Chinese Chemical Letters Vol. 16, No. 8, pp 1059-1062, 2005 1059
http://www.imm.ac.cn/journal/ccl.html

Preparation of Functional Magnetic Nanoparticles and its Application in Diagnostic Analysis

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Abstract: The magnetic nanoparticles modified with carboxyl functional group were synthesized and characterized. These nanoparticles covalently bound with hepatitis B surface antibody (HBsAb), were used to detect hepatitis B surface antigen (HBsAg) in immunovoltammetry. The detection limit was found to be 0.06 ng/mL, which is much higher than that of enzyme-linked immunosorbent assay (ELISA) used in clinical analysis.

Keywords: Functional magnetic nanoparticles, synthesize, characterization, immunoassay, electrochemical detection

In the recent years, nanosized magnetic particles have been paid considerable attention in the fields of medicine and biotechnology[1,2]. Magnetic particles have been widely used in the immobilization of enzyme[3], immunoassay[4], bioseparation[5], biosensor[6], targeting drug[7], environmental analysis[8,9] and so on. Functional magnatic nanoparticles were made up from the organic polymer compound and inorganic magnetic materials by co-polymerization. They not only possess magnetic property, but also bear the functional groups on their surface, such as carboxyl, amino, hydroxyl and aldehyde. Many bioactive substances such as antibody, enzyme, protein and nucleic acid have been bound to them by use of coupling reagents.

The detection of HBsAg has been studied extensively. The enzyme-linked immunosorbent assay (ELISA), as a most conventional method, is widely used in clinic. However, its sensitivity is limited due to less amount of antibody adsorbed on polyethylene plate, and the analytical procedure is also time-cost. It is necessary to search more sensitive and simple method for clinical diagnose. In this paper, carboxyl magnetic nanoparticles are synthesized for detecting HBsAg, its effective magnetic separation and sensitive electrochemical measurement is reported. Firstly, the nanoparticles modified with HBsAb capture HBsAg and second HBsAb labeled with horseradish peroxide. The magnetic nanoparticles with "sandwich" complex are then separated magnetically from sample solution. The enzyme labelled complex catalyzes the reaction of 2-amino-hydroxybenzene and H_2O_2 in the buffer solution. Finally, the electroactive product of 3-amino phenoxazine is detected with differential pulse

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voltammetry (DPV).

Experimental

Preparation of magnetic nanoparticles

35.5 g polyglycol, 33 mL 0.16 mol/L $FeCl_2$, 12 mL 0.06% H_2O_2 and 40 mL distilled water were added to a three neck flask with gentle stirring under nitrogen atmosphere. 3 mol/L NaOH was dropped into the flask to adjust pH to 11 and the temperature was maintained at 50℃ during the reaction period. After 4 h the Fe_3O_4 nanoparticles were separated by applying magnetic filed and washed with distilled water. The diameters of Fe_3O_4 nanoparticles were 8～10 nm measured with transmission electron microscopy (TEM).

3 mL magnetic Fe_3O_4 nanoparticles, 7 g polyglycol-4000, 60 mL ethanol and 15 mL distilled water were dispersed 10 min by ultrasonic in a three neck flask, then 20 mL styrene, 2 mL crylic acid, 0.8 mL divinylbenzene and 3 g acetyl benzoyl peroxide were added with vigorous stirring. The reaction temperature was kept at 75℃ for 10 h under nitrogen atmosphere. Finally, a brown product of magnetic nanoparticles modified with carboxyl functional group was separated magnetically and washed with distilled water and ethanol[10].

Characterization

The morphology of the magnetic nanoparticles was examined using a JEM-2010 TEM. (**Figure 1**) The result shows that the diameter of nanoparticles is about 90～100 nm. The magnetic measurement was carried out at a PPMS－9 physical property system. **Figure 2** shows magnetization curve of the nanoparticles. From the curve, the saturation magnetization (M_s) of 1.26 emu/g and the remanent magnetization (M_r) of 0.01 emu/g, could be determined, illustrating superparamagnetic property of the nanoparticles. The carbonyl group of the nanoparticles was confirmed by an adsorption peak of 1720.32 cm^{-1} using a Vector-22 Fourier transform infrared (FTIR) spectroscopy.

Binding of antibody onto magnetic nanoparticles

20 mg of carboxyl magnetic particles were added to 1 mL of 0.1 mol/L phosphate buffer solution (PBS). The solution was then shaken for 15 min with 0.5 mL of carbodiimide solution (0.025 g/mL). Finally, 0.5 mL HBsAb (0.1 mg/mL) was added, and the mixture was shaken for 2 h. The binding process was carried out at room temperature. The antibody-bound magnetic particles were separated magnetically from the mixture and washed with 0.1 mol/L Tris-HCl-0.05% Tween 20. 20 mg/mL HBsAb particles solution was prepared with 0.1 mol/L PBS for using.

Figure 1 TEM images of magnetic nanoparticles

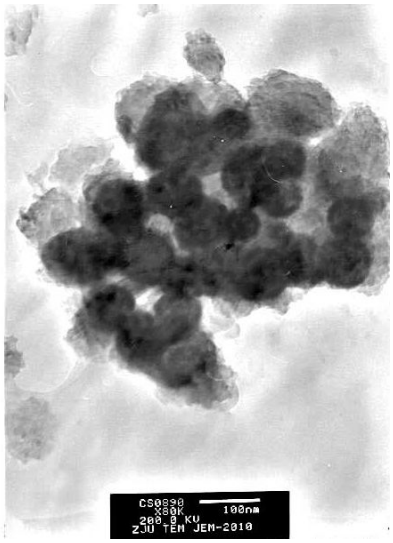
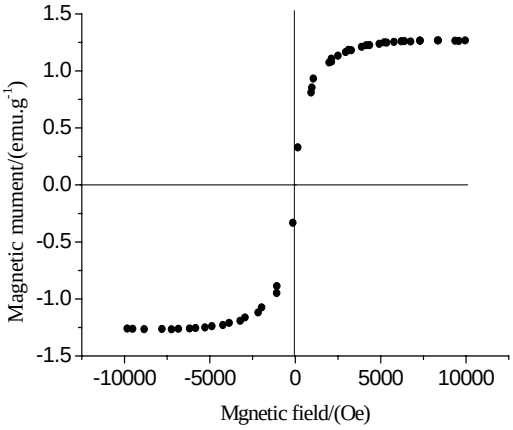


Figure 2 Magnetization curve for magnetic nanoparticles



Electrochemical detection of HBsAg

20 μL HBsAb particles solution were mixed with 50 μL diluted solutions of HBsAg and second HBsAb labeled with horseradish peroxide solution (1:2000). The mixture was kept in a water bath at 37℃ for 10 min, and then separated magnetically. The particles with "sandwich" complex were added in a mixed solution of 0.1 mL 1.0×10^{-2} mol/L 2-amino hydroxybenzene, 0.5 mL 4.0×10^{-3} mol/L H_2O_2 and 0.5 mL 0.1mol/L PBS. After the mixture was kept in a water bath at 37 ℃ for 30 min, 3-amino phenoxazine in the solution was detected at a gold film electrode in DPV. **Figure 3** shows that there is a pair of sharp peaks at –0.27 V and –0.32 V in the voltammograms. However, no peak is observed if the nanoparticles without immune complex are used (there is no antigen in the sample solution). **Figure 4** shows that the peak current is linear with the concentration of HBsAg in the range of 0.2～1.0 and 1.0～500 ng/mL. The linear correlation equations are y (μA) = 2.474*x* (ng/mL)+0.1313 and y (μA) = 0.06218*x* (ng/mL)+3.323, correlation coefficients are 0.9908 and 0.9961, respectively. The detection limit is found to be 0.06 ng/mL, while the detection limit of ELISA method is 1 ng/mL.

Conclusion

The carboxyl functional magnetic nanoparticles with superparamagnetic property are synthesized by dispersing polymerization method. The nanoparticles are bound with HBsAb for capturing HBsAg in samples. The DPV is used to detect electroactive product in buffer solution. The sensitivity of this novel method is much higher than that of ELISA in HBsAg detection. It is obvious that the high sensitivity is attributed to the

huge surface area of nanoparticles for capturing HBsAg and sensitization of electrochemical measurement. Also, this method is convenient and time-saving. It will be expected to enhance the sensitivity for detecting different antigens in diagnostic analysis.

Figure 3 Cyclic voltammograms of the electro-active product for immune particles reacted with (a) 0.0 and (b) 80 ng/mL antigen

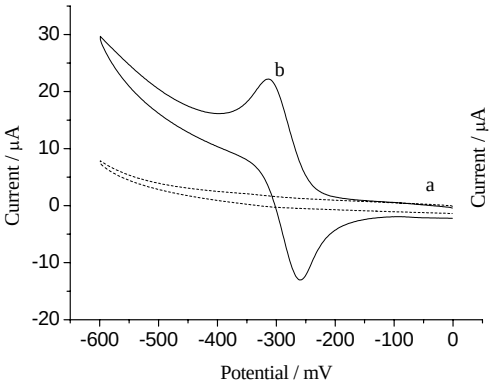
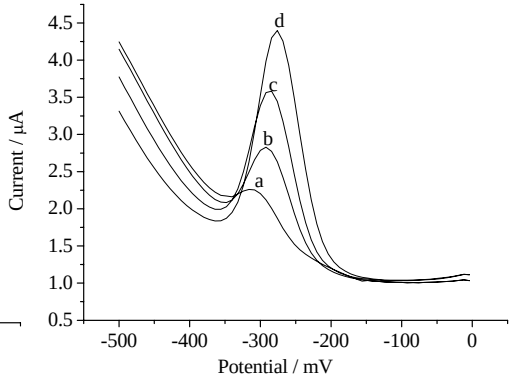


Figure 4 Differential pulse voltammograms for a. 0; b. 0.2; c. 0.5; d. 1.0 ng/mL of HBsAg



Acknowledgment

The work was supported by the National Natural Science Foundation of China (29975024，20275034), the Key Project of Science and Technology of Zhejiang Province (2003C21024) and the Instrumental Analysis Foundation of Zhejiang Province (04062). The authors thank the Instrumental Analysis Center of Zhejiang University for special measurements.

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Received 27 Augest, 2004