i = \omega \qquad (1)$$

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where c_i and m_i represent the ionic concentrations and absolute values of the effective ionic mobilities of all ionic constituents and the numerical value of the Kohlrausch function ω is locally invariant with time [2]. If a volume element of the capillary is originally filled with a carrier electrolyte AB (consisting of a co-ion A and counter ion B) at a concentration c_A, it will contain after some time a mixture of sample components and carrier electrolyte when sample components pass, but finally the original situation will be restored again. If a mixture of a component i and the carrier electrolyte AB passes through such a volume element, the following equation is valid:

$$c_A^C = c_A^S + c_i^S k_i \tag{2}$$

with

$$k_i = \frac{m_i + m_B}{m_A + m_B} \cdot \frac{m_A}{m_i}$$

The superscripts C and S refer to the composition of the pure carrier electrolyte AB zone and the sample zone, respectively. The concentration of the counter ion B is determined by the electroneutrality condition.

For the zone conductivity σ can be derived:

$$\sigma^S = \sigma^C + c_i b_i \tag{3}$$

where

$$b_i = F(m_i + m_B)(1 - m_A/m_i)$$

Applying a conductivity detector in capillary electrophoresis, a detector response, directly related to σ^S-σ^C, can be expected being linearly proportional to $b_i c_i$ and hence the spatial [3] peak area will be proportional to the product of b_i and the injected amount Q_{inj}.

Generally, the measured peak area will be expressed on a temporal basis [3] and it can be expected that for CZE both without and with electroosmotic flow (EOF) the measured peak area A_i will be proportional to:

$$b_i Q_{inj} t_i \tag{4}$$

As the migration time t_i is reversely proportional to m_i and $m_i + m_{EOF}$ (without and with EOF, respectively) at a given voltage the measured peak area A_i will be proportional to:

$$b_i Q_{inj}/m_i \text{ or } b_i Q_{inj}/(m_i + m_{EOF}) \tag{5}$$

The relationship between measured peak area A_i and b_i/m_i, $b_i/(m_i + m_{EOF})$ or $b_i t_i$ must be linear, passing through the origin, whereas the products $A_i m_i/b_i$, $A_i(m_i + m_{EOF})/b_i$ and $A_i/b_i t_i$ should be a constant for all different ionic species for an equimolar sample composition. It must be remembered that generally in chromatographic

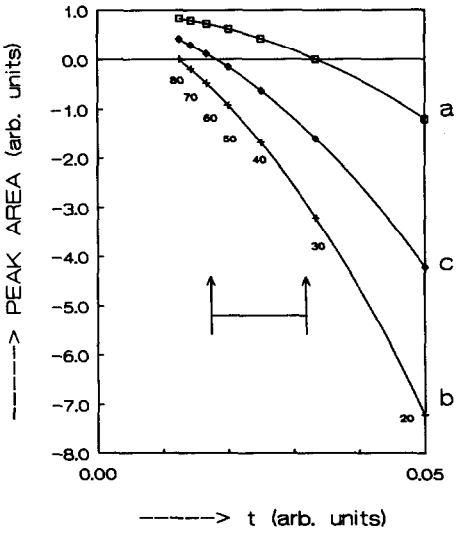

Fig. 1. Calculated relationship between temporal peak area and migration time (t, arbitrary units) for ionic species with effective mobilities of $80 \cdot 10^{-5}$ to $20 \cdot 10^{-5}$ cm^2/V · s assuming effective mobilities for co- and counter ions of (a) $30 \cdot 10^{-5}$ and $30 \cdot 10^{-5}$, (b) $80 \cdot 10^{-5}$ and $30 \cdot 10^{-5}$ and (c) $55 \cdot 10^{-5}$ and $30 \cdot 10^{-5}$ cm^2/V · s. The numbers refer to the effective mobilities, $m \cdot 10^5$ cm^2/V · s, of the ionic species.

techniques one has to work with spatial peak area as the components move with equal speed through the detector.

In Fig. 1 the peak areas (arbitrary units), calculated according to eqn. 5 (without EOF) for a given Q_{inj}, are given as a function of the migration times for ionic species with effective mobilities varying from $80 \cdot 10^{-5}$ to $20 \cdot 10^{-5}$cm^2/V · s and assuming effective mobilities for m_A and m_B of (a) $30 \cdot 10^{-5}$ and $30 \cdot 10^{-5}$, (b) $80 \cdot 10^{-5}$ and $30 \cdot 10^{-5}$ and (c) $55 \cdot 10^{-5}$ and $30 \cdot 10^{-5}$ cm^2/V · s. It can be clearly seen from Fig. 1 that the peak area changes sign at a mobility m_i equal to m_A and increases with larger differences between m_i and m_A. Further, the relationship is not linear,

TABLE I

MEASURED PEAK AREAS A_i (ARBITRARY UNITS), MIGRATION TIMES t_i (s) AND RATIOS c_i/c_{st} CALCULATED WITH THE EQUATION ACCORDING TO REF. 1

Carrier electrolyte: (I) 0.01 *M* MES–histidine at pH 6; (II) 0.005 *M* chloride–Tris at pH 7.1

Ionic species	I			II		
	A_i	t_i	c_i/c_{st}	A_i	t_i	c_i/c_{st}
Formate	53.1	113	1.83	10.5	280	0.14
Acetate	37.0	148	1.67	26.7	338	0.42
Propionate	29.4	164	1.46	33.8	369	0.58
Butyrate	25.5	175	1.36	39.1	390	0.70
Pentanoate	20.6	185	1.16	42.4	405	0.79
Hexanoate	16.9	195	1.00	51.0	425	1.00

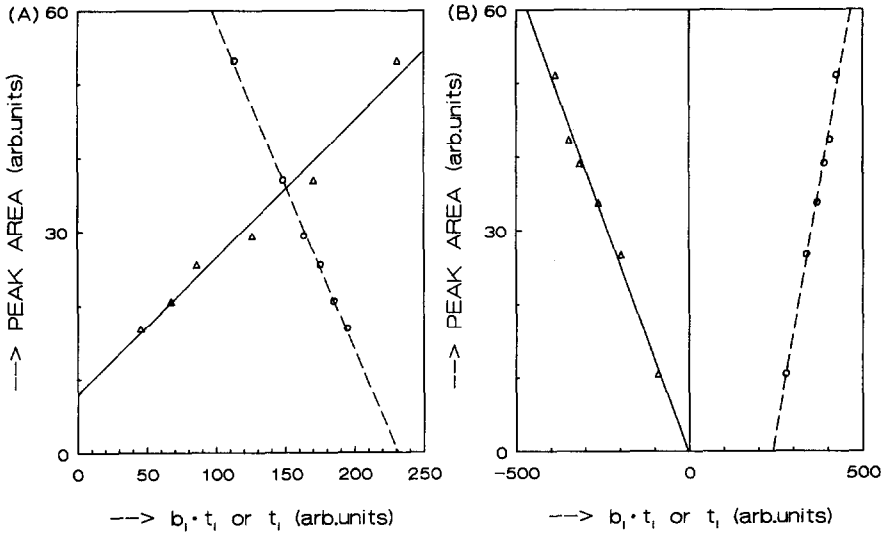

Fig. 2. Relationship between measured peak area [1] and $b_i t_i$ (solid line) and t_i (dashed line) for the electrolyte system (A) 0.01 *M* MES adjusted to pH of 6 by adding histidine and (B) 0.005 *M* HCl adjusted to pH 7.1 by adding Tris. The sample consisted of a mixture of formate, acetate, propionate, butyrate, pentanoate and hexanoate. Applying the correction factor b_i a linear relationship passing through the origin is obtained according to eqn. 4.

although for a fairly small mobility range (the arrows indicate broadly the mobility range of formate to hexanoate) it is nearly linear.

Table I gives the measured peak areas and migration times according to Huang *et al.* [1] and the calculated ratios c_i/c_{st}, with hexanoate considered as a standard (st), using the equation $c_i = c_{st} A_i t_i / A_{st} t_{st}$ as used by Huang *et al.* [1]. It can be concluded from Table I that this equation cannot be used in general, as the quotients have to be unity for an equimolar sample composition.

In Fig. 2A and B the relationships between peak area, as measured by Huang *et al.* [1], and the calculated $b_i t_i$ or t_i are given. We applied eqn. 4 (and not eqn. 5) because we did not know if the EOF was fully suppressed by the addition of tetradecyltrimethyl ammonium bromide (TTAB). It can be clearly seen that the linear relationship between temporal peak area and migration time (dashed line), as measured by Huang *et al.* changes into a linear relationship nearly passing through the origin on applying the correction factor b_i (solid line), as expected from theory. The results in Fig. 2B are better than those in Fig. 2A (large intercept on the ordinate) because the accuracy of the effective mobility 2-(N-morpholino)ethanesulphonic acid (MES) is very critical to the calculated value of b_i. In the calculation of the factor b_i the mobilities have been corrected according to the Debye–Hückel–Onsager theory.

It is obvious that a relationship between peak area A_i and retention time t_i, not linear and not passing through the origin, is not practical to be used for an internal standard, in contrast with the use of the relationship between peak area A_i and the product $b_i t_i$.

For a UV detector the measured absorbance A will be

$$A = \varepsilon c l \tag{6}$$

where ε is the molar absorption coefficient (l/mol · cm) and l is the effective path length in the detector (cm). For the carrier electrolyte this means

$$A^{C} = (\varepsilon_A + \varepsilon_B) c_A^{C} l \tag{7}$$

For a sample zone the absorbance will be

$$A^{S} = (\varepsilon_A + \varepsilon_B) c_A^{S} l + (\varepsilon_B + \varepsilon_i) c_i^{S} l \tag{8}$$

The UV signal of a sample zone, using eqn. 2, will be

$$A = A^{C} - A^{S} = c_i^{S} l [(\varepsilon_A + \varepsilon_B) k_i - (\varepsilon_i + \varepsilon_B)] \tag{9}$$

For non-UV-absorbing counter ions and sample ions, applying indirect UV detection with UV-absorbing co-ions, the UV signal is proportional to $c_i k_i$.

Analogously to conductivity detection, the spatial peak area will be proportional to $k_i Q_{inj}$ and the measured peak area A_i on a temporal basis to $k_i Q_{inj} t_i$. Further, the expression $A_i / k_i t_i$ has to be a constant in a given electrolyte system for all components at an equimolar sample composition.

EXPERIMENTAL

Instrumentation

For all quantitative experiments with a conductivity detector we used a laboratory-built capillary electrophoresis system with an on-column conductivity detector as described previously [4]. As the apparatus is a closed system, the EOF is fully suppressed. The sampling takes place into a broadened part of the capillary tube (0.55 mm I.D.) connected with two feeders (0.4 mm diameter), perpendicular to the capillary tube. A constant d.c. power supply with a maximum potential of 20 kV was used. Peak areas were determined using the integration program CAESAR. The detector electronics were connected with an IBM XT PC via a LabMaster (Scientific Solutions, Solon, USA).

For all quantitative CZE experiments with a UV detector we used the P/ACE System 2000 HPCE (Beckman, Palo Alto, CA, USA) applying UV detection at 254 nm. All experiments were carried out at 25°C using an original Beckman capillary of 57 cm, with a distance between injection and detection of 50 cm and an I.D. of 75 μm.

For all zone electrophoretic separations the injection took place at the inlet side. In the anionic mode the cathode was placed at the inlet and the anode at the outlet side, and *vice versa* for the cationic mode.

Reagents and samples

All chemicals were of analytical-reagent grade. Before preparing the sample solutions, all chemicals were dried at 105°C.

TABLE II

EFFECTIVE MOBILITIES $-m_i \cdot 10^{-5}$ (cm^2/V · s), CALCULATED FACTORS b_i (ARBITRARY UNITS), MEASURED PEAK AREAS A_i (ARBITRARY UNITS) AND CALCULATED VALUES OF K (= $A_i m_i / b_i$) FOR DIFFERENT BACKGROUND ELECTROLYTES

Component	m_i	b_i	A_i	K
0.01 M MES + imidazole (pH 7)				
Chloride	74.45	79.30	218.47	205.1
Chlorate	62.61	65.00	209.08	201.4
Fluoride	53.22	52.75	201.93	203.7
Formate	52.43	51.68	200.34	203.3
Acetate	38.54	30.50	163.76	206.9
Propionate	33.36	20.94	136.66	217.7
Benzoate	29.93	13.75	112.66	245.2
0.01 M acetic acid + imidazole (pH 7)				
Chloride	74.17	56.84	122.71	160.1
Chlorate	62.35	40.93	103.74	158.0
Fluoride	52.97	26.87	78.77	155.3
Formate	52.18	25.62	72.51	147.7
Propionate	33.13	−12.19	−53.70	145.9
Benzoate	29.71	−21.61	−100.35	137.9
0.01 M HCl + imidazole (pH 7)				
Chlorate	62.35	−20.14	−31.74	98.3
Fluoride	52.97	−38.88	−68.82	93.8
Formate	52.18	−40.60	−72.95	93.8
Acetate	38.31	−77.67	−180.98	89.3
Propionate	33.13	−96.60	−253.93	87.1
Benzoate	29.71	−111.75	−316.45	84.2

RESULTS AND DISCUSSION

In order to check the relationship between temporal peak area and mobilities for both conductivity (eqn. 5) and indirect UV detection (eqn. 9), we measured the temporal peak area with conductivity detection and indirect UV detection, in both the anionic and cationic modes.

In Table II, the effective mobilities, m_i, calculated factors, b_i, temporal peak areas, A_i (measured with a closed CZE apparatus with an on-line conductivity detector [4]), and calculated values of K are given. The factors K (= $A_i m_i / b_i$) are virtually constant in the three different electrolyte systems, although a disadvantage of the sample injection used in our apparatus is that although linear calibration graphs are obtained for both isotachophoretic and CZE experiments, the effective injection volumes for the different components are not completely identical [4], and moreover the separation power of the apparatus used was too small to separate the whole sample mixture in a single experiment. Of course the values of the factors K for the different systems are different due to different circumstances. The three different electrolyte systems consisted of the co-ions MES, acetate and chloride at a pH of 7 adjusted by adding imidazole (anionic mode, constant electric current 10 μA). The sample components were chloride, chlorate, fluoride, formate, acetate, propionate and benzoate at a concentration of $5 \cdot 10^{-4}$ M.

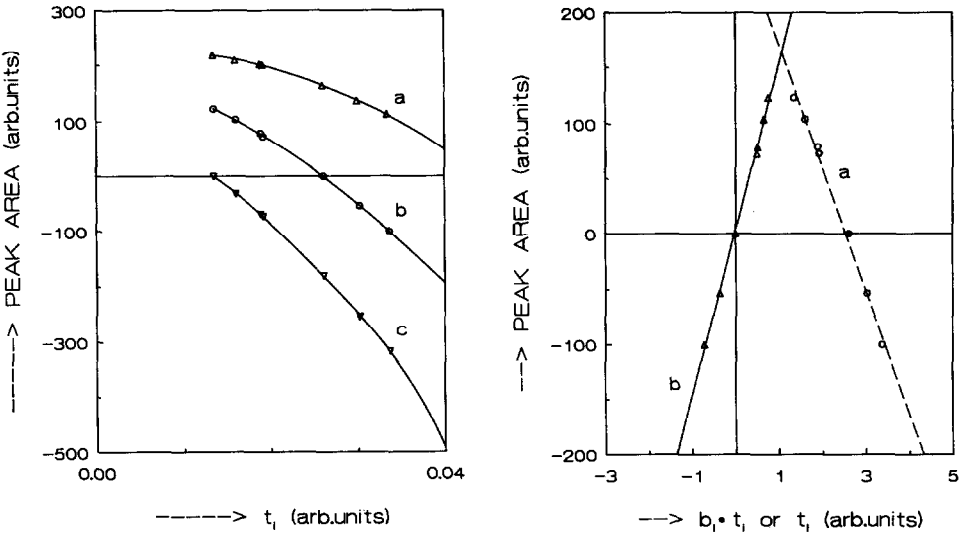

Fig. 3. Relationship between measured peak area and t_i for conductivity detection in a closed system, for three different electrolyte systems. The sign of the peak area changes at an effective mobility equal to that of the co-ion, confirming the theory (see Table II for data). Background electrolyte: (a) MES, (b) acetic acid and (c) hydrochloric acid, all at pH 7 adjusted by adding imidazole.

Fig. 4. Relationship between measured peak area and (a) t_i and (b) $b_i t_i$ for the electrolyte system 0.01 *M* acetic acid adjusted to pH 7 by adding imidazole. The sample consisted of a mixture of chloride, chlorate, fluoride, acetate, propionate and benzoate ($5 \cdot 10^{-4}$ *M*). It can be clearly seen that the relationship between peak area and t_i (dashed line) changes to a linear relationship passing through the origin if the correction factor b_i is used (solid line).

In Fig. 3 the measured peak areas in Table II are given as a function of time. The similarity with Fig. 1 is obvious.

In Fig. 4 the measured peak areas of one of the electrolyte systems in Table II are given as a function of both (a) the migration time t_i and (b) $b_i t_i$. It can be clearly seen that relationship (a) changes into (b), a linear relationship passing through the origin.

To check the relationship between peak area and mobilities for indirect UV detection with non-UV-absorbing components and counter ions, experiments were carried out in both the anionic and cationic modes. In the anionic mode we used three background electrolytes, 0.01 *M* benzoic acid, 0.01 *M* nicotinic acid and 0.01 *M* sulphosalicylic acid adjusted to pH 8 by adding Tris [tris(hydroxymethyl)aminomethane]. The sample mixture consisted of chloride, chlorate, fluoride, acetate, propionate and MES ($5 \cdot 10^{-4}$ *M*), applying pressure injection times of 5, 10 and 15 s. All experiments were carried out with a constant voltage of 25 kV. In order to supress the EOF for the greater part, 0.05% methylhydroxyethylcellulose (MHEC) was added to all solutions.

In Table III the calculated effective mobilities and calculated factors k_i, the measured migration times t_i, measured peak areas A_i and calculated values of K ($= A_i/k_i t_i$) are given. The factor K should be a constant for all components in the same

TABLE III

EFFECTIVE MOBILITIES $-m \cdot 10^5$ ($cm^2/V \cdot S$), CALCULATED FACTORS k_i ACCORDING TO EQN. 2, MEASURED MIGRATION TIMES t_i (min), MEASURED TEMPORAL PEAK AREAS A_i (ARBITRARY UNITS) AND CALCULATED VALUES OF $K (= A_i/k_i t_i)$ FOR THREE DIFFERENT PRESSURE INJECTION TIMES

Wavelength of UV detection, 254 nm; capillary length, 57 cm; distance between injection and detection, 50 cm; constant voltage, 25 kV.

Background electrolyte	Component			Pressure injection time								
				5 s			10 s			15 s		
		m_i	k_i	t_i	A_i	K	t_i	A_i	K	t_i	A_i	K
0.01 *M* benzoic acid adjusted to pH 8 with Tris	Chloride	74.17	0.722	3.13	3.85	1.70	3.17	7.42	3.24	3.18	11.11	4.84
	Chlorate	62.35	0.757	3.87	4.83	1.65	3.92	9.85	3.32	3.92	14.29	4.81
	Fluoride	52.97	0.796	4.67	5.90	1.59	4.73	11.64	3.09	4.71	17.78	4.74
	Acetate	38.31	0.896	7.34	11.02	1.68	7.38	21.73	3.29	7.26	32.30	4.97
	Propionate	33.13	0.952	9.09	14.25	1.65	9.11	28.26	3.26	8.90	41.15	4.86
	MES	24.24	1.104	15.96	26.95	1.53	15.76	55.51	3.19	14.97	79.90	4.83
	Average					1.63			3.23			4.84
	Standard deviation					0.063			0.082			0.075
0.01 *M* nicotinic acid adjusted to pH 8 with Tris												
	Chloride	74.17	0.733	3.46	3.51	1.38	3.39	6.98	2.81	3.36	10.99	4.46
	Chlorate	62.35	0.769	4.38	4.87	1.45	4.26	9.45	2.89	4.20	14.18	4.39
	Fluoride	52.97	0.808	5.41	6.23	1.42	5.21	11.94	2.84	5.12	17.80	4.30
	Acetate	38.31	0.909	9.34	12.58	1.48	8.62	23.05	2.94	8.30	33.43	4.43
	Propionate	33.13	0.966	12.37	16.92	1.42	11.07	30.63	2.86	10.50	44.10	4.35
	MES	24.24	1.121	27.21	43.37	1.42	23.07	72.24	2.79	20.34	98.11	4.30
	Average					1.43			2.85			4.37
	Standard deviation					0.034			0.055			0.066
0.01 *M* sulphosalicylic acid adjusted to pH 8 with Tris	Chloride	74.17	0.890	3.59	1.32	0.41	3.34	3.16	1.06	3.25	4.51	1.56
	Chlorate	62.35	0.934	4.68	1.70	0.39	4.24	4.01	1.01	4.09	5.42	1.42
	Fluoride	52.97	0.982	5.95	2.34	0.40	5.22	5.09	0.99	4.98	7.14	1.46
	Acetate	38.31	1.105	11.61	5.00	0.39	8.93	9.49	0.96	8.18	12.45	1.38
	Propionate	33.13	1.174	17.16	7.91	0.39	11.93	13.36	0.95	10.64	17.33	1.39
	Average					0.40			1.00			1.44
	Standard deviation					0.0089			0.044			0.073

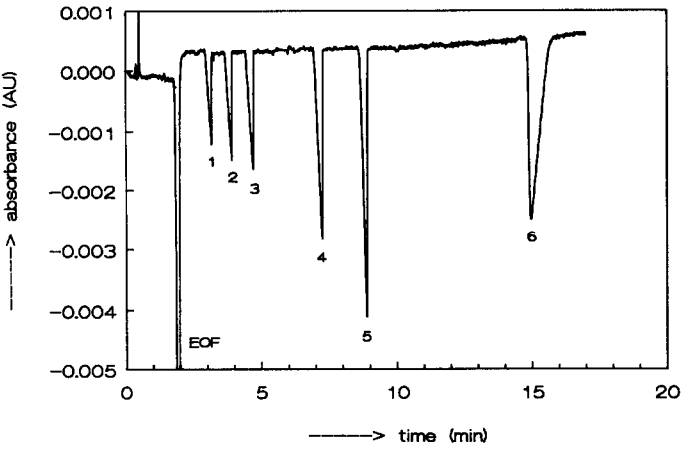

Fig. 5. Electropherogram for the separation of (1) chloride, (2) chlorate, (3) fluoride, (4) acetate, (5) propionate and (6) MES in the indirect UV mode ($5 \cdot 10^{-4}$ *M*, pressure injection time 15 s). Carrier electrolyte, 0.01 *M* benzoic acid adjusted to pH 8 by adding Tris; wavelength, 254 nm; anionic mode, applied voltage 25 kV.

TABLE IV

CALCULATED EFFECTIVE MOBILITIES $-m_i \cdot 10^5$ ($cm^2/V \cdot s$), CALCULATED FACTORS k_i ACCORDING TO EQN. 2, MEASURED MIGRATION TIMES t_i (min), MEASURED TEMPORAL PEAK AREAS A_i (ARBITRARY UNITS) AND CALCULATED VALUES OF K ($=A_i/k_i t_i$) FOR THREE DIFFERENT PRESSURE INJECTION TIMES

Pressure injection time (s)	Component	m_i	k_i	t_i	A_i	K
5	Hexanoate	26.09 (0.115)	1.064 (0.002)	4.69 (0.017)	11.54 (0.181)	2.31 (0.038)
	Pentanoate	27.65 (0.112)	1.035 (0.002)	4.88 (0.015)	11.72 (0.181)	2.32 (0.035)
	Butanoate	29.51 (0.119)	1.003 (0.002)	5.12 (0.019)	12.20 (0.089)	2.37 (0.019)
	Propionate	32.58 (0.099)	0.959 (0.001)	5.58 (0.021)	13.34 (0.200)	2.49 (0.037)
	Acetate	37.31 (0.102)	0.905 (0.001)	6.49 (0.029)	15.12 (0.133)	2.57 (0.025)
	Formate	51.67 (0.112)	0.803 (0.001)	12.71 (0.127)	24.01 (0.625)	2.35 (0.054)
	Average					2.40 (0.105)
10	Hexanoate	26.50 (0.050)	1.056 (0.001)	4.61 (0.032)	23.53 (0.262)	4.83 (0.074)
	Pentanoate	28.05 (0.035)	1.028 (0.001)	4.79 (0.032)	23.97 (0.292)	4.87 (0.076)
	Butanoate	29.73 (0.068)	1.000 (0.001)	5.00 (0.039)	24.94 (0.306)	4.99 (0.080)
	Propionate	32.59 (0.064)	0.959 (0.001)	5.41 (0.046)	26.86 (0.374)	5.18 (0.100)
	Acetate	37.21 (0.064)	0.906 (0.001)	6.23 (0.060)	29.96 (0.370)	5.31 (0.078)
	Formate	51.38 (0.082)	0.804 (0.000)	11.64 (0.224)	48.20 (0.731)	5.15 (0.162)
	Average					5.06 (0.189)
15	Hexanoate	26.76 (0.109)	1.051 (0.002)	4.63 (0.010)	35.61 (1.480)	7.31 (0.302)
	Pentanoate	28.28 (0.088)	1.024 (0.002)	4.81 (0.010)	36.08 (1.004)	7.33 (0.203)
	Butanoate	29.84 (0.092)	0.998 (0.001)	5.01 (0.013)	37.49 (0.985)	7.50 (0.190)
	Propionate	32.56 (0.113)	0.959 (0.001)	5.40 (0.013)	40.02 (0.760)	7.73 (0.141)
	Acetate	37.17 (0.116)	0.907 (0.001)	6.21 (0.017)	44.90 (1.423)	7.97 (0.250)
	Formate	51.54 (0.097)	0.804 (0.001)	11.71 (0.083)	72.40 (3.469)	7.69 (0.323)
	Average					7.59 (0.256)

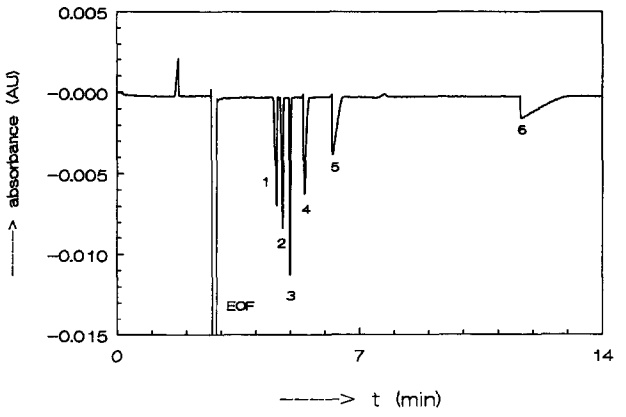

Fig. 6. Electropherogram for the separation of (1) hexanoate, (2) pentanoate, (3) butanoate, (4) propionate, (5) acetate and (6) formate in the indirect UV mode ($5 \cdot 10^{-4}$ *M*, pressure injection time 10 s). Carrier electrolyte, 0.01 *M* benzoic acid adjusted to pH 8.5 by adding Tris; wavelength, 254 nm; cationic mode, applied voltage 25 kV.

carrier electrolyte. It can be concluded from Table III that the factor K is indeed a constant for all components and is linearly related to the amount of the components injected.

Fig. 5 gives an example of the separation of a mixture (in the anionic mode, pressure injection time 15 s) with indirect UV detection. The carrier electrolyte was 0.01 *M* benzoic acid at pH 8. The sample consisted of (1) chloride, (2) chlorate, (3) fluoride, (4) acetate, (5) propionate and (6) MES at a concentration of $5 \cdot 10^{-4}$ *M*. In order to obtain an impression of the velocity of the EOF (remember that the EOF is suppressed for the greater part by the addition of 0.05% MHEC to all solutions), we created an EOF peak directed from outlet to inlet by injecting water at the outlet side by electromigration injection at 10 kV for 5 s (the length from injection to detection is only 7 cm for this EOF marker; m_{EOF} is about $14 \cdot 10^{-5}$ cm^2/V · s; without MHEC m_{EOF} is about $67 \cdot 10^{-5}$ cm^2/V · s). Note the fronting and tailing shapes of the peaks with high and low effective mobilities, respectively.

In the cationic mode we applied an electrolyte system consisting of 0.01 *M* benzoic acid adjusted to pH 8.5 by adding Tris. The applied voltage was 25 kV. The sample consisted of formate, acetate, propionate, butanoate, pentanoate and hexanoate at a concentration of $5 \cdot 10^{-4}$ *M*, applying pressure injection times of 5, 10 and 15 s. In Table IV the effective mobilities and calculated factors k_i, the measured migration times t_i, measured peak area A_i and calculated factors K ($= A_i/k_i t_i$) are given. The effective mobilities of the components are calculated from the migration times of the EOF and of the components as described previously [5].

In order to study the reproducibility of the method, all experiments were carried out five times and the average values are given in Table IV. Standard deviations are given in parentheses. It can be concluded from these data that the reproducibility is good for the migration times and the calculated effective mobilities and factors k_i. The reproducibility of K values is poorer (standard deviation *ca.* 1–2%) owing to the

inaccuracy of the measured peak areas, possibly caused by the injection method and/or inaccuracy of the peak-area determination.

Fig. 6 gives an example of the separation of the mixture in Table IV (in the cationic mode, pressure injection time 10 s) in the indirect UV mode. The carrier electrolyte was 0.01 *M* benzoic acid at pH 8.5 adjusted by adding Tris. The strong tailing effect for formate due to the absence of a self-correcting effect of the zones in CZE can be clearly seen.

CONCLUSIONS

For conductivity and indirect UV detection (for non-UV-absorbing components, applying a non-UV-absorbing counter ion and a UV-absorbing co-ion) there is a defined relationship between measured temporal peak areas and effective mobilities, independent of the kind of ionic species. Data measured for several components in several electrolyte systems confirmed the derived relationship.

The relationship between temporal peak area and the product of a correction factor (b_i for conductivity detection and k_i for indirect UV detection) and migration time t_i is linear, passing through the origin. Applying an internal standard, this relationship can be used in quantitative CZE analysis with calibration graphs being superfluous.

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