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Tunable thick porous silica coating fabricated by multilayer-by-multilayer bonding of silica nanoparticles for open-tubular capillary chromatographic separation

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ABSTRACT

A simple coating procedure employing a multilayer-by-multilayer process to modify the inner surface of bare fused-silica capillaries with silica nanoparticles was established. The silica nanoparticles were adsorbed onto the capillary wall via a strong electrostatic interaction between amino functional groups and silica particles. The thickness of the coating could be tuned from 130 to 600 nm by increasing the coating cycles from one to three. Both the retention factor and the resolution were greatly increased with increasing coating cycles. The loading capacity determined by naphthalene in the column with three coating cycles is 152.1 pmol. The effects of buffer concentration and pH value on the stability of the coating were evaluated. The retention reproducibility of the separation of toluene was 0.8, 1.2, 2.3, and 4.5%, respectively, for run-to-run, day-to-day, column-to-column, and batch-to-batch, respectively. The chromatographic performance of these columns was evaluated by both capillary liquid chromatography and open-tubular capillary electrochromatography (OT-CEC). Separation of aromatic hydrocarbons in the column with three coating cycles provided high theoretical plate numbers (up to 269,280 plates m^{-1} for toluene) and short separation time (<15 min) by using OT-CEC mode. The method was also used to separate egg white proteins. Both acidic and basic proteins as well as four glycoisoforms were separated in a single run.

1. Introduction

Capillary electrochromatography (CEC) combines the high efficiency of capillary electrophoresis (CE) with the high selectivity and diversity of applications of liquid chromatography (LC). Three main modes of CEC that are generally distinguished are packed [1,2], monolithic [3], and open-tubular column CEC (OT-CEC) [4]. Among all the CEC columns, open-tubular column has important advantages over packed capillary column including the ease of preparation, the absence of bubble formation, and simple instrumental handling [5].

In a typical OT column, a single layer of stationary phase is deposited onto the capillary inner wall, leading to relatively low capacities and relatively poor resolution of analytes [6]. In order to resolve this shortcoming, some approaches including sol–gel-derived phases [7,8], etching [8,9], porous layers [10–20], and nanoparticle phases [21–28] have been developed to increase the surface area of the OT columns. Since polymer based materials can provide a thick coating and can be formed relative easily, most of the published works were focused on fabricating a coating onto capillary column via a polymerization reaction [29]. Compared to other materials, nanoparticles have the merits of large surface area and ease of preparation. Therefore, nanoparticles of silica [30], gold [26,31–35], titanium dioxide [36–38], and grapheme oxide nanosheets [39–41] have been immobilized onto the inner surface of capillary column. However, although these types of nanoparticles have been used successfully for chromatographic separation, in most cases, columns were coated with only a single layer. Some of the columns were coated with a few layers of nanoparticles [28,30,34,37–42]. However, the thickness of the coating was still very thin. The reason for that is properly due to the preparation of surface bonded porous multilayers of nanoparticles within capillary columns is a considerable challenge. As a result, the phase ratios of these open-tubular columns are still not high enough even

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though the nanoparticles possess high surface area. Thus, in order to fabricate the OT column with higher phase ratio, it is necessary to develop a method to prepare a thick coating with nanoparticles.

Among all the nanoparticles mentioned above, silica nanoparticles are the object of intense research due to their promising physical, mechanical and chemical properties. Moreover, their applications have been intensely proposed in several analytical chemistry fields. Previously, porous silica layer was prepared by dynamic coating [42], static coating [43–45], liquid phase deposition [30], or sol–gel method [46]. However, the layer thickness, obtained by all of these coating procedures, was either too thin or uncontrollable.

Layer-by-layer (LBL) method is a powerful method to design and application of function-specific films at the nanoscale level [47]. By using this method, capillary column coated with multilayers of gold nanoparticles was prepared by Liu et al. [48]. Compared to the methods of coating Au nanoparticles onto the capillary wall via chemical bonding [22] or evaporation [49] that only a thin layer of gold nanoparticles was coated, a thick film like a porous layer was obtained by using LBL method. Moreover, by using LBL method, the merits of nanoparticles were preserved. Although LBL method is a powerful method used to prepare a thick coating with controllable thickness, however, normally only one layer is formed after each modification cycle. Therefore, a numerous modification cycles should be done to fabricate a thick coating. Recently, Forster et al. reported fabricating a silica coating with the thickness up to 500 nm using a sol–gel method [50]. However, the thick coating could be achieved only in the capillary with an inner diameter smaller than 15 μm. In this paper, we described the preparation of a thick coating with tunable thickness in capillary column through a multilayer-by-multilayer (ML-B-ML) deposition of silica nanoparticles. Compared with typical LBL method that only one layer was formed in each coating cycle, multilayers were formed in each coating cycle by using ML-B-ML method. As a result, coating thickness up to 600 nm could be prepared in the capillary of 75 μm I.D. by only three coating cycles. Moreover, the thickness of the shell could be tuned from 130 to 600 nm through simply changing the coating cycles. To the best of our knowledge, deposition of silica nanoparticles via ML-B-ML method has never been employed for applications in open-tubular separation.

2. Experimental

2.1. Materials

Octadecyltrichlorosilane was purchased from Acros Organics. (3-Aminopropyl)diethoxymethylsilane (3-AMDS) was purchased from Aladdin Chemistry (Shanghai, China). Analytical grade thiourea, toluene, naphthalene, biphenyl, 2-methylnaphthalene, acenaphthene, and HPLC-grade methanol (MeOH) were all purchased from Shanghai Chemical Reagent, Inc. of Chinese Medicine Group (Shanghai, China). Avidin, ovotransferrin, ovalbumin, ovomucoid, ovoflavoprotein, and lysozyme were obtained from Sigma (St. Louis, MO). All chemicals were used without any further purification. Water used in all of the experiments was Robust pure water purchased from a supermarket. Fused silica capillary (75 μm i.d. × 365 μm o.d.) was purchased from Yongnian Rui-feng Fiber Plant (Handan, China).

2.2. Preparation of capillary column coated with silica nanoparticles

There are four main steps in fabrication process: (i) A bare fused silica capillary was flushed with 1 M NaOH for 1 h, water for 5 min, 0.1 M HCl for 30 min, water for 5 min, and acetone for 5 min, respectively. (ii) After drying with nitrogen, the capillary was flushed with 200 μL of 3-AMDS toluene solution (1 vol%) to modify the inner surface of the capillary column through a covalent interaction between hydroxy groups and silane groups. The capillary was kept at room temperature (25 °C) for 30 min and then flushed with nitrogen for 5 min. (iii) Then the capillary was flushed with 0.1 M HCl for 1 h to protonize the amine group. (iv) To produce a layer of silica nanoparticles, the capillary was subsequently flowed with 500 μL of silica sol (HS-20, Zhejiang Yuda Chemical Industry Co. Ltd. The silica nanoparticles are stabilized with H^+ and the pH value is 1.5–3. Particle size: 15–30 nm. The content of silica in the solution is 20 ± 1% (w/w).). The pH value of silica sol was adjusted to pH 3 by the addition of 0.1 M NaOH solution. The silica sol was kept in the capillary column for 2 h and then washed with water for 5 min. More coating cycles could be done by repeating operations of (ii) to (iv). To be used for chromatographic separation, thus prepared capillary column was further derivatized with octadecyltrichlorosilane by passing a toluene solution containing 10% (v/v) octadecyltrichlorosilane through the capillary, which was kept in the column for 2 h and heated at 105 °C. The excess octadecyltrichlorosilane was removed from the capillary column by pumping with toluene and then flushing with ethanol and distilled water.

2.3. Chromatographic separation

A Beckman MDQ P/ACE system (Beckman, Fullerton, CA) with an on-column DAD detector was used for all OT-CEC and OT-CLC experiments. Stock solutions of 1.0 mg mL^{-1} of each sample were prepared in MeOH. Standard working solution was prepared by diluting the standard stock solution with MeOH-H_2O (50/50, v/v). A stock background electrolyte was prepared by dissolving an exact amount of phosphate in water. The pH value of the phosphate solution was adjusted to the range of 3.0–9.0 by the addition of 0.1 M NaOH or 1.0 M phosphoric acid solutions. The mobile phase was obtained by mixing the phosphate solution with the appropriate amount of water and MeOH. Chicken eggs were obtained from the local supermarket. The egg white and egg yolk were separated. The egg white was diluted with an 8-time volume of phosphate buffer solution (20 mM, pH 7.6). Prior to carrying out the chromatographic separation, all solutions were filtered through a 0.22 μm membrane filter (Schleicher & Schuell, Dassel, Germany) and degassed in an ultrasonic bath for 15 min before use.

2.4. Characterization

The general morphology of the capillary wall coated with silica nanoparticles was characterized by field emission scanning electron microscopy (FESEM, Hitachi S-4800 II, 15 kV).

3. Results and discussion

Fig. 1 shows the typical SEM images of capillary column coated without and with different layer of silica nanoparticles. It can be seen that the inner surface of fused silica capillary column is very smooth after being treated with 1 M NaOH. After coating, the capillary wall became a little rough. From the cross section of the column, it can be found that a uniform coating was formed. The thicknesses of the coating were determined to be ~160, ~350, and ~600 nm for one cycle, two cycles, and three coating cycles, respectively. It was surprised that about six layers of silica nanoparticles were formed after only one coating cycle. As a comparison, normally only one layer of silica nanoparticles could be absorbed when polyelectrolytes such as PDADMA (poly(diallyldimethylammonium chloride)) were used as matrix to incorporate nanoparticles. Therefore, to achieve a thick coating, tens or even hundreds of polyelectrolytes layers are required