Journal of Chromatography, 452 (1988) 591–600
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 492

USE OF A DOUBLE-DETECTOR SYSTEM FOR THE MEASUREMENT OF MOBILITIES IN ZONE ELECTROPHORESIS

J. L. BECKERS, Th. P. E. M. VERHEGGEN and F. M. EVERAERTS*
Laboratory of Instrumental Analysis, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven (The Netherlands)

SUMMARY

A double-detector system with which mobilities can be determined by capillary zone electrophoresis is described. Ionic species are detected by two detectors, mounted at a fixed distance from each other. In zone electrophoresis the velocity of an ionic species is proportional to its mobility. The time needed for an ionic species to pass both detectors is reversely proportional to that mobility, provided that the electric field strength is constant. From the ratio of the times required by a sample ionic species and a standard ionic species (with a known mobility) to pass from the first to the second detector, the mobility of the sample ionic species can be calculated. During the analyses parameters such as temperature, pH and ionic strength are constant, which simplifies the calculations substantially. From experiments with some standard ionic species, mobilities have been obtained comparable to those in the literature with a relative standard deviation of about 1%.

INTRODUCTION

Mobilities have often been determined with electrophoretic methods, such as isotachophoresis (ITP) and zone electrophoresis (ZE). In ITP, all ionic species migrate in their respective zones with an equal velocity v if the steady state has been reached. Hereby an electric field strength E is created, determined by their effective mobilities m according to

$$v = mE \tag{1}$$

Using a conductivity detector, an isotachopherogram will be obtained in which the step heights contain information concerning the conductivity of the zones and, from this, mobilities can be calculated. Because the pH, temperature and concentrations vary in the different zones, corrections for, *e.g.*, activities must be calculated in an iterative manner.

Several workers have succesfully determined mobilities using ITP data[1–7]. Although absolute mobilities and pK values could be calculated from thermometric ITP data, using the concept of the isoconductor[8], it appeared to be more difficult to

0021-9673/88/$03.50

obtain reproducible and accurate data using ITP conductivity data. Although a conductivity detector (a.c./d.c. mode) has superior qualities with regard to analytical applications, not pure zone conductivities but impedances are measured with a conductivity detector whereby, *e.g.*, double layers and the coating play an important part. Sometimes the step heights vary substantially on adding certain non-conducting surface-active additives whereas the zone conductivities can be considered to be nearly equal[9].

In ZE, all ionic species also migrate with a velocity $v = mE$. If retention times are measured for ionic species ($t_{R,i}$) and a standard ionic species ($t_{R,St}$) with a known mobility (m_{St}), the effective mobilities (m_i) can be calculated by the following relation:

$$m_i = t_{R,St} m_{St} / t_{R,i} \tag{2}$$

assumed the E gradient to be constant. In this way ionic species can be identified in ZE.

In capillary ZE (CZE) the sample is often introduced by means of an injection valve or by dipping one end of the capillary tube in the sample solution, whereby the sample is introduced using a gravity flow or by electromigration*.

After sampling, in the first stage of the ZE separation process the sample concentration must be adjusted to that of the background electrolyte by means of a moving boundary procedure. An advantage of this procedure is that diluted sample solutions will be concentrated[11]. However, after this procedure the sample ought to be eluted by the background electrolyte until a nearly constant E gradient is created. Only from this moment can we really speak of zone electrophoresis and only from this moment can we use the retention times properly. Because this first stage in CZE can last tens of seconds, retention times measured from the beginning of the analysis often differ considerably[12] and inaccurate mobilities can result.

In order to cope with these problems, the use of a so-called double-detector system (DDS) is introduced in this paper, with which mobilities can be determined and ionic species can be identified. Using a DDS it is only of importance that at the time of detection, for both detectors, all components are separated and migrate zone electrophoretically. By detecting the zones with two detectors mounted at a fixed distance from each other, the mobilities can be determined related to a standard ionic species by

$$m_i = m_{St} t_{St} / t_i \tag{3}$$

where t_{St} and t_i are the times required by the standard and sample ionic species to migrate from the first to the second detector.

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* It must be borne in mind that in electromigration the sample introduced does not have the same composition as the original sample solution, because this sample introduction follows a moving bounary procedure[10]. By applying electroendosmotic flow this effect is often negligible.

THEORETICAL

In order to optimize the positions of the measuring electrodes of a ZE apparatus we simulated the ZE process for the separation of four cations by means of a numerical solution of the basic transport equation:

$$\frac{dc}{dt} = -\frac{d(mEc)}{dx} + D \cdot \frac{d^2c}{dx^2} \tag{4}$$

where c is the concentration of the ionic species and x is the position in the separation tube.

The electric field strength was calculated from the electric current density i by

$$i = E\sum_j c_j m_j F \tag{5}$$

whereby F is the Faraday constant. The conditions of the simulation are given in Table I. The values for the capillary diameter, electric current and mobilities of the background electrolyte ionic species are chosen arbitrarily.

The diffusion constant D was calculated using the equation[13]

$$D = mkT/ez \tag{6}$$

where k is the Boltzmann constant, T is the absolute temperature and ez is the charge of the ionic species.

Concentration effect of the sample

In order to concentrate the sample in a narrow band, a very dilute sample (0.00025 *M*) is introduced into the capillary tube over a length of 8 mm. The concentration effect of the sample was simulated and shown in Fig. 1 for one of the sample ions, K^+, after 0.1 and 1.0 s.

It can be clearly seen that a concentration by a factor of about 10 occurs,

TABLE I

CONDITIONS FOR THE SIMULATION OF A ZONE ELECTROPHORETIC SEPARATION

Electric current, 20 μA; capillary diameter, 0.25 mm I.D.

Species	*Ion*	*Concentration (M)*	*Mobility (10^5 cm²/V · s)*
Background electrolyte	M^+	0.01	30
	X^-	0.01	20
Sample ions	K^+	0.00025	76.2
	Na^+	0.00025	51.9
	Li^+	0.00025	40.1
	TEA^+	0.00025	33.0

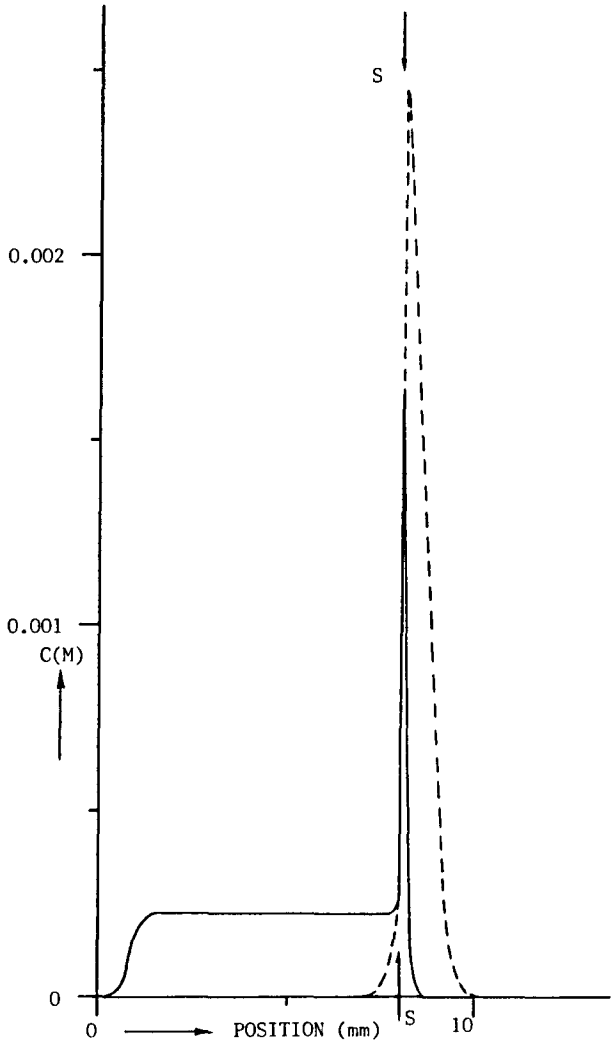

Fig. 1. Concentration effect for one of the ionic species (K^+) in the sample in the zone electrophoretic simulation. The sample is introduced at a concentration of 0.00025 *M* and is concentrated to about 0.0025 *M* beyond the sampling point S. The concentration profiles are given after 0.1 and 1 s (dotted line) of the separation. On the horizontal axis the position in the capillary tube is given (in mm), the original position of sampling being between 0 and 8 mm.

which means that the sample band will be about 0.8 mm in length. It is remarkable that the concentration effect occurs beyond the position of injection S, which means that a large *E* gradient exists at the position of sampling according to Kohlrausch's law. For this reason a larger diameter in our apparatus was chosen at the position of sampling in order to decrease this effect. Further, the concentration profiles (see Fig. 1) are diffuse at the front side and steep at the rear side as the sample ionic species have a higher mobility than the background electrolyte ionic species M^{+11}.

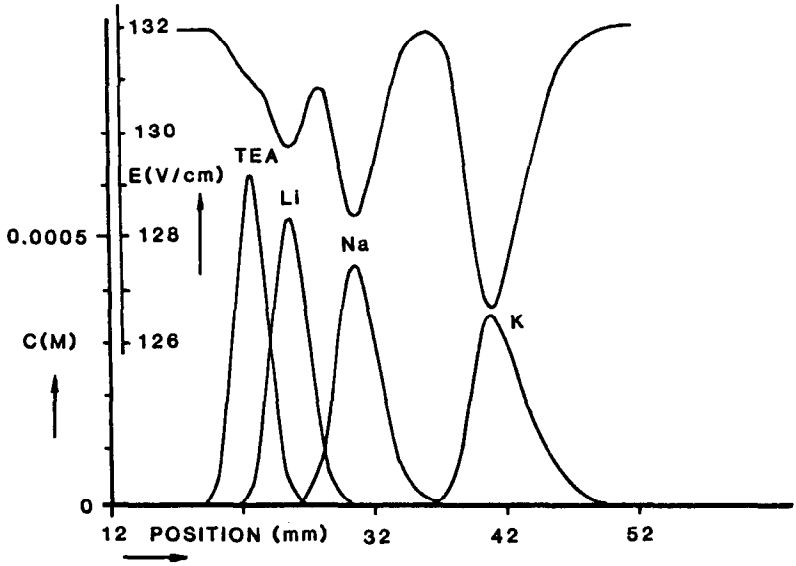

Fig. 2. Concentration profiles and E gradient for the simulation of a zone electrophoretic separation of four cations after 36 s. On the horizontal axis the position in the capillary tube is given in mm. The original position of sampling lies between 0 and 8 mm.

Length of the capillary tube

Because the length of the capillary tube must be chosen in such a way that all ionic species migrate in a zone electrophoretic way (*i.e.* the E gradient must be constant within, *e.g.*, 1%), the minimal distance between the sampling point and first detector was determined by means of a simulation. In Figs. 2 and 3 the concentration

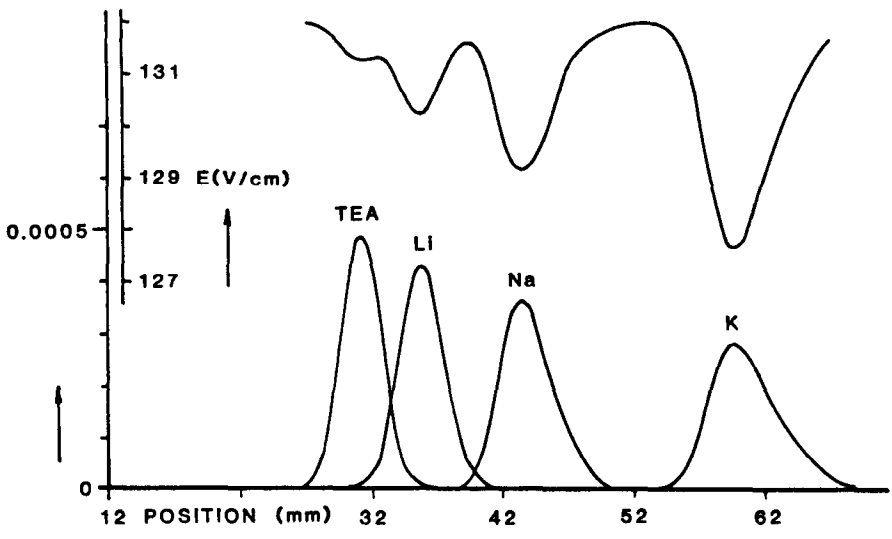

Fig. 3. Concentration profiles and E gradient for the simulation of a zone electrophoretic separation of four cations after 55 s. See Fig. 2 for further details.

profiles and E gradient as a function of the position in the capillary tube after 36 and 55 s are given (see Table I). It must be noted that although the last two zones are almost separated, the E gradient shows only a slight indication of the presence of two ionic species. Further, the K^+ zone shows a large difference in E gradient although its concentration is the lowest of the four cations. This means that the E gradient is strongly dependent on both the concentrations in the zones and the difference between the mobilities of the ionic species and the background ions.

From Figs. 2 and 3 it can be concluded that by about 3 cm from the sampling point (at position 38) all sample ionic species have been separated.

EXPERIMENTAL

In order to examine the utility of the principle of the DDS, analyses were performed with a separation and detection unit constructed as described elsewhere[14]. The capillary tube has an I.D. of 0.25 mm and contains three sets of measuring electrodes. The distance from the sampling point to the first electrode set is about 3 cm and the distances between the electrode sets are about 10 mm. The sample is introduced into a broadened part (0.55 mm I.D.) of the capillary tube. The sampling volume is about 2.4 μl.

In the electrode compartments the electrolyte solution contacts Pt–Ir electrodes. For detection we used a.c. electronics[15], which appears to be sufficiently stable at low noise levels[11]. For the determination of mobilities we used the described apparatus with all valves closed so that the linear electroendosmotic flow would be eliminated. Using an open capillary tube, usually with very narrow bore capillaries, the determined velocity of the ionic species will be the sum of the electric field strength-induced velocity and the electroendosmotic flow velocity.

RESULTS

The apparatus was constructed in order to check if mobilities can be measured with sufficient accuracy. The separation capability is small because of its dimensions. Therefore, separations can be carried out of about four ionic species that have sufficient differences in their mobilities such as K^+, Na^+, Li^+ and TEA^+ for cation separations. Chloride, formate, acetate, propionate and *p*-aminobenzoate were chosen for the anion separations. For dilute samples good separations could be obtained, even at the first detector.

For both anion and cation separations we used as a background electrolyte a solution of 0.01 M morpholinoethane sulphonic acid (MES) and 0.02 M histidine at pH 6.4. The mobilities of the ions in this solution are low, so a large E gradient can be obtained at a low current density. All experiments were carried out at a constant electric current of 10 μA.

For the experiments we used the first and the third electrode set (both equipped with a.c. measuring electronics) as the DDS. The E gradient was measured with d.c. potential gradient electronics connected to the second set.

In the first instance we measured the effect of the concentration of the sample ions. Symmetric peaks could be obtained for concentrations of about 10^{-5} M and lower. At these concentration levels it could be concluded from the second detector

TABLE II

MIGRATION TIMES AND CALCULATED "ABSOLUTE" MOBILITIES FOR THE CATIONS K^+, Na^+, Li^+ AND TEA^+ USING THE DDS

Migration time (mm)*				*Calculated mobility (10^5 cm²/V · s)*			
K^+	Na^+	Li^+	TEA^+	K^+	Na^+	Li^+	TEA^+
36.3	53.7	68.7	82.2	76.78	51.90	40.57	33.91
36.5	53.7	68.0	82.4	76.36	51.90	40.99	33.82
36.0	52.7	67.9	81.4	75.98	51.90	40.28	33.60
43.7	63.5	82.5	99.3	75.42	51.90	39.95	33.19
43.2	63.7	82.2	99.3	76.53	51.90	40.22	33.29
43.2	63.0	82.2	99.0	75.69	51.90	39.78	33.03
43.4	63.2	82.5	99.2	75.75	51.90	39.76	33.07
41.3	60.3	77.3	92.8	75.78	51.90	40.49	33.72
40.5	59.9	76.3	91.2	76.76	51.90	40.74	34.09
41.3	59.2	76.8	91.8	74.39	51.90	40.01	33.47
35.2	52.2	67.8	82.4	76.97	51.90	39.96	32.88
36.1	53.9	69.5	84.5	76.20	51.90	39.58	32.55
35.1	51.7	67.8	82.1	76.45	51.90	39.58	32.68
35.5	52.4	66.8	80.7	76.61	51.90	40.71	33.70

* Required to pass from the first to the second detector, taken from the electropherograms in millimeters.

that the fluctuations in the E gradient were less than about 1.5% for the most mobile ionic species K^+ and even less for the others.

In the Tables II and III the migration times (in mm) required to pass the DDS are given for both cations and anions. Of course, using a DDS, retention times are of no importance.

TABLE III

MIGRATION TIMES AND CALCULATED "ABSOLUTE" MOBILITIES FOR THE ANIONS CHLORIDE (I), FORMATE (II), ACETATE (III), PROPIONATE (IV) AND *p*-AMINO-BENZOATE (V) USING THE DDS

Migration time (mm)					*Calculated mobility (10^5 cm²/V · s)*				
I	*II*	*III*	*IV*	*V*	*I*	*II*	*III*	*IV*	*V*
40.3	56.4	76.5	88.5	104.8	78.68	56.22	41.45	35.83	30.26
42.9	59.0	80.6	94.0	111.2	77.88	56.62	41.45	35.54	30.04
43.4	59.9	82.0	95.0	112.0	78.32	56.74	41.45	35.78	30.35
43.0	59.3	82.0	94.8	113.0	79.04	57.32	41.45	35.85	30.08
42.2	59.0	81.3	94.2	110.8	79.86	57.12	41.45	35.77	30.41
42.7	59.0	81.5	93.5	111.2	79.11	57.26	41.45	36.13	30.38
41.8	56.8	79.0	90.8	107.8	78.34	57.65	41.45	36.06	30.38
42.4	58.5	81.2	93.7	110.7	79.38	57.53	41.45	35.92	30.40

TABLE IV

MOBILITIES DETERMINED BY THE USE OF A DDS IN ZONE ELECTROPHORESIS

The values for the cations and anions are averages from 14 and 8 experiments, respectively (see Tables II and III). For literature values, see refs. 4 and 13.

Ionic species	*Average mobility (10^5 cm^2/V · s)*			*Standard deviation (10^5 cm^2/V · s)*
	Measured	*After correction for pH*	*From literature*	
K^+	76.12	76.12	76.2	0.66
Na^+	51.90	51.90	51.9	0.00
Li^+	40.19	40.19	40.1	0.44
TEA^+	33.36	33.36	–	0.46
Chloride	78.83	78.83	79.1	0.60
Formate	57.06	57.23	56.6	0.46
Acetate	41.45	42.40	42.4	0.00
Propionate	35.86	36.96	37.1	0.17
p-Aminobenzoate	30.29	31.29	31.6	0.14

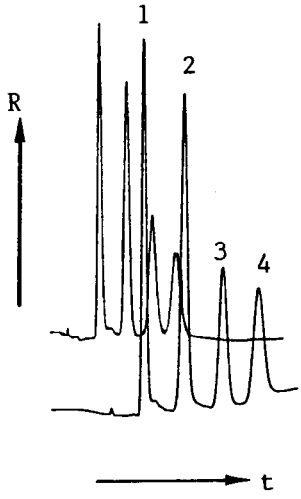

Fig. 4. A zone electrophorogram for the separation of the four cations, (1) K^+, (2) Na^+, (3) Li^+ and (4) TEA^+ (tetraethylammonium), with the use of a DDS. The detector response *R* correlates with increasing conductivity.

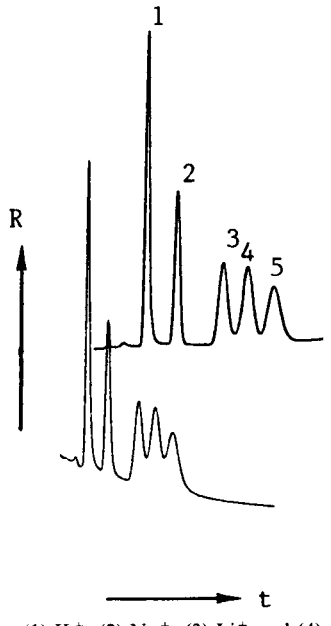

Fig. 5. A zone electropherogram for the separation of five anions, (1) chloride, (2) formate, (3) acetate, (4) propionate and (5) *p*-aminobenzoate, using a DDS. The detector response *R* correlates with increasing conductivity.

In practice, the "effective mobilities" measured, are related to the absolute mobilities by

$$m_{\text{eff,i}} = \alpha_i \gamma_i m_{0,i} \tag{7}$$

where $m_{\text{eff,i}}$ is the effective mobility, α_i is the disociation constant, γ_i is the activity coefficient and $m_{0,i}$ is the absolute mobility. In order to calculate the effectie mobilities of all ionic species from the migration times using eqn. 3, we have to know the effective mobility of one of the ionic species, which can be considered as a standard. For the cation separations Na^+ and for the anion separations acetate ions were taken as standards.

In a zone electrophoretic system (*i.e.*, at constant ionic strength, pH and temperature) for monovalent, fully ionized species the activity coefficients and dissociation constants can be considered to be equal. This means that using the absolute mobility of the standard ionic species (eqn. 3) the absolute mobility of the sample ionic species can be obtained at that temperature.

Further, all ionic species show a very similar dependence of their absolute mobilities on temperature (about 2% per °C). Therefore, using the absolute mobility of the standard at 25°C, the absolute mobilities at 25°C of the sample ionic species can be obtained. To check this, the first experiments were carried out with the apparatus thermostated at 25°C. The results were identical with and without thermostating.

For weak anions we used as the standard acetic acid (pK_a = 4.756) and calculated its mobility with a correction for the pH. The calculations of "absolute" mobilities obtained in this way are given in the Tables II and III. It is remarkable that, although the migration times differ considerably, the calculated mobilities fit very well. Differences in migration time can be caused by pressures in the system due to the use of syringes to introduce the sample and background electrolyte. Using a peristaltic pump or gravity flow to fill the compartments more constant migration times were obtained.

In Table IV the average determined "absolute" mobilities are compared with those in the literature. For the weak anions a correction was made for the influence of the pH of the system.

In Figs, 4 and 5 examples of zone electrophorograms are given for a cation and an anion separation, respectively, obtained with the DDS.

CONCLUSION

Using a DDS in CZE, ionic mobilities can be determined with values comparable to those given in the literature with a relative standard deviation of about 1%. A great advantage over the ITP determination of mobilities is that in CZE parameters such a temperature, pH and ionic strenth are nearly constant, which simplifies substantially the calculations. The question of whether a DDS can be used for identification in ZE depends on the stability and sensitivity of the measuring electronics, especially with the use of longer capillary tubes. Of course, a double-beam laser or a double-beam UV system can be used as a DDS.

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