ELSEVIER

Journal of Chromatography A, 772 (1997) 73–79

JOURNAL OF
CHROMATOGRAPHY A

System peaks in capillary zone electrophoresis
I. Simple model of vacancy electrophoresis[1]

Petr Gebauer*, Petr Boček

Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, CZ-611 42 Brno, Czech Republic

Abstract

The paper introduces the concept of vacancy electrophoresis where instead of normal electrophoretic zones, system peaks (concentration vacancies) migrate and are separated. The electrolyte set-up to achieve this requires a reversal of background electrolyte and sample with the capillary filled by the sample and the background electrolyte injected instead. A simple theory of electromigrational zone dispersion is used to describe the origin and properties of a system peak corresponding to a concentration vacancy. Model experiments confirm the theoretical considerations. The potential practical use of vacancy electrophoresis is discussed.

Keywords: Vacancy electrophoresis; System peaks; Organic acids

1. Introduction

It is a frequent phenomenon in capillary separation methods that besides the peaks of the injected substances, peaks that result from some natural properties of the system are also obtained; they are usually called 'system peaks'. The practice of capillary zone electrophoresis (CZE) has shown that in this technique also reproducibly obtainable peaks may appear that do not correspond to the zone electrophoretic migration of any of the sample components. Although they may be of different origin, there is also the tendency to call them collectively 'system peaks'.

The most comprehensive explanation to date of the origin of system peaks in CZE was presented by Poppe [1] who described the behavior of a multi-component electrophoretic system as an eigenvalue problem. From the results it can be concluded that when neglecting the effects of H^+/OH^-, an n-component system (including both positive and negative ionic constituents of both the background electrolyte (BGE) and the injected sample) creates $n-1$ eigenzones from which one is the immobile (if not driven by electroosmosis) concentration disturbance created by the introduction of the sample and $n-2$ zones are (in dependence on the system composition) either sample zones or system zones. This indicates that in simple systems of moderate pH the presence of a minimum of three components (e.g., one counter-ion and two co-ions) is necessary to obtain an electromigrating zone. Extensive investigations on system peaks by Beckers [2,3] have shown that in acidic or alkaline systems the problem may be more complex. A series of experiments where water was injected into a histidine–acetic acid BGE of varying pH has demonstrated that by decreasing the pH of the BGE, a system peak appears besides the immobile sample peak. Similar

*Corresponding author.

[1]First presented on 23 July 1996 in a lecture held at the Laboratory of Instrumental Analysis, University of Technology, Eindhoven, Netherlands.

0021-9673/97/$17.00 © 1997 Elsevier Science B.V. All rights reserved
PII S0021-9673(97)00116-7

observations were described by Gross and Yeung [4] who reported a pronounced system peak in an acidic sodium salicylate–salicylic acid binary BGE. Although it is expected that such system peaks originate from some effect of H^+ ions at low pH, an exact examination of this phenomenon is still missing.

Obviously, the problem of system peaks in capillary electrophoresis requires further research. The aim of this paper is to contribute to the understanding of the origin and properties of system peaks by presenting a simple theoretical model and experimental evidence of vacancy electrophoresis where the electropherogram is formed by a series of system peaks instead of sample peaks.

2. Theoretical

To describe a migrating vacancy in the concentration of one component in a system comprising two (or more) components of like charge is to understand it as the reversal of the migration of a sample zone in a BGE. The following simple model system was selected for the theoretical treatment. It involves two anions (coions) A^- and B^- and one cation R^+ (counterion). Both anions are assumed to belong to strong acids; the cation may be of a weak base. The pH is assumed to be in the safe region where the effects of H^+/OH^- ions can be neglected. Electromigration is assumed to be the only transport phenomenon, i.e., diffusion, electroosmosis and other effects are not taken into account. For simplicity, the solutions in the entire separation space (including the sampled zone) are assumed to have the same value of the Kohlrausch regulating function [5].

Fig. 1 explains the parallels between the normal and reversed system by showing the starting configurations for both cases. Fig. 1a illustrates the usual configuration at the start of a normal zone electrophoretic run. The BGE is formed by a solution of R^+A^- (zone 1) and a pulse of the sample solution containing the analyte B^- and (in the general case also) the BGE coion A^- is introduced (zone 2). The numbers designating the zones have practical meaning: they indicate the number of components of like charge present in the actual zone. The lower panel of Fig. 1a shows the concentration profiles of both components A and B; the sample zone is formed by a rectangular concentration pulse of component B demarcated by the concentration boundaries 1–2 and 2–1. Fig. 1b shows the reversed configuration. Here the sample solution is used as the BGE (zone 2) and instead of the sample, the BGE from Fig. 1a (zone 1) is used. As seen from the lower panel of Fig. 1b, the 'sample' zone is here formed by a rectangular concentration vacancy of component B demarcated by the concentration boundaries 2–1 and 1–2.

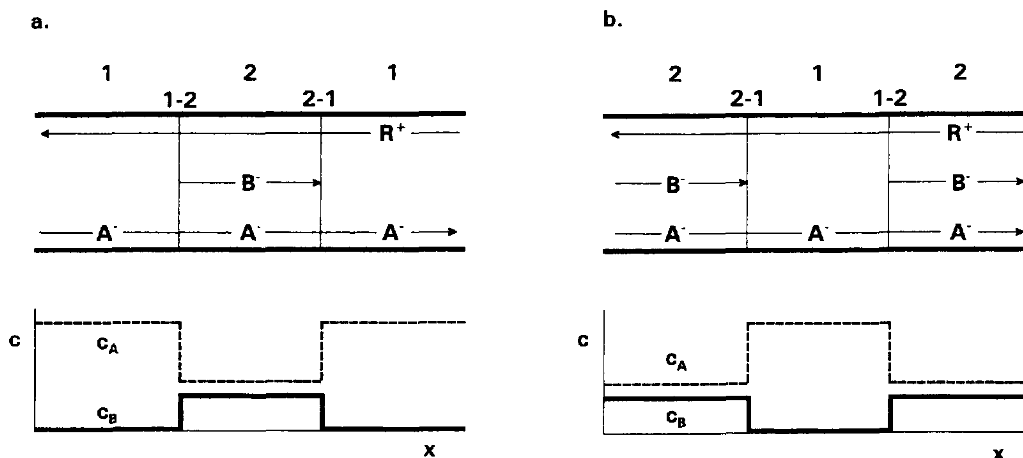

Fig. 1. Scheme of the two model systems. (a) Normal zone electrophoresis. Into the BGE (zone 1), a solution of the sample ion B containing also the BGE coion A is introduced as zone 2. (b) Reversed system (vacancy electrophoresis). The capillary is filled with the sample solution (zone 2) and the BGE (zone 1) is introduced as the sample.

Selecting now the composition of zone 2 the same for both mentioned cases (Fig. 1a and b) and assuming that zone 1 is adjusted to the ω-value (value of the Kohlrausch regulating function [5]) of zone 2, the composition of zone 1 is obviously the same in both cases, too. The starting configurations in both cases thus involve two zone boundaries exactly the same that in principle migrate independently of each other and can be therefore described by the same set of equations.

Let us look first at the normal or classical case in Fig. 1a. The evolution of the concentration profile of the zone of component B during zone electrophoresis in such a simple case has been described long ago [6] (see also [7]). Consider one possible case where the electrophoretic mobility (unsigned quantity) of the sample (B^-) is lower than that of the BGE coion (A^-), $\mu_B < \mu_A$. In such case, when electric current is passed through the system so that the ions present move in the directions indicated by the arrows in Fig. 1a, the front zone boundary 2–1 is a self-sharpening one migrating with the velocity

$$v_{2-1} = \frac{\mu_B i}{\kappa_2} \tag{1}$$

where κ_j is the specific conductivity of zone j and i is the electric current density. The rear zone boundary 1–2 is a diffuse one, spread by electromigrational dispersion into a continuously growing transition zone where the concentration of B is variable (this zone is denoted further on by the subscript 'tr'). The velocity of the rear edge of this zone (at which the composition of the transition zone approaches that of zone 1, i.e., component B vanishes) is

$$v_{1-tr} = \frac{\mu_A \mu_B \mu_R i F \omega}{\kappa_1^2} \tag{2}$$

where $\omega = c_{A,1}/\mu_A + c_{R,1}/\mu_R$ with $c_{i,j}$ being the analytical concentration of component i in zone j; F is the Faraday constant. Similarly the velocity of the front edge of the transition zone (where its composition approaches that of zone 2) is

$$v_{tr-2} = \frac{\mu_A \mu_B \mu_R i F \omega}{\kappa_2^2} \tag{3}$$

The front edge of the transition zone reaches the migrating boundary 2–1 after an electrophoresis time

$$t_0 = \frac{l}{v_{tr-2} - v_{2-1}} \tag{4}$$

(l is the length of the sample zone) when the original concentration plateau of component B vanishes. At times $t > t_0$ the entire sample (component B) migrates within the transition zone and its concentration profile is given by

$$c_{B,tr} = \frac{\mu_A \mu_B \mu_R \omega}{(\mu_A - \mu_B)(\mu_B + \mu_R)} \left[1 - \sqrt{\frac{i}{F} \frac{\mu_B}{\mu_A \mu_R \omega} \frac{t}{x}} \right] \tag{5}$$

where t is the time from the start of electrophoresis and x is the longitudinal coordinate (selected so that for the left boundary of zone 2 $x=0$ at $t=0$). The concentration profile of component B along the transition zone is a convex curve between zero (corresponding to its rear edge) and $c_{B,2}$; for its shape for a model example, see Section 4 (Fig. 2).

The front boundary of the transition zone remains sharp and its position at time $t > t_0$ is given by

$$\sqrt{x} = \sqrt{x_0} + (\sqrt{t} - \sqrt{t_0}) a \sqrt{\frac{i}{F} \frac{\mu_B}{\mu_A \mu_R \omega}} \tag{6}$$

(with $a=1$ and x_0 being the position of the front boundary of the transition zone at $t=t_0$) whereas its rear edge moves still with the constant velocity v_{1-tr} (Eq. (2)). Note that for the present case with $c_{B,1}=0$ Eq. (2) can be rewritten to

$$v_{1-tr} = \frac{\mu_B i}{\kappa_1} \tag{7}$$

which corresponds to the velocity of a single ion B in the BGE (zone 1).

Let us look now at the vacancy shown in Fig. 1b. Most of the above equations can be directly used for the description of this reverse case. The difference is that here the rear boundary of zone 1 (boundary 2–1) is self-sharpening, migrating with velocity v_{2-1} (Eq. (1)). The front boundary of zone 1, 1–2, is here the diffuse one which, on migrating, creates a transition zone with a convex concentration profile of component B. The velocities of its front and rear edges are v_{tr-2} (Eq. (3)) and v_{1-tr} (Eq. (2)), respectively. The rear edge meets the migrating boundary 2–1 after an electrophoresis time

$$t_0 = \frac{1}{v_{2-1} - v_{1-tr}} \quad (8)$$

What vanishes at time t_0 in this case is the 'total vacancy', i.e., the part of the system with zero concentration of component B. At times $t > t_0$ component B is again present in the entire electrophoretic column. The zone corresponding to the 'vacancy' is still present but is now characterized completely as a transient one containing both A and B forming a moving concentration gradient of B with $c_{B,tr}$ decreasing from front to rear. The concentration profile of this zone is again described by Eq. (5) with the only difference that for the starting point $x=0$, $t=0$, the right boundary of zone 1 is taken as reference. The rear boundary of the transition zone remains sharp; for its position at time $t > t_0$ again Eq. (6) is valid but here with

$$a = 1 + \frac{Fc_{B,2}(\mu_B + \mu_R)}{\kappa_2} \frac{\mu_A - \mu_B}{\mu_B} \quad (9)$$

because the background electrolyte (here zone 2) also involves component B. The front edge of the transition zone moves with the constant velocity v_{tr-2} (Eq. (3)); note that this edge is the point where the 'zone' is vanishing; its velocity, however, cannot be expressed in such an easy way as in the previous case by Eq. (7).

3. Experimental

A laboratory-made set-up equipped with a 50-cm long coated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 75-μm I.D. with a separation length of 38 cm was used for the experiments. The inner capillary surface was coated with 6% polyacrylamide gel using the procedure described by Hjertén [8] except that γ-methacryl–oxypropylsilane was dissolved in ethanol instead of water. The capillary was either freely hung in air at room temperature (25°C) or placed in a laboratory-made thermostating chamber connected to a water bath thermostat set to 25°C with a precision better than 0.05°C. Sampling was performed using a simple hydrodynamic sampling device with $\Delta h = 2$ cm. The sample zones were detected using an 875 UV (Version 5) detector from Jasco (Tokyo, Japan). The detector was equipped with a laboratory-made capillary holder where the detection window was sandwiched between two quartz lenses. The detector output was connected to a two-channel 4200 Line Recorder (Laboratorní přístroje, Prague, Czechoslovakia). As the power supply, a CZE 1000 R model from Spellmann (Plainview, NY, USA) was used. All chemicals used were of analytical-reagent grade. The solutions were prepared in distilled water; their pH was measured by a 240 model pH meter from Corning (Sudbury, UK). The theoretical calculations were made using a simple program written in QBASIC.

4. Results and discussion

To illustrate the analogy between normal zone electrophoresis and vacancy electrophoresis, a simple model system composed in accordance with Fig. 1a and b was investigated. Its components are shown in Table 1. The composition of zone 2 was 0.005 *M* in both formate and picrate and 0.02 *M* in histidine; this provides an ω value of $9.21 \cdot 10^8$ mol V s m^{-5}. The composition of zone 1 corresponding to this value is 0.01137 *M* formate and 0.02137 *M* histidine.

Fig. 2a shows the evolution of the zone of B with time, as calculated using Eqs. (1)–(6). This case represents normal zone electrophoretic migration of component B, as it corresponds to Fig. 1a. The originally rectangular concentration profile changes gradually into a triangular tailing one which undergoes electromigration dispersion at its rear side. Fig. 2b shows the calculated development of the migrating vacancy (missing B) corresponding to Fig. 1b. Also in this case the originally rectangular profile is distorted by electromigration: its rear boundary remains sharp and its front part is dispersed by electromigration dispersion. The plateau of zero

Table 1
Components of the model system

Model component	Substance	Ionic mobility [9] (10^{-9} m^2 V^{-1} s^{-1})
A	Formate	57.1
B	Picrate	31.7
R	Histidine	29.6

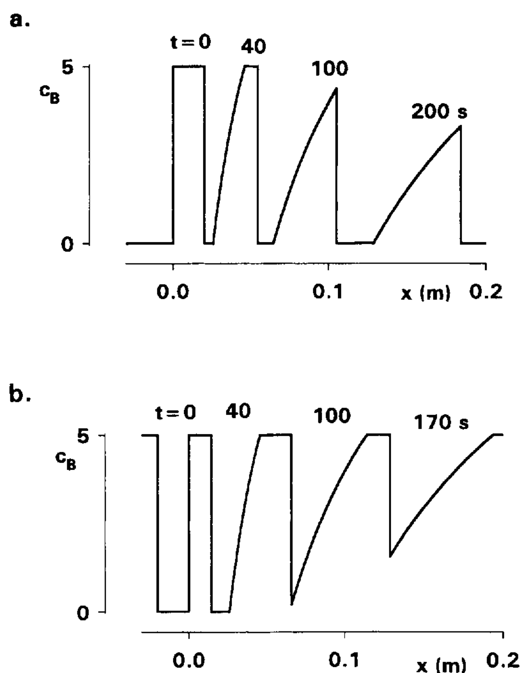

Fig. 2. Time evolution of the longitudinal concentration profiles of component B, c_B (in mol m^{-3}) for both investigated cases: (a) normal zone electrophoresis, (b) vacancy electrophoresis. x = longitudinal coordinate, t = time. For explanation, see text.

concentration of B disappears soon and the vacancy is flattened and broadened during continued electromigration.

Fig. 3 shows the related experiments. Fig. 3a brings the record of a zone of picrate migrating in the formate–histidine BGE. The sample was overloaded to an extent necessary for obtaining the asymmetric peak profile; the tailing peak shape corresponds exactly to the theory. Fig. 3b brings the migration of a vacancy (picrate missing). A pulse of the formate–histidine BGE was sampled into the formate–picrate–histidine electrolyte and a well established migrating negative peak in the picrate detection signal was recorded. Its existence and shape correspond to the theory perfectly: it is a fronting peak with a sharp rear boundary. Very similar results may be expected for the case where the sample differs in its ω value from that of the BGE because the analyte/system zone, when migrating into the BGE, is re-adjusted to its ω value. Differences in quantitative parameters (detection time, peak size) may be the result but the qualitative character of the record should remain the same.

The questions arise now whether it is possible to find a system where the generation of multiple

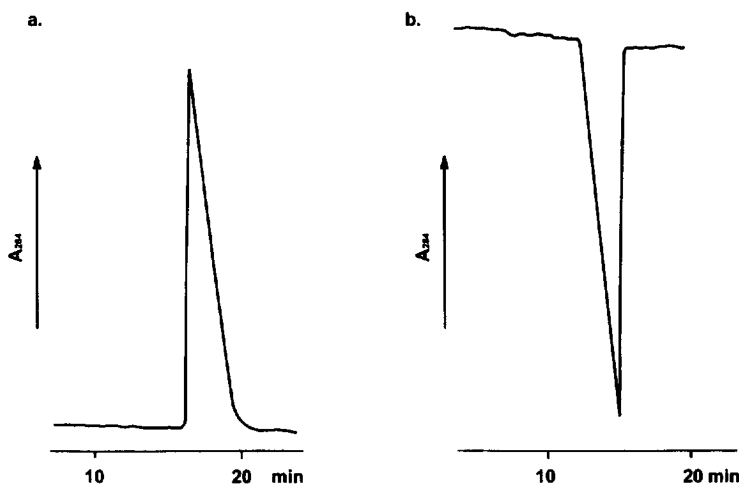

Fig. 3. Experimental UV-absorption records of the systems from Fig. 2. The electrolytes used were: solution 1: 0.01137 M formic acid + 0.02137 M histidine; solution 2: 0.005 M picric acid + 0.005 M formic acid + 0.02 M histidine. (a) BGE = solution 1, sample = solution 2; (b) BGE = solution 2, sample = solution 1. The experiments were performed using a non-thermostated capillary at a constant voltage of 10 kV. Sampling was performed for 40 s. The range of the detector (detection wavelength 284 nm) was set to 0.16 AU and that of the recorder to 5 mV.

system peaks is possible, and/or, what will be the behavior of systems where the BGE is formed by a mixture of more than two components of like charge. Both questions have the same answer. By way of analogy and in agreement with present knowledge (see Section 1) it can be expected that zone electrophoresis of a sample provides one system peak for each but one BGE component that is missing in the sample (or even present here in a concentration different from that required to match with the ω value of the BGE).

Fig. 4 gives experimental evidence of the possibility to generate multiple system peaks in CZE. Fig. 4a shows the experimental record of the separation of a 5-component sample in phosphate buffer where five normal (positive) and well resolved electrophoretic peaks were obtained. Fig. 4b shows the reversed experiment where the separation capillary was filled with the above sample serving now as the BGE and vice versa. A set of five migrating system peaks (vacancies) was the result. These vacancies are detected as negative peaks in the detection signal of the quite strongly absorbing sample solution. Note that the resolution of the vacancy zones is almost as good as that of the normal electrophoretic zones, thus indicating the possible practical use of vacancy electrophoresis. Both the migration order and the relative magnitudes of the negative peaks fit quite well with those of the peaks from Fig. 4a. What does not correspond in Fig. 4a and b are the relative detection times of the 5 components. This is, however, in accordance with the theory (see Section 2, comment after Eq. (9)) because the migration velocity (apparent mobility) of a system peak is generally not directly related to the mobility of a single component but is a function of the composition of the BGE (this is in accordance with the investigations on the mobility of a system peak in a BGE with binary coions made by Beckers [3]).

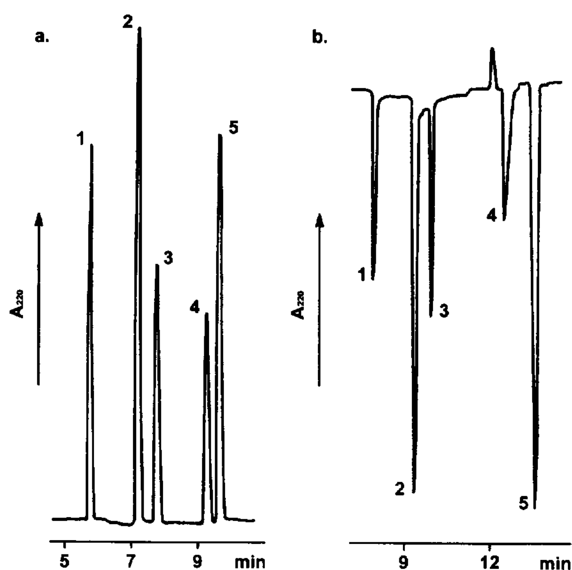

Fig. 4. Experimental UV-absorption records of the 5-component system. The electrolytes used were: solution 1: 0.015 *M* sodiumdihydrogen phosphate adjusted to pH 7 with NaOH; solution 2: salicylic acid, sulfosalicylic acid, phthalic acid, picric acid, (0.0015 *M* each), 0.003 *M* $NaNO_3$, 0.014 *M* trishydroxymethylaminomethane; solution 3: solution 2 diluted with solution 1 (1:4, v/v). (a) BGE=solution 1, sample=solution 3; (b) BGE=solution 3, sample=solution 1. The experiments were performed with the setup using a thermostated capillary at a constant voltage of 7.5 kV. Sampling was performed for 30 s. The range of the detector (detection wavelength 220 nm) was set to (a) 0.005 AU and (b) 0.04 AU and that of the recorder to (a) 0.1 mV and (b) 10 mV. The peaks on the records correspond to (1) nitrate, (2) sulfosalicylate, (3) phthalate, (4) salicylate and (5) picrate.

5. Conclusion

A simple theoretical approach based on electromigration zone dispersion helps to describe and understand the origin and properties of system zones ('system peaks') in zone electrophoresis. This approach throws clear light on the problem of system peaks in practice, provides easy insight into the behavior of systems and offers the description of system peaks by simple equations. Predictions of the theory presented agree with model experiments. The considerations can be extended to more complicated cases where multiple system zones ('system peaks') may occur. The general condition of generation of zones and system zones in BGEs of moderate pH can be formulated on a easy-to-understand phenomenological basis as follows. In a BGE with two or more coions, zone electrophoresis of a sample provides one normal zone for each sample component that is missing in the BGE and one system zone (vacancy) for each but one BGE component that is missing in the sample. The possibility of generating multiple system (vacancy) peaks in zone electrophoresis was demonstrated by a 5-component model sample in

phosphate buffer: by filling the capillary with the sample solution and by sampling pure buffer, 5 system zones were generated with a well-evaluable detection record. The concept of the vacancy electrophoresis based on sample–BGE reversal may have practical potential, e.g., at difficult analyzable sample components where conditioning of the entire capillary with the sample solution may help significantly. Another possibility may be samples with a difficult matrix: by involving it into the BGE, its negative effect may be mostly eliminated. A more detailed investigation of the behavior and practical aspects of vacancy electrophoresis and other electrolyte set-ups generating multiple system peaks is under investigation.

Acknowledgments

This work was supported by the Grant Agency of the Czech Republic (Grants No. 203/94/0998 and 203/96/0124) and the Grant Agency of the Academy of Sciences of the Czech Republic (Grant No. 431404).

References

[1] H. Poppe, Anal. Chem. 64 (1992) 1908–1919.
[2] J.L. Beckers, J. Chromatogr. A 662 (1994) 153–166.
[3] J.L. Beckers, J. Chromatogr. A 679 (1994) 153–165.
[4] L. Gross, E.S. Yeung, J. Chromatogr. 480 (1989) 169–178.
[5] F. Kohlrausch, Ann. Phys. Chem. 62 (1897) 209–239.
[6] H. Weber, Die Partiellen Differential-Gleichungen der Mathematik und Physik, Vol. I, Vieweg, Braunschweig, 1910, p. 503.
[7] P. Gebauer, M. Deml, J. Pospíchal, P. Boček, Electrophoresis 11 (1990) 724–731.
[8] S. Hjertén, J. Chromatogr. 347 (1985) 191–198.
[9] J. Pospíchal, P. Gebauer, P. Boček, Chem. Rev. 89 (1989) 419–430.