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Tuning capillary surface properties by charged polymeric coatings

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ABSTRACT

Separation of proteins in capillary electrophoresis (CE) is often hindered by their interactions with the silanols groups on the inner surface of the fused silica capillary. In particular, the positive charges of alkaline proteins establish electrostatic interactions with the negative charges of the surface, leading to peak broadening and loss of separation efficiency. Moreover, uncontrolled electroosmotic flow (EOF), severely impacts on analyte mobility causing either loss of resolution or loss of efficiency. Among several strategies, coatings are widely employed to mask capillary surface silanols and so to reduce protein adsorption and EOF.

Here we report on the synthesis and characterization of a novel family of adsorbed polymeric coatings, which provide improved performance in terms of prevention of protein adsorption and EOF regulation. In particular, we have added to the polymer backbone, made of *N, N*-dimethylacrylamide, different ionizable monomers (weak acrylamido acids and bases commercially available with the trade name of *Immobilines*) to confer a net positive or negative charge to the polymer chain depending on the buffer pH. As a consequence, the separation of alkaline protein is drastically improved in different pH conditions, because the interactions with the inner capillary wall were completely prevented by electrostatic repulsion. The content of these ionogenic monomers can be adjusted, permitting a perfect control of the surface charge density, so EOF is finely and precisely regulated. We also investigated the conformational variation of the polymer on the surface by changing buffer pH using Dual Polarization Interferometry (DPI). The coating procedure is very simple and fast as it consists in the adsorption of a diluted aqueous solution of the polymer on the capillary walls. In addition, the coating is very stable under harsh conditions, can be used for several runs without any re-conditioning or re-coating steps and it is compatible with MS volatile buffers.

1. Introduction

Capillary electrophoresis (CE) is a well established powerful technique that provides fast and efficient separations of a wide class of charged species, including pharmaceutical products and biopolymers such as proteins [1] [2], peptides [3], polysaccharides [4] and DNA by combining the advantages of electrophoretic separation principles with automation, high-throughput and miniaturization [5] [6].

However, the separation efficiency of compounds in CE is often compromised by their interaction with the silanol groups on the surface of the inner capillary. In particular, the positive charges of alkaline proteins tend to interact with the negative charges of the surface, leading to peak broadening and loss of separation efficiency. Furthermore, uncontrolled electroosmotic flow (EOF), severely impacts on analyte mobility causing either loss of resolution or loss of separation efficiency, due to short or to excessively long migration times, respectively. Hence, an effective regulation of the capillary surface properties is mandatory to obtain efficient and reproducible separations.

Among several strategies, coatings are widely employed to mask capillary surface silanols and so to reduce protein adsorption and EOF [7] [8] [9] [10]. A coating for CE applications must provide a homogeneous surface and it must be stable to shear forces. In addition, species present in the running buffer such as urea and detergents or protein analytes should not detach the coating from the surface or bind to the coating itself.

The use of charged coatings to overcome the issue of protein adsorption onto the capillary wall is a widely exploited approach [11]. In particular, relative large molecular size polymers are known to form a thick layer on the wall through hydrogen bonds or

Abbreviations: EOF, electroosmotic flow; DMA, N,N-dimethylacrylamide; pI, isoelectric point; BGE, background electrolyte; DPI, Dual Polarization Interferometry; I.D, internal diameter; 6-EACA, 6-aminocaproic acid; AcOH, acetic acid; GMA, glycidyl methacrylate; MAPS, [3-(methacryloyl-oxy)propyl]trimethoxysilane; BSA, bovine serum albumine; MES, 2-(N-morpholino)ethanesulfonic acid.

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electrostatic interactions [12]. To generate the so called Successive Multiple Ionic Polymer Layer (SMIL) [13], for instance, ionic and cationic polymers have been adsorbed on the surface to form alternate layers. Although this procedure revealed to be versatile and easy to perform, it has several drawbacks. First of all, due to the high charge density of the polymer, the EOF is not adjustable and the analyses are performed using a strong anodic EOF. Secondly, successive polymer layers can cause capillary clogging while the process requires an overnight coating procedure, several washing steps and a long conditioning before the analysis. Furthermore, there are situations in which the coating can be removed from the surface, for example when strong basic conditions or organic solvents are used as running buffers. On the contrary, a covalently bound coating exploits the presence of an anchoring group, such as a silane moiety, to modify permanently the inner surface of the capillary. This method is, usually, more difficult to perform and control, but the obtained coating shows a superior performance in several applications, above all CE-MS analysis [14]. Recently, we have introduced a class of hydrophilic polymeric coatings which self-adsorbs onto fused silica capillary by a combined physi-chemisorption mechanism. Their main feature is the ability to regulate EOF intensity and to prevent protein adsorption on the capillary wall [15]. These poly(*N,N*-dimethylacrylamide)-based copolymers display the simultaneous presence of chemically reactive groups (*N*-acryloyloxysuccinimide or glycidyl methacrylate) and silane groups in the backbone, which, after a thermal incubation, condensate with the surface silanols resulting in formation of highly stable films.

Here we report on a recent evolution of this family of adsorbed polymeric coatings, which provides improved performance in terms of prevention of protein adsorption and EOF regulation. In particular, we have added to the backbone of one of the polymer previously reported, different ionizable monomers (weak acrylamido acids and bases commercially available with the trade name of *Immobilines*) to confer a net positive or negative charge to the polymer chain, depending on the buffer pH. As a consequence, the separation of alkaline or acidic protein is improved in different pH conditions because the interaction with the capillary wall is completely prevented by electrostatic repulsion.

In this work, we also investigate the conformational variation of the polymer on the surface by changing buffer pH. A similar study was conducted by Spuhler and collaborators [16] in a different context. The availability of Immobilines with different pK_a values, allows the synthesis of several different polymers able to confer different properties to the capillary wall. Moreover, the copolymerization of both weak acrylamido acids and bases yields an amphoteric coating that adopts a net positive or negative charge simply by changing buffer pH. The content of these ionogenic monomers can be adjusted, permitting a perfect control of the surface charge density when the capillary wall is bathed in a given buffer pH.

The coating procedure is another main advantage of these polymers: it is very simple and fast as it consists in the adsorption of a diluted aqueous solution of the pre-synthesized polymer on the capillary wall, which can be performed online directly into the CE instrument. In addition, the coating is very stable under harsh conditions, can be used for several runs without any re-conditioning or re-coating steps and it is compatible with MS volatile buffers.

2. Materials and methods

2.1. Chemicals

Ethanol (EtOH), NaOH, HCl, ethanolamine, ammonium sulfate, tris(hydroxymethyl)aminomethane (Tris), bicine, phosphoric acid (H_3PO_4), 6-aminocaproic acid (6-EACA), acetic acid (AcOH), acrylamide, 2-(*N*-morpholino)ethanesulfonic acid (MES) *N,N*-dimethylacrylamide (DMA), glycidyl methacrylate (GMA), [3-(methacryloyl-oxy)propyl]trimethoxysilane (MAPS), acrylamido buffers (Immobiline) pK_a 3.6, 7.0, 8.5, 10.3, tetrahydrofuran (THF), azobisisobutyronitrile (AIBN), ammonium sulfate ($(NH_4)_2SO_4$), sodium chloride, sodium phosphate monobasic, acetonitrile, aluminum oxide, petroleum ether, lysozyme, cytochrome C, and ribonuclease A were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Gel permeation chromatography analyses were performed using a Jasco 880 system connected to a UV detector Jasco Uvidec-100-III, using Schodex columns OHpak SB-G (guard column), OHpak SB-804 M HQ, OHpak SB-803 HQ, and OHpak SB-802.5 HQ (New York, NY, USA). The spectra were analyzed using the Chrom-NAV Chromatography data system software (Jasco, MD, USA).

Fused silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA).

Separations were performed using a P/ACE^{TM} MDQ Capillary Electrophoresis System with UV detector from Beckman Coulter Inc. (Brea, CA, USA).

Dual polarization interferometry (DPI) measurements were conducted using an Analight Bio 200 (Farfield Group, Biolin Scientific, Manchester, UK) running Analight Explorer software. Analight chip were pretreated using a HARRICK Plasma Cleaner, PDC-002 (Ithaca, NY, USA) connected to an oxygen line.

2.2. Synthesis of poly(DMA-GMA-MAPS-Acrylamido buffer)

In a three-neck round bottom flask, 20 mL of anhydrous THF was degassed by purging argon for 20 minutes. DMA (3.67 g; 37.02 mmol; filtered over aluminum oxide to remove the inhibitor), GMA (0.23 g; 1,60 mmol) acrylamido buffer (1 mL of 200 mM stock solution; for the amphoteric polymer add 1 mL of 200 mM stock solution of Immobiline pK_a 8.5 and 0.5 mL of 200 mM stock solution of Immobiline pK_a 3.6) and MAPS (0.10 g; 0.42 mmol) were added under argon flow. Subsequently, AIBN (0.01 g; 0.08 mmol) was added under argon flow, and the solution was polymerized at 65 °C for 2 h. The reaction was stopped by cooling to room temperature; the polymer solution was diluted 1:1 by adding 20 mL of anhydrous THF and precipitated in 400 mL of petroleum ether. The product was collected as a white powder by filtration with a Buckner funnel and dried under vacuum at room temperature.

The structure of the polymers is reported in Fig. 1.

2.3. Polymer Characterization

The size of each polymer was characterized using Gel Permeation Chromatography (GPC) in tandem with a UV detector. The GPC setup consists of three Shodex aqueous GPC columns in series: OHpak SB-G (guard column), OHpak SB-804 HQ, OHpak SB-803 HQ and OHpak SB-802.5 HQ. Each column is packed with a polyhydroxymethacrylate gel and connected in series with a decreasing exclusion limit. The columns were maintained at 40 °C throughout each run. The relative molecular mass is calculated using a calibration curve obtained using polyacrylamide standards with molecular mass ranging from 22 KDa to 400 KDa.

Dry copolymers samples were diluted using the GPC mobile phase (GPC buffer: 100 mM NaCl, 50 mM NaH_2PO_4, titrated to pH 3.5 using H_3PO_4, 10%v/v acetonitrile) to a concentration of 0.25 mg/mL and the samples were run three times through the GPC system to test for reproducibility. Each run injected 20 μL of sample to be analyzed and the flow rate through the system was held at a constant 0.35 mL/min.

The GPC analyses of poly(DMA-GMA-MAPS-Immobiline pK_a 7.0), poly(DMA-GMA-MAPS-Immobiline pK_a 8.5), poly(DMA-GMA-MAPS-Immobiline pK_a 10.3) indicate the polymer have,

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