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RESEARCH ARTICLE

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Preparation and characterization of PS/pAPBA core-shell microspheres

Abstract Polystyrene microspheres with an average diameter of 55 μm were prepared by suspension polymerization *via* oxidation of the monomer by ammonium persulfate. Poly-3-aminophenylboronic acid was grafted onto the surfaces of the polystyrene microspheres to form polystyrene/poly-3-aminophenylboronic acid core- shell micospheres. The samples were characterized by scanning electron microscopy, Raman spectroscopy, X-ray photoelectron spectroscopy and nitrogen adsorption/desorption method. The results show that poly-3-aminophenylboronic acid was successfully grafted to the surfaces of the polystyrene microspheres by aromatic ring electron-pairing interaction. The surfaces of the core-shell microspheres possessed a porous structure, with the average pore diameter of 30.2 nm and the BET surface area of 193.26 m^2/g.

Keywords polystyrene, poly-3-aminophenylboronic acid, core-shell microspheres, structure characterization

1 Introduction

Polystyrene (PS) microspheres have gained broad applications in biomedicine due to advantages, such as high density and strong ability of adsorption [1–3]. However, their surfaces are inert. PS microspheres only passively adsorb active substances. The study of the modifying surface has gained increasing interest for expanding their application [4–7]. Zhang *et al*. [4] grafted acrylonitrile to the surfaces of PS microspheres and enhanced the polarity of the surfaces. Sun *et al*. [5] grafted electrostatic layer-by-layer assembly protein films on the surfaces of PS microspheres forming core-shell structured of PS-protein particles. The particles displayed high catalytic activity and stability. They may have potential promise in fabricating mediator-free biosensors and bioreactors.

As a functional material, poly-3-aminophenylboronic acid (pABPA) can be grafted on various supports [8] forming stable thin films [9, 10]. These films were broadly applied to selective separation and molecular recognition for bio-molecules [8, 9, 11, 12]. Recently X-ray photoelectron spectroscopy (XPS) has been used for the surface characterization of various samples. Qiu *et al*. modified Pt nanoparticles or bulk with the thin film of polymer (*N*-viny-2-pyrrolidone) and investigated the carbonyl charge transfer from Pt metal to polymer (*N*-viny-2-pyrrolidone) using XPS. Tan *et al*. modified sulfonated cation-exchange membrane and Nafion membrane using polyaniline and the obtained composite membranes were characterized with XPS [14, 15].

In this paper, pABPA was polymerized on the surface of PS microspheres forming core-shell particles in aqueous solution. The method is simple, feasible and the reacting conditions are in mild. To our best knowledge, this result is reported for the first time and can be established as a foundation for the application of the core-shell particles in biochemistry.

2 Experimental

2.1 Materials and apparatus

Styrene (analytical reagent), 2,2′-azobisisobutyronitrile (AIBN, analytical reagent), ammonium persulfate (APS, analytical reagent), poly(vinyl alcohol) (PVA,average degree of polymerization = 1750, molecular weight = 75000–79000), 3-aminophenylboronic acid (APBA, purity > 98%), Lysozyme and Papain (Sigma), Trypsin Gibco. Water was doubly distilled. Styrene was deposed using NaOH (5 wt%) for removing polymerization inhibitor, and

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then washed with water before use. All other reagents were used without further purification.

Scanning electron microscopy (SEM) micrographs were taken using JSM-6390LV SEM (Japan, Electronic Co.). SEM samples were sputtered with gold. Fourier Transform-Raman (FT-Raman) scattering spectra were recorded on a Nicolet Nexus FT-IR/Raman spectrometer (United States Nicolet). XPS analyses were performed using an Escalab MKII X-ray photoelectron spectrometer, using nonmonochromatized Al Kα X-ray as excitation source (United States). N_2 adsorption/desorption isotherms were measured at liquid-nitrogen temperature (77.4 K) using a Nova 1000 analyzer. Samples were degassed at 80°C for 6 h before measurements. Elemental analysis results were obtained with a PE-2400 CHN elemental analyzer (United States). The absorption spectra were carried on UV-visible spectrophotometer (Shanghai Optical Instrument Co., China).

2.2 Preparation of the PS microspheres

PS microspheres were polymerized by suspension polymerization. In brief, 10 mL of styrene containing 0.3 g of AIBN (as initiator), 40 mL of 3% poly(vinyl alcohol) aqueous solution and 250 mL of water were added into a 500 mL tri-neck flask with a machine stirrer, a condenser and a thermometer. The flask was immersed in water bath and the polymerization was carried out at 82–85°C for about 2 h. At the same time, stirring rate was controlled at 350 rpm. The final mixture was cooled to room temperature and filtered. The collected product was washed with water and methanol before being dried at room temperature.

2.3 The surface modification of the PS microspheres

Two separate aqueous solutions APBA and ammonium persulfate were prepared as 100 and 50 mM, respectively. Blank core-shell microspheres were prepared by placement of 1 g of dried PS microspheres and 10 mL of an APBA solution in a 25 mL open vessel. After 30 min of stirring, 10 mL of an ammonium persulfate solution was added for initiating polymerization. The reaction was carried out for 60 min at 23°C. The polymerized microspheres were separated through filtration and washed repeatedly with water. After that, the obtained products were dried at room temperature. Protein imprinted core-shell microspheres were prepared in a similar manner but containing 1 g/L protein in the APBA solution.

2.4 Protein adsorption on the core-shell microspheres

Adsorption experiments were conducted with imprinted and blank core-shell microspheres. Protein solutions (100 g/L) were prepared in PB/0.1% Tween-20 (pH 7.0). 10 mL of the protein solutions and 0.1 g of dried microspheres were added to a 25 mL vessel. Then, the vessel was sealed and shaken at room temperature. After 60 min of the incubation, the microspheres were filtered through a porous poly (vinylidene fluoride) membrane (hydrophilic membrane, millipore pore size = 0.45 μm). The amount of protein adsorbed on the surfaces of microspheres was calculated from the differences in protein concentrations before and after incubation. Protein concentrations were measured with a UV-visible spectrophotometer at 280 nm. In the control experiments, PB/0.1% Tween-20 without protein were carried out to eliminate the influence of the pAPBA on detection of the protein because pAPBA also absorbs at 280 nm.

3 Results and discussion

3.1 Morphology of the microspheres

As seen in Fig. 1(a), the obtained PS microspheres have an average diameter of 55 μm with a narrow size distribution. Compared with core-shell microsphere, the PS microsphere has a slick surface [see Fig. 1(b) and (c)]. In addition, Fig. 1(d) represented the images of broken core-shell microsphere and the interface of the core-shell. From these figures, it is believed that pAPBA polymerized on the surface of PS microspheres forming core-shell structure.

3.2 Analysis of Raman spectra

The FT-Raman spectra of PS and core-shell microspheres are shown in Fig. 2. As can be seen from the spectra, all the peaks of PS microsphere became obviously weak. The peak of the N–H stretching vibration appeared around 3340 cm^{-1} (arrow pointing to) in the spectrum of the core-shell microspheres. This shows that pAPBA grafted on the surfaces of PS microspheres.

3.3 Analysis of XPS

In the XPS of core-shell microspheres (Fig. 3), appearances of the peaks (B1s, N1s and O1s) were able to prove the grafting success of pAPBA on PS microspheres. The observation is in good agreement with the results of TEM and FI-Raman.

In order to understand the interface interaction between the core and shell, C1s XPS spectra of the pAPBA powder and core-shell microspheres (Fig. 4) were recorded. From the spectra, C1s XPS spectrum of the pAPBA powder was in symmetry. But for the core-shell microspheres, there is a shoulder peak on higher binding energy side. The shoulder peak is from the C1s of the core-shell interface [13]. We attributed this chemical shift to the special chemical microenvironment on the interface of the core-shell. Bossi *et al*. [10] thought that pAPBA grafted tightly to the PS

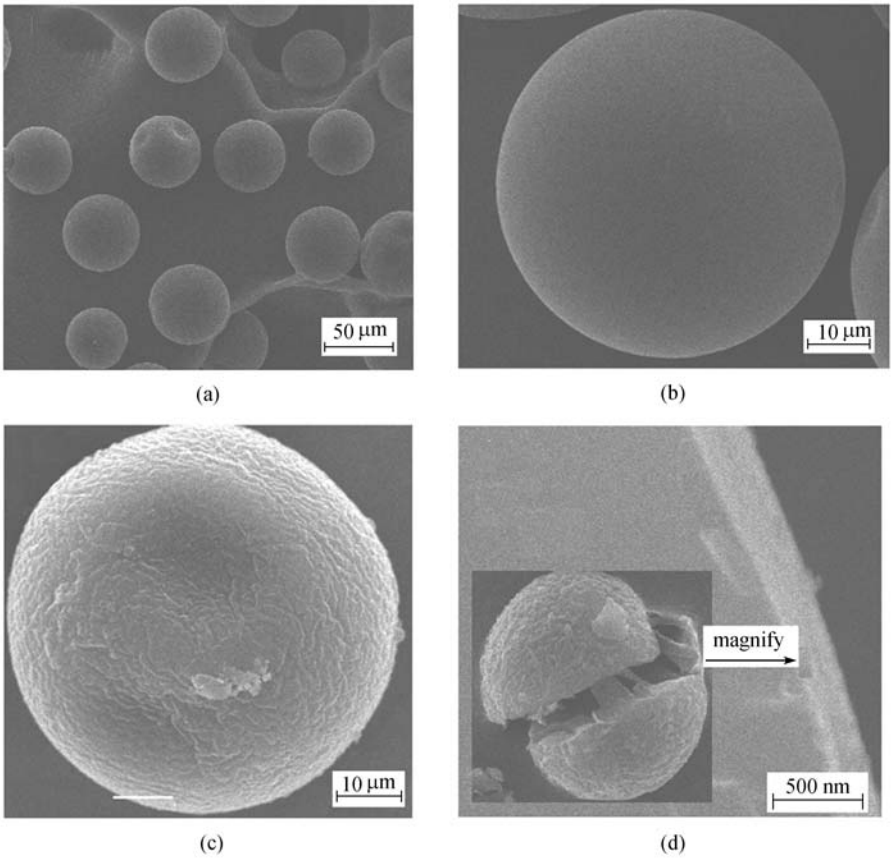

Fig. 1 SEM micrographs of (a) PS microspheres, (b) single PS microsphere, (c) single PS/pAPBA core-shell microsphere, and (d) core-shell interface

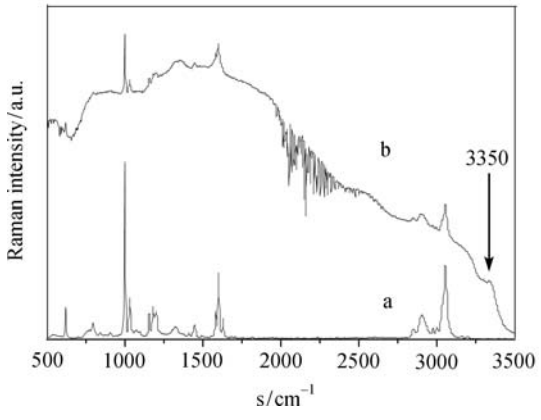

Fig. 2 FT-Raman spectra of the samples
(a) PS microspheres; (b) PS/pAPBA core-shell microspheres

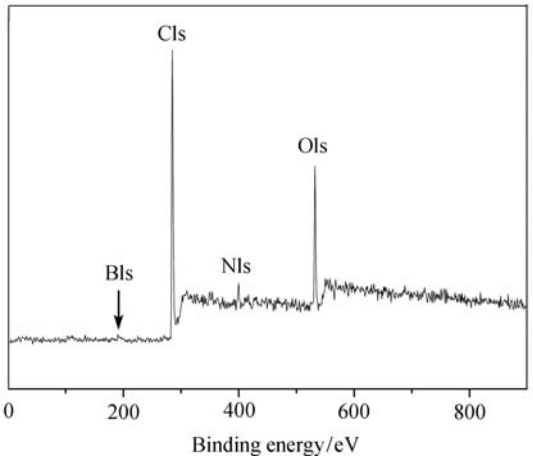

Fig. 3 XPS spectrum of the PS/pAPBA core-shell microspheres

surface by an aromatic ring electron-pairing interaction. Therefore, we speculated that the electron density around the carbon atoms due to the electron-pairing interaction between aromatic rings decreased and the C1s binding energy in the benzene ring increased [14, 15].

3.4 Elemental analysis of nitrogen

The data of elemental analysis shows that nitrogen content is 0.03 wt% in the sample of the core-shell microspheres. And the pAPBA content is derived to be 0.29 wt%. This indicated that only a few of pAPBA were grafted on PS

Table 1 Adsorption of proteins on blank and imprinted core-shell microspheres *

Protein	Adsorption amount/($\mu g \cdot g^{-1}$)		
	Lysozyme	Trypsin	Papain
Blank core-shell microspheres	804	820	1549
Imprinted core-shell microspheres	1875	2678	2545

* Adsorption was performed in 10 mL of 50 mmol/L sodium phosphate buffer/0.1% Tween-20 (pH = 7.0) with a protein concentration of 0.1 g/L and 0.1 g of dried microspheres for 1 h at room temperature.

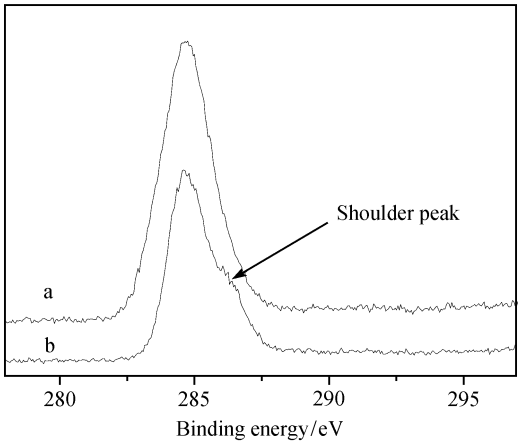

Fig. 4 C1s XPS spectra of (a) pAPBA powder and (b) PS/ XPS spectrum of the PS/pAPBA core-shell microspheres

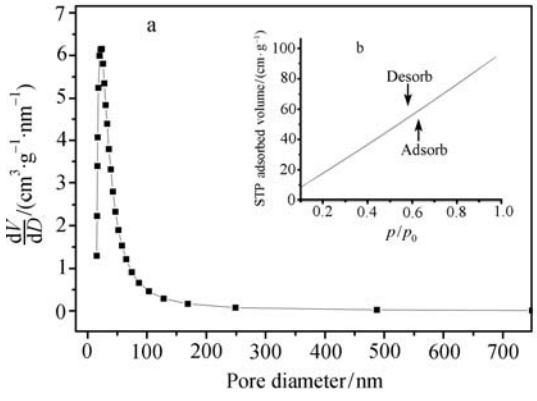

Fig. 5 Pore size distribution plot (a) and nitrogen adsorption/desorption isotherm (b) of PS/pAPBA core-shell microspheres

microspheres. The result is in agreement with the Raman spectra results (weak peaks of pAPBA). Furthermore, based on the structure of the pAPBA, the mass fraction of a molecular layer pAPBA on core-shell microspheres with a diameter of 55 μm was roughly calculated to be 7.0×10^{-5}. The ratio of the calculated value to the measured value (0.29%) is 1∶41. Thus, it is enough to form an integrate shell on the surface of PS microsphere.

3.5 Physical adsorption/desorption of nitrogen

From the pore size distribution plot and nitrogen adsorption/desorption isotherm of the core-shell sample (see Fig. 5), the adsorption and desorption isotherms almost fully overlap and form a beeline. Such adsorption is of Brunane Ⅱ, that is, gas physical adsorption on solid surface. The pore size distribution is narrow and the pore diameter is 30.2 nm. The calculated BET surface area is 193.26 m^2/g. The results show that the shell is a sort of porous thin film and the large surface area is from a mass of middle micropore on the surface of the microspheres.

3.6 Special adsorption of the protein imprinted core-shell microspheres

The adsorption experiments were conducted using imprinted and blank core-shell microspheres. The experimental data were presented in Table 1. The imprinted microspheres possess special adsorption for templates due to their recognition sites. Compared with other imprinted material, the imprinted microspheres have higher adsorption ability [16].

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