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Competitive magnetic immunoassay for protein detection in thin channels

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ABSTRACT

Functional magnetic nanoparticles are prepared and characterized for protein detection in a magnetic separation channel. This detection method is based on a competitive immunoassay of magnetic separation in thin channels using functional magnetic nanoparticles. We used protein A–IgG complex to demonstrate the feasibility. Free IgG and fixed number of IgG-labeled microparticles were used to compete for limited sites of protein A on the magnetic nanoparticles. Several experimental parameters were investigated for protein detection. The deposited percentages of IgG-labeled microparticles at various concentrations of free IgG were determined and used as a reference plot. The IgG concentration in a sample was deduced and determined based on the reference plot using the deposited percentage of IgG-labeled microparticles from the sample. The linear range of IgG detection was from 5.0×10^{-8} to 1.0×10^{-11} M. The detection limit was 3.69×10^{-12} M. The running time was less than 10 min. Selectivities were higher than 92% and the relative errors were less than 7%. The IgG concentration of serum was determined to be 3.6 mg ml^{-1}. This measurement differed by 8.3% from the ELISA measurement. The recoveries of IgG spiked in serum were found to be higher than 94%. This method can provide simple, fast, and selective analysis for protein detection and other immunoassay-related applications.

1. Introduction

Protein detections are important to many biochemical and clinical analyses [1–3], and are routinely used as references for medical diagnosis and clinical applications. Enzyme-linked immunosorbent assay (ELISA) is a very sensitive technique in protein detection, but it is also very laborious and time-consuming. Simple, fast, sensitive, and reliable methods for detecting proteins are still needed for various bio-related applications and deserve further investigation. Magnetic immunoassay has the advantages of being simple, fast, and selective. Protein immobilization on nanoparticles can provide more consistent immobilization and higher surface areas than that of direct immobilization on the channel. Therefore, functional magnetic nanoparticles combined with a magnetic immunoassay are very promising for use in biochemical and biomedical applications. This article reports a method of protein detection based on a competitive immunoassay of magnetic separation in thin channels using functional magnetic nanoparticles. Functional magnetic nanoparticles were prepared from a simple polymeric modification and protein conjugation of magnetite nanoparticles. The model complex of protein A–IgG was used for protein detection.

In this detection system, protein A-conjugated magnetic nanoparticles were delivered through a thin channel to form a deposition layer under magnetic fields. Then, the predeposited layer selectively captured the immunoglobulin G (IgG), which was injected into the system sequentially. Free IgG was used to compete with the fixed numbers of IgG-labeled microparticles for limited sites of protein A on the magnetic nanoparticles. The deposited percentages of IgG-labeled microparticles were plotted at various concentrations of free IgG as a reference plot for the fixed numbers of protein A-labeled magnetic nanoparticles. The IgG concentration in a sample was then deduced and determined based on the reference plot using the deposited percentages of the sample.

2. Experimental

2.1. Materials

Glycidyl methacrylate (GMA), polyvinylpyrrolidone (PVP), 2,2-azobisisobutyronitrile (AIBN), immunoglobulin G (rabbit IgG), protein A, N-hydroxysuccinimide, 1-ethyl-3-(3-dimethyl aminopropyl)carbidiimide, 1,6-hexamethylenediamine, and ethylene glycol dimethacrylate (EGDMA) were purchased from Aldrich (St. Louis, MO, USA). Ferric chloride, ferrous chloride, sodium hydroxide, and ethylene dimethacrylate were purchased from J.T. Baker (Philipsburg, NJ, USA). A permanent magnet assembled from rare

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earth magnets was used to generate magnetic fields for experiments. The maximum energy product of Nd–Fe–B magnets was 3.50×10^7 G Oe. The channel assembly was the same as that used in a previous study in the literature [4]. The channel length, breadth, and thickness used were 1.0, 0.02, and 0.015 cm, respectively. The calculated void volume was 0.0003 ml.

2.2. Particle preparation

Microparticles of polyglycidyl methacrylate (PGMA) were prepared by PVP-stabilized dispersion polymerization of glycidyl methacrylate [5]. Solution A was prepared by mixing 4.0 g GMA, 0.25 g AIBN, and 0.4 g EGDMA. Solution B was prepared by mixing 0.4 g PVP, 35 ml ethanol, and 35 ml H_2O. Solutions A and B were then mixed to prepare PGMA microparticles by stirring under nitrogen gas at 65 °C for 4 h. Magnetite nanoparticles were prepared by chemical precipitation of ferric and ferrous chlorides with sodium hydroxide. Polymeric modifications of magnetite nanoparticles are commonly used to improve particle stability in solutions and to provide particle functions for applications [5–10]. Surface modifications of magnetite nanoparticles using PGMA were prepared for core/shell nanoparticles. Core/shell nanoparticles were prepared by adding magnetites into solution A before mixing with solution B as in the PGMA preparation step, then stirring under nitrogen gas at 65 °C for 4 h. The amount of reagents in solutions A and B was adjusted to form different sizes of core/shell nanoparticles. One gram of core/shell nanoparticles was mixed with 2.0 g of hexamethyldiamine in 20 ml of ethanol at 65 °C for 12 h to convert the epoxy into amine groups. Proteins were conjugated with core/shell nanoparticles to form functional magnetic nanoparticles. Phosphate buffered saline solutions with pH of 7.02 were used as carriers for protein solutions. The coupling of protein and particles was prepared by mixing 0.1 mg of particles with 0.153 g 1-ethyl-3-(3-dimethyl aminopropyl)carbidiimide, 0.023 g N-hydroxysuccinimide, and 10^{-8} M protein in 10 ml PBS solution for 4 h. A multichannel syringe pump (KD Scientific, Boston, MA, USA) was used for carrier delivery.

2.3. Particle characterization

Hemacytometer and light microscopy (Olympus BX-50, Tokyo, Japan) were used for microparticle counting and concentration calculation. Atomic force microscopy (AFM) and transmission electron microscopy (TEM) were used to observe the size and shape of nanoparticles. The crystal structure was measured by an X-ray diffraction spectrometer (Shimazu XRD-7000, Kyoto, Japan). The magnetization curves of particles were studied by a superconducting quantum interference device (SQUID) magnetometer. The functional groups of epoxy and amine on particles were verified using FT-IR spectrometry. The deposited percentages of microparticles were calculated from dividing the number of deposited particles by the number of deposited and eluted particles.

3. Results and discussions

3.1. Preparation and characterization of particles

The sizes of magnetite (Fe_3O_4) nanoparticles were 40, 100, 200, and 350 nm as verified by AFM. The structures of these magnetite nanoparticles were confirmed by X-ray diffraction peaks on (2 2 0), (3 3 1), (4 4 0), (4 2 2), and (5 3 1). The sizes of core/shell nanoparticles after coating magnetite with PGMA were 80, 200, 400, and 860 nm, respectively. The AFM image of 80 nm core/shell nanoparticles is shown in Fig. 1. The average size was 80 ± 8 nm. The amount of polymer shell on the core/shell nanoparticles was about 65% as deduced from the mass loss of thermo-gravimetric analysis after heating the nanoparticles to 700 °C. The magnetization curves for different sizes of core/shell nanoparticles are shown in Fig. 2. The reasonably high values of saturated magnetization may be due to the crystal structure and high weight percentages of the magnetite

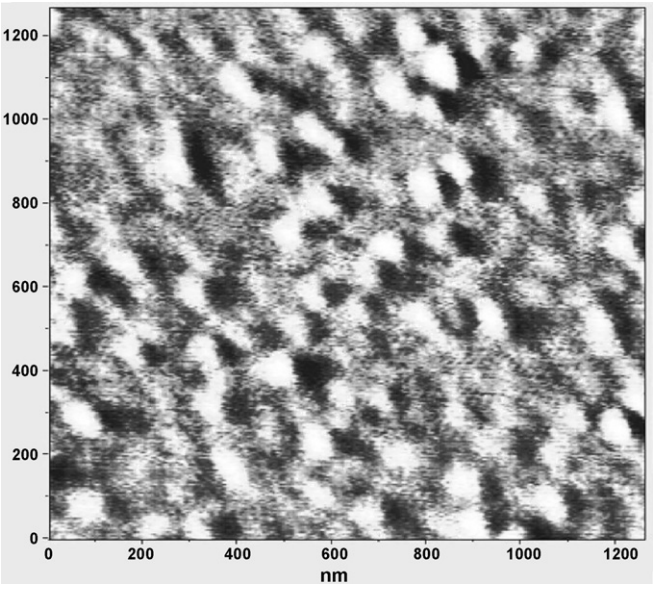

Fig. 1. The AFM image of 80 nm core/shell nanoparticles.

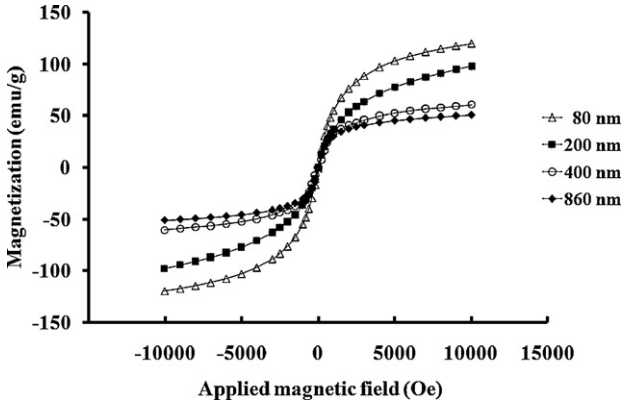

Fig. 2. Magnetization curve for different sizes of core/shell naoparticles at room temperature.

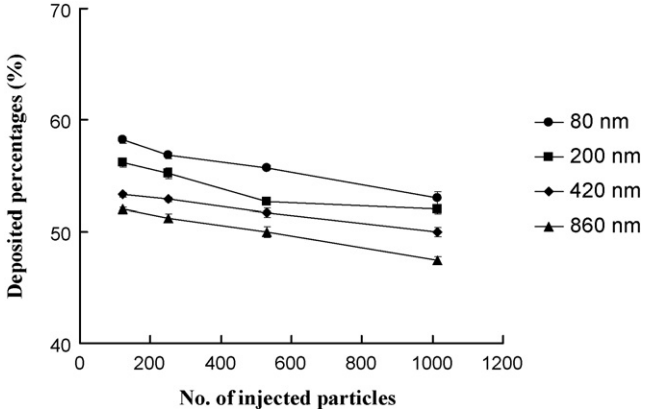

Fig. 3. Deposited percentages of IgG-labeled microparticles for different sizes of core/shell nanoparticles labeled with protein A at a flow-rate of 0.05 ml min^{-1}.

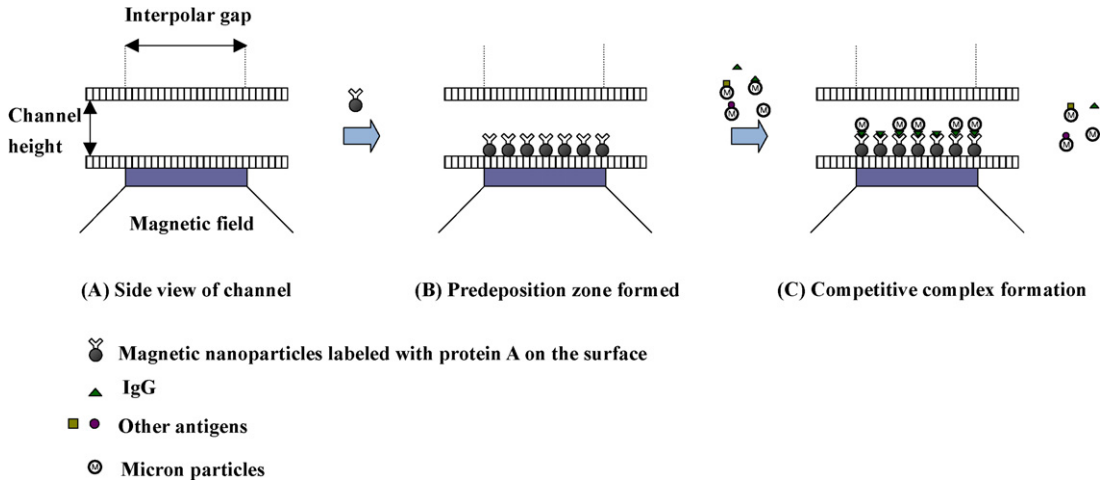

Fig. 4. Schematic diagram of the competitive immunoassay for IgG detection.

nanoparticles. The saturated magnetizations of the four nanoparticles increased as particle size decreased. The epoxy groups on the surface of particles were confirmed by a new band of infrared at 910 cm^{-1}. The conversion of the epoxy groups to amine groups was confirmed by the removal of the infrared band at 910 cm^{-1} with the assistance of infrared band changes at 1645 and 1271 cm^{-1}. The microparticles were spherical in shape with a mean size of 2.0 ± 0.2 μm as estimated by optical microscopy.

3.2. Method development and validation

The effects of flow-rate and nanoparticle size on the deposited percentages of microparticles were studied and optimized using the direct reaction of protein A–IgG complex on the particle surface. In this study, core/shell nanoparticles labeled with protein A were flowed through thin channels to form a deposition layer to capture the IgG on the microparticles. The deposited percentages of IgG-labeled microparticles increased as the flow-rates decreased for all sizes of magnetic nanoparticles. This is due to the longer reaction time for the affinity reaction of protein A and IgG at the lower flow-rates. The useful ranges of flow-rate are from 0.001 to 0.05 ml min^{-1}. The flow-rate at 0.001 ml min^{-1} was used when more depositions and detailed comparison were needed. On the other hand, a higher flow-rate at 0.05 ml min^{-1} was used when high speed and kinetic information were needed. The smaller core/shell nanoparticles had higher deposited percentages than the larger ones, as shown in Fig. 3. This may be due to the larger surface area and higher susceptibility of the smaller core/shell nanoparticles. The 80 nm core/shell nanoparticles were used for IgG detection in competitive immunoassay of protein A–IgG.

In Fig. 4, free IgG and fixed numbers of IgG-labeled microparticles competed for the limited number of sites of protein A deposited on the core/shell nanoparticles in the competitive immunoassay. The protein A-labeled core/shell nanoparticles were predeposited in the thin channel, as shown in Fig. 4B. Then the analytes were injected into the system. The free IgG and IgG-labeled microparticles were captured by the protein A-labeled core/shell nanoparticles, as shown in Fig. 4C. The deposited percentages of IgG-labeled microparticles varied as the concentration of free IgG changed for the fixed numbers of protein A-labeled nanoparticles. The reference plot was established for competitive immunoassay of protein A-labeled core/shell nanoparticles and IgG-labeled microparticles at various concentrations of free IgG at a flow-rate of 0.001 ml min^{-1}, as shown in Fig. 5. This reference plot provided the correlation between the free IgG concentrations and the deposited percentages of microparticles. The IgG concentrations in the samples were then deduced and determined based on the reference plot using the deposited percentages of IgG-labeled microparticles from the samples. The detection limit was 3.69×10^{-12} M based on three times the standard deviation of the blank measurement. The detection limit was equivalent to 0.554 ng ml^{-1}, which was 10 times lower than those of most detection methods for IgG [11–13]. The deposited percentages were linear from 5.0×10^{-8} to 1.0×10^{-11} M with a correlation coefficient equal to 0.992. The linear range was 10 times wider than those of other detection methods for IgG [11,12]. The deposited percentages of IgG-labeled microparticles were 12 times higher than those of microparticles labeled with albumin, amine, or the acid group in reaction with protein A-labeled magnetic nanoparticles. This result shows that the predeposited magnetic nanoparticles can selectively capture the targeted analytes.

A serum sample was diluted and mixed with IgG-labeled microparticles to react competitively with protein A-labeled nanoparticles. The deposited percentage of microparticles was found to be 49.3 ± 2.6%, which corresponded to 2.4×10^{-10} M IgG as estimated from the reference plot. The IgG concentration in the serum sample was determined to be 3.6 mg ml^{-1} after dilution correction. This measurement differed by 8.3% from the ELISA measurement. The recoveries of 1.0×10^{-9} and 3.3×10^{-10} M IgG spiked in serum were found to be 94 and 103%, respectively. In summary, this method can provide simple, fast, and selective analysis for protein detection and other immunoassay-related applications.

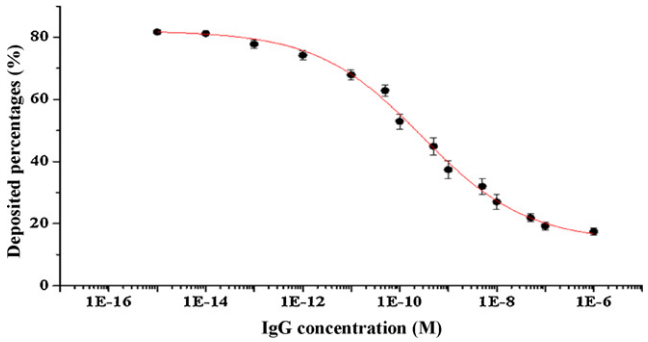

Fig. 5. A reference plot for deposited percentages of IgG-labeled microparticles at various concentrations of free IgG at a flow-rate of 0.001 ml min^{-1} ($n = 3$).

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References

[1] J.M. Nam, C.S. Thaxton, C.A. Mirkin, Science 30 (2003) 1884.
[2] C.R. Martin, D.T. Michell, Anal. Chem. 70 (1998) 322A.
[3] W.C. Chan, S. Nie, Science 281 (1998) 2016.
[4] C.B. Fuh, Y.S. Su, H.Y. Tsai, J. Chromatogr. A 1027 (2004) 289.
[5] D. Horak, P. Shapoval, J. Polym. Sci. A Polym. Chem. 38 (2000) 3855.
[6] C.C. Berry, S. Wells, S. Charles, A.S. Curtis, Biomaterials 24 (2003) 4551.
[7] P.K. Gupta, C.T. Huang, Life Sci. 44 (1989) 175.
[8] F. Sauzedde, A. Elaissari, C. Pichot, Colloid Polym. Sci. 277 (1999) 1041.
[9] J. Chatterjee, Y. Haik, C.J. Chen, J. Magn. Magn. Mater. 225 (2001) 21.
[10] I. Capek, Adv. Colloid Interface Sci. 92 (2001) 195.
[11] Y. Cao, X. Hua, X. Zhu, Z. Wang, Z. Huang, Y. Zhao, H. Chen, M. Liu, J. Immunol. Methods 317 (2006) 163.
[12] L. Wang, S. Hong, L. Wang, L. Dong, G. Bian, T. Xia, H. Chen, Spectrochim. Acta Part A 65 (2006) 439.
[13] Z. Peng, Z. Chen, J. Jiang, X. Zhang, G. Shen, R. Yu, Anal. Chim. Acta 583 (2007) 40.