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Application of pH-responsive poly(2-dimethylaminoethylmethacrylate)-*block*-poly(acrylic acid) coatings for the open-tubular capillary electrochromatographic analysis of acidic and basic compounds

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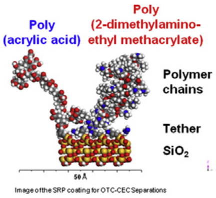
HIGHLIGHTS

- A new type of stimuli-responsive polymeric coating for OT-CEC has been prepared.
- The Y-shaped copolymer enables EOF magnitude and direction to be easily adjusted.
- Acidic and basic compounds analysed under different pH and ionic strength conditions.
- EOF-driven separations achieved with organic solvent free buffer electrolytes.
- Excellent repeatability over many cycles of use in the OTC-CEC mode achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

A new type of stimuli-responsive polymeric (SRP) coating has been prepared for use in open tubular capillary electrochromatography (OT-CEC), by grafting poly(2-dimethylaminoethylmethacrylate)-*block*-poly(acrylic acid) (PDMAEMA-*b*-PAA) as a Y-shaped block copolymer with two dissimilar chain compositions onto the inner walls of aminopropyl-modified silica capillaries. The grafting process introduced weakly charged functional groups from the PAA and PDMAEMA, enabling the generation of

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Abbreviations: APS, (3-aminopropyl)silyl; APTMS, (3-aminopropyl)trimethoxysilane; ATRP, atomic transfer radical polymerization; BGE, background electrolyte; $CHCl_3$, chloroform; DCM, dichloromethane; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; EOF, electroendosmotic flow; HP-CEC, high-performance capillary electrochromatography; HPLC, high-performance liquid chromatography; OT, open-tubular; OT-CEC, open-tubular capillary electrochromatography; SEM, scanning electron microscopy; PAA, poly(acrylic acid); PDMAEMA, poly(2-dimethylaminoethylmethacrylate); PDMAEMA-*b*-PAA, poly(2-dimethylaminoethylmethacrylate)-*block*-poly(acrylic acid); P*t*BuA, poly(*tert*-butyl acrylate); SRP, stimuli-responsive polymer; TFA, trifluoroacetic acid; TLC, thin layer chromatography.

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electroendosmotic flow with magnitude and direction adjustable by changing the pH of the running buffer electrolyte. This stimuli-responsive PDMAEMA-*b*-PAA block copolymer was found to provide excellent resolution of various acidic and basic compounds, leading to efficient analyte separation. When operated in the OT-CEC mode, separation selectivities could be readily manipulated via differential contributions from chromatographic and electrophoretic mechanisms, simply by changing the pH or the ionic strength of the running buffer electrolyte.

1. Introduction

High-performance capillary electrochromatography (HP-CEC) is a hybrid technique that combines the attributes of both chromatography and electrophoresis to achieve unique compound selectivities in analytical separations [1,2]. In the chromatographic mode, compounds are selectively distributed between the stationary and the mobile phases according to their intrinsic hydrophilicity, hydrophobicity and other physicochemical characteristics, resulting in different migration times with selectivity tuned by changing the properties of the stationary phase or the composition of the mobile phase. In the electrophoretic mode, a compound migrates according to its relative electromobility, (μ_a), which is the sum of intrinsic electrophoretic mobility of the analyte, (μ_e) and the electrophoretic mobility of the electroendosmotic flow, EOF (μ_{eof}). The chromatographic and electrophoretic contributions to the overall retention processes can be synergistic or opposed, leading to fine control over the overall separation selectivity and retention for charged compounds due to these differential mobilities [3–6]. Further, one of the important merits of HP-CEC compared to high-performance liquid chromatography (HPLC) is that improved peak efficiencies can also be achieved due to the plug profile of the EOF as opposed to the parabolic flow profiles observed with pressure-driven systems.

Previously, separations in HP-CEC have been carried out in one of four formats as: (i) capillaries packed with micro-particles, (ii) open-tubular (OT) columns, (iii) monoliths, and (iv) microfluidic platforms [7]. The open-tubular capillary electrochromatography (OT-CEC) format offers inherent advantages of facile methods of preparation, avoidance of bubble formation, as well as the opportunity to use higher electric field strengths without creating electrical current instabilities caused by excess Joule heating [8]. In OT-CEC, the stationary phase is present as a thin coating on the inner walls of a fused silica capillary, immobilized either by chemical tethering or by dynamic adsorption. Although OT-CEC has become a useful technique, in particular for gaining insights into electromigration processes *per se*, OT-CEC separations can suffer from low stationary phase to mobile phase volume ratios, which limit analyte loadability and in some cases resolution. Nevertheless, this technique has attracted attention for the analysis of impurities in pharmaceutical samples, neurotransmitters and non-steroidal anti-inflammatory drugs as well as other low molecular weight analytes [9–13] with relatively high levels of run-to-run, day-to-day, and column-to-column precision.

The chemical immobilization of preformed stimuli-responsive polymers of known composition and moderate molecular weight onto the internal surface walls of a capillary represent a promising approach to produce stationary phases for OT-CEC separations. This new approach has the inherent advantages of providing capillary wall coatings of defined thickness with retention and resolution controlled by an external stimulus that directly impacts on the properties of the stationary phase *per se* in a pre-determined manner. By employing preformed stimuli-responsive polymers of known composition and moderate molecular weight as the coating with OT-CEC columns, this approach has the potential to overcome some of the problems associated with capillaries packed with inhomogeneous micro-particulate stationary phases [14,15] or porous layer open tubular (PLOT) columns [16,17], derived by random polymerisation of monomers that generate *in situ* monolithic coatings, and thus provide an avenue to achieve more robust control of the EOF.

Hitherto, various block copolymers have been used as covalent coatings for OT-CEC, but often these materials, particularly when employed in the PLOT format, exhibit tailing/broad peaks, which necessitate the use of organic solvent modifiers as components of the buffer background electrolytes (BGEs). For example, the block copolymer poly(maleic anhydride-*alt*-styrene)$_{119}$-*block*-poly(-styrene)$_{558}$ has been employed as a covalent coating for the OT-CEC separation of five aromatic amines but with relatively broad bandwidths [18]. In other investigations, the amphipathic block copolymer poly(*tert*-butylacrylate)$_{127}$-*block*-poly-(glycidylmethacrylate)$_{86}$ was synthesized by atom transfer radical polymerization (ATRP), and used as a covalent capillary coating of *ca.* 10 nm thickness for the OT-CEC separation of steroids [19]. More recently, the diblock copolymer, poly(butyl methacrylate)$_n$-*block*-poly(-glycidylmethacrylate)$_m$, also made by ATRP methods, was used as a covalent coating with block ratios (n/m) ranging from 71/9, 16/62, 14/4 to 0/63 for the OT-CEC separation of 1,4-dihydropyridines, such as amlodipine, nicardipine and nitrendipine [20]. Collectively, these findings suggest that the use of block copolymers of high molecular weight as OT-CEC stationary phases leads to separations with slow mass transfer kinetics and reduced separation efficiencies, although analyte selectivities may be improved in comparison to bare silica capillaries if organic solvents are employed in the BGE [16]. In contrast, in our work, no organic solvent modifiers are required in the BGE, yet good resolution can still be achieved.

The use of stimuli-responsive polymers (SRPs), coated onto the inner surface of a capillary as a stationary phase has the advantage that the properties of the stationary phase can be tuned by an external stimulus and the swollen (solvated) properties of the polymer chains provide a higher stationary phase volume fraction. These factors make SRPs suitable for specific separation applications. A variety of cationic, anionic and neutral polymers, derived from a single class of monomer, have previously been investigated as stationary phases in OT-CEC [21,22]. With neutral polymer-modified coatings, negligible EOF is generated due to the absence of charged groups, apart from the residual non-derivatized silanols [23].

As documented in the current investigation, with hetero-block-co-polymeric coatings generated from two different classes of monomers, such as poly(acrylic acid) (PAA) and poly(2-dimethylaminoethylmethacrylate) (PDMAEMA), the charged moieties of these polymers can generate a zeta potential suitable to maintain a stable and high EOF. The charged moieties of these stationary phases can also interact via electrostatic mechanisms with complementary groups present in the analytes. For many