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Short communication

Voltage pre-conditioning technique for optimisation of migration-time reproducibility in capillary electrophoresis

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Abstract

An additional tool for pre-conditioning a capillary electrophoretic system is described which involves the application of a short-term voltage (30 kV for 120 s) prior to analysis. Using this technique, migration-time reproducibility was improved to ≤0.25% R.S.D. ($n = 10$). This report comprises the first demonstration of the utility and capillary-to-capillary reproducibility of such a technique. Given the buffering capacity of the electrolyte this is not due to pH stabilisation; other possible mechanisms are discussed.

1. Introduction

While accuracy of results is the goal for a particular analysis under development, the ultimate goal of validation is to ensure that a process achieves its intention precisely and reliably. Reproducibility is therefore a key point in any assay validation. In particular reproducibility of migration time is of importance in peak identification, for repeated fraction collection [1] and for assessing success of method transfer [2]. In CE the apparent mobility of an analyte, and therefore its migration time, is dependent not only upon its own electrophoretic mobility but also upon that of the electroosmotic flow (EOF) since the apparent mobility is an additive quantity. Given an adequate buffering capacity of the electrolyte and stable capillary thermostatting, observed variations in migration times are due to fluctuations of the EOF rather than gross differences in the electrophoretic mobility of the analytes from run to run. The error associated with the migration time of anionic compounds, in normal polarity capillary zone electrophoresis (CZE), is affected to a greater degree than that of cationic species. Further, where a significant EOF is needed, e.g. in SDS-MECC, its reproducibility is of greater importance to the reproducibility of the assay.

The observed hysteresis of the EOF vs. pH response [3,4] and the history of the capillary wall is often of greater importance in determining the degree of EOF present than the immediate pH of the run buffer. Even transient changes or coating on the capillary wall can affect the EOF locally leading to changes in migration times of analytes. Efforts to clean the capillary wall between runs has generally led to the use of NaOH washes, although when operating at low pH it is more reasonable to use washes and pre-treatments with a similar pH [1]. This short study comprises the first report of the efficacy of the use of voltage pre-conditioning. This technique may be considered along with other pre-

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treatment techniques–e.g. alkali, run buffer or acid wash–as another tool which may be employed in the optimisation of migration times and especially in optimisation of EOF reproducibility. This is of particular importance when operating at a high pH where the EOF is less reproducible than at low pH [5].

2. Experimental

2.1. Equipment and chemicals

All separations were performed using the HP^{3D}CE instrument (Hewlett-Packard, Waldbronn, Germany). All chemicals and analytes were supplied by Sigma. CZE separations were carried out using a 100 m*M* borate buffer pH 8.5 and a capillary of 64.5 cm total length, 56 cm effective length and an internal diameter of 75 μm. Samples were detected using a diode-array detector at 254 nm with a bandwidth of 20 nm. Spectra were also collected during the runs for peak identification. Sample was injected for 5 s at 35 mbar and run at 30 kV with capillary temperature thermostatted to 25°C. The test sample included acetaminophen (neutral, peak 1, M_r 151.16), nicotinic acid (pK_a 4.85, peak 2, M_r 184.19), *o*-acetylsalicylic acid (pK_a 3.49, peak 3, M_r 180.15) and 2,4-dihydroxybenzoic acid (pK_a 4.3, peak 4, M_r 154.12).

3. Results and discussion

The CZE conditions provided a well resolved separation of the 4 sample components (Fig. 1a,b). Various preconditioning strategies were used which are summarised in Table 1. The relative standard deviation (%R.S.D.) for migration times for the four peaks are presented in Table 2. In all cases these are %R.S.D. from 10 runs. In this case and contrary to accepted prejudices about the utility of NaOH wash cycles, the omittance of an alkali wash in treatment I improves the migration time reproducibility when compared to that obtained with the alkali wash (II). Continuous use of the system

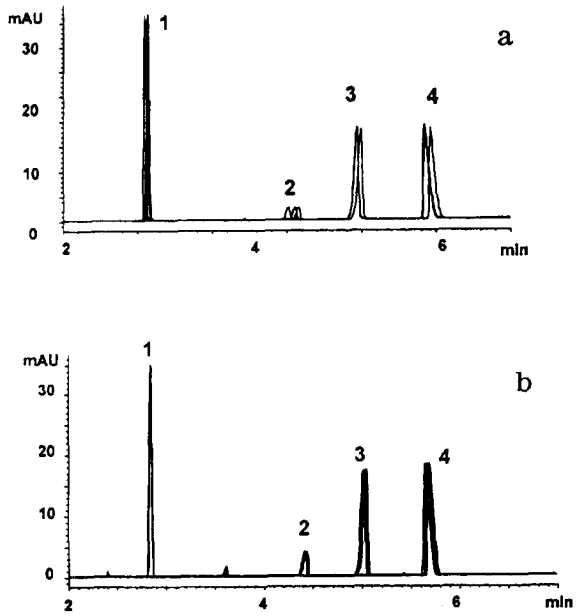

Fig. 1. Separation of analytes (a) using pre-treatment regimen V and (b) pre-treatment regimen VI (see Table 1). All other conditions are as in text. Peaks: 1 = acetaminophen (91 μm/ml), 2 = nicotinic acid (60 μg/ml), 3 = acetyl salicylic acid (150 μg/ml), 4 = dihydroxybenzoic acid (120 μg/ml).

with no washes or replenishment (III) results in a degradation of the migration-time reproducibility, however, washing with buffer from the inlet vial (IV) improves the migration-time reproducibility. In contrast, washing with buffer from an external vial which has not been exposed to an applied voltage (V) produces a less reproducible migration time. When inlet and outlet vials were replenished, the capillary washed with fresh buffer and the entire electrolyte system exposed to a short burst of high voltage (VI) the migration-time reproducibility is markedly improved. The combination of buffer replenishment and voltage conditioning in this instance produced highly reproducibly migration times (Fig. 1).

A repetition of this pre-treatment regimen produced %R.S.D. ($n = 10$) of 0.25% for each of the four peaks, and when used in a fresh capillary with no previous analytical exposure the %R.S.D. ($n = 10$) was 0.11, 0.18, 0.29, 0.29% for peaks 1 to 4 respectively.

These data suggest that exposure to a voltage

Table 1
Treatment numbers and corresponding pre-conditioning regimens

Treatment	Pre-conditioning regimen
I	1-min wash with 0.1 *M* NaOH/replenish inlet and outlet vial 3-min buffer wash (inlet to waste)
II	No NaOH wash Replenish inlet and outlet vials 3-min buffer wash (inlet to waste)
III	No NaOH wash No replenishment No wash
IV	No NaOH wash No replenishment 1-min buffer wash (inlet to waste)
V	No NaOH wash No replenishment 1-min buffer wash (external vial to waste)
VI	No NaOH wash Replenish inlet and outlet vials 1-min buffer wash (inlet to waste) Apply 30 kV for 120 s

prior to an analytical run stabilises the separation system. Although the pH of an electrolyte might be expected to change over the course of time with the application of an electric potential [6,7], the operating pH of this system is within the $pK_a \pm 1$ range of boric acid (p*K* 9.24) which offers maximal buffering capacity. Bello et al. [5] have demonstrated that the applied electric field and the accompanying radial electric field strongly affect the EOF. After demonstrating the decreased reproducibility of the EOF with an increase in pH, they suggested that a probable cause is the interaction of cations with the fused-silica capillary wall. This they ascribe to adsorption and diffusion enhanced by the radial electric field. Cohen and Grushka [8] have also described the irreproducibility of the EOF at higher pH values and suggest that additives such as amines can completely stabilize the EOF. Their assumption being that the amines shield the wall from adsorption of other impurities in the buffer that might otherwise be adsorbed onto the wall and subsequently change the EOF. These reports [5,8] together with the observations reported here suggest that voltage conditioning causes the impacting of cations, or an interaction with buffer contaminants, such that the wall is saturated with these and therefore stabilised prior to operation. The metal-ion chelating effect of EDTA has been demonstrated, in separations of nucleotides, to improve peak shapes. This is presumably due to the

Table 2
Migration-time reproducibility (%R.S.D.) of peaks 1 to 4 ($n = 10$)

Treatment	R.S.D. (%)			
	Peak 1	Peak 2	Peak 3	Peak 4
I	1.98	3.10	3.53	4.12
II	0.70	1.11	1.21	1.47
III	1.63	3.57	4.06	3.91
IV	0.78	0.85	1.03	2.35
V	1.84	3.00	3.38	3.34
VI	0.15	0.21	0.25	0.25

preferential interaction of EDTA with metal ions over the interaction of nucleotides with metal cations [4,9]. If we make two presumptions from these data and observations, i.e. (1) the voltage conditioning procedure stabilises the system by depleting the buffer of contaminants while simultaneously impacting these onto the capillary wall, therefore saturating and stabilising the wall, and (2) the presence of EDTA, in some buffer solutions, removes the metal ions to the extent that their interaction with nucleotides is eliminated and peak shape is improved, then this suggests that even in ostensibly pure buffer salts there are sufficient contaminant metal ions to affect the separation. If the irreproducible EOF presumed to be due to impacting cations is due to impacting metal ions, this suggests that the addition of EDTA to buffers may have some beneficial effects in "mopping up" these cations, although this was not investigated here.

4. Conclusions

This short report describes a novel tool for preconditioning regimens which may be of use in optimising migration-time reproducibility. The application of a voltage to an electrolyte system prior to sample injection and separation provides better migration-time reproducibility than when the buffer is used freshly. The mechanism is thought to derive from an interaction between cations and/or contaminants present in the buffer and the internal capillary wall. The usefulness of this technique on other CE systems remains to be evaluated.

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