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Neutral hydrophilic coatings for capillary electrophoresis prepared by controlled radical polymerization

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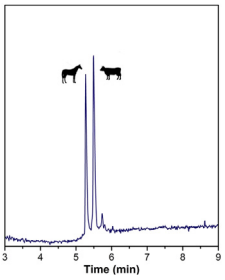
HIGHLIGHTS

- A controlled radical polymerization scheme is devised for stable, non-fouling CE coatings.
- Polymers are surface-initiated from α-bromoisobutyryl groups under mild conditions.
- High separation efficiency and complete solute recovery are obtained for lysozyme.
- Clog-free capillaries inherently result from the surface-confined grafting method.

GRAPHICAL ABSTRACT



Cytochrome C variants on a poly(acryloylaminoethanol)-coated capillary

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ABSTRACT

In the present study, porous silica particles as well as impervious fused-silica wafers and capillary tubes were modified with hydrophilic polymers (hydroxylated polyacrylamides and polyacrylates), using a surface-confined grafting procedure based on atom transfer radical polymerization (ATRP) which was also surface-initiated from α-bromoisobutyryl groups. Initiator immobilization was achieved by hydrosilylation of allyl alcohol on hydride silica followed by esterification of the resulting propanol-bonded surface with α-bromoisobutyryl bromide. Elemental analysis, IR and NMR spectroscopies on silica micro-particles, atomic force microscopy, ellipsometry and profilometry on fused-silica wafers, as well as CE on fused-silica tubes were used to characterize the chemically modified silica substrate at different stages. We studied the effect of monomer concentration as well as cross-linker on the ability of the polymer film to reduce electroosmosis and to prevent protein adsorption (*i. e.*, its non-fouling capabilities) and found that the former was rather insensitive to both parameters. Surface deactivation towards adsorption was somewhat more susceptible to monomer concentration and appeared also to be favored by a low concentration of the cross-linker. The results show that hydrophilic polyacrylamide and polyacrylate coatings of controlled thickness can be prepared by ATRP under very mild polymerization conditions (aqueous solvent, room temperature and short reaction times) and that the coated capillary tubes exhibit high efficiencies for protein separations (0.3–0.6 million theoretical plates per meter) as well as long-term hydrolytic stability under the inherently harsh conditions of capillary isoelectric focusing. Additionally, there was no adsorption of lysozyme on the coated surface as indicated by a complete recovery of the basic enzyme. Furthermore, since polymerization is confined to the inner

Abbreviations: EOM, electroosmotic mobility; ATRP, atom transfer radical polymerization; AFM, atomic force microscopy; AAE, *N*-acryloyl-aminoethanol; AAP, *N*-acryloyl-aminopropanol; HEMA, 2-hydroxyethyl methacrylate; HEA, 2-hydroxyethyl acrylate; Me_6TREN, tris[2-(dimethylamino)ethyl]amine; bpy, bipyridine; PIPPS, piperazine-*N*, *N*′-bis(3-propanesulfonic acid); CIEF, capillary isoelectric focusing; MOPS, 3-(*N*-morpholino)propanesulfonic acid; Hb, hemoglobin.

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capillary surface, simple precautions (*e.g.*, solution filtration) during the surface modification process are sufficient to prevent capillary clogging.

1. Introduction

Fused-silica capillary tubes used in GC and micro-bore LC have also found extensive use in the modern version of electrophoresis, CE [1,2]. In contrast with chromatography, chemical modification of the fused silica capillary has been aimed primarily at eliminating unwanted interactions between the inner wall of the CE tube and the sample undergoing separation. Many chemical modifications schemes used to modify silica-based chromatographic substrates have been readily extended to electrophoretic capillaries [3–5]. Strictly speaking, the type and number of active surface functionalities on silica surfaces (silanols, siloxanes, etc.) depend on factors such as synthesis method, thermal history and the presence of humidity. Additionally, the irregular porous structure of silica micro-particles governs the accessibility of reagents to the active silica sites during surface modification. This kinetic factor aside, the chemistries of porous and flat silicas are essentially equivalent. It is also accepted that wafer surfaces are geometrically and chemically equivalent to the inner surface of fused-silica capillaries; they are both flat at the molecular level. Surface coverage resulting from organic groups grafted on a flat silica surface should be significantly denser than that of the same groups on a curved surface (particulate porous silica) since the later is sterically more constrained resulting in dissimilar group conformations [6].

One of the greatest challenges in the current practice of CE is the requirement of surface deactivation of capillaries toward protein adsorption. Such unsolved bottleneck arises from the nonspecific interactions (ion exchange, hydrogen bonding, dispersion, etc.) between the inner capillary wall and the solute. Such unwanted interactions are responsible for excessive peak tailing, incomplete solute recovery and unreliable quantification. As a result, the high speed, separation efficiency, selectivity and versatility, minimal sample size requirement and easy automation of CE cannot be fully exploited in the case of many proteins. Researchers have suggested that a polymeric film that furnishes a hydrophilic, stable and adsorption-resistant (non-fouling) barrier between the capillary wall and the solute is required in protein analysis [7,8]. Such materials are also of great interest in many other fields such as medical sciences, biosensors, contact lenses technology, enzyme-based immuno assays, to name just a few [9].

The attachment of polymers to solid surfaces via chemical bonding is an important strategy to modify the substrate properties in such a manner that the nonspecific interactions between the protein molecules and silica surface are minimized [7,10]. Anchoring of a polymer onto a silica surface has been achieved by two main strategies, the “grafting-to” and the “grafting-from” methods. The grafting-to approach involves the attachment of a prefabricated polymer from solution, via the formation of a covalent bond between polymer active groups and matching groups on the substrate surface. Water-soluble polymers such as poly(-ethylene glycol) and poly(vinyl alcohol) are typical examples of preformed polymers used in the grafting-to technique. Hydroxyl groups of the polymer react with surface-immobilized active species, such as glycidyl [11]. Despite its experimental simplicity, the grafting-to strategy is limited by strong steric hindrance effects that worsen with increasing polymer size and eventually hinder contact of incoming polymer with surface reactive sites [10]. In the grafting-from approach, propagating polymer chains grow from surface-immobilized initiator groups. Among the various procedures for the grafting-from method, atom transfer radical polymerization (ATRP) is especially attractive for its remarkable control over the molar mass of the grafted polymer, great versatility (works well with a variety of functionalized monomers), compatibility with water and the possibility of mild polymerization temperatures [12–14]. In the ATRP-based grafting-from approach, chain transfer and thermal self-initiation processes are essentially negligible and polymeric chains grow exclusively from the surface; *i.e.*, the process is both surface-initiated and surface-confined. The initiator moieties are most usually anchored to the silica surface via silane coupling chemistry. Halogenated ATRP initiator groups, such as benzyl chloride or, to a much greater extent, 2-bromo-isobutanoyl have been used in the past to grow neutral hydrophilic films on fused silica capillaries. Wirth and her research group reported the first covalent bonding of a polymer film for CE by ATRP in 1998 [15]. Surprisingly, very few papers have been published about ATRP applied to CE since then [16–18].

Although acrylamide has been the most commonly used monomer to make polymeric coatings for CE [4,7], the limited stability at moderately high pH of polyacrylamide has been a well-known fact from traditional slab gel electrophoresis [19]. The slow deamidation of the polymer under this condition leads to a considerable deterioration of the coating, evidenced by the formation of fully-dissociated carboxylic groups that cause strong electroosmosis, polymeric layer swelling and analyte band distortion. The use of *N*-substituted acrylamide derivatives such as *N*-acryloylamino-ethoxyethanol, has resulted in coated capillaries with superior resistance to hydrolysis and hence improved long-term CE separations at alkaline pH [20]. *N*-acryloyl-aminoethanol (AAE) [21–23] and its relative *N*-acryloyl-aminopropanol (AAP) [24,25], also *N*-substituted acrylamide derivatives, should provide durable coatings as well. It appears that the *N*-substitution with hydroxyl-terminated chains produces polymeric coatings that are not only hydrolytically more stable, but have also higher hydrophilicity compared to polyacrylamide [19,24,25]. When it comes to electrophoretic performance, hydrophilicity turns out to be of paramount importance since a high hydrophilicity of the polymer effectively precludes proteins to compete with water for its potentially adsorptive sites [26]. It has been suggested that any monomer less hydrophilic than acrylamide should be considered unsuitable to produce a good quality gel “since acrylamide is itself already at the border-line between hydrophilicity and hydrophobicity” [25].

There is a definite need for hydrophilic coatings of improved hydrolytic stability that enable the use of CE at its full potential, and such improved materials are frequently targeted at protein separations. In the present work we explore the combination of several promising synthetic schemes to modify the fused-silica surface of capillaries for a lasting resistance to protein adsorption. More specifically, our work attempts to bring together the best of three worlds: (i) a stable anchorage of the polymerization initiating group to the inner wall of the capillary tube by means of Si–C linkages formed by hydrosilylation; (ii) a stable polymeric film whose strength arises from the *N*-substitution on acrylamide; and (iii) a surface-confined *in-situ* polymerization method (ATRP) that