ELSEVIER

Journal of Chromatography A, 772 (1997) 81–89

JOURNAL OF CHROMATOGRAPHY A

System peaks in capillary zone electrophoresis II. Experimental study of vacancy peaks

Claudia Desiderio[a], Salvatore Fanali[a], Petr Gebauer[b], Petr Boček[b,*]

[a]*Istituto di Cromatografia del Consiglio Nazionale Ricerche, Area della Ricerca di Roma, P.O. Box 10, 00016 Monterotondo Scalo, Rome, Italy*

[b]*Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, CZ-611 42 Brno, Czech Republic*

Abstract

An uncoated capillary with selected background electrolytes (BGEs) was used to study the migrating vacancies of UV-absorbing components of the buffer. Picric and salicylic acids were chosen as the UV-absorbing anionic species, to be used either as samples in normal capillary electrophoresis (CE) mode (direct CE) or as BGE components (reverse CE). To study the effect of BGE composition, 2-morpholinoethanesulfonic acid and monochloroacetate, with lower and higher mobilities, respectively, were selected as two different BGE co-ions of picrate and salicylate. In normal CE mode, anionic species provided tailing or fronting peaks (representing fronting or tailing migrating zones), depending on whether their effective mobilities were lower or higher than the mobility of the BGE co-ion, respectively. In reverse CE, the effect was the opposite. Picrate and salicylate vacancies were represented by fronting or tailing dips (migrating as tailing or fronting zones) when their mobilities were higher or lower than that of the BGE co-ion, respectively. The electroosmotic peak may be negative or positive, according to the original value of the Kohlrausch regulating function of the sample. Finally, the difference between migrating vacancies and indirect detection is demonstrated and explained, showing indirect detection of a mixture of potassium and sodium acetate detected simultaneously with a benzoic acid vacancy.

Keywords: System peaks; Vacancy electrophoresis; Organic acids

1. Introduction

It is well known in capillary electrophoresis (CE) with UV absorbance detection that, in many cases, the analysis record shows not only the peaks corresponding to the sampled analytes but also some additional peaks that do not belong to any species of the sample. This represents a serious problem, especially when working in the indirect detection mode where interpretation of the analysis records is a much greater puzzle [1]. Indirect UV-absorbance detection is a simple method where the migrating analytes displace a suitable UV-absorbing component of the background electrolyte (BGE) used and are detected as negative peaks. To identify the individual peaks, a well-known spiking procedure is currently used, based on the enrichment of the sample with one component, analysis of the enriched sample and recognition of the peak that becomes larger. Unfortunately, in many cases, strange results are obtained where, when spiking the sample with one component, the resulting detection record shows changes in the magnitude of several peaks.

Over the last several years, the above-mentioned phenomena were paid increased attention. The term "system peak" was introduced to denote a peak not corresponding to any injected analyte. Several related papers have been published, especially by

*Corresponding author.

0021-9673/97/$17.00 © 1997 Elsevier Science B.V. All rights reserved
PII S0021-9673(97)00117-9

Poppe [2], Bruin et al. [3] and Beckers [1,4–6]. Recently, a simple approach for explaining system peaks has been presented [7], considering the system peak as a migrating vacancy. This approach seems to be capable of clearly explaining and determining the origin and properties of system peaks or dips. It has been shown that by using a BGE containing more co-ionic species, the injection of a "dummy" sample with these species missing can produce the corresponding number of migrating vacancies, i.e., system peaks.

In this paper we present a systematic experimental study related to this model. In particular, we discuss the calibration dependencies of model samples, showing how a change in the concentration of an analyte in the injected sample may convert a vacancy peak into a normal peak and vice versa. We also describe the effect of the ionic strength of a sample upon the electroosmotic peak (frequently called the water peak). Furthermore, the existence and origin of peaks corresponding to zones of non-absorbing co-ions from the sample (so-called Kohlrausch peaks) is demonstrated and explained. Finally, a clear demonstration is given of the principal difference between indirect detection and migration of vacancies.

2. Experimental

2.1. Chemicals and reagents

Tris(hydroxymethyl)aminomethane (Tris), monochloroacetic acid (ClAc), salicylic acid, picric acid, benzoic acid, sodium acetate and potassium acetate were purchased from Carlo Erba (Milan, Italy). Sodium hydroxide and 2-morpholinoethanesulfonic acid monohydrate (MES) were from Fluka (Buchs, Switzerland). Double distilled water was boiled before use in the preparation of solutions. The concentrated solutions were all 0.04 *M* and were diluted with water or with the running BGE, to the final desired concentration. The BGEs used for experiments were MES–Tris (1:2, v/v) or monochloracetic acid (ClAc)–Tris, pH 8.0. For reverse CE, the above-described buffers were supplemented with salicylic and picric acids (each 0.8 m*M*), or with 10 m*M* benzoic acid.

2.2. Instrumentation

An automated capillary electrophoresis apparatus (Bio-Rad CE3000), equipped with a multi-wavelength UV detector, was used for experiments. Electropherograms were monitored at a wavelength of 206 nm. Electrophoretic separations were performed in an uncoated capillary (Polymicro Technologies, Phoenix, AZ, USA) of 50 cm total length (45.5 cm effective length)×0.05 mm I.D., inserted into a Bio-Rad laboratory-assembled cartridge. A constant voltage of 20 kV was applied. Samples were injected by pressure at the anodic end by applying 5 p.s.i. s (1 p.s.i.=6894.76 Pa), corresponding to a 0.67-cm long injection zone. Between runs, the capillary was washed with water (60 s), 0.1 *M* sodium hydroxide (60 s) and water (70 s), before being purged with the BGE (70 s). The carousels and the capillary were both thermostated at 25°C. Electroosmotic flow measurements were made by injecting mesityloxide.

3. Results and discussion

Based on preliminary experiments, we selected picric and salicylic acids as the UV-absorbing components of the BGE. In order to reveal the asymmetry of the electromigration dispersion, i.e., fronting or tailing of the peaks and vacancies, experiments were performed with two different BGEs containing diverse anionic components that had effective mobilities that were lower (MES) or higher (ClAc) than those of the picrate and salicylate UV-absorbing ions. The pH values and the composition of the BGEs used are described in Table 1. Their pH value (of around 8) ensured full ionization of all the ionic species studied, used either as BGE components or as injected analytes. Table 2 reports on the effective mobility and p*K* values of the ionic species investigated in this paper.

The use of a fused-silica uncoated capillary allowed us to study the behavior of the system in the sample injection zone. The strong cathodic electroosmotic flow (EOF) drove the anionic zones through the capillary and the detection cell. We did not use any EOF marker, since it could influence the processes and hide the effects we wanted to study,

Table 1
Overview of the background electrolytes used

BGE	Composition	pH	μ_{EO}
Direct MES	5.0 m*M* MES, 10.0 m*M* Tris	8.04	55.8
Reverse MES	5.0 m*M* MES, 10.0 m*M* Tris 0.8 m*M* Picric acid 0.8 m*M* Salicylic acid	8.06	53.0
Direct ClAc	5.0 m*M* ClAc, 10.0 m*M* Tris	8.06	59.2
Reverse ClAc I	5.0 m*M* ClAc, 10 m*M* Tris 0.8 m*M* Picric acid 0.8 m*M* Salicylic acid	8.02	57.6
Reverse ClAc II	10.0 m*M* ClAc, 10 m*M* Tris 10.0 m*M* Benzoic acid	8.06	56.4

For each BGE, its composition, pH and electroosmotic mobility, μ_{EO} (10^{-9} m^2 V^{-1} s^{-1}), are given.

however, in separate runs, the EOF was measured (each time with new BGE) by injecting water. The EOF data are also in Table 1. In most cases, a peak or dip, marking the EOF, was visible in the electropherograms, corresponding to the migration time of the EOF marker.

Due to the presence of EOF, it has to be stressed here that the migration order and asymmetry of anionic zones is revealed by the detector in an inverse way. The slower anion (picrate) showed both the peak and the vacancy with the shorter migration time than the faster anion (salicylate). Moreover, in the direct CE, the tailing anionic zones gave fronting peaks, and the fronting anionic zones gave tailing peaks. The opposite occurred in reverse CE.

Fig. 1 Fig. 2 depict the measurements of picrate and salicylate in direct (peaks) and reverse (vacancies) CE mode, respectively, using the BGE system with the faster co-ion, the ClAc–Tris buffer. It can be clearly seen in Fig. 1a–d that the direct injection of picrate and salicylate gave well-developed fronting peaks. The corresponding zones inside the capillary are, of course, tailing. This result is in complete agreement with the rule that is valid for strong electrolyte systems, which states that species having larger mobilities than the BGE co-ion show fronting zones. Since the anionic species involved are fully ionized, this rule can be applied here.

The migrating vacancies of picrate and salicylate

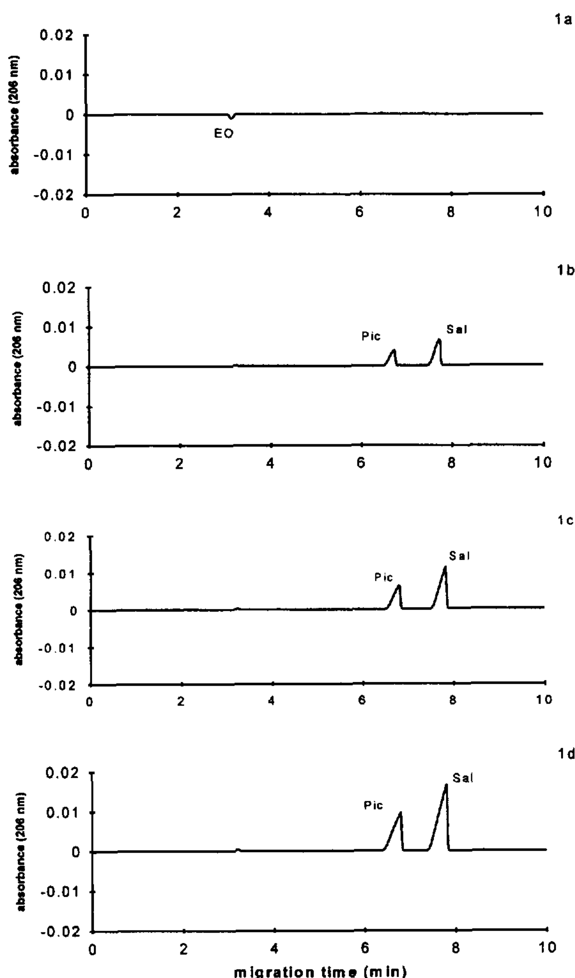

Fig. 1. Direct CE analysis of picric (Pic) and salicylic (Sal) acids in direct ClAc BGE. (a) Injection of water; (b)–(d) injection of 0.2, 0.4 and 0.8 m*M* (each) picric and salicylic acids, respectively.

Table 2
Ionic mobilities at infinite dilution

Ionic species	μ	p*K*
$Monochloroacetate^{-}$	−41.1	2.865
$Picrate^{-}$	−31.5	0.708
Salicylic acid	−35.4	3.110
Acetic acid	−42.4	4.760
Benzoic acid	−33.6	4.203
MES	−28.0	6.095
Tris	29.5	8.100
Sodium	51.9	14.000
Potassium	76.2	14.000

Values of μ (10^{-9} m^2 V^{-1} s^{-1}) and p*K* for the ionic species used are from References [3,5,8]

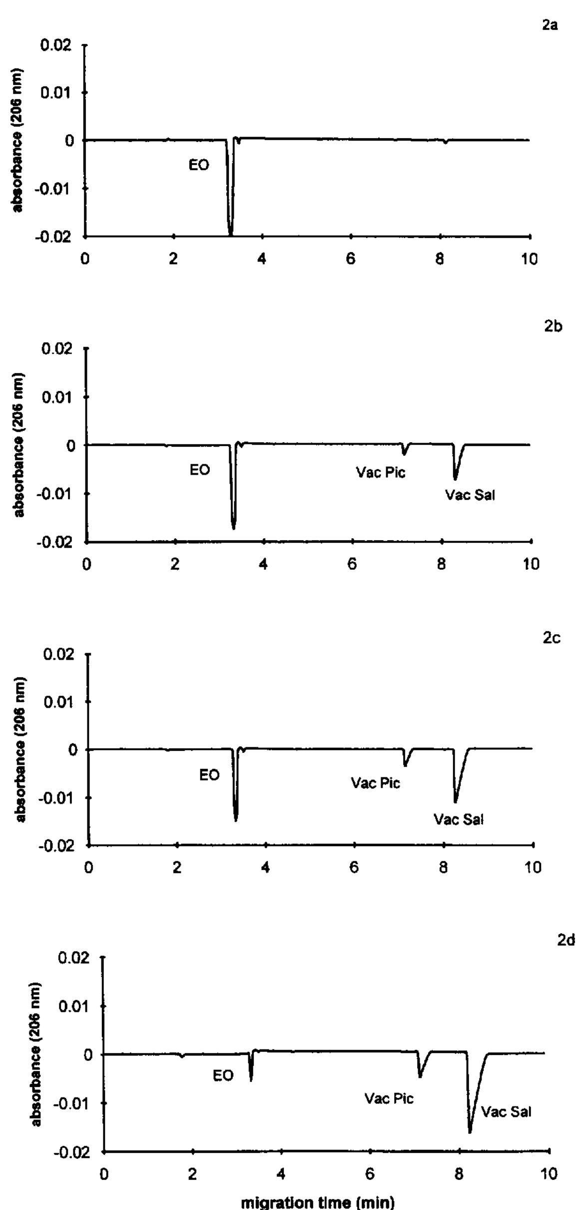

Fig. 2. Reverse CE analysis of picric acid (Vac Pic) and salicylic acid (Vac Sal) vacancies. BGE: reverse ClAc I. (a) Injection of water; (b)–(d) injection of 1.25, 2.5 and 5 m*M* ClAc–Tris (1:2, v/v) buffer, respectively. EO=Electroosmotic flow.

are shown in Fig. 2. For reverse CE experiments, the ClAc–Tris buffer was supplemented with both picric and salicylic acids (0.8 m*M* each) and the injected sample did not contain any picrate or salicylate and was just a standard solution of ClAc+Tris (1:2 in molarity) at different concentration levels (0–5 m*M* ClAc). Injection of pure water (Fig. 2a) gave a strong dip, as it corresponds to the adjustment of the used BGE to the value of the Kohlrausch regulating function (KRF) [9] in the sample zone. For pure water, this value is nearly zero, since it corresponds to the concentration of H^+ and OH^- only and the adjusted concentration level of the BGE is therefore very low. When injecting a solution of ClAc and Tris at increasing concentrations, the adjusted value of the concentration of UV-absorbing BGE at the sample position becomes larger and the EOF peak (dip) becomes smaller.

The sample of ClAc and Tris produces a starting pulse where the picrate and salicylate are not present at all, so that the sample pulse represents a vacancy for these species. From the description of the migration of such a vacancy [7], it follows that the magnitude of this vacancy is given by the length of the Kohlrausch-adjusted sample zone. The larger the amount of ClAc injected, the longer is the adjusted zone of the migrating vacancy when it leaves the original sample position and the larger are the negative peaks representing the vacancies. Since the two species that are present in the BGE used here are missing in the sample, we must consider the sample as two coinciding vacancies. These vacancies migrate independently of each other and show mutually different migration velocities. The result is obvious: Two dips (negative peaks), corresponding to the vacancies (where the selected species is missing) of picrate and salicylate appear in the detection trace. As predicted by the theory, the migrating vacancies show an asymmetry that is inverse to the positive peaks of the selected species under the same electrolyte conditions. Obviously, the tailing dips of vacancies correspond to the fronting peak of the anionic zones.

Fig. 3 Fig. 4 show the direct and reverse CE measurements of picric and salicylic acids in the BGE containing the slower-migrating co-ion (MES). As expected for the presence of a slower-migrating BGE co-ion, the peaks were tailing and, thus, the sample species were migrating as fronting zones and their peak magnitudes were directly proportional to the injected concentrations (see Fig. 3).

Fig. 4 represents the reverse (vacancy) CE analysis of picric and salicylic acids in the reverse

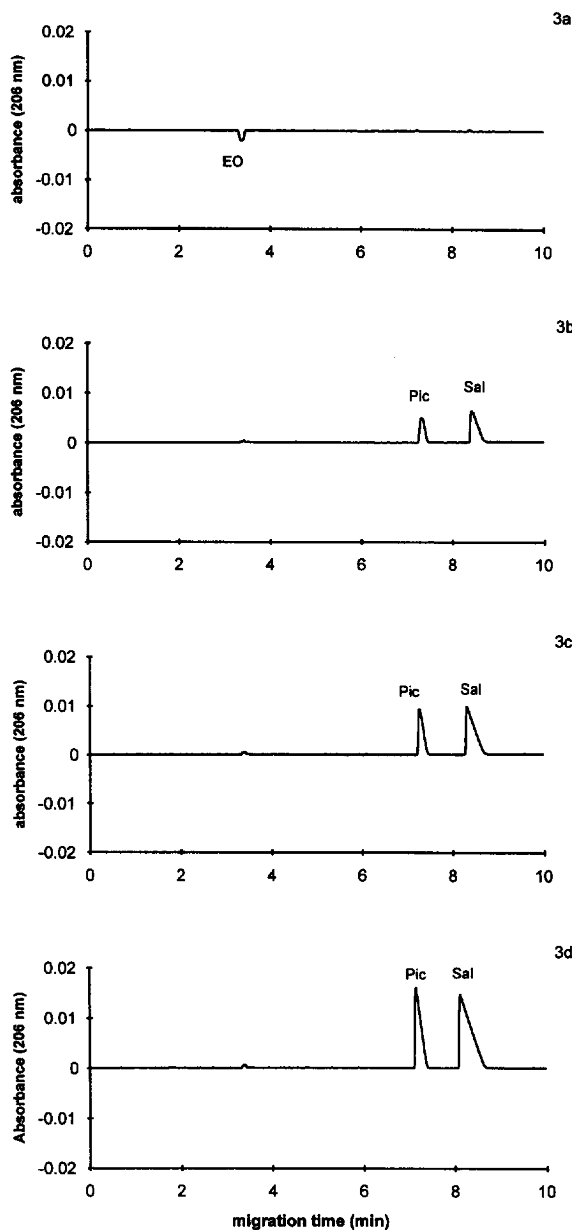

Fig. 3. Direct CE analysis of picric and salicylic acids in direct MES BGE. (a) Injection of water; (b)–(d) injection of 0.2, 0.4, 0.8 m*M* (each) of picric acid and salicylic acid, respectively.

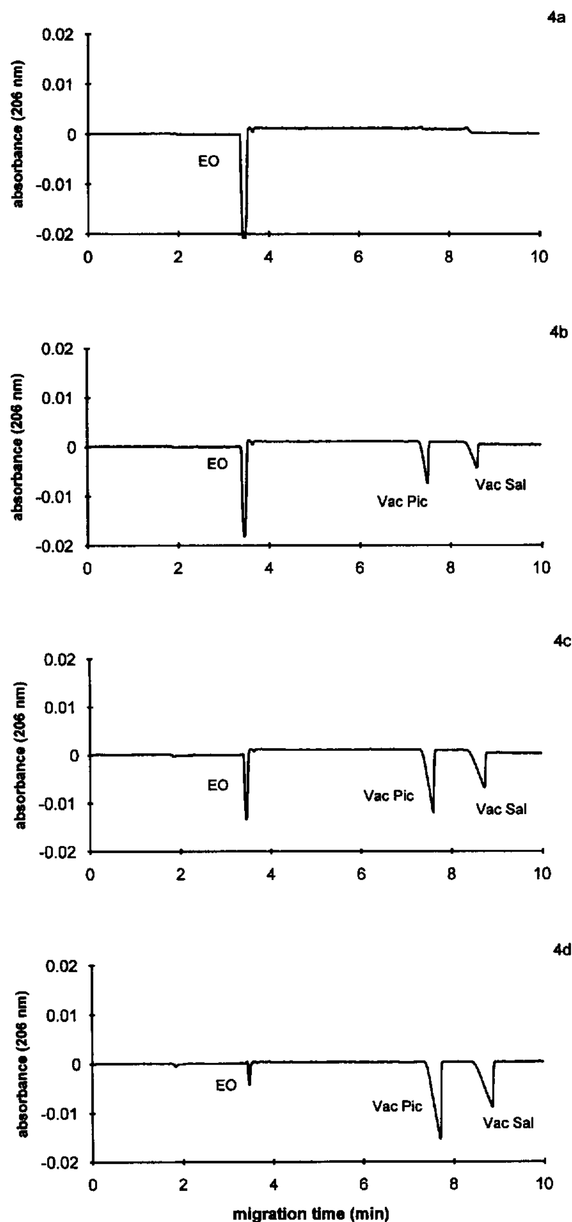

Fig. 4. Reverse CE analysis of picric acid and salicylic acid vacancies. BGE: reverse MES. (a) Injection of water; (b)–(d) injection of 1.7, 3.4 and 5 m*M* MES–Tris (1:2, v/v) buffer, respectively.

MES BGE. The results can be discussed in a similar way to those obtained in the analysis with the reverse ClAc I BGE. It can also be outlined here that the vacancies again showed an inverse dip shape to that of the corresponding peaks in the direct analysis. The injected samples in Fig. 4 were water (Fig. 4a) and MES+Tris (1+2 in molarity) buffer at selected concentrations (1.7–5 m*M* MES). It could also be observed that, for the vacancies, a correlation exists between the injected concentration of MES and the

magnitude of the vacancies' dips; the lower the concentration of MES, the smaller were the vacancy dips. Also, the injection of water revealed a big dip marking the EOF; its existence can be explained again by the KRF adjustment.

It can be summarized at this point that in an uncoated capillary and in normal CE mode, anionic species exhibit tailing or fronting peaks (and thus fronting or tailing zones, respectively) when their effective mobilities are lower or higher than the mobility of the BGE co-ion, respectively. In the case of reverse CE, the situation is reversed. When the BGE contains absorbing species with effective mobilities that are higher than that of the BGE co-ion, their vacancies will be fronting dips. If the ionic species possess lower mobilities than that of the co-ion, their vacancies are tailing dips.

The basic knowledge presented above allows us both to create and understand more complicated systems and/or detection patterns. Two of them are shown in Fig. 5 Fig. 6, depicting the electropherograms of reverse CE analyses of picric and salicylic acid vacancies in the reverse MES and reverse ClAc I BGEs, respectively (see Table 1). In Fig. 5, the injected samples were formed by the direct MES buffer spiked with increasing concentrations of picric acid (0–2.4 m*M*), while in Fig. 6, direct ClAc buffer samples were used, containing different concentrations of salicylic acid. In both cases, the injection of increasing concentrations of one of the absorbing anions resulted in a stepwise reduction in its vacancy dip, up to the level where the vacancy disappeared and then continuing in a growing positive peak. The inversion of peak shape between vacancy and normal zone is clearly seen in both Figs. 5 and 6. In Fig. 5, e.g., the fronting picrate vacancy changes into a tailing picrate peak (positive) with increasing picrate concentration in the sample. Both Figs. 5 and 6 also demonstrate that the vacancy of the anion that was not spiked in the sample hardly changed at all. Note that for these examples, the rules that were mentioned previously (valid for strong electrolyte systems) apply, according to which shapes of the peaks and vacancies are controlled by the mobility difference between the BGE co-ion and the analyte ion.

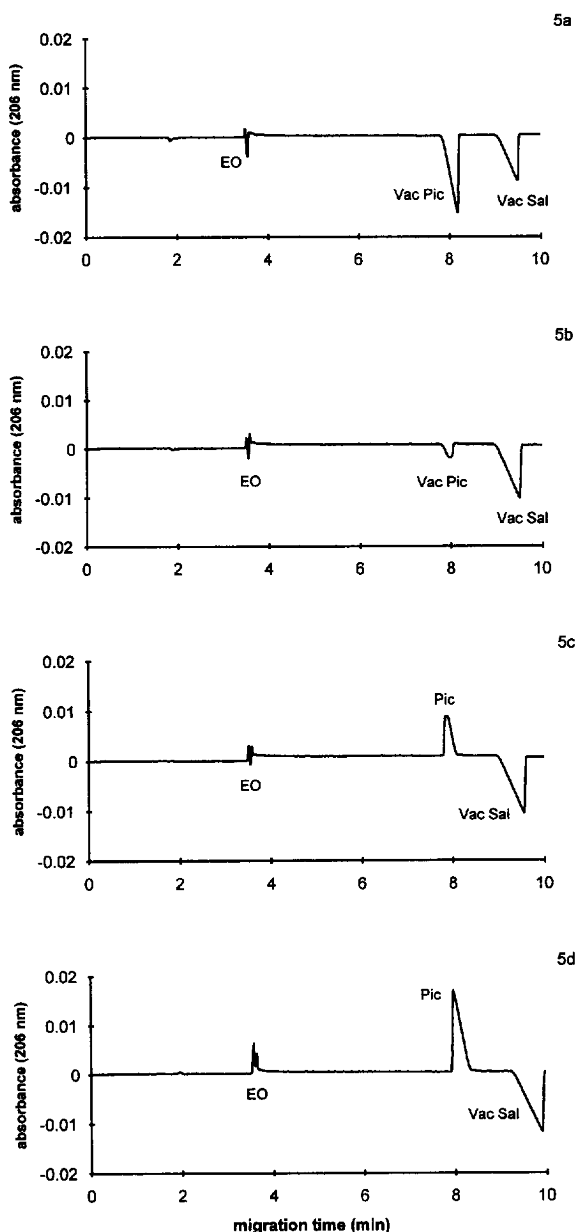

Fig. 5. Effect of the concentration of picric acid in reverse CE analysis. BGE: reverse MES. (a) Injection of 5 m*M* MES, 10 m*M* Tris; (b)–(d) injection of 0.8, 1.6 and 2.4 m*M* picric acid, respectively, in 5 m*M* MES, 10 m*M* Tris.

Another type of "ghost peak" observed in CZE systems with EOF is the so-called water, or EOF, peak that appears when the concentration discontinuity at the original sample location is driven through the detector. Fig. 7 shows the effect of the sample's concentration on the sign and magnitude of the EOF peak. In this case, the BGE was reverse

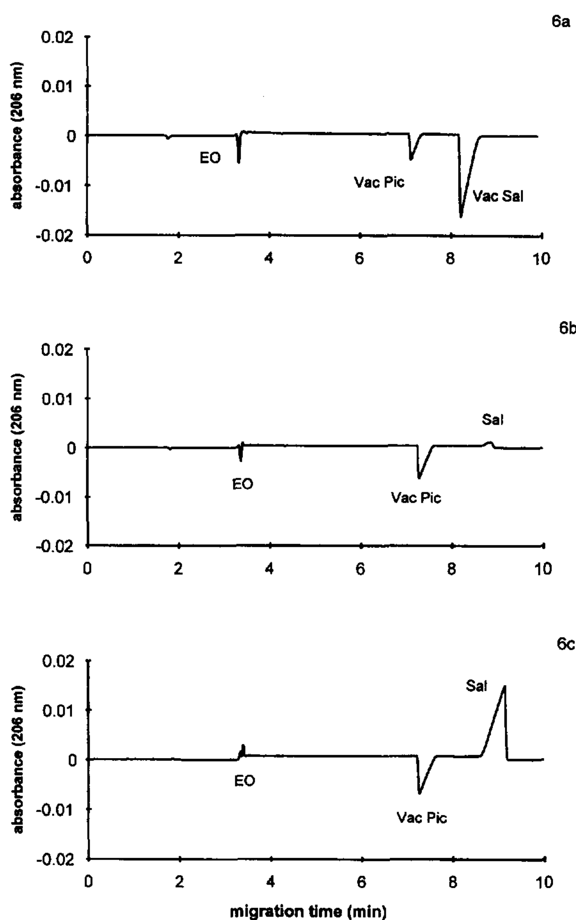

Fig. 6. Effect of the concentration of salicylic acid in reverse CE analysis. BGE: reverse ClAc I. (a) Injection of 5 m*M* ClAc, 10 m*M* Tris; (b, c) injection of 0.8 and 1.6 m*M* salicylic acid, respectively, in 5 m*M* ClAc, 10 m*M* Tris.

ClAc II buffer and various concentrations of direct ClAc served as the sample. It is particularly interesting to observe that the negative EOF peak started to become smaller and smaller on injection of higher concentrations of ClAc, and became positive when the concentration of sample exceeded that of the running BGE. This effect can be explained by the adjustment of the BGE to the KRF value in the sample zone. The EOF peak was negative when the ClAc concentration injected was below the total BGE concentration ($<$20 m*M*) and was positive when the sample concentration was higher ($>$20 m*M*). Fig. 7 also shows that, as for the previous experiments, the benzoic vacancy dip became bigger, on increasing the concentration of the BGE injected.

One of the phenomena that are not commonly understood is that a substantial difference exists between system (vacancy) peaks and indirect detection peaks. Fig. 8 demonstrates this difference, showing an analysis record for an equimolar mixture of potassium and sodium acetate (as the sample), using reverse ClAc II as the BGE. Indirect detection is represented here by the peak of acetate (Ind Ace), which migrates as a fronting zone (tailing peak) and displaces the UV-absorbing benzoate (the peak is negative). Note that this peak represents a migrating species (acetate) that was really present in the sample. The system peak is represented by the vacancy of benzoate (Vac Ben), detected as a negative tailing peak. This peak does not represent any species present in the sample, but one (benzoate) that is absent in the sample while being present in the BGE. The movement of the stationary zone (original sample location) due to electroosmosis is represented by the EOF peak. The sign (positive or negative) and the magnitude of this peak correspond to the adjustment of the BGE concentration to the KRF of the sample. Finally, two real cationic zones migrate in the capillary, since potassium and sodium were injected. The concentration of cations in their zones (adjusted to the KRF of the BGE) cause a local increase in the concentration of the BGE co-ions, as their mobilities are higher than that of Tris. The sign of these two "Kohlrausch" peaks (Koh K, Koh Na) is, therefore, positive.

4. Conclusions

The concept of migrating vacancies is capable of explaining all types of peaks and dips in capillary electrophoresis with UV-absorbance detection. Injection of a sample into the capillary creates a pulse with a composition that is different from the BGE in front of and behind it.

If there is a component of the BGE that is absent in the sample pulse, then the pulse represents a vacancy of such a component and, after applying voltage across the capillary, this vacancy migrates as an individual zone and may be detected. If there are more components missing in the sample and present

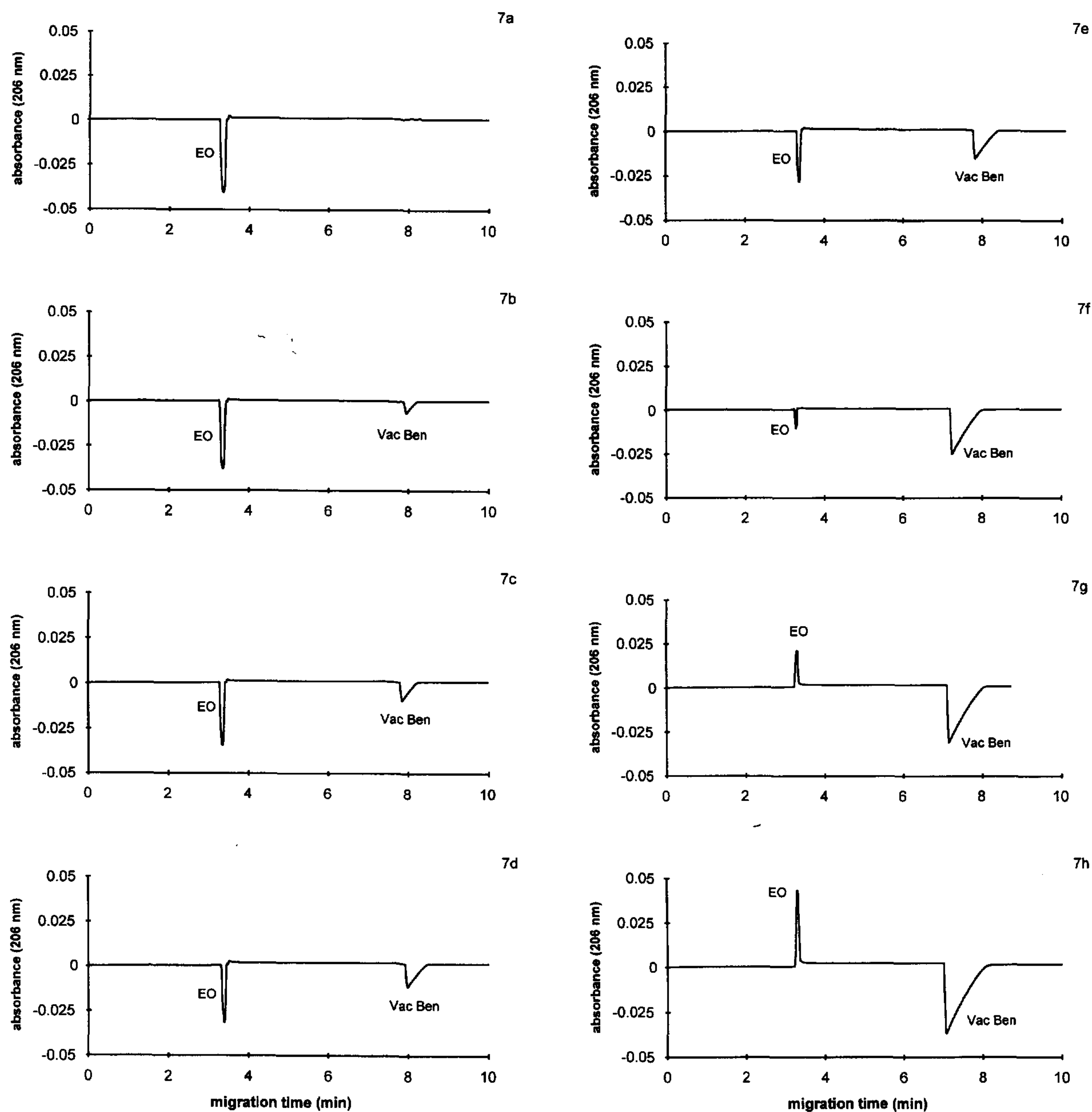

Fig. 7. Effect of sample concentration on the EOF peak and benzoic acid vacancy (Vac Ben) in reverse CE analysis. BGE: reverse ClAc II. (a) Injection of water; (b)–(h) injection of 2.5, 5, 7.5, 10, 20, 30 and 40 m*M* ClAc–Tris (1:2, v/v), respectively.

in the BGE, then the sample represents a vacancy for each individual species that is absent and these vacancies migrate as individual zones and may be mutually separated and detected. The magnitudes of the vacancies created by a sample are proportional to the injected amount of ionic species present in the sample. If a species present in the BGE is also contained in the sample, but at a different concentration, then it may create a migrating vacancy or a positive (normal) peak, depending on the ratio of its concentration in the sample and the BGE. By changing the concentration of such a species in the

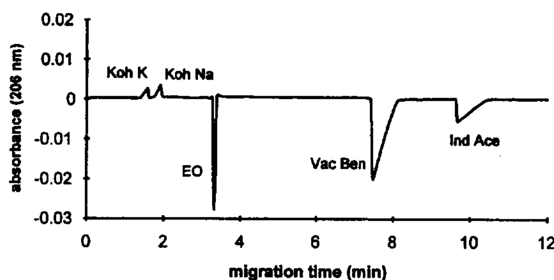

Fig. 8. Indirect detection of a mixture of potassium acetate and sodium acetate (1:1, v/v; 5 m*M* each). BGE: reverse ClAc II. Koh K=Kohlrausch peak of potassium; Koh Na=Kohlrausch peak of sodium; Vac Ben=vacancy of benzoic acid; Ind Ace=indirectly detected peak of acetic acid; EO=electroosmotic flow.

sample (from zero up to values higher than that in the BGE), the vacancy changes into a positive peak. The asymmetry of a migrating vacancy is opposite to that of a positive peak of the given species under the same electrolyte conditions. Hence, if a positive zone is tailing, the related vacancy is fronting and vice versa.

The original sample pulse also creates a zone that is demarcated by stationary boundaries and does not move, due to the applied electric field. Provided that there is no EOF, this zone is stationary and the migrating sample ions are replaced with those of the BGE at a concentration adjusted to the KRF of the sample. In the presence of electroosmosis, the above zone is driven by EOF, and may be detected and serve as a marker for measuring the EOF. Depending on the original KRF of the sample, the EOF peak may be negative or positive. The indirect detection peak of a species and that of its migrating vacancy may appear to be very similar, with both being negative, however, there is a fundamental difference between them. A negative peak on indirect detection of a species corresponds to a species that was present in the sample, whereas a negative peak of a migrating vacancy corresponds to a species that was absent in the sample.

Acknowledgments

This work was supported by the Consiglio Nazionale delle Ricerce, Italy, the Grant Agency of the Czech Republic (grant Nos. 203/94/0998 and 203/96/0124), the Grant Agency of the Academy of Sciences of the Czech Republic (grant No. 431404) and by the Czech Ministry of Education (grant No. VS-96021).

References

[1] J.L. Beckers, J. Chromatogr. A. 679 (1994) 153–165.
[2] H. Poppe, Anal. Chem. 64 (1992) 1908–1919.
[3] G.J.M. Bruin, A.C. van Asten, X. Xu, H. Poppe, J. Chromatogr. 608 (1992) 97–107.
[4] J.L. Beckers, J. Chromatogr. A. 662 (1994) 153–166.
[5] J.L. Beckers, J. Chromatogr. A. 696 (1995) 285–294.
[6] J.L. Beckers, J. Chromatogr. A. 741 (1996) 265–277.
[7] P. Gebauer and P. Boček, J. Chromatogr. A, 772 (1997) 73-79.
[8] T. Hirokawa, K. Nakahara, Y. Kiso, J. Chromatogr. 470 (1989) 21–41.
[9] F. Kohlrausch, Ann. Phys. Chem. 62 (1897) 209–239.