Mathematical model of segmented capillary electromigration performances depending on stationary phase physicochemical properties

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Abstract. Segmented capillaries comprising two segments with different surface charges of polymer layers are prepared. One segment with a neutral surface charge is responsible for separation and another one with negatively charged surface provides the electro-osmotic flow adjustment. The average electro-osmotic flow rate is found to correlate with the length of negatively charged segment. A mathematical model is suggested to describe electromigration performances of segmented capillaries depending on the segments length ratio also taking into account the stationary phase/coating with definite surface properties (\(\xi\)-potential) or electroosmotic flow rate.

1. Introduction.
The improvement of separation techniques in electromigration analytical procedures relates to obtaining stationary phases \([1, 7]\) on the internal surface of fused silica capillaries, dynamic coatings \([2]\), pseudo-stationary phases \([3-5]\), additives \([6]\) yielding complexes, ion pairs, solvation, micelles, etc. All the considered approach have certain advantages and drawbacks described in numerous reviews and monographs \([1,2,6,7]\).

So, a disadvantage of stationary phases inside capillaries is the complexity of obtaining a uniform stationary phase in the whole bulk or along the entire inner surface of the capillary \([7]\). In the case of dynamic coatings, the reproducibility of their uniform deposition upon the internal surface of the capillary is limited by the capillary conditioning procedure and deteriorates with each consecutive deposition or rinsing \([2]\). If a dynamic coating is permanently present in a buffer solution, e.g. at DNA sequencing, the reproducibility of separation results is methodically limited by 100 experiments \([1]\).

The development of pseudo-stationary phases in capillaries generally relate to self-organization of magnetic particles slurries into an integral system upon magnetic field application. However, the main problem in this case determined by non-steady behavior of magnetic nanoparticles as a stationary phase due to instability of suspending nanoparticles is still not addressed \([3-5]\).

The addition of special additives into buffer solutions allows the optimization of separation selectivity and efficiency; however this approach is limited by the instability of \(\xi\)-potential at the inner surface of
the capillary. This drawback intrinsic to dynamic coatings [2,6] is determined by the complexity of adjustment of ionization and adsorption activity adjustment for surface silanol groups in fused silica capillaries [1,2,7]. Various kinds of silanol groups have different ionization constants and adsorption activity those results in the instability of electric performances of the capillary surface [7, 8]. Even a small adsorption of admixtures or separated substances significantly deteriorates the separation quality at capillary electrophoresis. For this reason the development of approaches to shielding silanol groups on the surface of capillaries is crucial for the further improvement of electromigration analytical procedures [1,2,7].

In our earlier studies the surface functional composition and surface structure of atactic polystyrene coatings on fused silica plates [9,10] and electromigration performances of capillaries with polystyrene coatings [11] were characterized in order to obtain uniform polymer layers (stationary phases) in capillaries with adjustable hydrophilic-hydrophobic properties. Certain features and trends of polystyrene coating surface energy changes with the polymer concentration variation in the solution were revealed [9], correlations between electromigration performances of polystyrene-coated fused silica capillary columns and components of polystyrene coating surface energy were established [11] and optimal concentration and temperature-kinetic conditions for obtaining polystyrene coatings with adjustable electokinetic performances were defined [9,10,11]. However, the developed methods for the synthesis of stationary phases, like most of the existing approaches to covalent stationary phases in capillaries, afford either electro-osmotic flow (EOF) stabilization within a wide range of background electrolyte pH values or enhance the separation efficiency [2,7,12,13]. In order to address both of these goals, in [12,14] a novel promising approach was suggested on the basis of the development of segmented capillaries (SC) comprising two segments with different polymer layer surface charges, e.g. neutral-anionic or neutral-cationic pairs. The increased separation efficiency in SC is determined by the involvement of a segment with a neutral (non-charged) surface providing the retention and separation selectivity in the absence of irreversible adsorption in couple with another segment bearing a negative (or positive) surface charge affording the adjustment and, if necessary, increase of EOF. In order to study the effect of the ratio between the lengths of the considered segments upon SC electromigration performances, single polymer (PS or PSSA) modified and segmented (involving a neutral polystyrene (PS) and negatively charged polystyrenesulfonic acid (PSSA)) segments onto the internal surface of fused silica capillary followed by a comparative analysis of EOF marker mobility.

2. Method
2.1. Polymer coating synthesis
PS and PSSA polymer coatings on the capillary internal surface were synthesized by the consecutive filling of fused silica capillaries with the corresponding reaction mixtures followed by chemical and thermal initiation of polymerization reactions according to the procedures earlier described in [9-11]. The internal surface of SC was modified using a similar procedure taking into account the requirement to provide the target PSSA:PS ratios 2:1±5% and 1:2±5% with the capillary length up to the detection window \( l_0 = 45 \) cm (Fig. 1.).
2.2. Characterization of electromigration performances

EOF rate was measured using a capillary electrophoresis installation Nanophor-01 (Institute for Analytical Instrumentation, Russian Academy of Sciences) using a standard procedure [1] for the characterization of surface electric properties of fused silica capillary columns and ξ-potential on the sorbent surface. The electro-osmotic mobility $\mu_{eo}$ was determined according to the equation:

$$\mu_{eo} = \frac{l_0}{E \cdot t}$$

(1)

where $l_0$ is the capillary length to the detector, $t$ is EOF marker migration time (s) and $E$ is the electric field strength[1].

Electromigration performances of the prepared modified and segmented capillaries were comparatively studied by measuring EOF.

2.3. Model separation of cations

Preliminary studies were performed in respect of the efficiency of modified capillaries with negatively charged polymer coatings in the separation of inorganic cations (K$^+$; Na$^+$; Mg$^{2+}$; Ca$^{2+}$) in comparison with non-modified fused silica capillary. The selection of inorganic cations mixtures for this research was determined by the problems relating to separation efficiency using standard complexating reagents affecting the resulting peak symmetry [6].
3. Results
3.1. Effect of segment lengths on EOF

Electrophoregrams for EOF marker DMSO in segmented capillaries are shown in Fig. 2.

Figure 2. EOF electrophoregrams in segmented capillaries with PS:PSSA segments ratio 1:2 (a) and 2:1 (b). Effective length 45 cm, total length 50 cm, internal diameter 75 \( \mu \text{m} \), mobile phase — Bicine (0.03 M, pH 9.0), working voltage 15 kV, EOF marker – dimethylsulfoxide, spectrophotometric characterization at \( \lambda = 214 \pm 2 \text{ nm} \).

The obtained EOF marker profiles in both single polymer coated and segmented SC demonstrate the peak symmetry reproducible retention time.

The data on EOF marker mobility measured in modified and segmented capillaries suggest that EOF rate linearly grows with PSSA (negative): PS (neutral) segments length ratio.

Figure 3. EOF rate change depending on PS/PSS-filled capillary segments length ratio. Effective length 45 cm, overall length 50 cm, internal diameter 75 \( \mu \text{m} \), mobile phase 0.03 M Bicin pH 9.0, working voltage 15 kV, EOF marker – dimethylsulfoxide, spectrophotometric detection at \( \lambda = 214 \pm 2 \text{ nm} \).

The analysis of linear correlation in Fig. 2 allowed us to develop a mathematical model to describe and predict EOF rate \( \mu_{\text{eo}} \) in segmented capillaries depending on the segments lengths and stationary phase with known surface properties including \( \xi \)-potential, EOF rate, etc.

\[
\mu_{\text{eo}} = \frac{\mu_{\text{eo1}} I_1}{I_0} + \frac{\mu_{\text{eo2}} I_2}{I_0}
\]  

(2)
where \( \mu_{eo1} \) and \( \mu_{eo2} \) are EOF rates in the columns 1 and 2 respectively, \( l_1, l_2 \) – segment length; \( l_0 \) – overall column length. According to the suggested model, EOF rate \( \mu_{eo} \) in SC is equal to the average value between the intrinsic rates \( \mu_{eo1} \) and \( \mu_{eo2} \) for each of the modified capillaries. The obtained data are in agreement with the results reported in [12] regarding monolithic and printed columns for capillary electrochromatography.

### 3.2. Model separation of cations

The efficiency of inorganic anions mixture separation was comparatively studied for a non-modified capillary with anionic coatings and non-modified fused silica capillaries (i.e. capillary elecrophoresis CE technique) (Fig. 4.).

#### Figure 4. Electrophoregrams of cations mixture separation using a non-modified capillary (a) and hollow anionic capillary column (b). Separation conditions: Nanophor 01 installation, internal diameter of capillaries 50 \( \mu \)m; sample injection within 5 s at 0.05 bar pressure; voltage – 25 kV; background electrolyte – 40 mM benzimidazole, 20 mM tartaric acid, 10 mM 18-crown-6; UV-detection at 267 nm. The observed peaks relate to \( K^+ \) (1); \( Na^+ \) (2); \( Mg^{2+} \) (3); \( Ca^{2+} \) (4) 0.05 M solutions.

The obtained data indicate that the application of modified capillary with anionic coating provides a decrease in migration time and dispersion of \( K^+ \), \( Na^+ \), \( Mg^{2+} \) and \( Ca^{2+} \) peaks as well as an enhanced resolution in comparison with non-modified fused silica capillaries. More detailed data on the comparison of efficiency and selectivity of cations and transport proteins in non-modified, single polymer coated and segmented capillaries will be published in the nearest time.

### 4. Conclusion

Generally, the obtained results indicate that the suggested approach based on the development of capillary columns involving segments bearing different surface charges responsible for various functions (retention/separation and electroosmotic flow stabilization) is promising to provide reproducible steady performances for enhanced electrophoretic separation of various target mixtures.

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