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Short communication

Capillary zone electrophoresis in phosphate buffer – known or unknown?

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Abstract

It has been shown recently that the analysis records in capillary electrophoresis may involve regions with extremely strong electromigration dispersion of peaks. Such a fundamental effect is due to the existence of more centers of symmetry in a given electrolyte system. This paper shows that even such a simple and frequently used electrolyte system as phosphate buffer may exhibit more than one center of symmetry. By using the peak shape diagram approach we have revealed that neutral and alkaline phosphate buffers have two centers of symmetry and one center of extreme dispersion. Model experiments confirmed this new important discovery. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Background electrolyte composition; Peak shape; Phosphate buffers; Inorganic anions; Inorganic cations; Organic acids

1. Introduction

Phosphate buffer is one of the most commonly used background electrolytes (BGEs) used in the contemporary practice of capillary zone electrophoresis (CZE). It has been, e.g., used by Jorgenson and Lukacs [1,2] in their pioneering 1981 work that launched CZE to become a widespread modern analytical method. The overall experience with this easy to prepare and easy to use buffer is excellent and therefore it is one of the first-choice BGEs used when starting to solve a new separation problem. Nobody has, however, so far investigated phosphate buffer in detail with regard to its electromigrational properties [3] that control peak width and peak shape of analyte zones and that may be the source of system peaks and potential disturbance of some analyte zones. It is known that electromigrating

system peaks originate from the presence of multiple BGE co-ions: $n+1$ co-ions correspond to n system peaks [4–6]. Recently it was shown that the solvent ion can play the role of a regular BGE co-ion which results in the presence of system peaks even in simple BGEs with one co-ionic substance when operating in acidic or alkaline medium [7,8]. The knowledge about the number and position(s) of system zones is important for practice because analyte zones co-migrating with system zones exhibit extremely strong (so-called schizophrenic) dispersion [6,9]. This paper presents theoretical and experimental results of the investigation of phosphate buffers from the above mentioned viewpoints and reveals the existence of some unexpected and so far unknown features.

2. Experimental

The theoretical calculations were made using

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simple programs written in QBasic. For the calculation of the peak shape diagram (PSD) values, the algorithm as described in Refs. [6,7] was used, adapted to be applicable also to multivalent anions. The velocity slope values were calculated assuming a current density of 1000 A m^{-2} . The mobilities and $\text{p}K$ values used for the calculations (taken from Ref. [10]) are shown in Table 1.

For CZE experiments, an automated capillary electrophoresis instrument P/ACE 5010 system (Beckman Instruments, Fullerton, CA, USA) equipped with a UV detector set to 214 nm was used. Electrophoretic separations were performed in a coated capillary of 47 cm (40 cm effective length) \times 0.1 mm I.D. (Polymicro Technologies, Phoenix, AZ, USA). The inner surface of the capillary was coated with linear polyacrylamide by the method described in Ref. [11] and modified as in Ref. [12]. Analyses of anions were performed with the cathode at the injection side. The thermostating temperature was 25°C . Samples were injected for 10 s at a pressure of 3.45 kPa. Between runs, the capillary was washed at 138 kPa for 1 min with the BGE. All measurements were performed at a constant voltage (U) of -20 kV . The time (t) axis of the experimental records was transformed to time-based mobility units (lL/tU).

All chemicals used were of analytical purity and were purchased from Lachema Chemapol (Brno, Czech Republic). The sample contained 0.7 mM sodium iodate, 1.3 mM potassium periodate and 1 mM each of sodium nitrate, picric acid, salicylic acid and sulfanilic acid. BGEs were prepared by mixing

stock solutions of KH_2PO_4 , K_2HPO_4 and H_3PO_4 to get the required pH. Deionized water from an aqua purificator G 7749 (Miele, Gütersloh, Germany) was used for the preparation of all solutions.

3. Results and discussion

One of the features that make phosphate buffers so universal is that their working range extends from acidic to alkaline medium. This is due to the fact that phosphoric acid is a trihydric acid with $\text{p}K_a$ values 2.12, 7.47 and 12.36 that ensure good buffering over the entire useful pH range. This range is characterized by the prevailing presence of monovalent and divalent phosphate anions in its acidic and alkaline part, respectively, see Fig. 1. Our theoretical interest was first aimed at investigating the electromigration dispersion (EMD) properties of acidic, neutral and alkaline phosphate solutions to find the regions of zone symmetry/asymmetry and of potential system zones. The PSD method [3,4,7] showed to be an excellent way to perform this study. As the model BGE, 10 mM potassium phosphate was selected at four pH values that correspond to stoichiometrically important compositions corresponding to 5, 10, 15 and 20 mM K^+ (pH 2.55, 4.76, 7.22 and 9.30, respectively, see Fig. 1). For each of these BGEs, the PSD diagram was calculated and an analysis was run with a six-component model sample (see Experimental).

Table 1
Mobilities and $\text{p}K_a$ values of the substances used for calculations and experiments

Substance (abbreviation)	Mobility ^a	$\text{p}K_a$
Picrate (Pic)	31.7	0.71
Iodate	41.9	0.77
Periodate	56.5	1.55
Nitrate	75.4	0.00
Salicylate (Sal)	35.3	2.94
Sulfanilate	33.7	3.13
Phosphate (1) ^b	31	2.00
Phosphate (2) ^b	55	7.22
Potassium	76.5	

^a In $10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$.

^b Values corrected to average ionic strength (0.02).

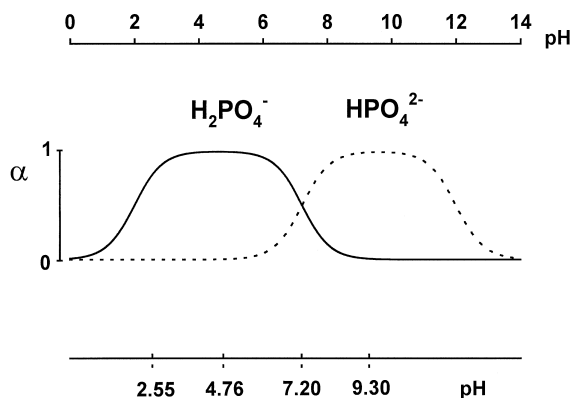


Fig. 1. Distribution diagram of monovalent and divalent phosphate anions in the aqueous pH region. The pH values on the bottom axis relate to Figs. 2–5.

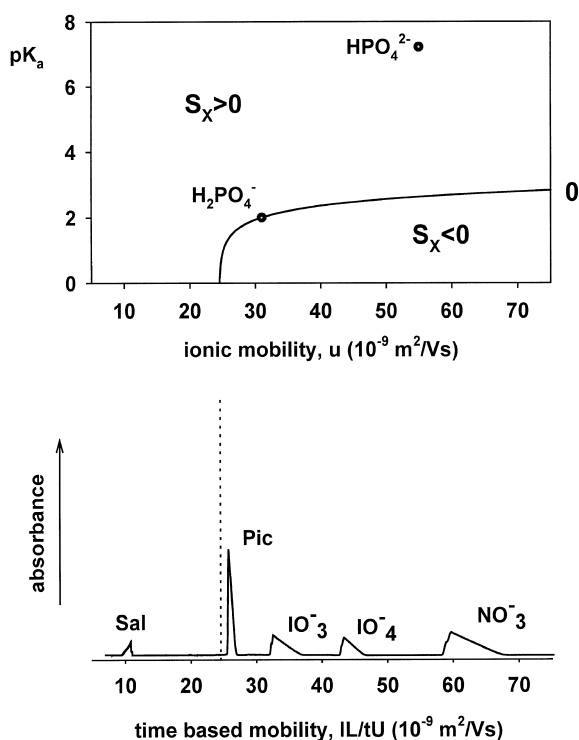


Fig. 2. Upper panel: peak shape diagram (pK_a vs. ionic mobility) of phosphate buffer at pH 2.55 (10 mM phosphate+5 mM K^+). The points correspond to monovalent and divalent phosphate anions ($H_2PO_4^-$ and HPO_4^{2-} , respectively). The curve marked "0" involves points of substances whose zones show zero velocity slope, $S_x=0$. For explanation, see text. Lower panel: experimental UV-absorbance detection record of the model sample in this phosphate buffer. For details, see Experimental.

Fig. 2 shows the results for the acidic system at pH 2.55 (10 mM phosphate+5 mM K^+). The PSD (upper panel) is presented in a simplified form containing only the contour corresponding to the zero velocity slope, $S_x=0$. This curve, comprising also the point corresponding to the monovalent phosphate anion ($H_2PO_4^-$), predicts anions of strong acids (pK_a near 0) to provide sharp symmetric zones around ionic mobility values of $u=25 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. This is confirmed experimentally (see lower panel of Fig. 2) where only picrate provided a sharp and almost symmetric peak. The other (high-mobility) ions exhibit broad fronting zones (corresponding to the $S_x<0$ region in the PSD) whereas the low-mobility salicylate migrated as a slow tailing zone. Sulfanilate was too slow at this pH and did not

practically migrate (its peak is absent). Note that the separation record is transformed to time-based mobility units to be comparable with the mobility coordinate of the PSD.

Fig. 3 relates to the modestly acidic system at pH 4.76 (10 mM phosphate and 10 mM K^+). The PSD shows a shift between the regions of tailing ($S_x>0$) and fronting ($S_x<0$) zones that demonstrates in particular by a shift in the $S_x=0$ contour towards higher mobilities. Its intersection with the horizontal axis (which corresponds to a symmetric peak of a strong anion) is at approx. $32 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. The experimental record confirms this by the picrate peak that corresponds to an already slightly tailing zone and especially by the very sharp sulfanilate zone (net mobility $32 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$).

Fig. 4 gives an answer to the question how the transition between the regions of predominating existence of monovalent and divalent phosphate

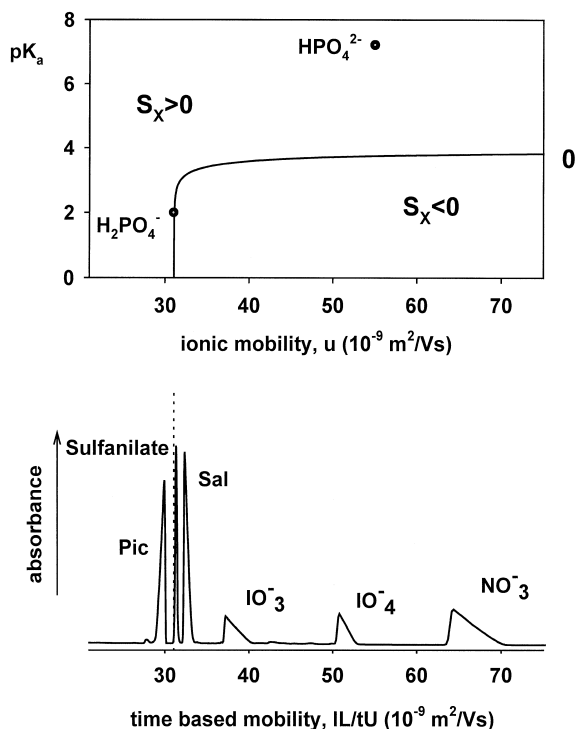


Fig. 3. Peak shape diagram and related experimental record of the model sample for phosphate buffer at pH 4.76 (10 mM phosphate+10 mM K^+). The description of both panels is the same as in Fig. 2.

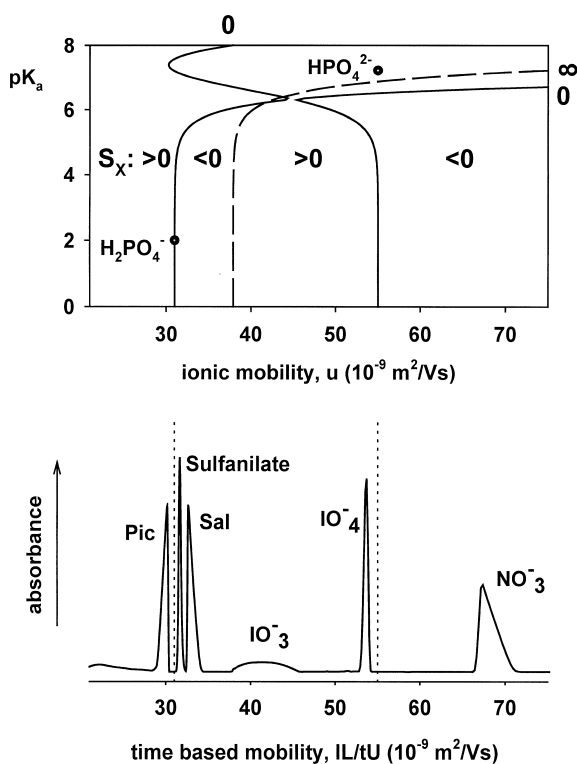


Fig. 4. Peak shape diagram and related experimental record of the model sample for phosphate buffer at pH 7.22 (10 mM phosphate+15 mM K^+). The description of both panels is the same as in Fig. 2. The dashed curve marked “ ∞ ” in the PSD (upper panel) involves points of substances whose zones are predicted to have infinite velocity slope, $S_x = \infty$.

(around phosphate's second pK_a , see Fig. 1) affects the EMD behavior of the phosphate buffer. The calculated PSD for the neutral system at pH 7.22 equal to the pK_2 of phosphate (10 mM phosphate and 15 mM K^+) brought a surprise: there are two zero- S_x contours and one contour corresponding to infinite S_x . This indicates that the system has two regions of tailing zones ($S_x > 0$), two regions of fronting zones ($S_x < 0$), two regions of zone symmetry (see the $S_x = 0$ contours intersecting the mobility axis at approx. 32 and $55 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$) and one region of zone schizoprenia (see the $S_x = \infty$ contour intersecting the mobility axis at approx. $38 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$). Obviously, phosphate buffer at this pH behaves like a buffer with two co-anions. The experiment completely confirms the theoretical predictions. The analyte peaks clearly demonstrate the

presence of two separate tailing to fronting transitions (from picrate to salicylate and from periodate to nitrate). The existence of two centers of symmetry is also obvious: one is close to sulfanilate and the other one is close to periodate. The existence of the schizophrenic region is nicely shown by the extremely dispersed iodate peak. Analytes migrating within this region may be so strongly dispersed that they are even lost for regular detection.

The last investigated phosphate buffer corresponds to modestly alkaline phosphate at pH 9.30 (10 mM phosphate and 20 mM K^+). The PSD (see upper panel of Fig. 5) shows binary co-ionic character also for this system where already the divalent phosphate anion (HPO_4^{2-}) prevails (see Fig. 1). The point corresponding to this highly mobile anion is found already on the low-mobility zero- S_x contour and this

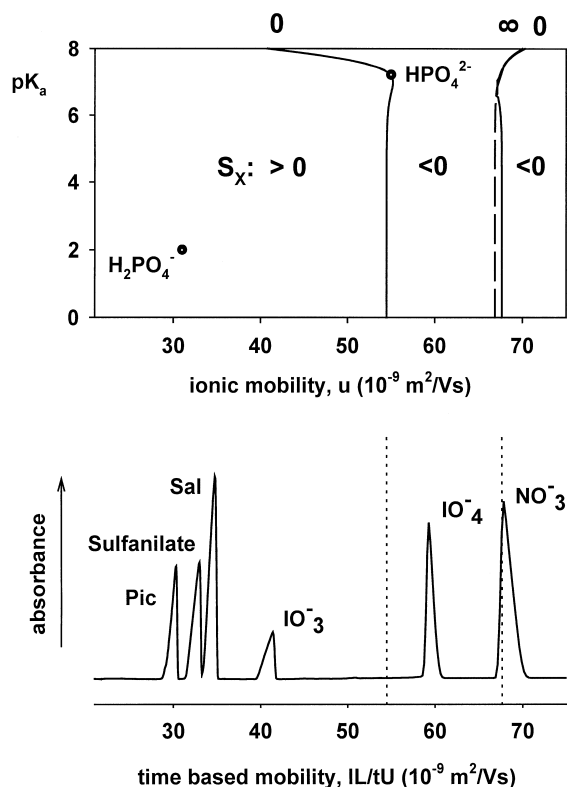


Fig. 5. Peak shape diagram and the related experimental record of the model sample for phosphate buffer at pH 9.30 (10 mM phosphate+20 mM K^+). The description of both panels is the same as in Fig. 4.

affects the partition between regions of tailing and fronting zones. Therefore most of the anions of the model sample show tailing peaks (picrate, sulfanilate, salicylate and iodate, see bottom panel of Fig. 4). Nitrate coincides with both the high-mobility zero- S_x contour and with the $S_x=\infty$ contour that are very close one to the other; this is probably the reason why the nitrate peak is not as sharp as would be expected.

4. Conclusion

Phosphate buffer, although one of the most commonly used BGEs in CZE, may affect crucially the migration behavior of analytes and within neutral and weak alkaline pH it may even deteriorate the analytical results. In this region, phosphate buffer behaves as a binary co-ion electrolyte with two regions of zone symmetry and one region of zone schizophtenia. At pH 7.2, e.g., there are two centers of symmetry at around 32 and $55 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and a region of schizophtenia at around $38 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. This must be taken into account when analyzing mobile anions to correctly interpret the separation records and/or to avoid unwanted zone dispersion and even the loss of the detected peaks.

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