



ORIGINAL ARTICLE

Electrophoretic behavior of charge regulated zwitter ionic buffers in covalently and dynamically coated fused silica capillaries



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Abstract In this work, the electrophoretic behavior of zwitterionic buffers is investigated in the absence of electroosmotic flow (EOF). Electro mobilization of capillary contents is noted when zwitterionic buffers are employed as the background electrolyte at a pH where the buffering moiety carries a net charge. The bulk flow of capillary contents was demonstrated via monitoring the migration of a neutral marker as well as a free and micellar negatively charged marker and SDS–protein complexes. This electrolyte-driven mobilization (EDM) was investigated in detail using 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer over a wide pH range (pH 4.0–8.0). Results confirmed that at a pH where HEPES molecules carry a net negative charge, a bulk flow toward the anode is observed. This was attributed to the migration of HEPES ions toward the anode along with their hydration shells. The relatively large difference in size and solvation number between the ionic buffering moiety and its counter-migrating ions (Na^+ or H^+) resulted in such a net movement. Results indicated that at constant voltage, plotting the measured current versus buffer pH can be used for determination of the isoelectric point of the zwitterionic buffering moiety. Furthermore, this novel mobilization modality was demonstrated using five different HEPES analogs over pH range 5.0–8.0. More in depth investigations are required in order to explore the applicability of EDM in coated capillaries of different wall chemistries and dimensions.

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1. Introduction

A large number of zwitterionic organic buffers “Good’s buffers”^{1,2} are commercially available. They have a good water solubility, low conductivity and low susceptibility to electrolysis at the electrodes.^{1,3–5} Zwitterionic buffers cover a wide pH range including the physiological pH which made them an ideal choice for applications involving biomolecules. Their use in downstream purification,⁶ characterization⁷ and

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analysis^{8–10} of biotechnology-derived therapeutics has been demonstrated by our group to eliminate the need for extensive sample pre-treatment. Zwitterionic buffers have been also employed in capillary electrophoresis (CE) applications employing liposome-coated capillaries.^{11–13} However, an in-depth investigation of the electrophoretic behavior of these buffers has not been reported.

Electroosmotic flow (EOF) is the bulk flow of uncharged liquids relative to a stationary charged surface under the effect of an applied electric field. Since 1809, when EOF was first discovered it has been recognized as the main mobilization force in capillary electrophoresis (CE) and micro-total analysis systems (μ TAS).¹⁴ EOF generates a constant, pulse-free flow that drags analytes, regardless of their charge in the same direction. On the other hand, EOF is sensitive to changes in the chemical composition of the surface, buffer pH and composition and temperature.¹⁵ Preventing the adsorption of analytes, proteins in particular to the wall of fused silica (FS) capillaries require the use of coated capillaries. In such cases the EOF is either partially or completely eliminated.^{8,16,17} However, the retention of some EOF has been one of the characteristics of an ideal coat in order to maintain the pulse-free mobilization.^{16–18} EOF is the most common “electrokinetic pump” and its use in CE and microchips for separations has been extensively investigated.^{14,19,20} However, partial or complete elimination of EOF due to capillary wall coating has limited the usefulness of EOF pumps, as explained above. In the literature, establishing of a new electrokinetic pump has always been dependent on modifying the chemistry of the capillary wall.^{14,19,21}

In this study, the development of a wall-independent, electrolyte-driven mobilization (EDM) will be investigated in covalently and dynamically coated capillaries. The use of zwitterionic organic buffers as background electrolytes (BGE) at pH range where a net negative charge is carried by the organic buffering moiety is explored. The EDM will be demonstrated using both neutral and negatively charged markers of different chemical nature and in capillaries of different dimensions.

2. Experimental

2.1. Chemicals, solutions and samples

All tested zwitterionic buffers were prepared (50 mM) in MilliQ water. Tested buffers are as follows: 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 4-(2-hydroxyethyl)piperazine-1-propanesulfonic acid (EPPS), 4-morpholineethanesulfonic acid (MES), 2-hydroxy-4-morpholinepropanesulfonic acid (MOPSO) and 2-hydroxy-3-[tris(hydroxymethyl) methylamino]-1-propanesulfonic acid (TAPSO). For each buffer, a suitable amount of the buffering moiety was weighed and dissolved in MilliQ water. The pH was adjusted to the required value by careful, drop wise addition of either 0.1 M NaOH or 0.1 M HCl. The volume was then completed so that the final concentration of buffering moiety is 50 mM. All buffers were obtained from Sigma, USA. A CE-grade neutral marker, benzyl alcohol (BA) was obtained from MicroSolv, USA and used without dilution in all experiments. A negatively charged marker, orange G (OG) was obtained from Beckman Coulter, USA and was prepared as 4% v/v OG in 50 mM HEPES buffer (pH 7.0).

2.2. Instruments

An Agilent HP CE 7100 system with a diode array detector (DAD) and a high velocity air flow thermostating was used (Agilent Technologies, Germany). System and data analysis were carried out using Chemstation software (Agilent Technologies, Germany). Ultra-pure water was obtained using a MilliQ UF-Plus system (Millipore, Germany) with a resistivity of at least 18.2 M Ω cm at 25 °C and TOC value below 5 ppb.

2.3. Capillaries

Zeroflow polyacrylamide coated capillaries were purchased from Microsolv (USA) while bare FS capillaries were purchased from Agilent Technologies (Germany). Capillaries were of 50 and 75 μ m id, 325 μ m od and total and effective length of 33.0 and 24.5 cm, respectively. Upon purchasing the Zeroflow coated capillaries, they were tested for lack of EOF according to manufacturer specifications. In agreement to the provided analysis certificate, no peak was detected for a neutral marker when phosphate buffer (pH 7.5) was used. Before each run, polyacrylamide coated capillaries were preconditioned with MilliQ water then with BGE for 5 min each.

In experiments where a dynamic neutral coat was required, bare FS capillaries were treated with polyethylene oxide (PEO) polymer according to a previously optimized protocol developed by our group.⁸ Briefly, capillaries were pre-conditioned with 0.1 M NaOH and 0.1 M H₃PO₄ for 2 min each then MilliQ water for 5 min. Capillaries were then flushed with 0.10% PEO solution (average $M_w \sim 100,000$) that was prepared in the BGE.

2.4. Analysis conditions

Marker samples were injected hydrodynamically at 50 mbar for 10 s. All experiments were carried out at 25 °C and detection was performed at 214 nm. The type and pH of buffer, voltage and polarity are as indicated in each experiment. Throughout the manuscript, positive polarity (normal polarity) indicates that the position of the detector is at the cathodic end while negative polarity (reversed polarity) indicates that the detector is at the anodic end of the capillary, as illustrated in each figure.

3. Results and discussion

The electro-driven bulk movement of the zwitterionic BGE was investigated using FS capillaries of different surface properties. HEPES buffer (pH 7.0) was taken as a model to demonstrate the suggested mobilization phenomenon. HEPES was selected since it is the most widely used member of Good's buffers in biological applications, especially those involving electrophoretic separations as discussed earlier.

3.1. Ion speciation of HEPES

HEPES is a zwitterionic compound with an isoelectric point $pI \approx 5.0$. It contains one sulfonate group that is completely dissociated over almost the whole pH range and two basic nitrogens at positions 1 (N_1) and 4 (N_4) of the piperazine ring

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