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Dynamic layer-by-layer self-assembly of multi-walled carbon nanotubes on quartz wool for on-line separation of lysozyme in egg white

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ABSTRACT

The multi-walled carbon nanotubes (MWNTs) coated quartz wool (MWNTs/QW) prepared by dynamic layer-by-layer self-assembly was used as solid-phase extraction (SPE) absorbent for on-line separation and preconcentration of lysozyme in egg white. The coating procedures were performed continuously in a flow system operated by a set of sequential injection devices. The quartz wool was placed in a microcolumn forming a loose packing to guarantee the minimized flow impedance and the intimate contact between proteins and absorbent surface. Various parameters affecting SPE efficiency including the volume, pH, ionic strength and flow rate of sample and eluent were systematically studied. The feasibility of the proposed method was validated by successfully applied to the separation of lysozyme in egg white.

1. Introduction

Lysozyme is the most important protein in egg white (3.5%) composing of 129 residues (14.3 kDa) [1]. It is also a special component of honey and the preservative of wine, and can prevent the growth of acid-forming bacteria. Therefore, it is important to determine the concentration of lysozyme in real samples. Because of the low content of lysozyme in real samples and the interferences of complex matrixes, the separation and preconcentration steps are necessary prior to analysis. The separation methods of lysozyme include ultrafiltration[2], precipitation[3], chromatography[4], reverse micelles extraction[5], solid-phase extraction (SPE) [6] and so on. Among these methods, SPE has the advantages of simple operation, rapidness, less environmental pollution, and easy combination with modern analytical techniques.

The research and applications of carbon nanotubes (CNTs) have been growing fast in chemistry, physics, materials, and life technologies fields due to their unique electrical, mechanical, optical and chemical properties since their discovery in 1991 [7–9]. Their large surface areas, unique surface property and high biocompatible nature offer excellent potential as solid-phase extraction absorbent for the separation and pre-concentration of a large variety of analytes, such as organic pollutants, metal ions, organometals, proteins and so on [10]. However, due to the aggregation tendency of carbon nanotubes, previous attempts for applying them in a flow-through mode had limited success because of limited sorption capacity and high backpressure in cartridges or lab-made-packed columns [11]. The disadvantage of using CNTs as flow-based SPE absorbent also includes continuous leaking of CNTs, resulting in poor analytical performance. In order to take advantage of the specific features of CNTs and to improve the analytical performance of on-line SPE system, the new CNTs-based adsorbent needs to be developed to overcome the above shortcoming.

In the past several years, the considerable attention has been paid to using CNTs as building blocks for novel high-performance materials [12,13]. In order to achieve optimized overall performance for on-line SPE, the matrix for CNTs should have the following characteristics: (1) homogeneous size; (2) lower flow resistance; (3) moderate mechanical strength; (4) high specific surface area. Quartz wool (QW) is a widely available material with high specific surface area and sufficient charge density, and is mainly used as small plugs placed in an adsorption column or a chromatography tube to hold the adsorbent in place. It has been also used for extraction of metal ions and organic pollutions [14–16], immobilization of biomacromoleculars [17] and cell separation [18]. In the present study, quartz wool was used as the support of multi-walled carbon nanotubes (MWNTs), to provide an economic, simple and efficient method for protein separation. In our previous work, the MWNTs coated SiO_2 beads had been prepared by layer-by-layer (LBL) self-assembly for protein separation and preconcentration [19]. The CNTs composites were also successfully used as SPE absorbent for metal ions and chromatographic stationary phase for the separation of aromatic compounds [20,21].

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Theoretically, the quartz wool could also be coated by MWNTs via LBL self-assembly of charged polyelectrolytes and oxidized MWNTs based on electrostatic interactions. However, it is nearly impossible to perform the LBL self-assembly strategy on quartz wool in the same way as on SiO_2 beads or glass slide. Because the quartz wool is very thin and long, the polyelectrolyte functionalized quartz wool tends to gather together and forms small lumps, which made it hard for the inner quartz wool to get functionalized further. In addition, there was visible loss after only few cycles. Taking into account the fact that the conventional static LBL adsorption is a laborious and time-consuming process, a new and reproducible assembly strategy needs to be developed.

The dynamic layer-by-layer technique is a growing approach for the functionalization of capillary, microporous membrane and hollow fiber [22–24]. The dynamic LBL self-assembly method could enhance the regularity of the assembled layers and allow reduction of the deposition cycles for same separation factor [25]. The aim of the present study was to apply the dynamic LBL self-assembly method to facilitate the preparation of MWNTs coated quartz wool (MWNTs/QW). The assembly process was successfully accomplished by alternatively dynamically filtrating poly(diallyldimethylammonium chloride) (PDDA) and poly(sodium 4-styrenesulfonate) (PSS) solution through the quartz wool packed microcolumn in a flow system operated by a sequential injection (SI) system. The polyelectrolytes layers were used to minimize the untoward interactions of proteins with the silanol groups on QW and supply sufficient charge for the deposition of MWNTs on the outermost layer. The practical applicability of the obtained MWNTs/QW was then validated by on-line solid-phase extraction of lysozyme in egg white in the same SI system.

2. Experimental

2.1. Chemicals and biologicals

Lysozyme (LYS, Ultra pure grade, 0663, AMRESCO Inc., USA) and albumin from chicken egg white (OVA, A5253, Sigma–Aldrich, USA) were employed as received without further treatment. Poly(diallyldimethylammonium chloride) solution (PDDA, 20 wt% in H_2O, 409,030, Mw = 400,000–500,000) and poly(sodium 4-styrenesulfonate) solution (PSS, 30 wt% in H_2O, 561,967, Mw = 200,000) were purchased from Sigma–Aldrich Company (USA). Quartz wool (Diameter: 5–8 μm, Solid Special Function Materials Co. Ltd., China) and large inner-diameter multi-walled carbon nanotubes (MWNTs, Timestub™, Chengdu Organic Chemicals Co. Ltd., Chinese Academy of Sciences, China. Purity >90%, outer diameter: 30–60 nm, inner diameter: 20–50 nm, length: 1–10 μm, special surface area >200 $m^2\ g^{-1}$, ash < 5%) were pretreated before use as described in Section 2. Other chemicals employed include sodium chloride, ethanol, nitric acid, concentrated sulfuric acid and 30% (v/v) hydrogen peroxide were at least of analytical reagent grade and purchased from Guangzhou Chemical Reagent Factory, China. The unstained protein molecular weight marker (SM0431, Thermo Fisher Scientific Inc., USA) was a mixture of 7 native proteins ranging in size from 14.4 kDa to 116 kDa. (β-galactosidase, 116.0 kDa; bovine serum albumin, 66.2 kDa; ovalbumin, 45.0 kDa; lactate dehydrogenase, 35.0 kDa; Rease Bsp98l, 25.0 kDa; β-lactoglobulin, 18.4 kDa; lysozyme, 14.4 kDa). The gel was stained with Coomassie brilliant blue G250 (Fluka) after electrophoresis separation. Purified water (SYSU Yixianquan, Guangzhou, China) was used throughout.

NaCl aqueous solution (0.5–2.0 mol L^{-1}) was prepared by dissolving the appropriate amount of NaCl in purified water. The PDDA and PSS were diluted to 0.2% (v/v) with 0.5 mol L^{-1} NaCl solution, and preserved in the dark for future use. The protein stock solutions (1.0 mg mL^{-1}) were prepared by dissolving appropriate amount of proteins in purified water and the working standard solutions were obtained by stepwise dilution of the stock solutions. Phosphate buffers (PBS) of various pH values were prepared by mixing appropriate amounts of the Na_2HPO_4 solution (0.05 mol L^{-1}) and the NaH_2PO_4 solution (0.10 mol L^{-1}) according to the required pH value. The piranha solution used for cleaning quartz wool was prepared freshly by slowly mixing 5 mL of hydrogen peroxide (30% v/v) into 15 mL of concentrated sulfuric acid.

2.2. Apparatus

The surface morphology of materials was investigated using an optical microscope (Bresser MicroSet, Meade Instruments Europe, German) and a scanning electron microscope (SEM, Quanta 600, FEI). The UV/vis spectra were recorded with a T6 UV/vis spectrophotometer (Beijing Purkinje General Instrument Co. Ltd., China). A MicroCSP3000 sequential injection pump and a C25-3188EMH multiposition valve (FIAlab Instruments, USA) were used for dynamic LBL self-assembly of polyelectrolytes and MWNTs on quartz wool and also for on-line separation and preconcentration of proteins. The pH values were measured with a pHS-3C pH meter (Shanghai Weiye Instrument Factory, China). A CQ-6 ultrasonic system (Hucao Ultrasonic Instrument, Shanghai, China) was utilized for dispersing MWNTs. The various samples containing proteins were assayed by SDS-PAGE in a BG-verMINI electrophoresis cell with a BG-Power600i electrophoresis power supply (Baygene Biotech Co. Ltd., China).

2.3. Purification and oxidization of MWNTs

Before use, the MWNTs were pretreated and oxidized according to the published procedures with minor adjustment [26]. 0.1 g of MWNTs were placed in a beaker with 10 mL of ethanol (50% v/v), stirred for 10 min to wet the MWNTs sufficiently, then collected by filtration and rinsed with purified water. The wetted MWNTs were afterwards dispersed into concentrated HNO_3 (60 mL, 2.2 mol L^{-1}) in an ultrasonic bath for 30 min in order to oxidize the coexisting graphitic nanoparticles and amorphous carbon. The mixture was thereafter allowed to stand for 8 h at room temperature. The oxidized MWNTs were filtered with a MF-Millipore™ membrane (diameter: 5 cm, pore aperture: 4.5 μm, thickness: 0.1 mm) and thoroughly rinsed with purified water for at least 3 times until the effluent was neutral. This procedure removed impurities from the surface of the carbon nanotubes and induced the forming of negatively charged surface through the ionization of acidic surface groups (i.e., the carboxylic and hydroxyl groups). Finally, the collected MWNTs were resuspended in water with the aid of ultrasonic agitation for 30 min to form a stable solution (0.5 mg mL^{-1}).

2.4. Pretreatment of quartz wool

The quartz wool was first immersed in anhydrous alcohol for 30 min and then dried under 60 °C. Then the piranha solution was used to remove residues and impurities from the quartz wool. Fifteen milliliter of concentrated sulfuric acid was poured into a clean and dry glass beaker, and 5 mL of hydrogen peroxide was added to the acid very slowly. After mixing, the dried quartz wool was put into the mixed solution immediately. After 20 min, the quartz wool was taken out and rinsed with purified water thoroughly, and dried under 60 °C.

2.5. Preparation of solid-phase extraction microcolumn

The entire sequential injection system consists of a 2.5 mL syringe pump, an 8-port multiposition valve, a UV/vis

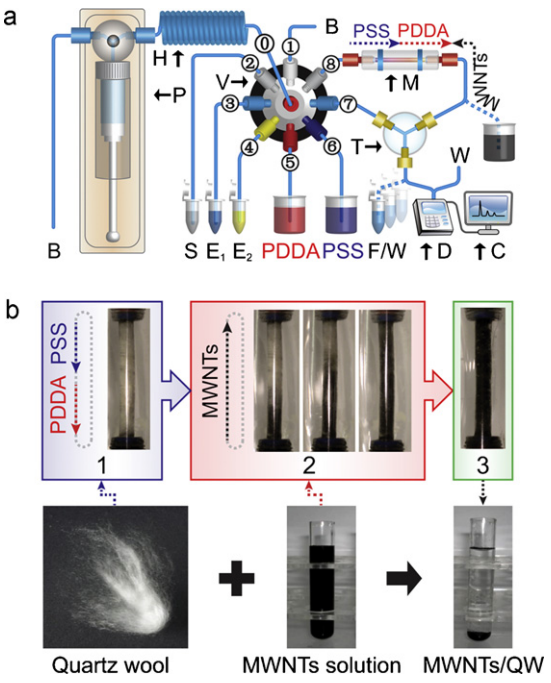

Fig. 1. (a) Flow manifold of the sequential injection system incorporating a microcolumn for dynamic coating of polyelectrolytes and MWNTs and on-line solid-phase extraction of proteins. P, sequential injection pump; H, holding coil; V, 8-port selection valve; M, microcolumn; T, 3-way connector; D, UV/vis spectrometer; C, computer; B, buffer solution; S, sample; E_1, weak eluent; E_2, strong eluent; F, collected fractions; W, waste. (b) Dynamic assembled procedure of a MWNTs/QW filled microcolumn: (1) a quartz wool filled microcolumn functionalized by alternate LBL depositions of positively charged poly(diallyldimethylammonium chloride) solution (PDDA) and negatively charged poly(sodium 4-styrenesulfonate) solution (PSS); (2) flowing and coating of MWNTs on the surface of QW; (3) an as-synthetic MWNTs/QW filling column.

spectrophotometer, and a holding coil with a capacity of 2.5 mL, as illustrated in Fig. 1a. All the external channels were made of PTFE tubing (outer diameter: 1.59 mm, inner diameter: 0.75 mm) and connected to the sequential injection system with flangeless nuts and ferrules. The solid-phase extraction microcolumn was made of poly(methyl methacrylate). The column length, effective column length, outer diameter and inner diameter were 50, 28, 20 and 2.5 mm, respectively. The appropriate amount of quartz wool (ca. 30 mg) previously treated with piranha solution was packed into the microcolumn. Two sets of flangeless nuts and ferrules were used to hold the quartz wool in place and connect the microcolumn to a sequential injection system for LBL self-assembly of polyelectrolytes and MWNTs on quartz wool (as shown in Fig. 1b). The dynamic LBL self-assembly procedure started with PDDA coating after thoroughly rinsing the quartz wool by passing through a sufficient amount of purified water. In a typical coating procedure, 1.5 mL of water and 1.0 mL of PDDA solution was orderly aspirated into the holding coil at a flow rate of 50 $\mu L\,s^{-1}$ from port 1 and 5, and then 0.8 mL of PDDA solution was slowly dispensed through port 8 into the microcolumn at 5 $\mu L\,s^{-1}$ followed by a 10 min of stopped flow to keep the quartz wool in the original concentration of polyelectrolytes solution. The rest of the polyelectrolytes solution and water was afterwards dispensed through port 8 at 20 $\mu L\,s^{-1}$ to eliminate the loosely retained polyelectrolytes on the surface of quartz wool. The PSS coating would be done in the same way from port 6 as PDDA coating. Afterwards, a program of the sequential injection system was set to perform certain cycles of these procedures by switching between the PSS and PDDA solution repeatedly to obtain desired number of bilayers, and then the free end of the tube connected on the microcolumn was placed into MWNTs solution. The pump was set to aspirate the MWNTs solution into the microcolumn repeatedly at 15 $\mu L\,s^{-1}$ until all the surface of quartz wool in the microcolumn was covered with MWNTs, while the carrier liquid was dispensed through port 7 at 50 $\mu L\,s^{-1}$ to waste. The assembly procedures were completed when the excess MWNTs could be seen flowing through the filling.

2.6. Solid-phase extraction of proteins

During the solid-phase extraction process, the effluent containing proteins after passing through the microcolumn was quantified with an UV/vis spectrometer at 280 nm in a flow cell, in order to evaluate the retention efficiency of proteins on the MWNTs/QW. Afterwards, the proteins eluted from the microcolumn were quantified in the same way. In this case, the solid-phase extraction of proteins with MWNTs/QW as absorbent includes the following steps: before the extraction operation is started, 1.3 mL of carrier buffer, 100 μL of LYS solution (10 $\mu g\,mL^{-1}$), 1.0 mL of carrier buffer solution and 100 μL of OVA solution (25 $\mu g\,mL^{-1}$) were aspirated into the holding coil at a flow rate of 50 $\mu L\,s^{-1}$, then being directly dispensed through port 7 to flow through the UV/vis spectrometer at 30 $\mu L\,s^{-1}$ for measurement of the peak high generated from the proteins without undergoing the SPE process. Afterwards the channel between the multiposition valve and the detector was rinsed with purified water (1.5 mL) at 50 $\mu L\,s^{-1}$ until a stable baseline was obtained. Thereafter, 500 μL of carrier buffer and 2.0 mL of sample solution were aspirated from port 2 into the holding coil at a flow rate of 50 $\mu L\,s^{-1}$, and were then dispensed through port 8 to flow through the microcolumn at 30 $\mu L\,s^{-1}$ to facilitate the adsorption of the LYS onto the surface of the MWNTs/QW. Prior to the elution of the adsorbed proteins, some of the loosely retained interfering components on the column surface were eliminated by a prewashing procedure, by flushing the microcolumn with 1.0 mL of purified water. The syringe pump was then set to successively aspirate carrier buffer (2.0 mL) and eluent 1 (1.0 mol L^{-1} NaCl, 100 μL) into the holding coil and then dispensed the stacked zones into the microcolumn through port 8 at 30 $\mu L\,s^{-1}$ to elute the retained LYS. The eluate was then either transferred directly into the detector for quantification or collected for further investigations. Finally, 200 μL of eluent 2 (2.0 mol L^{-1} NaCl) and 2.0 mL of carrier buffer were employed to clean up the microcolumn, eliminate any possible carry-over and preconditioned the microcolumn for subsequent operating runs.

3. Results and discussion

3.1. Preparation of MWNTs coated quartz wool

The commercial quartz wool used in this study is covered by many tiny particles (as shown in Fig. 2a), therefore a pretreatment procedure with piranha solution is necessary before LBL self-assembly. After acid-treatment, the surface of quartz wool is clean and negatively charged, but the quartz wool fibers also became thinner and shorter, thus the pretreatment time should be less than half an hour to maintain the size and strength of quartz wool.

The conventional LBL self-assembly process is alternating deposition of oppositely charged polyelectrolytes on solid support by using only beakers and tweezers. The variations of this solution-dipping deposition method including the spin-coating and spraying methods which were introduced more recently. But it is hard to obtain even coating on quartz wool in these ways because the quartz wool fibers are too light, thin and long to be dispersed well and collected completely in each step, and there was visible loss

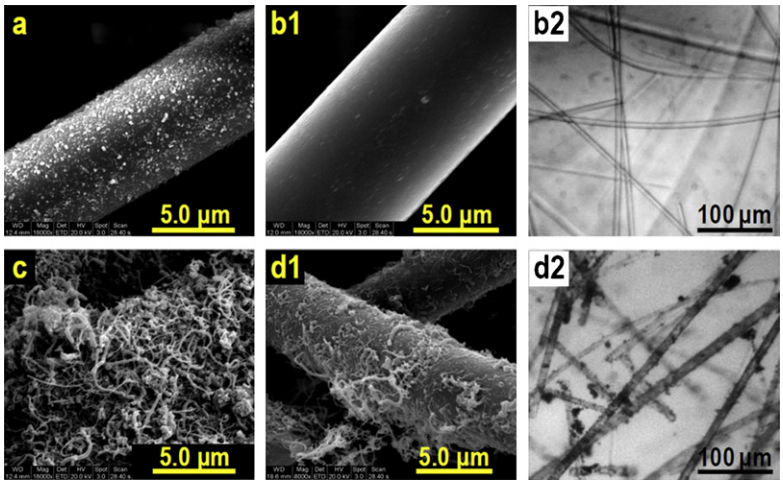

Fig. 2. SEM images of QW (a), purified QW (b1), MWNTs-COOH (c), MWNTs/QW (d1) and microscope images of purified QW (b2), MWNTs/QW (d2).

after only few cycles. In addition, the polyelectrolyte coated quart wool fibers tend to gather together and form small pieces, which made it even harder for the inner quartz wool fibers to get functionalized further.

Therefore, the mechanism of dynamic assembly was introduced into this study in which the whole LBL self-assembly procedure was performed in a microcolumn on-line, in situ and dynamically by a sequential injection system, which allowed the polyelectrolytes and acid-cut short MWNTs flew through and coated onto the surface of quartz wool thoroughly, at the same time, led to a surface charge reversal on the quartz wool surface.

The procedure of dynamic assembly is described in Section 2. Surface oxidation of MWNTs renders them negatively charged. Thus, positively charged polyelectrolyte PDDA is necessary as partner polyelectrolyte for making multilayers structure. Although this LBL self-assembly can be obtained by alternative adsorption with the positively charged polymer and the MWNTs, the negatively charged polymer offer additional degree of control of the structure and allow stronger connectivity of MWNTs with the matrix. Additionally, in order to minimize substrate effects and economize on the consumption of MWNTs, precursor layers on quartz wool were deposited with polyelectrolytes and the MWNTs were deposited on top of all coating layers.

In addition, it has been reported that the thickness and charge of coating film increase linearly with the increasing number of bilayers, while the permeability of gas through the coating layers decreases [27,28], which indicates that an appropriate range of coating bilayer number is important for reducing the substrate effects and increasing the retaining amount of MWNTs. In our study, we compared several columns with different coating bilayer numbers including 5, 8, 12, 15 and 20. It showed that the non-specific absorption was almost completely eliminated after 8 cycles, but the even and dense coating of MWNTs was achieved after 15 cycles. Thus, the cyclic number of the LBL self-assembly of polyelectrolytes on quartz wool was set as 15 to fit both of the retaining efficiency and time consuming, which also makes the silanol (—Si—OH) groups on the surface of the quartz wool are fully covered and no unspecific adsorption happens. During the whole assembly period, the quartz wool stayed in place and no obvious pressure was observed. The quartz wool wasn't flushed out or pushed tight by liquid flow, which shows its great potential as support material of SPE absorbent.

It is important to use completely dispersed MWNTs-COOH solution in this step, so the MWNTs-COOH could be carried by water and flew through the whole column, instead of being blocked or filtered by the quartz wool at one end of the column, which could made the packed material half black and half white. For this reason, the acid treatment time of MWNTs was prolonged to more than 8 h in order to make the MWNTs dispersed easily in water after oxidized with nitric acid. The assembly of the MWNTs-COOH onto the polyelectrolytes coated quartz wool was done by aspirating the MWNTs-COOH solution from the free end of the microcolumn as shown in Fig. 1a and b into the microcolumn repeatedly. The MWNTs-COOH were adsorbed and retained on the surface of quartz wool because of the electrostatic interaction between the MWNTs-COOH and PDDA, while the water used to disperse the MWNTs flew through the microcolumn at the same time. When the whole packing materials were fully black, and the effluent started turning grey, the MWNTs coating was completed. The 30 mg of quartz wool could be completely covered by MWNTs with only 6 mL of MWNTs-COOH solution, which contains about 3 mg MWNTs, giving an assembly ratio of approximately 10% (w/w). The prepared coating layer was stable for at least 2 weeks without the necessity for any regeneration between runs. When the LBL self-assembly is done, the remained polyelectrolytes and MWNTs attached inside the tubings could be striped away by soaking in acetone.

3.2. Characterization of the MWNTs/QW

Scanning electron microscopy (SEM) observations of the as-received quartz wool and acid-treated quartz wool showed the removal of impurities from quartz wool after treated with piranha solution (Fig. 2a and b1). The diameter of the quartz wool was about 5–8 μm, which had not been changed much after cleaning for 20 min with piranha solution. The length of quartz wool fibers could be up to several centimeters, but after being pressed into the microcolumn, most of the fibers were broken into short segments with less than 5 mm length (Fig. 2b2 and d2). Fig. 2c is the SEM images of dried MWNTs-COOH, which shows the MWNTs aggregated densely, but they are dispersed after being coated onto the quartz wool as shown in Fig. 2d1. There are some blank areas and some cross-linked structures could be found on the surface of fibers, which might caused by the overlap of the fibers and the MWNTs in solution gathered on the overlay regions instead of penetrating into them during the dynamic LBL self-assembly.

Fig. 3 presents the infrared (IR) transmission spectra of quartz wool before and after the MWNTs coating. Both of the spectra show two typical absorption peaks of Si—O—Si bonds at 1100–1200 cm^{-1} [29], which hardly changed after the LBL self-assembly. It seems that the introduction of polyelectrolytes and MWNTs did not made visible differences on IR spectra of quartz wool, which was likely because that it is the silica carrier making up most of the mass

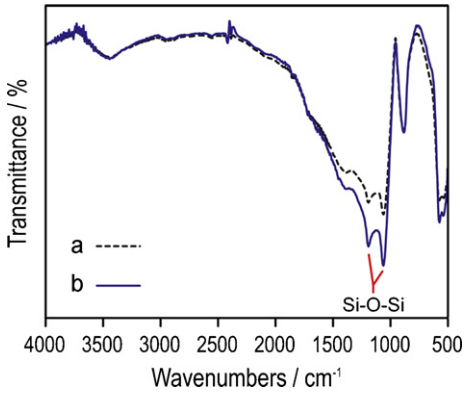

Fig. 3. Infrared spectra of the purified QW (a) and MWNTs/QW (b).

and volume of the adsorbent, rather than the polyelectrolytes and MWNTs.

3.3. *Experimental parameters affecting the sorption/elution of lysozyme on MWNTs/QW*

The MWNTs used in this work were firstly purified and oxidized with nitric acid to introduce the carboxylic groups, and then the MWNTs-COOH ionized in water to provide the necessary negative charge to assemble on the positively charged surfaces. The PDDA and PSS have been always used as polyelectrolytes for self-assembled monolayers, which were used here for covering the unwanted groups on quartz wool before the MWNTs were adhered onto the quartz wool by PDDA.

As described in our previous work [26], the sorption of proteins on MWNTs-COOH is mainly dominated by electrostatic interaction, therefore the sorption of proteins on MWNTs/QW should be affected by changing some experimental parameters like pH value and ionic strength. Some important factors tending to govern the absorption efficiency of proteins on the MWNTs/QW were thus investigated.

Firstly, it is necessary to control the ionic strength and pH value of the sample medium at appropriate levels for the sorption of LYS onto the MWNTs/QW composites.

Fig. 4a and b illustrates the dependence of retention efficiency of LYS and OVA on the ionic strength and the pH value of the sample medium. The ionic strength was determined by adjusting the concentration of NaCl in solution and the pH value was controlled by Na_2HPO_4–NaH_2PO_4 buffer solution. Fig. 4a illustrates that high sorption efficiency of LYS was readily achieved in an aqueous medium with an ionic strength $\leq$0.01 mol L^{-1}. Then the nearly linear decreasing of sorption efficiencies happened with the increasing of the ionic strength from 0.01 to 0.10 mol L^{-1}. When the ionic strength was up to 0.10 mol L^{-1}, there was only 40% of LYS could be adsorbed.

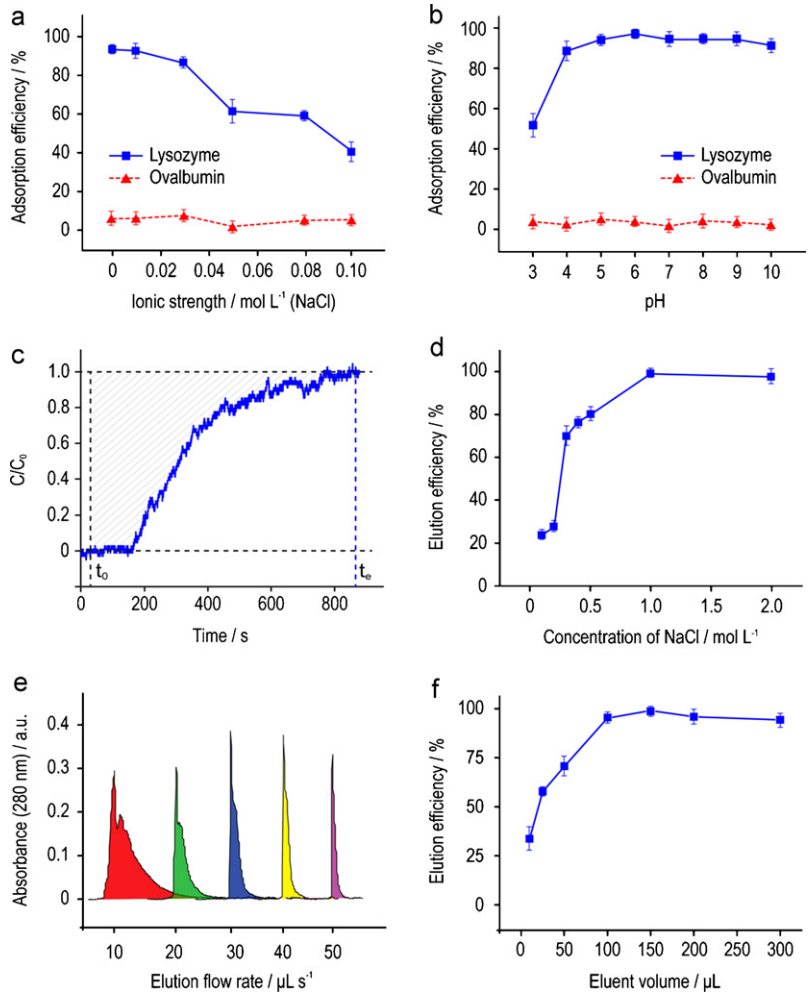

Fig. 4. Absorption studies of the LYS and OVA on MWNTs/QW. Effect of ionic strength (a), pH of phosphate buffer (b) on the adsorption efficiency; the breakthrough curve of LYS adsorption on the MWNTs/QW composites at pH 9 (c); effect of concentration of NaCl (d), eluent flow rate (e), eluent volume (f) on the elution efficiency. Experimental conditions: 2.0 mL of 10 μg mL^{-1} LYS solution and 2.0 mL of 60 μg mL^{-1} OVA solution.

Table 1
Characteristic SPE performance data of LYS (10 μg mL^{-1}) with MWNTs/QW, MWNTs/SiO_2 spheres [19] and MWNTs filled microcolumns.

Entry	MWNTs/QW	MWNTs/SiO_2	MWNTs
Maximum flow rate (μL s^{-1})	70	70	20
Actually used flow rate (μL s^{-1})	30	30	10
Sample processing frequency (h^{-1})	18	18	6
Sample/eluent volumes (mL)	2.0/0.1	2.0/0.1	2.0/0.2
Enrichment factors	30 ± 2	30 ± 2	15 ± 2
Amount of absorbent per column (mg)	30	30	9
Percentage of MWNTs in absorbent (%)	10	3	100
Amount of MWNTs per column (mg)	3	9	9
Dynamic sorption capacity on MWNTs (μg mg^{-1})	32 ± 2	18 ± 1	11 ± 1
RSDs (one column, %, 10 μg mL^{-1}, n = 11)	2.0 ± 0.5	2.4 ± 0.5	3.2 ± 0.6
RSDs (column to column, %, 10 μg mL^{-1}, n = 3)	5 ± 1	7 ± 2	15 ± 4
LODs (3σ, μg mL^{-1}, n = 11)	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01

Fig. 4b shows the sorption efficiency of LYS increased from 50% to 90% when the pH value of the sample medium was increased from 3 to 4 and after that, quantitative adsorption could readily be achieved. The adsorption of LYS was clearly affected by ionic strength and pH of sample medium, in contrast, OVA could not be adsorbed onto the MWNTs/QW no matter what concentration of NaCl and pH was. This proved that the electrostatic interaction is the main driving force for the sorption of LYS onto the MWNTs/QW composites. In practice, an ionic strength of 0.01 mol L^{-1} and a pH of 9 were employed to minimum the unwanted adsorption of other proteins because the isoelectric points (pI) of the most proteins in egg white are lower than 9.

The sampling flow rate and volume were also studied because they are two critical parameters for the performance of an on-line sorption system, which have significant effects on the sorption efficiency and processing time, and they also determine the lifetime of the system. In the present system, the sorption medium supported by quartz wool significantly reduced the flow resistance, and thus higher flow rates could be used to increase sampling throughput. The experimental results indicated that changing the sampling flow rate from 10–50 μL s^{-1} did not result in observable variations in the sorption efficiency of LYS, due to the fast sorption process of LYS on MWNTs. In practice, a relatively low sampling flow rate of 30 μL s^{-1} was employed to compromise with the responding speed of the UV spectrometer, since the smallest time interval of continuous detection is 0.5 s, which is too long to catch the peak points when the flow rate is high. At this flow rate, the lifetime of a MWNTs/QW packed microcolumn could be at least 2 weeks, while a MWNTs packed microcolumn could not last for more than 2 h.

Fig. 4c is the breakthrough curve of LYS adsorption on the MWNTs/QW composites at pH 9 (sampling flow rate: 30 μL s^{-1}, t_0 = 33 s, t_e = 878 s), from which it could be derived that the sorption capacity of LYS on the MWNTs/QW was 32 mg g^{-1} (96.4 μg LYS/3.0 mg MWNTs). Therefore the sampling volume could be up to 10 mL for 10 μg mL^{-1} of LYS solution, and the enrichment factors would be up to 150 with a eluent volume of 0.1 mL.

NaCl, the most commonly used eluent, was employed to recover the LYS retained on the surface of MWNTs/QW after the adsorption process. As shown in Fig. 4d the elution efficiency increased from 20% to 98% when the concentration of the NaCl solution increased from 0.1 to 1.0 mol L^{-1}. No additional enhancement of the elution efficiency was attained when the NaCl concentration was increased further up to 2.0 mol L^{-1}. Thus the appropriate concentration of 1.0 mol L^{-1} allowing the complete elution of the retained LYS was employed for subsequent investigations. The effect of the elution flow rate on the elution process is illustrated in Fig. 4e. It is obvious that the recorded peak shape was much sharper and well shaped as the elution flow rate increased from 20 to 50 μL s^{-1}. It could also be seen that the peak area and the peak height have different trends across the range of elution flow rates investigated. The peak area declined sharply as the elution flow rate increased, while the peak height peaked at 30 μL s^{-1} of flow rate and then dropped steadily. This dropping might be caused by the detection delay of the UV spectrometer. Thus an elution flow rate of 30 μL s^{-1} was employed for further investigations.

Generally, the volume of eluent governs not only the recovery efficiency of the retained species but also the enrichment factor.

Fig. 4f illustrates the dependence of the elution efficiency on the volume of NaCl solution. It shows that elution efficiencies of 30–98% for LYS could be achieved by varying the eluent volume within the range of 10–300 μL. A fairly high recovery rate of over 90% was obtained by using only 100 μL of 1.0 mol L^{-1} NaCl solution, so this eluent volume was used for further investigations.

3.4. Analytical performance of the present system

The present investigation aims at isolating basic proteins from complex real sample matrices with better analytical performance. The enrichment factors, dynamic sorption capacity, one-column repeatability and column-to-column reproducibility of LYS on MWNTs/QW were performed under the conditions optimized above and the analytical performance data of this system were compared with MWNTs/SiO_2 spheres and MWNTs filled microcolumns. The results are summarized in Table 1.

Under the optimized conditions, when employing 2.0 mL of LYS sample volume and 100 μL of eluent volume, an enrichment factor of 30 was achieved, along with a retention efficiency of 100% and an overall recovery rate of 90%. A limit of detection of 0.04 μg mL^{-1} was derived within a linear range of 0.2–16 μg mL^{-1}. A precision value of 2.6% RSD was obtained at a concentration level of 10 μg mL^{-1}. In addition, the results showed that the MWNTs/QW absorbance offered better dynamic sorption capacity than commonly used MWNTs-packed column and even MWNTs/SiO_2 spheres, which was because the MWNTs were dispersed sufficiently and then retained on the surface of quartz wool, so the MWNTs/QW adsorbent has larger specific surface area than accumulative MWNTs.

To evaluate the practical applicability of this MWNTs/QW packed microcolumn, a real sample of egg white was used here, in which the major protein components include ovalbumin (54%, pI = 4.6), ovaltransferrin (13%, pI = 6.7–7.2), lysozyme (3.5%, pI = 11) and ovomucin (3.5%). Chicken egg white (21.3 g) was separated from fresh eggs and diluted to 200 mL with purified water (protein content: ~10 mg mL^{-1}). The diluted egg white was homogenized in an ice-bath, filtered twice with gauze and then centrifuged at 10,000 rpm for 15 min. The supernatant fluid was reserved at 4 °C for further use. Before sampling, the supernatant fluid was 10-fold diluted with purified water (Fig. 5a) or Na_2HPO_4–NaH_2PO_4 buffer (0.01 mol L^{-1}, pH 9.0, Fig. 5b) respectively. The SPE process was performed as described in Section 2. The effluent was collected with several 500 μL of centrifuge tubes orderly instead of

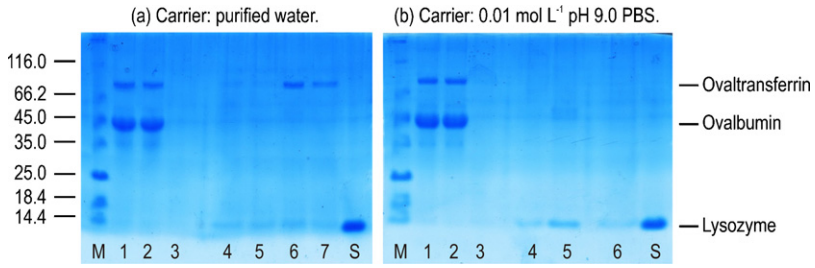

Fig. 5. The SDS-PAGE electrophoretograms. Lane M: protein marker (kDa); lane 1: egg white sample (total protein content: 1 mg mL^{-1}); lane 2: effluent passed through the microcolumn; lane 3: effluent pre-washed with abundant carrier solution; lane S: commercial pure LYS. (a) Carrier solution: purified water. Lanes 4 and 5: first eluate (sampling volume: 2 mL, eluent: 1.0 mol L^{-1} NaCl) and second eluate (eluent: 2.0 mol L^{-1} NaCl); lanes 6 and 7: first eluate (sampling volume: 4 mL, eluent: 1.0 mol L^{-1} NaCl) and second eluate (eluent: 2.0 mol L^{-1} NaCl). (b) Carrier solution: 0.01 mol L^{-1} pH 9.0 Na_2HPO_4–NaH_2PO_4 buffer solution. Lanes 4: eluate (sampling volume: 2 mL, eluent: 1.0 mol L^{-1} NaCl); Lanes 5 and 6: first eluate (sampling volume: 6 mL, eluent: 1.0 mol L^{-1} NaCl) and second eluate (eluent: 2.0 mol L^{-1} NaCl).

being dispensed into the detector. Usually the purified water was used for driving or separating fluid zones, but in order to maintain the volume and concentration of collected fractions, air was used here for dispensing the fluid flow and emptying the tubing and microcolumn. The proteins in collected fractions were then assayed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as detailed previously [30]. The SDS-PAGE electrophoretograms were showed in Fig. 5.

As can be seen from the electrophoretogram illustrated in Fig. 5a, the bands at 35 kDa, 45 kDa and 50 kDa were effectively removed in the eluate (lanes 4–7), but the bands of LYS are very vague, which might be caused by competitive adsorption of ovaltransferrin on MWNTs/QW during extraction since the bands of ovaltransferrin at ca. 75 kDa were observable in lanes 6 and 7.

Fig. 5b shows the ovaltransferrin bands were completely removed after extraction at pH 9.0 and a clear LYS band at 14.4 kDa in lane 5 could be seen. This observation well illustrated the practical applicability of present procedure for the effective separation and extraction of LYS from biological samples with complex matrix components.

4. Conclusion

We have demonstrated the feasibility for preparation of MWNTs coated quartz wool with dynamic LBL self-assembly and its application on separation of LYS in egg white through electrostatic interaction between the MWNTs and LYS. The coating procedures were performed continuously in a sequential injection system. The quartz wool was placed in a microcolumn to form a loose packing as support of MWNTs which guarantees the minimized flow impedance and the intimate contact between proteins and absorbent surface. The goal of performing protein extraction by current method with less workload, easy adjustment and better performance was achieved. The use of MWNTs/QW as absorbent for microcolumn packing in a flow system further improved the sorption efficiency of the absorbent, and the flow impedance was significantly alleviated which offered high sampling throughput while maintaining favorable sorption property. The results are promising and warrant further investigation. Furthermore, this method has good potential to be used for studying the interaction online between proteins and a wide range of other nanoparticles (e.g., graphene, AuNPs, quantum dots and so on), which could be attached on the support surface.

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