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Quaternized cellulose-supported gold nanoparticles as capillary coatings to enhance protein separation by capillary electrophoresis

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ABSTRACT

Gold nanoparticles (Au NPs) were synthesized and stabilized by using water-soluble quaternized cellulose (QC) as support matrix through a straightforward and environmentally friendly aqueous-phase approach. The structure and morphology of QC-supported Au NPs (QC-Au NPs) were investigated systematically by UV–visible, FT-IR, x-ray diffraction and TEM measurement. The Au NPs with mean diameter of about 7 nm were shown to efficiently redisperse in water due to the strong interaction between QC and Au NPs, and the solutions were quite stable after storage for nearly 4 months at room temperature. QC-Au NPs were subsequently used as novel physically adsorbed coatings for protein separation by CE. The separation performance was significantly improved in the capillary coated by QC-Au NPs compared with that of the uncoated capillary or QC coated capillary. A small quantity of Au NPs (Au content of 4.6%) was adequate for the obvious improvement of coating ability. The theoretical plate number of lysozyme in QC-Au1 NPs coated capillary was 2.9 times as much as that in QC coated capillary. We have demonstrated the separation of six model proteins with RSD of migration time less than 2.79% and RSD of peak area less than 4.81%. Furthermore, QC-Au NPs was applied to the analysis of closely related proteins and biological samples. With simplicity, high resolution and reproducibility, the proposed method shows potential for applications in proteomics and clinical diagnosis.

1. Introduction

Protein adsorption on the inner surface of the capillary is known to hinder the application of CE in protein analysis [1,2]. This is particularly pronounced for basic proteins which have opposite charges to the negatively charged fused-silica capillary wall. Protein adsorption may result in peak broadening, peak tailing, analyte loss, reduction of separation efficiency, resolution and reproducibility, or even the fouling of the column [3–5]. Various methods have been adopted for the reduction of protein adsorption, including pH value adjustments, the use of high ionic strength, background electrolyte additives such as amines and surfactants, covalent coatings, physically adsorbed coatings and hybrid coatings [2,6]. The use of extreme pH values and high ionic strength may limit selectivity or lead to protein irreversible denaturation [7]. The preparation of covalently coated capillary is laborious and time consuming, and the coatings cannot be regenerated [8,9]. In contrast, the application of physically adsorbed coating has emerged as the most common approach in recent years, due to its versatility, simplicity, speed of coating and regenerability in capillary coating [4]. A great many of polymers have been explored as physically adsorbed coatings to minimize protein adsorption. They could cover the silanol groups to sterically shield the adsorption sites, or alter the surface charge to electrostatically repel the charged protein [2,10,11].

On the other hand, gold nanoparticles (Au NPs) have attracted considerable attention in many fields including analytical chemistry and separation science owing to their unique physical and chemical properties such as long-term stability, high surface area-to-volume ratio, and ease of chemical modification [12–14]. Au NPs have been used in chromatographic and electromigration techniques, with applications as modifiers in stationary phases for capillary gas chromatography [15–17] and capillary liquid chromatography [18,19], as covalent coatings or non-covalent coatings in CE [20,21] and microchip CE [22], as pseudostationary phases in capillary electrochromatography [23–25]. A considerable amount of work has focused on DNA separations in CE, employing Au NPs as components of the separation media [26–31]. In 2006, Tseng and coworkers reported that DDAB-capped Au NPs could be acted

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Table 1
Preparation and properties of QC-Au NPs.

Sample ID	c_{QC} (mg/mL)	Au content (%)	ξ-potential (mV)	Particle Size (d, nm)	
				XRD	TEM
QC	/	/	21.4	/	/
QC-Au1	5	4.6	13.2	7.5	6.9 ± 1.9
QC-Au2	3	7.4	14.8	5.9	6.5 ± 2.0
QC-Au3	1	19.3	14.3	6.5	6.9 ± 2.5
QC-Au4	0.5	32.4	16.0	7.2	7.1 ± 2.5

as coating materials in CE for protein separation for the first time [32]. Cationic surfactant, i.e. didodecyldimethylammonium bromide (DDAB), was used to protect the Au NPs from agglomeration and to prevent the adsorption of basic proteins. Interestingly, it was found that mixing the DDAB-capped Au NPs directly with neutral high molecular-mass poly (ethyleneoxide) (PEO) led to the formation of complex AuNPPs and improved the separation efficiency. Compared with PEO, CTAB and PVA, the addition of Au NPs into 1.6% PDDAC solution (background electrolyte, BGE) was shown to significantly enhance the separation efficiency and speed of analysis for the separation of the proteins [33]. It can thus be deduced that a combination of Au NPs and polymers may be beneficial for protein separation by CE.

Previously, water-soluble quaternized cellulose (QC) has been homogeneously synthesized from cellulose in NaOH/urea aqueous solution [34,35], and studied as a physically adsorbed coating in CE for protein separation [36]. The results indicated that QC was efficient for reduced adsorption of basic proteins, reduced analysis time and improved separation performance. Moreover, QC was proved to be an ideal protective agent for the preparation of Ag NPs with high catalytic efficiency and antibacterial activities [37]. In this work, a straightforward and environment friendly approach is reported for the synthesis and stabilization of QC-Au NPs. QC-Au NPs were applied to protein separation by CE as new capillary coatings. The effects of QC-Au NPs in the separation of closely related proteins such as the PTMs of bovine lactoferrin (glycosylation) and the lysozyme of different genus were investigated; an analysis of lysozyme from biological samples was also provided.

2. Materials and methods

2.1. Materials.

Cellulose was supplied by Hubei Chemical Fiber Group Ltd. (Xiangyang, China). The viscosity-average molecular mass (M_η) was determined to be 5.1×10^4 g/mol. Water-soluble QC was homogeneously synthesized in NaOH/urea aqueous solution [34], the substitution degree (DS) of the quaternary ammonium group was determined to be 0.73 by using elemental analysis. Human transferrin, lactoferrin from bovine milk, Cytochrome c from bovine heart, lysozyme from chicken egg white, ribonuclease A from bovine pancreas, lysozyme from human milk were purchased from Sigma–Aldrich (St. Louis, MO, USA). Bovine pancreatic trypsin inhibitor was purchased from Ruibio-bio Company (Germany). Chymotrypsinogen was purchased from Yuanye Biology Technology (Shanghai, China). All of the chemical regents ($HAuCl_4$, $NaBH_4$ etc.) were purchased from Sinopharm Chemical Reagent Co., Ltd., and used without further purification. Deionized water (Millipore) was used for all experiments.

2.2. Preparation of QC-Au NPs

Au NPs were synthesized by the directly reduction of $AuCl_4^-$ ions in QC solution using $NaBH_4$ as the reducing agent. Typically, 0.5 mL of $HAuCl_4$ solution (2.428×10^{-2} mol/L) was added to 10 mL of QC (3 mg/mL) solution in a reaction vessel, followed by constant stirring. Then, 0.375 mL $NaBH_4$ solution (0.1 mol/L) was added immediately and further stirred for 1 h. The resulting solution was then dialyzed with the regenerated cellulose tubes (M_w cutoff 8000) against distilled water for 5 days. The final solution was lyophilized to obtain the QC-Au nanocomposites and coded as QC-Au2. According to Table 1, a series of QC-Au NPs was obtained by varying the concentration of QC. QC-Au NPs were then dissolved in phosphate buffer solution, and used for UV–visible, zeta potential and transmission electron microscopy (TEM) testing.

2.3. Characterization of QC-Au NPs

The zeta potential of the QC-Au NPs solution was performed on a Nano-ZS ZEN3600 (Malvern Instruments, U.K.) at 25 °C. UV–visible spectra were performed on UV-visible spectroscopy (UV-6, Mapada, China) using quartz cuvettes with an optical path of 1 cm. FT-IR spectra of QC-Au NPs were recorded on a Nicolet 5700 Fourier transform infrared spectrometer. The test specimens were prepared by the KBr-disk method. X-ray diffraction (XRD) was conducted on a XRD diffractometer (XRD-6000, Shimadzu, Japan). The XRD patterns with Cu Kα radiation ($\lambda = 0.15406$ nm) at 40 kV and 30 mA were recorded in the region of 2θ from 30 to 85° with a step speed of 1°/min. TEM images were observed on a JEM-2100 (HR) electron microscope, using an accelerating voltage of 200 kV. TEM samples were prepared by dipping a copper grid with a carbon film into the QC-Au NPs solution. More than 300 particles were randomly counted to determine the average particle size and size distribution for each sample.

2.4. EOF measurement and CE separation of proteins

A Beckman P/ACE MDQ system instrument (Beckman Coulter Instruments, Fullerton, CA, USA) was applied for the measurement with a DAD detector working at 210 nm. The fused silica capillaries with 75 μm i.d. and 365 μm o.d. (Yongnian Optical Fiber, Hebei, China), and 47 cm of total length (40 cm effective length) were rinsed with 0.1 mol/L NaOH for 20 min, and followed by deionized water for another 10 min. The capillary was subsequently flushed with running buffer (25 mmol/L phosphate buffer at different pH values containing QC-Au NPs or QC) under a pressure of 20.0 psi for 5 min. After pre-run for 10 min for balance, the electroosmotic mobility was measured at 20 °C. The protein solution was injected for 3 s at 0.5 psi and then run at a constant voltage at 20 °C. The proteins were dissolved in deionized water with a concentration of 0.25 mg/mL.

3. Results and discussion

3.1. Preparation and characterization of QC-Au NPs

Following the introduction of $HAuCl_4$ into the QC solution, $AuCl_4^-$ was absorbed onto the polymer chains by replacing the Cl^- derived from QC [38]. The addition of $NaBH_4$ led to an immediate change in the coloring of the mixture solution from light yellow