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Preparation of Boronic Acid-Functionalized Magnetic Nanoparticles by Polarity-regulating Molecular Self-assembly

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Abstract. Boronic acid-functionalized magnetic nanoparticles were prepared and characterized in this investigation. At first, the single-layer oleic acid-coated Fe₃O₄ magnetic nanoparticles were prepared by the chemical coprecipitation method and then the nanoparticles were surface-modified by a layer of poly(4-vinylphenylboronic acid) by the polarity-regulating molecular self-assembly technique to obtain the boronic acid-functionalized magnetic nanoparticles (Fe₃O₄@OA@BA). The resulted nanoparticles were characterized by Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analyzer (TGA), transmission electron microscope (TEM) and vibrating sample magnetometer (VSM). The results show that the molecules of oleic acid can form the inner layer chemisorption on the surface of Fe₃O₄ and the double-layer coating structure on the outer layer. The poly(4-vinylphenylboronic acid) coated nanoparticles with content about 26% have an average size of 15 nm and the saturation magnetization of 43 emu/g. Finally, the adsorption effect of Fe₃O₄@OA@BA on bovine serum albumin (BSA) was studied. Under optimal adsorption conditions, the adsorption capacity on BSA is 135 μg/mg, which embodies the superiority of molecular self-assembly to prepare functionalized nanoparticles.

1. Introduction

In recent years, functional magnetic nanoparticles have been attracted extensive attention due to their potential applications in the fields of biomacromolecular separation, enzyme immobilization, targeted drug delivery, magnetic resonance imaging, and immunoassay, etc. [1,2]. Nanoparticles are prone to agglomeration because of their high specific surface area and surface energy. However, as a common surface modifier oleic acid can reduce the surface energy effectively which has strong interaction forces among magnetic nanoparticles and prevent the agglomeration of nanoparticles [3-6]. In addition, Fe₃O₄ magnetic particles have strong magnetic properties and excellent biocompatibility and can be linked to functional biomolecular groups, making them be used widely in the nucleic acid analysis, targeted drugs, enzyme immobilization, etc. [7-9]. On the other hand, boronic acid chromatography materials have been used to detect and separate cis-diol molecules for a long time. Phenylboronic acid and its derivatives can also be combined with polyhydroxy compounds, such as glucose, polysaccharides, nucleic acids, and proteins, to form reversible complexes. Therefore, they can be used for identification, detection, and separation of these substances [10]. Liu et al. synthesized dendrimers with magnetic particles as carriers and modified a large amount of phenylboronic acid groups onto the surface of the magnetic nanoparticles [11]. And they were applied to accumulate three glycoproteins, horseradish peroxidase (HRP), transferrin (TRF) and anti-alpha fetoprotein (anti-AFP).



Compared with the previous materials, the enrichment efficiency was improved significantly. Based on the distillation precipitation polymerization and click chemistry, Zhang et al. prepared boronic acid modified magnetic hybrid materials which can successfully separate and collect the target glycoprotein from egg white [12]. In this work, we prepared boronic acid-functionalized magnetic nanoparticles by the polarity-regulating molecular self-assembly technique [13]. The amphiphilic monomer of 4-vinylphenylboronic acid was used to build up a self-assembled molecular layer on the surface of magnetic nanoparticles, in which the vinyl groups were inward and the boronic acid groups outward surrounding the core of nanoparticles. The possible structure of the nanoparticles is shown in Fig.1. Such a core-shell structure will have rich functional groups on the surfaces of nanoparticles for ease of use.

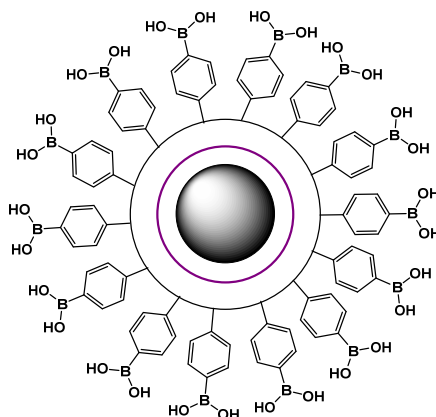


Figure 1. The structure of boronic acid-functionalized magnetic nanoparticles

2. Experimental Section

2.1. Reagents and Instruments.

The reagents used were of analytical grade. Oleic acid (OA) and $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ were bought from Tianjin Fuchen and Damao Chemical Reagent Factory, respectively. Ammonia, absolute ethanol, acetic acid, sodium acetate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, standard pH buff reagents (4.10, 6.86, 9.81) and bovine serum albumin (BSA) were bought from Sinopharm Chemical Reagent Co., Ltd. Phosphate buffer solutions (PBS, pH=6.0, 7.2) were prepared by dissolving $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ in double-distilled water. Acetic acid and sodium acetate were used to prepare a buffer solution of pH 3.3. Potassium persulfate was from Shanghai Degussa Catalyst Co., Ltd. The monomer of 4-vinylphenylboronic acid was from TCI Chemical Industrial Development Co., Ltd. The Tensor 27 Fourier transform infrared spectroscopy (Bruker, Germany) was used to record the IR spectra. The magnetic properties of the nanoparticles were measured by the PPMS-9 vibrating sample magnetometer (American Quantum Design Co., Ltd.). The TG209F1 thermogravimetric analyzer (Netzsch, Germany) was used under nitrogen protection and with the heating rate of $20^\circ\text{C}/\text{min}$. The JEM-2100 high-resolution transmission electron microscope (Hitachi, Japan) was used to observe the morphology of nanoparticles. The UV1800 spectrophotometer (Shimadzu, Japan) was used to measure the UV-vis spectra.

2.2. Procedure

2.2.1 Preparation of Boronic Acid-functionalized Magnetic Nanoparticles

In a three-necked flask, 0.75 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 2.18 g of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ were dissolved in 100 mL of double-distilled water in a nitrogen atmosphere. Then 5 mL of ammonia was added to the solution and stirred at 1000 r/min at a temperature of 70°C . After 20 min, a solution of 3 mL of oleic acid (dissolved in 30 mL of absolute ethanol) was added and the reaction was continued for 60 min. After that, it was allowed to stand for 30 min at room temperature to obtain the double-layer oleic acid coated magnetic nanoparticles ($\text{Fe}_3\text{O}_4@\text{BOA}$). The samples were washed with double-distilled water

and absolute ethanol alternately to get the single-layer oleic acid coated magnetic nanoparticles ($\text{Fe}_3\text{O}_4@\text{OA}$).

A mixture of 10 mg of $\text{Fe}_3\text{O}_4@\text{OA}$ and 25 mg of 4-vinylphenylboronic acid was sonicated for 30 min in a 250 mL water-ethanol (3:2) dispersion medium. The mixture was allowed to stand at room temperature under nitrogen for 3 h. Then a 3 mL solution of potassium persulfate (1 mg/mL) was added very carefully to avoid stirring the solution at a temperature of 70°C and *in-situ* polymerization was carried out for 3 h. The resulted nanoparticles were washed with absolute ethanol and double-distilled water alternately and dried to obtain boronic acid-functionalized magnetic nanoparticles ($\text{Fe}_3\text{O}_4@\text{OA}@\text{BA}$).

2.2.2 Adsorption of BSA by $\text{Fe}_3\text{O}_4@\text{OA}@\text{BA}$

In 100 mL of double-distilled water, 10 mg of $\text{Fe}_3\text{O}_4@\text{OA}$ was ultrasonically dispersed for 20 min. Then a 50 mL of BSA solution (1 mg/mL) was added and pH buff solutions were used to adjust the values of pH. After stringing for a while, the solution was kept static for 2 h for the absorption of BSA. After removing the nanoparticles, the UV-vis absorbance spectra of the clarified liquid layer were measured for calculating the adsorption amount of BSA.

3. Results and Discussion

3.1 FT-IR

FT-IR spectra of double-layer, single-layer oleic acid coated and boronic acid-functionalized magnetic nanoparticles are shown in Fig.2. All of them have a broad absorption peak at 3430 cm^{-1} , which is the stretching vibration of -OH. Meanwhile, the peaks at 2930 and 2850 cm^{-1} are the asymmetric and symmetric stretching vibration of $-\text{CH}_2$ in oleic acid and the stretching vibration peaks of $-\text{COO}$ are at 1630 and 1410 cm^{-1} , whereas the peak at 586 cm^{-1} is caused by the stretching vibration of Fe-O bonds. These results indicate that the nanoparticles contain oleic acid components [14]. Moreover, there is a stretching vibration absorption at 1710 cm^{-1} from the C=O bonds of oleic acid at the surface of the nanoparticles as shown in Fig.2(a). However, for single-layer oleic acid-coated ones in Fig.2(b), no obvious stretching vibration absorption of C=O bonds is observed because of the formation of chemical bonds between the carboxyl groups in the oleic acid molecule and the hydroxyl groups existed on the surface of nanoparticles. In Fig.2(c), the peaks at 1630 and 1520 cm^{-1} are the stretching vibration absorption of C=C skeleton in the benzene ring. The peaks at 1020 and 830 cm^{-1} are caused by the in-plane and out-plane deformation vibration absorption of C-H in benzene ring also. The stretching vibration of the B-O and B-C bonds can be seen at 1360 and 1080 cm^{-1} , respectively [15,16]. The appearances of the characteristic peaks indicate that the molecules of 4-vinylphenylboronic acid are coated on the surface of $\text{Fe}_3\text{O}_4@\text{OA}$ successfully.

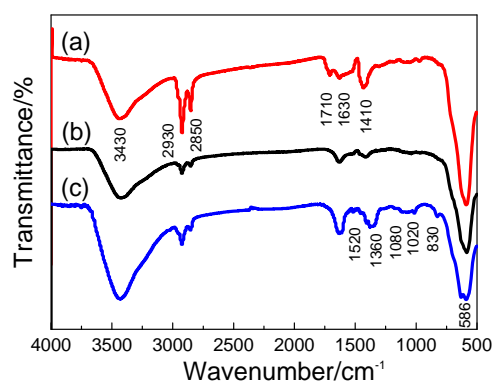


Figure 2. FTIR spectra of nanoparticles. (a) $\text{Fe}_3\text{O}_4@\text{BOA}$; (b) $\text{Fe}_3\text{O}_4@\text{OA}$; (c) $\text{Fe}_3\text{O}_4@\text{OA}@\text{BA}$

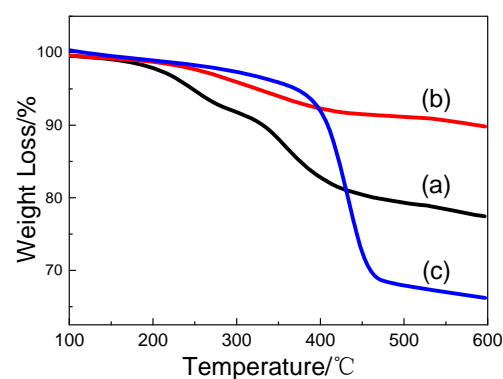


Figure 3. TGA of nanoparticles. (a) $\text{Fe}_3\text{O}_4@\text{BOA}$; (b) $\text{Fe}_3\text{O}_4@\text{OA}$; (c) $\text{Fe}_3\text{O}_4@\text{OA}@\text{BA}$

3.2 TGA

Fig.3 is showing the TGA curves of double-layer, single-layer oleic acid coated and boronic acid-functionalized magnetic nanoparticles. It can be seen from Fig.3(a) that the curve has two distinct weight loss stages. The first one is during the temperature of 200-300°C, due to the loss of the physical adsorption layer for oleic acid, and the value is only 7%. The second one ranging from 300-500°C has a weight loss of 10% due to the chemisorbed oleic acid in the inner layer. The curve of $\text{Fe}_3\text{O}_4@\text{OA}$ in Fig.3(b) also has a weight loss of 10%, which is consistent with the loss of the double-layer oleic acid in the inner layer. However, the temperature is slightly lower than that of the previous one due to only one coating layer existing on the surface of nanoparticles. In this case, the heat consumption is reduced. For $\text{Fe}_3\text{O}_4@\text{OA}@\text{BA}$ in Fig.3(c), the curve has only one weight loss stage and the value is about 36%. Therefore, the amount of coated poly (4-vinylphenylboronic acid) layer is about 26%, which tells us that the molecules of 4-vinylphenylboronic acid are coated on the surface of the nanoparticles.

3.3 Morphology and Magnetic Performance Analysis

Fig.4 is a TEM photograph for $\text{Fe}_3\text{O}_4@\text{OA}@\text{BA}$. The relatively regular shape can be seen from it and the average size is about 15 nm, which is slightly larger than that of $\text{Fe}_3\text{O}_4@\text{OA}$ [17].

Magnetic hysteresis loops are shown in Fig.5, the remanence and coercive force of the three nanoparticles are zero, so they all have superparamagnetism. The saturation magnetization of Fe_3O_4 is 62 emu/g. When coated with a layer of oleic acid, the value is slightly reduced to 59 emu/g due to the presence of non-magnetic components. When a layer of poly (4-vinylphenylboronic acid) is wrapped further, this value is decreased to 43 emu/g.

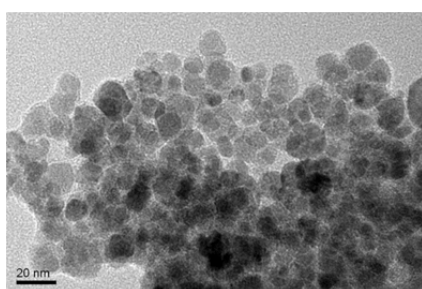


Figure 4. TEM image of $\text{Fe}_3\text{O}_4@\text{OA}@\text{BA}$

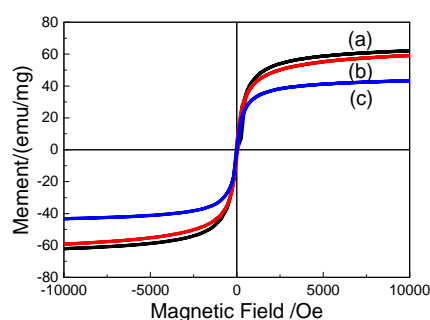


Figure 5. Hysteresis loops of (a) Fe_3O_4 ; (b) $\text{Fe}_3\text{O}_4@\text{OA}$; (c) $\text{Fe}_3\text{O}_4@\text{OA}@\text{BA}$

3.4 Adsorption of BSA by $\text{Fe}_3\text{O}_4@\text{OA}@\text{BA}$

The molecules of BSA contain aromatic amino acids with conjugated double bonds such as tyrosine, phenylalanine, and tryptophan. The UV-vis absorption spectrum appears a peak at 278 nm as shown in Fig.6, which is caused by the $\pi \rightarrow \pi^*$ electronic transfer of aromatic heterocycle in BSA [18]. A linear relationship between absorbance and concentration of BSA is inserted in Fig.6. Thus, the absorption amount of BSA on the nanoparticles can be calculated by measuring the absorbance of related solutions.

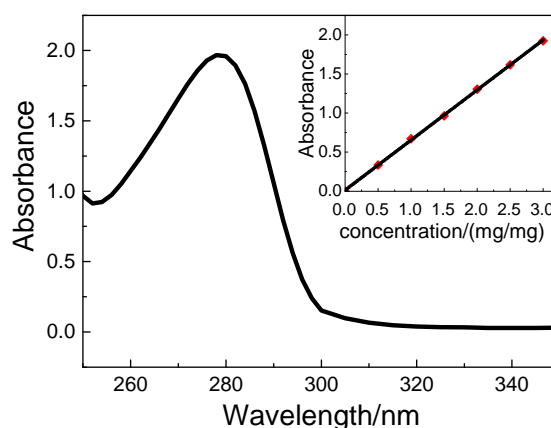


Figure 6. The UV-vis absorption spectrum of BSA. The inset is the plot of the absorbance at 278 nm against the concentration of BSA.

The effects of pH, adsorption time and initial concentration of BSA on the adsorption process were studied in this investigation. The results are shown in Fig.7. As the pH increases, shown in Fig.7(a), the adsorption amount is increased firstly and then decreased. When pH equals 6, a maximum adsorption amount appears. Usually, the variety of pH will lead to some changes in the configuration and surface properties of BSA, which may affect the adsorption behaviors of BSA. The experimental results show that the interaction between 3-aminophenyl boronic acid and BSA is mainly via tryptophan residues of BSA by fluorescence quenching method [19]. Since the isoelectric point (PI) of the tryptophan residue is around pH 6, the BSA molecule will exist in a single configuration in solution. Therefore, the carbonyl groups in tryptophan residues and the boronic acid groups in nanoparticles tend to form hydrogen bonds which will facilitate the adsorption process. When pH is adjusted to more than 6, BSA can be desorbed from the surface of functionalized magnetic nanoparticles because the boric acid groups are mainly in the form of $B(OH)_3^-$ in this case. The molecular structure of BSA is also changed, which affects the combination in turn [20]. In Fig.7(b), the adsorption amount of BSA is gradually increased and it is found that the adsorption process reaches equilibrium at room temperature for about 2 h. On the other hand, the adsorption amount of BSA is increased with the increasing concentration of BSA as shown in Fig.7(c). Based on the results the adsorption amount is reached $135\mu\text{g}/\text{mg}$ when the initial concentration of BSA is $1\text{ mg}/\text{mL}$. The result is showing an excellent adsorption performance for the prepared nanoparticles.

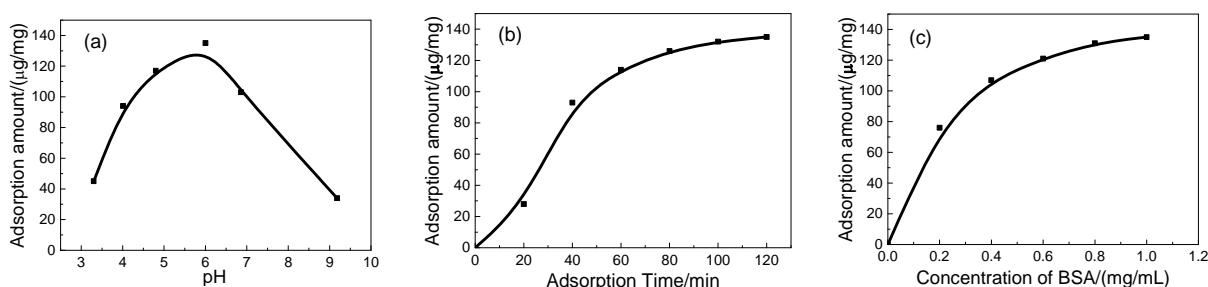


Figure 7. Effects of pH, adsorption time and initial concentration of BSA on the adsorption amount (a) pH ($C_{\text{BSA}}=1\text{ mg}/\text{mL}$, $t=2\text{h}$); (b) adsorption time ($\text{pH}=6$, $C_{\text{BSA}}=1\text{ mg}/\text{ml}$); (c) initial concentration of BSA ($\text{pH}=6$, $t=2\text{h}$);

4. Conclusion

This work is to conduct preliminary exploration on the preparation of boric acid-functionalized magnetic nanoparticles by the technique of polarity-regulating molecular self-assembly. The nanoparticles prepared by this method have abundant boric acid groups on the surface, so they can be

used for adsorption of biological macromolecules such as BSA easily. This is a successful attempt for preparation of functional nanoparticles by the technique. The following researches will try to explore the mechanism for the molecular self-assembly and optimize the preparation process. These should be helpful for manufacturing nanoparticles with excellent performance.

5. References

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