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A block copolymer covalent coating acting as surfactants in separation of 2-[hydroxy(4-nitrophenyl)methyl]-cyclopent-2-enone and 4-nitrobenzaldehyde by capillary electrophoresis

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ABSTRACT

An innovative block copolymer capillary coating P(MAn-alt-St)$_{127}$-b-PSt$_{592}$, synthesized by maleic anhydride and styrene, was developed as a new kind of coating for capillary electrophoresis. The covalent bond coating was effectively applied in the separation of raw material (4-nitrobenzaldehyde) and production (2-[hydroxy(4-nitrophenyl)methyl]-cyclopent-2-enone) in a Baylis–Hillman reaction using ammonium acetate with 20% tetrahydrofuran (v/v) as the buffer solution. Electroosmotic flow measurement gave an instantly stable value after 70 times continued injection in 5 days and showed that P(MAn-alt-St)$_{127}$-b-PSt$_{592}$ coatings could suppress electroosmotic flow effectively compared with the bare capillary. The effects of tetrahydrofuran and the pH of buffer on the separation were investigated. The characteristics of the coatings to form micelles similar to surfactants were detected by atomic force microscopy. Moreover, the superiority of this coating was further applied in the separation of four aromatic amines.

1. Introduction

Capillary electrophoresis (CE) is a separation technique with high efficiency, short analysis time, a small amount of sample and reagent consumption, which has been widely utilized for analysis. An understanding and control of the chemistry of capillary surface is essential to exploit the power of CE.

Since surfactant coatings are inexpensive, flexible and easy to apply in manipulating the surface charge of the capillary, they have become increasingly popular in capillary coatings [1–3]. Numerous types of surfactant coatings have been employed, including: "aggregate coating" and "semi-permanent coating" [4].

However, for "aggregate coatings", some of the surfactant monomers must be present in the running buffer to maintain the coatings; the major shortcoming is that free surfactant molecules in the background electrolyte could interfere with the separation or detection scheme. Furthermore, the presence of surfactant would suppress ionization in mass spectrometry (MS) detection, and thus would make these surfactant coated capillaries incompatible with this increasingly popular and powerful method. For "semi-permanent coating", attributed to form bilayer at the capillary wall [3], the coatings are sufficiently stable and the excess surfactants are removed from the capillary, so separations are performed with no surfactant in the running buffer. But perhaps the main drawback is their irreproducibility.

Many kinds of block copolymer have been used in capillary coatings as an alternative choice in recent years [5,6]. Some block copolymers have played an important role in the separation of proteins, deoxyribonucleic acids and oligonucleotides as separation medium [7,8]. The most interesting thing is that these block copolymer coatings have high sieving ability and can self-assemble to transient networks with appropriate mesh sizes or star like micelle structures [9] in selective conditions and the driving force mainly comes from the hydrophobicity in one of the blocks.

Among these block copolymers, only few of them such as the amphiphilic triblock copolymer $E_{99}P_{69}E_{99}$ (F127) forming micelles

Abbreviations: CE, capillary electrophoresis; MS, mass spectrometry; HPCE, high performance capillary electrophoresis; NMR, nuclear magnetic resonance; GPC, gel permeation chromatography; AFM, atomic force microscopy; SEM, scanning electron microscope; EOF, electroosmotic flow; *R*s, resolution; MEKC, micellar electro kinetic capillary chromatography; CMC, critical micellar concentration; RSDs, relative standard deviations; CTAB, cetyltrimethylammonium bromide; MAn, maleic anhydride; St, styrene; THF, tetrahydrofuran; SDS, sodium dodecyl sulfate; SDC, sodium deoxycholate; DMSO, dimethylsulfoxide; AIBN, azo-bis-isobutryonitrile; BTBA, (S)-benzyl dithiobenzoate; APTES, 3-aminopropyltriethoxysilane; HNE, 2-[hydroxy(4-nitrophenyl)methyl]-cyclopent-2-enone; NBA, 4-nitrobenzaldehyde.

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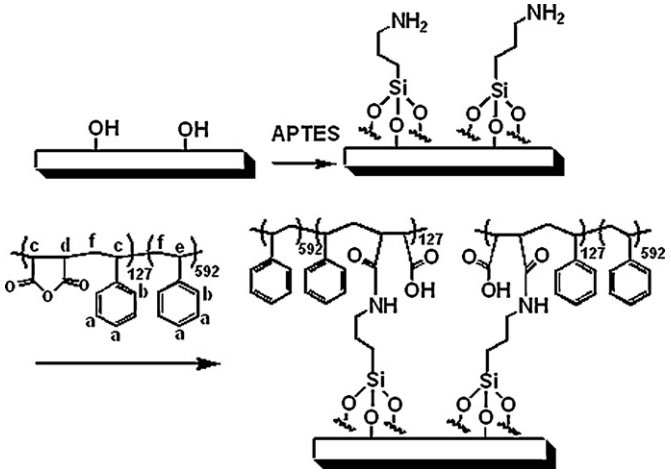

Scheme 1. Coating processes of P(MAn-alt-St)$_{127}$-b-PSt$_{592}$ block copolymer.

in solution [9] and block copolymer consisting of polyethylene glycols end-capped with micelle-forming fluorocarbon tails [10] used as surfactants in CE separation. Some other block copolymers also can form micelles on Si [11] or SiO_2 [12] but not in capillary. Unfortunately, these block copolymers are physically adsorbed or dynamically coated in capillary with low reproducibility. However, covalent bonding has been reported as an effective measure to improve reproducibility of polymer coatings [13–15]. Therefore, exploring new kinds of covalent bonded block copolymer acting as surfactants will be an active area of research.

In this work, a novel covalent bonded block copolymer coating in the capillary, which can be expected to play the role of surfactants in the CE separation, has been investigated. We have synthesized a well-defined block copolymer having alternating segments constituted by maleic anhydride (MAn) and styrene (St): P(MAn-alt-St)$_m$-b-PSt$_n$ (Scheme 1). The segment in the block copolymer can easily be hydrolyzed resulting in an amphiphilic block copolymer which can form uniform nanoscale structures by self-assembly. The architectural particles in nanoscale size were constructed by PSt segment as a hydrophobic head group and P(MAn-alt-St) segment as a hydrophilic tail, which was similar to the configuration of surfactants. Thus the innovative capillary coatings have been developed and applied in the separation of the raw material (4-nitrobenzaldehyde) (NBA) and production (2-[hydroxy(4-nitrophenyl)methyl]-cyclopent-2-enone) (HNE) [16] in the Baylis–Hillman reaction. In addition, the separation of four kinds of aromatic amines by the coated capillary has further confirmed that the copolymers indeed play the role as surfactants.

2. Experimental

2.1. Instrumentation

A 1229 high performance capillary electrophoresis (HPCE) analyzer (Beijing Institute of New Technology and Application, Beijing, China) was used to carry out all electrophoretic experiments. Unless stated otherwise, separations were performed at 25 °C in block copolymer coated capillaries (Yongnian Optical Fiber Factory, Hebei, China) of 75 μm I.D. × 70 cm (55 cm effective).

Capillary coating process was as follows: The bare capillary was washed [17] with 1.0 M NaOH for 2 h, followed with pure water, 1.0 M HCl for 10 min, then with pure water and acetone. After complete removal of acetone by nitrogen, tetrahydrofuran (THF) and 3-aminopropyltriethoxysilane (APTES) (50/50, v/v) were flowed through the capillary and kept at room temperature (25 °C) for 12 h. After the reaction, the modified capillary was washed repeatedly with chloroform for 5 min. The modified capillary was coated by rinsing with the block copolymer solution (30.0 mg block copolymer dissolved in 1.0 mL chloroform) for 1 h. Then it was placed at room temperature for 3 h before rinsing with methanol for 2 min. A detection window (about 2 mm width) was made directly by burning off the block copolymer inner capillary and polyimide coating outside.

Prior to injection, the coated capillaries were sequentially rinsed with water and running buffer for 2 min each. A sample was separated at +20 kV and siphoned for 10.0 s in 15.0 cm height. The separated bands were detected by UV absorption at 254 nm and acquired at 4 Hz. Peaks were identified by spiking relative standard HNE, NBA and aromatic amines in sample solutions and the peaks with increased height were considered to be the targets.

Elemental analysis was carried out on FLASH EA1112 (Thermo, America) and the characterization of polymer coating was detected by an S-4300 scanning electron microscope (SEM, Hitachi, Japan). Atomic force microscopy (AFM) phase images were obtained by a Nano-Scope III MultiMode AFM Digital Instruments at room temperature. Si tips were used with a resonance frequency of approximately 300 kHz and a spring constant of about 40 N m^{-1}. The scan rate was in the range 0.5–1.2 Hz. Three flat glass of similar material to the capillary were coated with block copolymer for AFM measurements in air.

Gel permeation chromatography (GPC) was performed on a set of a Hitachi L-2130 pump with a Waters 2410 refractive index detector, and a Waters 2487 ultraviolet detector with the combination of Hersteller MZ-Gel SDplus 5 μm, porosity 10^3, 10^4, 10^5, and 10^6 Å. THF was used as the eluent with a flow rate of 1.0 mL/min and polystyrene standards were used for calibration.

^{1}H nuclear magnetic resonance (^{1}H NMR) observation of the block copolymers was performed on Bruker Avance 400 spectrometer (Bruker biospin, Switzerland) (400 MHz). A 4.0-mg block copolymers dissolved in 0.5 mL deuterated chloroform containing 0.03% tetramethylsilane for NMR measurements.

2.2. Chemicals

APTES was purchased from Acros (New Jersey, USA). P-phenylenediamine, aniline, 4-bromoanilines, α-naphthylamine, NBA, THF, dimethyl sulfoxide (DMSO), chloroform, sodium dodecyl sulfate (SDS), cetyl trimethyl ammonium bromide (CTAB) and other chemicals were analytical reagent grade and obtained from Beijing Chemical Factory (Beijing, China). Sodium deoxycholate (SDC) was purchased from Beijing Aoboxing Biological Technology Ltd. (Beijing, China). Azo-bis-isobutryonitrile (AIBN) was purchased from Shanghai Chemical Plant (Shanghai, China) and MAn was obtained from Guangfu reagent (Tianjin, China). They were re-crystallized before use. Styrene (St) was got from Shanghai Chemical Plant (Shanghai, China) and (S)-benzyl dithiobenzoate (BTBA) utilized without further purification. HNE was synthesized in our laboratory [16]. DMSO was used as the neutral marker in the electroosmotic flow (EOF) determination.

2.3. Solutions preparation

All solutions were prepared in triply distilled water produced by a distillation apparatus model SZ-97 (Yarong Biochemical Instrument Co., Shanghai, China) and stored at 4 °C. CE running buffers, unless stated otherwise, were composed of 30.0 mM boric acid, and adjusted to pH 10.5 with 1.0 M NaOH or 30.0 mM ammonium acetate, adjusted to pH 7.0 with 1.0 M ammonia before THF was added. Before use, all the running buffers were filtered through a membrane filter with 0.45 μm pores and degassed by sonication for 2 min.