Journal of Chromatography A, 1499 (2017) 111–117

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Layer-by-layer self-assembly of polyelectrolyte multilayers on silica spheres as reversed-phase/hydrophilic interaction mixed-mode stationary phases for high performance liquid chromatography

Wenjie Zhao[a], Xiuming Jiang[a,*], Shujiao Ni[a], Yun Guo[b], Lijun He[a], Guoqiang Xiang[a], Shusheng Zhang[b]

[a] *School of Chemistry and Chemical Engineering, Henan University of Technology, Zhengzhou 450001, PR China*
[b] *College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou 450001, PR China*

ARTICLE INFO

Article history:
Received 26 October 2016
Received in revised form 9 March 2017
Accepted 30 March 2017
Available online 31 March 2017

Keywords:
Stationary phase
Layer-by-layer self-assembly
Polyelectrolyte multilayers
Reversed-phase/hydrophilic mixed-mode chromatography
Poly(ethylene-*alt*-maleate)

ABSTRACT

In this study, a series of stationary phases, comprising silica spheres and polyelectrolyte multilayers (PEMs) as the core and surface coating, respectively, were prepared via a layer-by-layer self-assembly approach for use in high-performance liquid chromatography. PEMs coatings were formed by the alternate deposition of positively charged poly(allylamine hydrochloride) and negatively charged poly(ethylene-*alt*-maleate) anions which were synthesized by esterification between poly(ethylene-*alt*-maleic anhydride) and *n*-alkyl alcohols ($C_nH_{2n+1}OH$, n = 4, 8, 10, 12). The chromatographic performance and retention mechanism of the new stationary phases were evaluated in simultaneous reversed-phase/hydrophilic interaction mixed-mode chromatography using different solute probes, such as alkyl benzene, *p*-hydroxybenzoic acid and its esters, anilines, phenols, and pyrimidines. The separation performance of the new stationary phases was optimized by tuning the *n*-alkyl chain length of poly(ethylene-*alt*-maleate) and bilayer numbers of PEMs. These results indicated that the new stationary phases demonstrate promise for the simultaneous separation of complex hydrophobic and hydrophilic samples with high selectivity.

1. Introduction

Conventionally, the separation of small molecules by chromatography is mainly based on a single mechanism, such as hydrophobic interactions for the separation of nonpolar compounds on a reversed-phase packing and hydrophilic interactions for the separation of polar compounds with high efficiency under the hydrophilic mode. Nevertheless, with the development of chromatography, mixed-mode chromatography (MMC) has emerged as a new type of chromatography that can utilize multiple interaction between the stationary phase and analytes in order to achieve good separation. Compared to conventional single-mode chromatography, MMC exhibits the main advantage of separating two or more completely different analytes using the same column with different separation modes [1–4]. Recently, various mixed-mode stationary phases, such as those used for reversed-phase or ion-exchange [5–9], hydrophilic or ion-exchange [10–13], reversed-phase or hydrophilic [14–21], and reversed-phase or normal-phase chromatography [22–24], have been reported for the separation of both small molecules and macromolecules. Wang et al. have prepared a mixed-mode C_{18}-Diol stationary phase and reported good performance for the simultaneous separation of acids, bases, and neutrals by both reversed-phase liquid chromatographic (RPLC) and hydrophilic interaction chromatographic (HILIC) [18]. Liu et al. have prepared a mixed-mode stationary phase based on click amino-modified silica for ion chromatography (IC) and HILIC, and applied in the separation of polar compounds including nucleosides, organic acids and bases [13]. Guo et al. have immobilized β-cyclodextrin on a silica surface for the mixed-mode separation of flavone glycosides by HILIC/RPLC [19].

Over the past few decades, the layer-by-layer (LbL) self-assembly of thin films has been attracting considerable interest because of its ability to exert nanometer control over film thickness and its wide variety of usable materials for coating planar and particulate substrates [25]. In brief, by the alternate immersion of the substrate into two aqueous solutions of oppositely charged materials, LbL thin films of the two materials are electrostatically bound together and can be built up to very large numbers of mul-

---

* Corresponding author at: Lianhua Road 100, Zhengzhou 450052, PR China.
E-mail address: jxm1965@haut.edu.cn (X. Jiang).

http://dx.doi.org/10.1016/j.chroma.2017.03.083
0021-9673/

tilayers (a pair of such layers is referred to as one bilayer). By thorough rinsing between each deposition step, excess material is washed away from the previous step while leaving sufficient surface charge for the next material to be further electrostatically deposited. Thus, uniform, dense, and robust stacks of layers can be deposited on the substrate surface with a wide variety of configurations. A desired film thickness can be achieved by the simple termination of the procedure after the desired number of bilayers (BLs) is attained. According to the procedure mentioned above, polyelectrolyte multilayers (PEMs) can be formed by the alternate deposition of layers of oppositely charged polyelectrolyte molecules onto a solid substrate. The choice of materials allows for the engineering of responsive and functional thin films for various applications, such as drug delivery [26,27], surface modification [27–29], biosensors [30,31], photonic devices [32], and nanoreactors [33]. However, to the best of our knowledge, few applications of PEM coatings on silica gel for use as HPLC stationary phases have been reported, attributed to the doubts of their stability under the long scour of the mobile phase [34]. Indeed, the strong stability of these coatings has permitted the successful preparation of stationary phases for electrochromatography and gas chromatography via the electrostatically driven deposition of polyelectrolytes onto the inner wall of a fused-silica capillary [35–39].

In this study, a series of polyanionic poly(ethylene-*alt*-maleate) esters (PEME) with different side-chain length of *n*-alkyl alcohols ($C_nH_{2n+1}OH$, $n = 4, 8, 10, 12$) was synthesized. By employing the layer-by-layer self-assembly technique, which involved the alternate immersion of silica spheres into positively charged polyallylamine hydrochloride (PAH) and negatively charged PEME aqueous solutions, various PAH/PEME multilayer HPLC stationary phases, namely SiO_2@(PAH/PEME-C_n)$_m$ ($n = 4, 8, 10, 12$; $m = 4, 6, 8$) (m represents the number of bilayers) were prepared. The chromatographic performance and retention mechanism of the new stationary phases were evaluated for simultaneous reversed-phase/hydrophilic interaction MMC using different solute probes, such as alkyl benzenes, *p*-hydroxybenzoic acid and its esters, anilines, phenols, and pyrimidines. In addition, the effects of the *n*-alkyl chain length and bilayer numbers on the chromatographic behavior were also investigated.

2. Experimental

2.1. Apparatus and materials

Silica gel (particle size of 5 μm, pore size of 100 Å, and specific surface area of 300 $m^2 g^{-1}$) was provided by the Lanzhou Institute of Chemical Physics, Chinese Academy of Science (Lanzhou, China). Poly(ethylene-*alt*-maleic anhydride) (PEMA, Sigma-Aldrich) was dried at 70–120 °C for 4 h before use. 1-Butyl alcohol, 1-octanol, 1-decanol, 1-dodecanol, and PAH were purchased from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China) and used without further purification. Unless specified otherwise, all chemicals and solvents were of analytical reagent grade and purchased from the Beijing Chemical Plant (Beijing, China). HPLC-grade methanol and acetonitrile were purchased from the Luzhong Reagent Plant of Shanghai (Shanghai, China). Water was purified using a Milli-Q purification system (Millipore, USA).

Elemental analysis (EA) was performed with a Flash EA 1112 elemental analyzer (Thermo Electron, USA). Fourier-transform infrared spectra (FTIR) were recorded with a Bruker Vector 22 instrument (Bruker, USA). NMR spectra were recorded with a Bruker DPX 400 spectrometer (Bruker, USA) using solvent peaks as reference. Brunauer–Emmett–Teller (BET) surface area, pore volume, and pore size distribution were measured on a NOVA 2000e surface area and pore size analyzer (Quantachrome, Florida, FL, USA) using nitrogen adsorption at 77 K in the range $0.02 \leq P/P_0 \leq 0.20$.

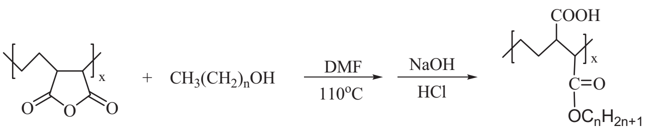

Fig. 1. Synthesis process of PEMA- $C_nH_{2n+1}OH$ (n = 4, 8, 10. 12).

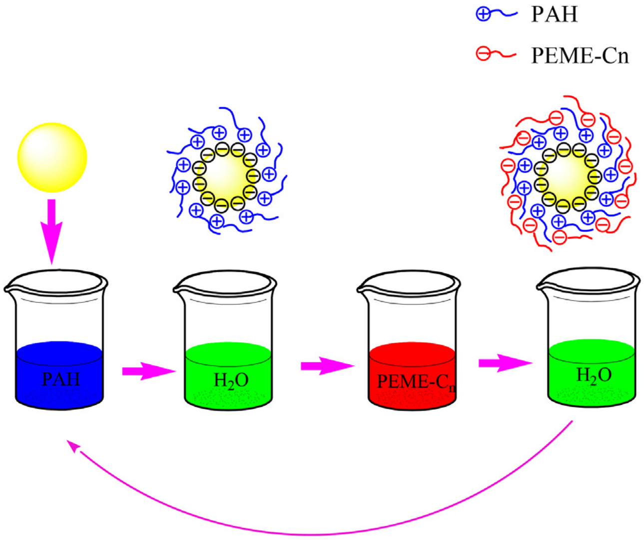

Fig. 2. Preparation process flow of polyelectrolyte multilayers coating using layer-by-layer self-assembly technique on silica spheres.

2.2. Preparation of PEME polyanions

PEME polyanions were prepared by the esterification of PEMA and *n*-alkyl alcohols ($C_nH_{2n+1}OH$, $n = 4, 8, 10, 12$). First, PEMA (10 mmol) and *n*-alkyl alcohols (11 mmol) were dissolved in dimethylformamide and charged into a pre-dried 2-neck round bottom flask. The mixture was stirred at 110 °C for 7 h under N_2. After cooling the reaction mixture to room temperature, the product was precipitated into distilled water and rinsed three times with petroleum ether to remove the residual *n*-alkyl alcohols. After the product was dissolved in a 1 mol L^{-1} sodium hydroxide solution, the product was precipitated again by using a 1 mol L^{-1} dilute hydrochloric acid solution to adjust the pH. The purified PEME polyanions were dried at 50 °C for 24 h in a vacuum oven before use. Fig. 1 shows the preparation process.

2.3. Preparation of SiO_2@(PAH/PEME-C_n)$_m$ stationary phases

Fig. 2 shows the schematic of the LbL self-assembly technique for polyelectrolyte coating. The silica gel surface exhibited a negative charge; hence, the first single layer should be assembled with PAH. Silica spheres were dipped in a 3.0 M PAH solution for 30 min, followed by rinsing with ultrapure water. The PAH adsorbed substrates were then dipped into a 3.0 M PEME aqueous solution 30 min, followed by rinsing with ultrapure water. Thus, the first bilayer was deposited on the silica surface. The alternating deposition of PAH and PEME was continued by repeating the steps described above until different numbers of bilayers were obtained.