Nanomagnetic Particles for SQUID-Based Magnetically Labeled Immunoassay

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Abstract—With the increasing importance of SQUID-based magnetically labeled immunoassay, the study on the synthesis of controllable sizes of magnetic nanoparticles plays a role to promote the accuracy of the immunoassay. In this work, Fe_3O_4 nano-particles coated with a suitable bio-probe (biotin) are synthesized through chemical co-precipitation process to probe the bio-target (avidin). Through the synthesis developed here, the particle hydrodynamic diameter can be adjusted from 30 to 90 nm, which provide candidates for probing various bio-targets in the future. The amount of the magnetically labeled avidin is then analyzed via measuring the saturated magnetization or the remanence of the sample by using a SQUID magnetometer.

Index Terms—Immunoassay, nanomagnetic particles, SQUIDs.

I. INTRODUCTION

WITH the rapid progress of nano-biotechnology, lots of new research topics have been created and have been attracting a great deal of interest from scientists and engineers. In this area, the exploration of nano-bio-diagnosis on antibody, antigen, cellular and/or genetic levels is both important and urgent because of the unique merits, like ultrahigh sensitivity. The popularly used types of bio-diagnosis, such as enzyme linked immunosorbent assay (ELISA) or fluorescence immunoassay (FIA), have disadvantages; for example, complicated processes or higher uncertainty in the accuracy due to the self-absorption/emission of detected bio-samples. To promote bio-diagnosis, numerous novel methods have been proposed and actively developed [1]–[5]. A promising candidate is the magnetically labeled bio-diagnosis because of its high efficiency, high convenience, high accuracy and versatile functions [6]–[8].

For magnetically labeled bio-diagnosis (MLD), the bio-probe (say biotin) is coated onto the surface of magnetic nano-particles, which are dispersed in water. The coated nano-particles in the magnetic fluid associate with the target bio-molecular (avidin) to form magnetic clusters. Then, the amount of the

target bio-molecule is detected by measuring the magnetic signals of the magnetic clusters [9]–[11]. At present, the magnetic properties such as magnetic relaxation, magnetic remanence, and ac magnetic susceptibility of these magnetic clusters are detected. To achieve a very high sensitivity to the magnetic characterization of the MLD system, the superconducting quantum interference devices (SQUID) is adapted as a sensor because the SQUID is the most sensitive device to measure magnetic moments in the world. Therefore, SQUID-based magnetically labeled diagnosis systems are the most promising candidate for MLD.

It was pointed out that the resolution and the accuracy for MLD greatly depend on the mean value and the dispersion of the nano-particle size. For example, magnetic particles with larger diameters (~ 20 nm) are used for the relaxation measurement in order to distinguish the Brownian relaxation time constant of a single particle from the Néel relaxation time constant [10]. For the ac susceptibility measurement, a higher uniformity in nanoparticle size is required. Hence, the study on synthesis methods to prepare highly homogeneous magnetic nanoparticles of controlled sizes is an important issue in developing MLD. In this work, we study the preparation of bio-compatible magnetic nano-particles for SQUID-based immunoassay.

II. EXPERIMENTAL DETAILS

A flow chart to illustrate the synthesis of the bio-compatible magnetic Fe_3O_4 nanoparticles is shown in Fig. 1 [12]. 50-ml 0.20-M ferrite solution containing stoichiometric ratio 1:2 ferrous sulfate hepta-hydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and ferric chloride hexa-hydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) was well mixed with an equal volume of aqueous dextran. The dextran (molecular weight = 74 000) was selected as a surfactant of Fe_3O_4 particles dispersed in water by taking the hydrophilic, biocompatibility, toxicity, and easy preparation into account for biomedical applications. Then appropriate amount of urea $\text{CO}(\text{NH}_2)_2$ was added into the mixed solution. The mixture was heated gradually up to 90 °C to decompose urea. The amount of decomposed urea was adjusted by controlling the heating duration after the temperature reached 90 °C. After that, while stirring rigorously, the mixture was titrated to raise the pH to around 10–11 by the addition drop wise of 3.5-M ammonium hydroxide at room temperature. The black Fe_3O_4 particles were synthesized in this step. Aggregates and excess unbound dextran were removed by centrifugation and gel filtration chromatography. The stable, purified water-based magnetic fluid containing dextran-coated Fe_3O_4 nano-particles was obtained with a concentration of about 10 mg Fe/ml. Then,

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Fig. 1. Flow chart for preparation of bio-compatible Fe_3O_4 magnetic nanoparticles dispersed in water.

biotin was bound onto the dextran on the Fe_3O_4 particles through the oxidation of dextran by adding NaIO_4 . Thus, biotin-dextran-coated Fe_3O_4 particles dispersed in water were obtained for use in detecting avidin. Finally, the unbound biotin was removed by dialyzing the biotin-dextran coated Fe_3O_4 nano-particle solution. The crystalline of magnetic particles was analyzed using powder x-ray diffraction (XRD) in a Siemens D-500N diffractometer with a wavelength of 0.15418 nm. The size distribution of Fe_3O_4 particles in water was evaluated by using laser scattering analysis of Microtrac Nanotrac-150.

III. RESULTS AND DISCUSSION

According to the synthesis process shown in Fig. 1, the Fe^{3+} in $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ reacts with the Fe^{2+} in $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ via

to form Fe_3O_4 particles. The OH^- in (1) is provided by decomposing urea at 90 °C via

Since the concentration of OH^- released from urea is not high enough to activate the Fe_3O_4 formation, ammonia solution is required to be titrated into the mixture. After the formation of Fe_3O_4 particles, dextran is attached onto the particles. The dextran acts not only a surfactant between the particles and water, but also serves as a connection between the bio-probe (biotin) and the magnetic particles.

Fig. 2. $\theta - 2\theta$ x-ray powder diffraction pattern of the synthesized magnetic particles. The indexes are for the Fe_3O_4 phase.

In order to associate dextran with biotin, the dextran on the particles is oxidized with NaIO_4 to change the hydroxyl group ($-\text{COH}$) into an aldehyde group ($-\text{CHO}$). Then, the aldehyde group ($-\text{CHO}$) in dextran can react to the amino group ($-\text{NH}_2$) in biotin to achieve bio-probes for avidin on magnetic particles, as shown in

where R in oxidized dextran sticks onto magnetic particles. Thus, we can obtain a homogeneous water-based biotin-dextran coated Fe_3O_4 magnetic fluid. Fig. 2 shows the XRD pattern for the synthesized magnetic particles. It is clear that the magnetic particles are single phase of Fe_3O_4 .

Since the sensitivity of MLD heavily relies on the average diameter and its standard deviation of Fe_3O_4 particles, the size distribution of Fe_3O_4 particles was measured. Fig. 3(a) gives a typical size distribution of the isolated biotin-dextran coated Fe_3O_4 particles. The mean value of the hydrodynamic diameter of Fe_3O_4 particles is 30.9 nm with a standard deviation of 21%. According to our studies, the average diameter of Fe_3O_4 nanoparticles can be adjusted from 25 to 90 nm by controlling the period of time for decomposing urea at 90 °C, as shown in Fig. 3(b). The flexibility in tuning particle size provides opportunities to investigate the most suitable size of magnetic nanoparticles for various MLD systems.

After adding the bio-target avidin into the magnetic fluid, the biotin-dextran coated Fe_3O_4 particles were attached together to form magnetic clusters because that avidin was able to associate with biotins on different Fe_3O_4 particles. As a result, the mean value of the magnetic clusters in the solution becomes larger

Fig. 3. (a) Size distribution of isolated biotin-dextran coated Fe_3O_4 nano-particles. (b) Mean diameter of Fe_3O_4 particles as functions of the decomposition time of urea. The inset in (a) illustrate two isolated biotin-dextran coated Fe_3O_4 nanoparticles. The arrows denote the magnetic dipole moments.

Fig. 4. Size distribution of Fe_3O_4 particles reacting with the detected bio-target avidin. The inset illustrates the magnetic clusters formed with avidin and biotin-dextran coated Fe_3O_4 particles.

as compared with that of isolated Fe_3O_4 particles. This is evidenced by the results shown in Fig. 4. It was found that the mean size increased from 30.9 nm for isolated particles to 54.4 nm for magnetic clusters when the biotin-dextran Fe_3O_4 particles reacted with avidin. It is noted that when various amounts of avidin are added to react with the biotin-dextran coated Fe_3O_4 particles, different values of the mean size of magnetic clusters are observed. Fig. 5(a) gives the experimental relationship between the mean size of magnetic clusters and the amount of the added avidin. The mean size of the magnetic clusters increases linearly with the increasing amount of avidin of 30 to 100 μg .

Fig. 5. Relationship between (a) the mean size of the magnetic clusters, (b) the saturated magnetization of the magnetic clusters and the amount of avidin. The inset in (b) shows the magnetic hysteresis curves for the filtrated biotin-dextran coated Fe_3O_4 with (solid line) or without (dashed line) binding to avidin.

In addition to the mean size of the magnetic clusters, the amount of the added avidin can be analyzed by detecting its magnetic properties, as is done in a magnetically labeled immunoassay. Here, the saturated magnetization and the magnetic remanence of the magnetic clusters were measured by using a SQUID magnetometer. To do this, the magnetic clusters have to be separated from isolated particles that do not react with avidin. This can be achieved by filtering the sample through a micro-filter with nano-sized holes of 50 nm in diameter. After drying the filtered sample, the magnetic hysteresis of the sample was measured. Since the mean size of the magnetic clusters is different for the samples added with various amounts of avidin, the amount of the residual magnetic clusters on the micro-filters is different, in turns, various values of saturated magnetization M_s should be observed. Two typical magnetic hysteresis curves for the samples with (solid line)/without (dashed line) reacting to avidin are shown in the inset of Fig. 5(b). The sample with the association to avidin exhibits a significant nonzero value of saturated magnetization. Fig. 5(b) plots the saturated magnetization of the samples as a function of the amount of added avidin. It is clear that the more the avidin, the higher the saturated magnetization.

Besides exhibiting a higher saturated magnetization, the sample with magnetic clusters should exhibit a higher value of magnetic remanence M_R . The value of M_R of each sample can be identified from its magnetic hysteresis curve taken by using a SQUID magnetometer. Fig. 6 shows M_R of the magnetic clusters as a function of the amount of the added avidin. A linear relationship was observed. The results shown in Figs. 5 and 6 reveal that the synthesized biotin-dextran coated Fe_3O_4 magnetic nanoparticles can be used as a labeling indicator in MLD.

Fig. 6. Magnetic remanence of magnetic clusters as a function of amount of avidin.

IV. CONCLUSION

Biocompatible Fe_3O_4 magnetic nanoparticles have been synthesized. We have further demonstrated that the biocompatible Fe_3O_4 magnetic nanoparticles can be used for SQUID-based magnetically labeled detection for bio-molecules, such as avidin. The amount of detected bio-target can be probed quantitatively by measuring the saturated magnetization or the magnetic remanence by using a SQUID magnetometer.

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