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Cationic polyelectrolyte functionalized magnetic particles assisted highly sensitive pathogens detection in combination with polymerase chain reaction and capillary electrophoresis

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ABSTRACT

Pathogenic bacteria cause significant morbidity and mortality to humans. There is a pressing need to establish a simple and reliable method to detect them. Herein, we show that magnetic particles (MPs) can be functionalized by poly(diallyl dimethylammonium chloride) (PDDA), and the particles (PDDA-MPs) can be utilized as adsorbents for capture of pathogenic bacteria from aqueous solution based on electrostatic interaction. The as-prepared PDDA-MPs were characterized by Fourier-transform infrared spectroscopy, zeta potential, vibrating sample magnetometry, X-ray diffraction spectrometry, scanning electron microscopy, and transmission electron microscopy. The adsorption equilibrium time can be achieved in 3 min. According to the Langmuir adsorption isotherm, the maximum adsorption capacities for *E. coli* O157:H7 (Gram-negative bacteria) and *L. monocytogenes* (Gram-positive bacteria) were calculated to be 1.8×10^9 and 3.1×10^9 cfu mg^{-1}, respectively. The bacteria in spiked mineral water (1000 mL) can be completely captured when applying 50 mg of PDDA-MPs and an adsorption time of 5 min. In addition, PDDA-MPs-based magnetic separation method in combination with polymerase chain reaction and capillary electrophoresis allows for rapid detection of 10^1 cfu mL^{-1} bacteria.

1. Introduction

Bacteria are widely present in our daily lives. Some of them are harmony with people, while some are harmful to people's health, which are called pathogenic bacteria. *Escherichia coli* O157:H7 (*E. coli* O157:H7), a Gram-negative pathogen, has been reported to cause haemorrhagic colitis, haemolytic uraemic syndrome, and other illnesses [1]. *Listeria monocytogenes* (*L. monocytogenes*), a Gram-positive pathogen, is associated with some serious invasive diseases like septicemia, meningitis, and meningoencephalitis [2]. Because of the hazards of pathogens to humans, exposure of humans to ready to eat foods and drinks contaminated by these pathogens should be prevented. The limits for these pathogens have been set to be less than 10^2 cfu g^{-1} in foods and drinks in the European Union. The strict criteria have increased the need for rapid and highly sensitive detection methods for bacteria.

The conventional plate-counting method is an effective way to get an accuracy result for bacteria detection. However, the method is usually time-consuming and labor-intensive. In order to reduce the detection time for bacteria, some other methods have been developed, such as biosensor-based [3,4] and polymerase chain reaction (PCR) based methods [5–7]. However, the detection limits for bacteria of these methods are usually in the range of 10^3–10^4 cfu mL^{-1}. Although real-time PCR-based method can effectively improve the detection sensitivity of bacteria with a detection limit lower than 10^2 cfu g^{-1} [8], the time-consuming culture enrichment was still a necessary step for high sensitivity, which limits the rapid detection of low concentration of bacteria. Therefore, development of new rapid and sensitive detection methods for bacteria is still challenging.

Magnetite (Fe_3O_4) particles possessing appealing magnetic properties, nontoxicity, easy preparation, and easy surface-functionalization have gained attractive attention in a variety of applications especially in biomedicine [9,10] and environmentology [11–13]. The magnetic responsiveness of functional magnetic particles (MPs) enables them to be conveniently separated from aqueous solutions by applying an external magnetic field. Some functional MPs have been applied for capture of pollutants in water, such as antibiotics [14,15] and heavy metal ions [16–19]. MPs functionalized with some particular groups, such as amine [20], *N*-methylimidazolium [21], pigeon ovalbumin [22] and amino acid [23] have also been utilized for pathogens capture and removal.

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Poly(diallyl dimethylammonium) chloride (PDDA) with highly hydrophilic and permanently charged quaternary ammonium groups is a water-soluble cationic polyelectrolyte. It is widely used in water treatment, paper manufacturing, mining industry, and so on [24]. It was also used to remove negatively charged perchlorate [25], chromate [26], and arsenic [27] from aqueous solution based on electrostatic interactions in a polyelectrolyte-enhanced ultrafiltration process. To the best of our knowledge, PDDA has not been reported for bacteria capture. The favorable attributes of MPs and PDDA inspired us to prepare PDDA functionalized MPs (PDDA-MPs). And the feasibility of PDDA-MPs as adsorbents for capture of pathogens can be expected since bacteria are negatively charged above their isoelectric points.

In this work, PDDA-MPs were synthesized by three reaction steps. They are characterized by Fourier-transform infrared spectrometry (FT-IR), zeta potential, vibrating sample magnetometry (VSM), X-ray diffraction spectrometry (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The positive charges on PDDA-MPs enable them to rapidly capture negatively charged bacteria from aqueous solution based on electrostatic interaction. And the magnetic property of PDDA-MPs enables them to be conveniently separated from aqueous solution. The adsorption isotherms of PDDA-MPs for pathogenic bacteria (*E. coli* O157:H7 and *L. monocytogenes*) were studied. Furthermore, PDDA-MPs were used for highly sensitive detection of bacteria in mineral water in combination with PCR and capillary electrophoresis (CE).

2. Experimental

2.1. Chemicals and reagents

PDDA, 20% in water, with an average molecular weight between 400,000 and 500,000 Da, was purchased from Sigma–Aldrich, Co. (MO, USA). Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ammonium hydroxide ($NH_3{\cdot}H_2O$, [25%, w/w]), tris (hydroxymethyl) amino methane (Tris), boric acid (H_3BO_3), sodium chloride (NaCl) and sodium hydroxide (NaOH) were purchased from Guangzhou Chemical Regent Factory (Guangzhou, China). Isopropanol was acquired from SK Chemicals (Ulsan, South Korea), Tetraethyl orthosilicate (TEOS) was acquired from Acros Organic (New Jersey, USA). Sodium acetate anhydrous (NaAc) was purchased from Tianjin Guangcheng Chemical Reagent Company (Tianjin, China). Ethylene glycol was purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). All these reagents were analytical grade or better. Hydroxypropylmethylcellulose (HPMC), the viscosity in 2% aqueous solution at 25 °C was 100–4000 mPa s, was purchased from Shanghai Sangon Biological Engineering & Technology (Shanghai, China). All aqueous solutions were prepared using water obtained from an Elga water purification system (ELGA, London, UK).

A GeneRuler 100-bp DNA ladder was purchased from Fermentas (Beijing, China) and consisted of 10 fragments ranging from 100 to 1000 bp with a total concentration of 0.5 mg mL^{-1}. Taq DNA polymerase (5 U μL^{-1}), 25 mM $MgCl_2$, 10 × PCR buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl), and deoxynucleotide triphosphate (dNTP) mixture (including dATP, dGTP, dCTP, and dTTP, where the concentration of each dNTP was 2.5 mM) were purchased from Takara Biotechnology (Dalian, China). The primers (Table 1) used for PCR were synthesized by Shanghai Sangon Biological Engineering & Technology Services (Shanghai, China). TIANamp bacteria DNA kit was acquired from TianGen Biotech (Beijing, China).

2.2. Preparation of PDDA-MPs

Three steps are involved in the preparation of PDDA-MPs, as shown in Fig. 1(a). Firstly, magnetic Fe_3O_4 particles were prepared using the solvothermal reduction method [28] with minor modifications. Briefly, 1.35 g $FeCl_3{\cdot}6H_2O$ was dissolved in 40 mL ethylene glycol to form a clear solution. Then 3.6 g NaAc was added to the solution and the mixture was stirred vigorously for 30 min. The solution was introduced to a Teflon-lined stainless-steel autoclave with 50 mL capacity and heated at 200 °C for 10 h. The resulting black products were cooled to room temperature and rinsed several times with ethanol. Finally, the products were dried at 60 °C under nitrogen atmosphere for 6 h.

Secondly, the magnetic Fe_3O_4 particles were coated with silica according to the sol–gel method [29]. The as-prepared magnetic Fe_3O_4 particles (approximate 0.3 g) were dispersed in 40 mL isopropanol with the aid of ultrasonication. Then, 2 mL deionized water and 0.5 mL $NH_3{\cdot}H_2O$ (25%) were added to the suspension. Afterwards, 0.3 mL TEOS was added to the mixture dropwise and the reaction was stirred for 8 h at room temperature. Finally, the particles were rinsed several times with ethanol and dried under nitrogen atmosphere at 60 °C. The magnetic silica particles were soaked in 1 M HCl for 24 h at 4 °C, then dried under nitrogen atmosphere at 60 °C. The silica shell can isolate the inner Fe_3O_4 from outer environment, protect the Fe_3O_4 from being oxidized and provide –OH as the reaction moieties after activating using HCl solution.

Thirdly, the magnetic silica particles were functionalized with PDDA. The PDDA coating solution was prepared by dissolving PDDA at 0.2% in water, whose ionic strength was adjusted to 1.5 M by addition of NaCl. The magnetic silica particles (approximate 0.3 g) were suspended in 20 mL 1 M NaOH and shaken for 15 min, then rinsed several times with deionized water. Afterwards, the particles were added to 20 mL 0.2% PDDA solution and shaken for 15 min. Finally, the products were collected by applying an external magnetic field and dried under nitrogen atmosphere at 60 °C.

2.3. Characterization

The FT-IR spectra of PDDA, SiO_2@Fe_3O_4, and PDDA-MPs were recorded on a Bruker FT-IR spectrometer (Bruker, Germany). The zeta potentials of SiO_2@Fe_3O_4 and PDDA-MPs at different pH values were measured on a Zetasizer Nano ZS (Malvern, Worcestershire, UK). The magnetization characterization of Fe_3O_4, SiO_2@Fe_3O_4 and PDDA-MPs were performed on a vibrating sample magnetometer (Quantum Design, San Diego, USA) at room temperature and an applied field of 10 kOe. The XRD measurements of Fe_3O_4, SiO_2@Fe_3O_4 and PDDA-MPs were recorded on a Bruker D8 Advance (Bruker AXS, Germany) using Cu Kα radiation with scattering angles (2θ) of 20–80° and a counting time of 10 s per increment. The TEM images of PDDA-MPs and the conjugates between PDDA-MPs and bacteria were carried out on a JEM-2100HR transmission electron microscope (JEOL, Tokyo, Japan). SEM images of PDDA-MPs were carried out on a ZEISS Ultra 55 field emission scanning electron microscope (Carl Zeiss, Oberkochen, Germany).

2.4. Preparation of bacteria samples

Gram-negative bacteria (*E. coli* O157:H7) and Gram-positive bacteria (*L. monocytogenes*) were chosen as the model pathogenic bacteria. *E. coli* O157:H7 strain was grown at 37 °C for 8 h on a rotary shaker at 200 rpm in Luria-Bertani broth (tryptone 10 g L^{-1}, yeast extract 5 g L^{-1} and NaCl 10 g L^{-1}, pH 7.5). *L. monocytogenes* (CMCC54007) was grown at 37 °C for 10 h on a rotary shaker at 200 rpm in nutrient broth (3 g L^{-1} beef extract, 5 g L^{-1} bacterial peptone and 5 g L^{-1} NaCl, pH 7.0).

Before use, the bacterial cells were all centrifuged at 6000 rpm for 5 min and isolated from the broth. Then the isolated bacterial cells were washed with Tris-borate buffer (50 mM, pH 7.0) three times. Different concentrations of bacteria were adjusted