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Journal of Magnetism and Magnetic Materials 293 (2005) 48–54
Journal of magnetism and magnetic materials
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Study of streptavidin coated onto PAMAM dendrimer modified magnetite nanoparticles

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Available online 2 March 2005

Abstract

Polyamidoamine dendrimer was synthesized on the surface of amino silane modified magnetite nanoparticles. After coating of streptavidin to these dendrimer-modified magnetite nanoparticles, an up to 3.4 times higher amount of streptavidin (SA) was measured compared to magnetite nanoparticles modified with only amino silane. The biotin-binding capacity of SA thus increased after dendrimer modification.

PACS: 87.68.+z; 75.70.Rf; 82.65.+r

Keywords: Magnetite nanoparticles (MNP); Polyamidoamine; PAMAM; Dendrimer; Streptavidin (SA); Biotin; Silane; ELISA

1. Introduction

In recent years, the design, synthesis, and application of nanosized biocompatible magnetic materials has opened up new perspectives of biological and biomedical applications, as for instance, in cell separation and magnetohyperthermia of tumor cells. In particular, biocompatible magnetic fluids (BMFs) have played a key role as a material basis for magnetic-active drug delivery systems. BMFs are composed of magnetic nanoparticles (MNP) surface-coated with organic molecules and dispersed as a stable colloid in physiological medium [1–5]. MNP are generally of core/shell structures coated with various polymers, biopolymers and organic materials as demonstrated in detail in Tedesco's works [3,6].

Dendrimers are a relatively new class of polymers with a well-defined, three-dimensional structure. They are synthesized from a polyfunctional core by adding branched monomers that react with the functional groups of the core, in turn leaving end groups that can react again. The number of terminal groups increases after each cycle or "generation" of the synthesis. A polyamidoamine (PAMAM) dendrimer can introduce

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doi:10.1016/j.jmmm.2005.01.042

a dense outer amine shell through a cascade type generation [7,8]. The outer amine concentration doubles with every layer generated and is limited only by steric interferences. A PAMAM coating may be used to reduce magnetite agglomeration, and the terminal groups on the periphery can be tailored to control composite solubility.

Magnetic particles are often sensitized with streptavidin (SA) to take advantage of its extremely high affinity interaction with biotin. Because biotin can be easily conjugated to many different diagnostic reagents, a variety of assays for different analytes in several formats can be developed [9]. Determining the amount of SA bound to the particles, and their capacity to bind biotin, are important steps in particle evaluation. This paper describes the direct formation of a PAMAM dendrimer on the surface of amino silane modified MNP and its application in SA binding. These kinds of MNP are prepared to take advantage of their extremely high affinity interaction with biotin [10,11]. The biotin binding capacities of SA coated PAMAM-MNP were also investigated in this paper.

2. Materials and methods

2.1. Materials

Ferric chloride ($FeCl_3 \cdot 6H_2O$), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), sodium hydroxide, glutaraldehyde (25%) aqueous solution, methylacrylate and ethylenediamine were purchased from Shanghai Chemical Reagent Corporation (China). Tween 20, bovine serum albumin (BSA), biotinylated horseradish peroxidase (biotin-HRP), biotin and d-biotin *p*-nitrophenyl ester were purchased from Sigma (Shanghai, China), 3-aminopropyltrimethoxysilane (APTS) was obtained from Fluka (Shanghai, China). SA (a 58-kd protein) was prepared from streptomycetes [12]. All reagents were used as received.

2.2. Preparation of magnetite nanoparticles

MNP was prepared according to the method of Mehta [13]. The Fe^{2+} and Fe^{3+} ions ($Fe^{3+}/Fe^{2+} = 2$) mixture solution was poured into the 4 M sodium hydroxide solution under N_2 protection while stirring at 80 °C for 60 min (pH 10). The obtained MNP were washed for several times with water and ethanol.

2.3. Nanoparticles modified with amino-silane

Fifty milliliter of 5% (wt%) MNP ethanol suspension was mixed with 150 mL ethanol in a 500 mL two-necked round-bottom flask equipped with a condenser. Ten milliliter of 3-aminopropyltrimethoxysilane [$NH_2(CH_2)_3Si(OCH_3)_3$, APTS] was added under stirring at 60 °C for 7 h. The product was washed for several times with methanol by magnetic separation (Fig. 1, step 1) and dried into powder at room temperature under vacuum. G0-MNP represents the magnetite nanoparticles modified only with APTS.

2.4. Magnetite nanoparticles modified with PAMAM dendrimer

G0-MNP of 0.5 g was dispersed in 50 mL methanol and 20 mL of methylacrylate added. The suspension was ultrasonicated and stirred for 7 h at room temperature. The particles were washed with methanol 5 times by magnetic separation, then 4 mL ethylenediamine was added and the suspension stirred for 5 h at 50 °C. The addition of methylacrylate and ethylenediamine was repeated to get the desired PAMAM dendrimer of generations (G1–G5) on the MNP surface (Fig. 1, step 2 and step 3). The PAMAM-MNP was then washed 3 times with methanol and 3 times with water.

2.5. Immobilization of streptavidin

PAMAM-MNP was dispersed into 10% glutaraldehyde in phosphate-buffer (0.05 M pH 7.4) solution with stirring for 3 h at room temperature. The suspension was washed with PBS buffer solution 3 times by magnetic separation. One milliliter of 0.85 mg/ml SA was added to 1 mL of 2.5 mg/mL PAMAM-MNP in phosphate-buffer (0.05 M pH 7.4, containing 0.05% Tween-20), the mixture was incubated for 10 h at 4 °C with

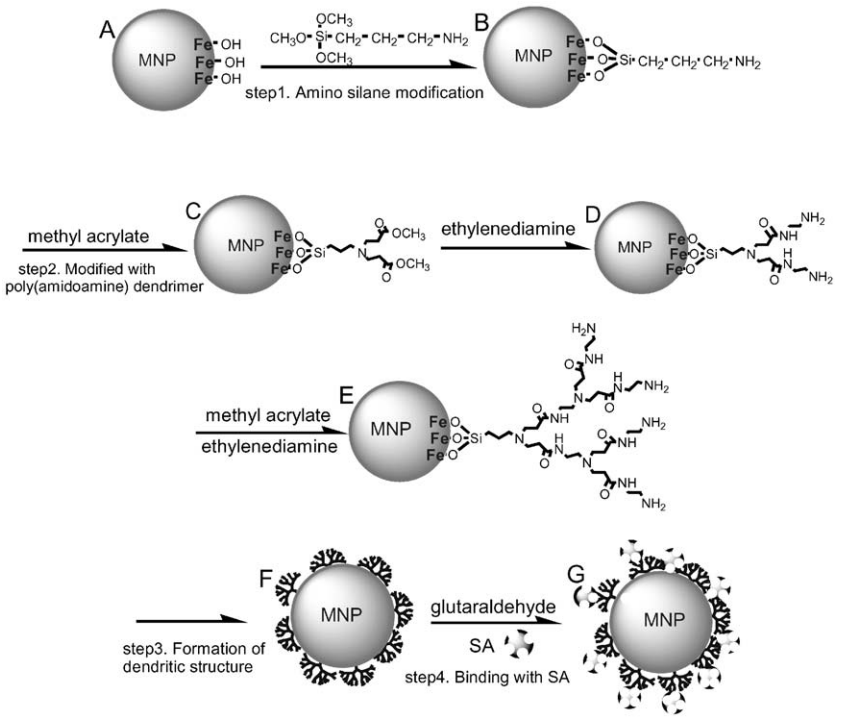

Fig. 1. MNP modified with PAMAM dendrimer and its binding with SA.

stirring. Excess ethanolamine (0.5 ml 1 M pH 7.4) was added and stirred for 2 h at room temperature to terminate the activated residual groups (Fig. 1, step 4). Unreacted SA was separated magnetically and the UV spectra of the supernatant determined at 282 nm.

2.6. Colorimetric measurements

In order to investigate the biotin binding capacity of SA-PAMAM-MNP, a colorimetric measurement was performed by adding excess d-biotin *p*-nitrophenyl ester (BNPE). Briefly, the SA-PAMAM-MNP (10 mg) was washed 3 times with 10 mL of 0.2 M acetate buffer (pH 5.0, containing 0.05% Tween 20), then 5 mL of the above buffer containing 2 mg BNPE in 0.1 mL dimethyl sulfoxide was added. After 30 min incubation under shaking, the particles were rinsed 5 times with 10 mL of the above buffer, magnetically separated and transferred to 1 mL of 0.1 M NaOH (Fig. 2). The BNPE bound particles hydrolyzed and produced a yellow dye which was analyzed at 400 nm in the supernatant. The visible absorption intensity is proportional to the *p*-nitryl phenol concentration.

2.7. Enzyme-linked immunosorbent assay (ELISA)

In order to determine the activity of SA-PAMAM-MNP, a competitive ELISA was performed. The SA-PAMAM-MNP were blocked by adding 0.5% BSA in 0.05 M PBS with stirring for 2 h at room temperature to prevent unspecific binding of the biotinylated horseradish peroxidase (BHRP); 0.05 mL of 0.5 mg/mL SA-PAMAM-MNP was mixed with 0.1 mL BHRP and 0.1 mL biotin with different concentration in 0.05 M PBS (containing 0.05% Tween 20 and 0.5% BSA), and the mixture was incubated with shaking for 30 min at room temperature. The nanoparticles were rinsed 5 times with the above buffer solution, and then 0.1 mL of the

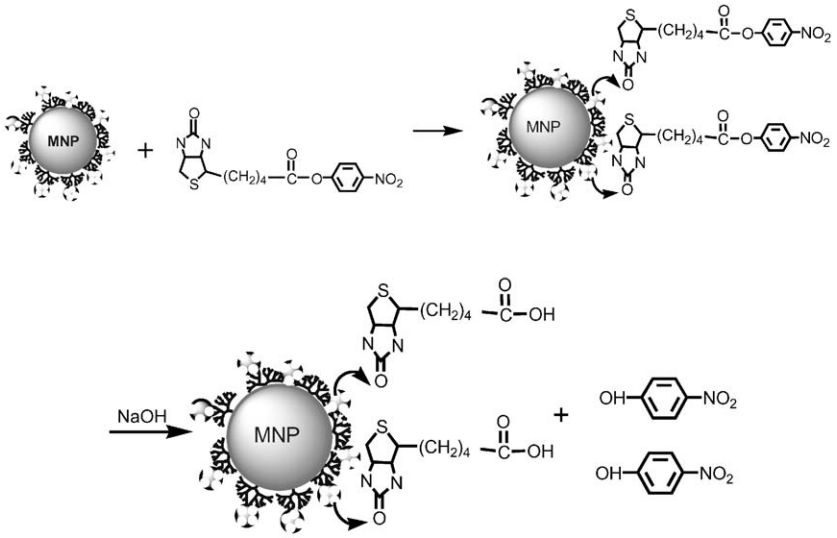

Fig. 2. SA-PAMAM-MNP bound with BNPE were hydrolyzed in the presence of NaOH to produce *p*-nitryl phenol.

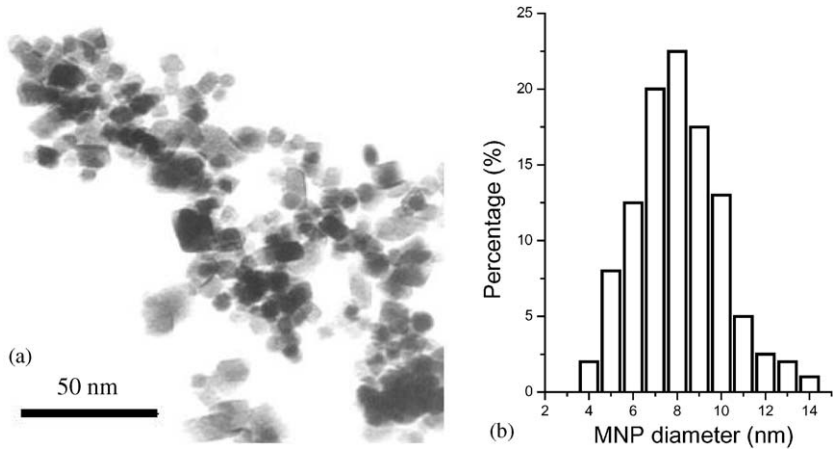

Fig. 3. (a) TEM Photography of the magnetite nanoparticles coated with APTS. (b) Diameter distribution of magnetite nanoparticles from TEM data.

substrate (0.01 mL of 1 mg/mL tetramethyl benzidine in dimethyl sulfoxide, 0.01 mL of 3% hydrogen peroxide and 0.09 mL of 0.05 M acetate buffer of pH 5.8 was added under shaking for 10 min. The enzymatic reaction was stopped by adding 0.1 mL of 1 M sulphuric acid. Aliquots of 0.1 mL of this solution were analyzed in a microtiter plate at 450 nm.

3. Results and discussions

3.1. Tem observation

The TEM photography in Fig. 3a shows mostly quasi-spherical MNP with an average diameter of 8.2 nm. The size distribution of the MNP is shown in Fig. 3b.

3.2. FT-IR characterization

The FT-IR spectra of the PAMAM-MNPs (i.e. G1–G5-MNP) were determined with a FTS135 infrared spectrometer (BIO-RAD, USA) as shown in Fig. 4. It can be seen that the stretching vibration of C–H absorption bands are at 2922 and 2851 cm^{-1}, the bending vibration of -NH_2 group is at 3440 cm^{-1}, the stretching vibration of acylamide group (C = O) is at 1732 cm^{-1}. The stretching vibration of Si–O at the surface of G0-MNP is at 995 cm^{-1}, which shifts to about 1011 cm^{-1} of the G1–G5-MNP due to the presence of highly electronegative -CO–NH- groups. The characteristic absorption band of the Fe–O bond is at 579 cm^{-1}. All data confirm that the PAMAM dendrimer is covalently bound to MNP.

3.3. Elemental analysis

The contents of C and N were characterized with 2400 SERIES II CHNS/O element analyzer (PERKIN ELMER, USA) as shown in Table 1. The contents of nitrogen and carbon on the surface of PAMAM-MNP increased from G0-MNP to G5-MNP. The measured nitrogen content increased less than twice from G1 to G5 because of steric interference during the dendrimer growth on the MNP surface. On the other hand, agglomerating of the nanoparticles is another factor that limits the growth of dendrimer.

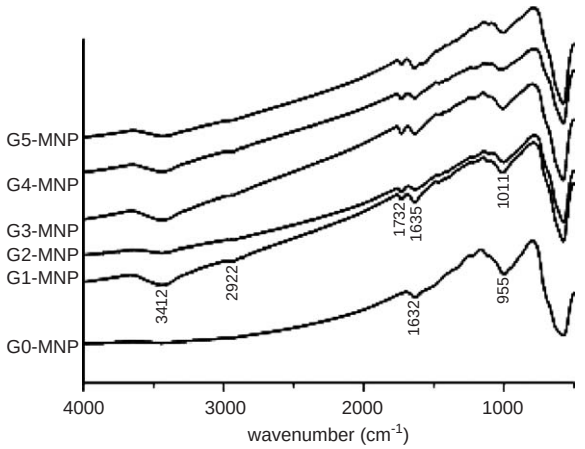

Fig. 4. FTIR spectra of PAMAM-MNP.

3.4. Streptavidin coated onto PAMAM-MNP and its biotin binding capacity

The amount of SA coated onto PAMAM-MNP can be calculated by Eq. (1):

$$\eta = \frac{m_{SA}(A_{SA} - A_{mag})}{m_{mag}A_{SA}}, \quad (1)$$

where η (μg/mg) refers to the SA amount coated on 1 mg PAMAM-MNP, m_{SA} (μg) is the total weight of SA, m_{mag} (mg) is the dry weight of PAMAM-MNP used to bind SA, A_{SA} refers to the absorption of initial SA solution at 282 nm, and A_{mag} is the absorption of supernatant of SA-PAMAM-MNP at 282 nm. The UV spectra are shown in Fig. 5. So, the SA amount coated onto 1 mg of MNP can be calculated (Table 2).

The first method used to determine the biotin-binding capacity of SA-PAMAM-MNP was based on the colorimetric measurement after adding

Table 1
Chemical analysis of surface elements in PAMAM-MNP samples

Sample	G0	G1	G2	G3	G4	G5
N content (wt%)	0.14	0.49	1.08	1.46	1.62	1.70
C content (wt%)	0.30	0.83	1.83	2.48	2.75	2.89

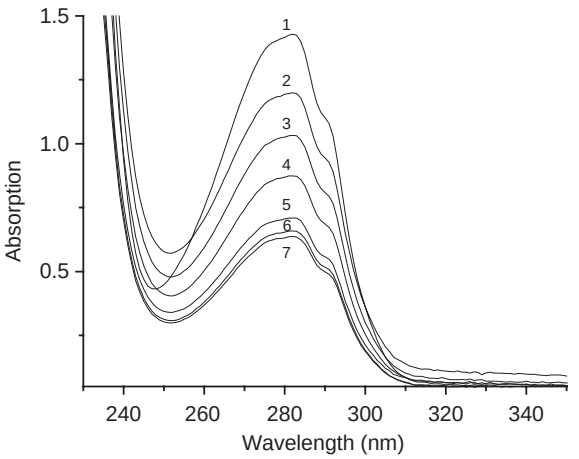

Fig. 5. UV spectra of supernatants of SA-PAMAM-MNP samples (1) SA; (2) SA-G0-MNP; (3) SA-G1-MNP; (4) SA-G2-MNP; (5) SA-G3-MNP; (6) SA-G4-MNP; (7) SA-G5-MNP.

Table 2
Biotin binding capability of SA-PAMAM-MNP determined by two methods

Sample	SA amount (μg/mg)	Bound biotin content (nmol/mg)	
		Colorimetric measurement	ELISA inhibition test
Blank[a]	0	0.78	—
G0-MNP	54.7	1.61	1.02
G1-MNP	94.2	3.10	2.16
G2-MNP	132.1	5.23	4.29
G3-MNP	171.3	5.95	4.93
G4-MNP	183.2	5.73	4.75
G5-MNP	188.4	5.58	4.56

[a]MNP coated with only BSA.

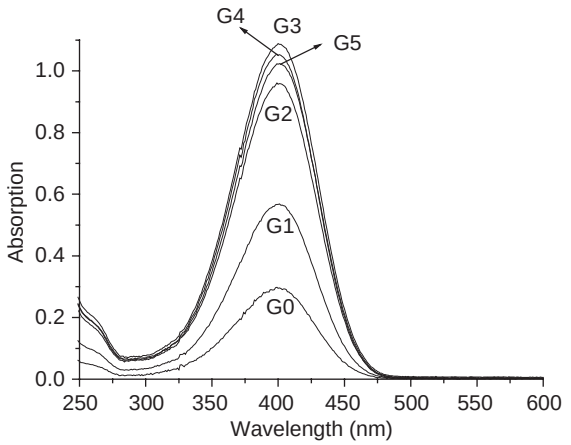

Fig. 6. The UV-vis spectra of the *p*-nitryl phenol, which was produced by hydrolysis of the BNPE bound with SA-PAMAM-MNP in the presence of NaOH (G0–G5 is the generations of PAMAM dendrimer).

excess BNPE (Fig. 2). After BNPE was hydrolyzed in the presence of NaOH to produce *p*-nitryl phenol, its visible spectrum was investigated at 400 nm (Fig. 6). Biotin binding capacity of SA-PAMAM-MNP was calculated by Eq. (2).

$$C = \frac{AV}{m\varepsilon} \times 10^6, \tag{2}$$

where C (nmol/mg) refers to the biotin binding capacity of SA-PAMAM-MNP, m (mg) is the dry weight of PAMAM-MNP used to bind with SA, A is the absorbance at 400 nm, ε is the molar absorption constant of *p*-nitryl phenol (18 300 L/mol), and V (mL) is the hydrolyzed volume of supernatant. The bound biotin amounts of SA on 1 mg PAMAM-MNP are shown in Table 2.

The second method used to determine the biotin binding capacity of SA-PAMAM-MNP was based on the competitive enzyme-linked immunosorbent assay (ELISA) by using biotinylated horse radish peroxidase (BHRP). In the case of binding inhibition tests, the SA-PAMAM-MNP in PBS solution containing BHRP was incubated with various amounts of the biotin as competitor. The reactive activity of SA-PAMAM-MNP was measured by comparison with standard inhibition

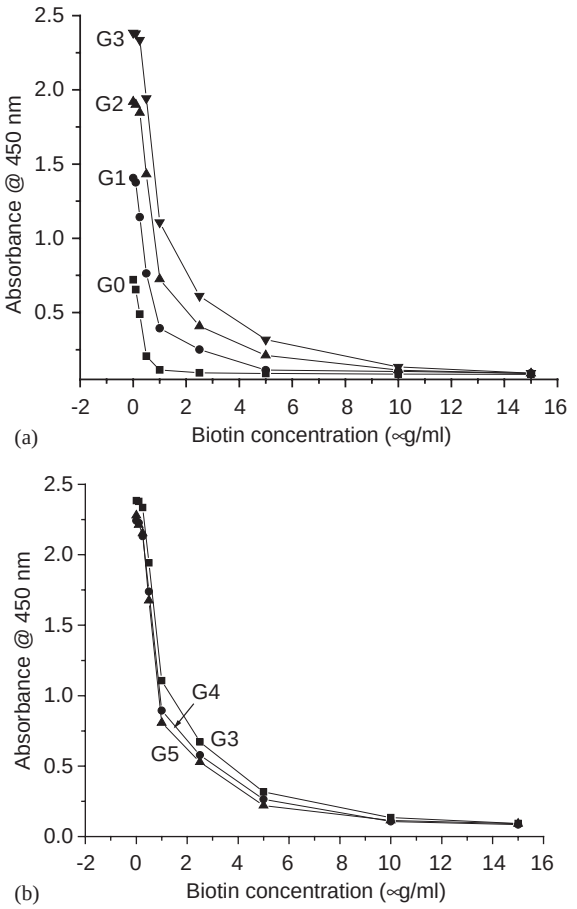

Fig. 7. Results of the binding inhibition test of the biotin and the BHRP by using SA-PAMAM-MNP. (a) G0–G3; (b) G3–G5.

curve, which was obtained using a given series concentrations of biotin (Fig. 7). The biotin-binding capacity is taken at the point of 95% inhibition of BHRP binding. The results were shown in Table 2.

If successful cascading dendrimer generation takes place (Fig. 1), the amount of coupled SA will double with every generation starting from the APTS-modified magnetite nanoparticles (G0-MNP) initiator. Experimentally, there was 3.4 times more SA coated on G5-MNP than on G0-MNP. The bound biotin capacity increased from G0 to G3 and was then reduced from G3 to G5. Since 1 mol SA can theoretically bind 4 mol biotin, SA coated on the PAMAM-MNP lost about 40–60% of its binding capacity due to steric interference with MNP. The G5-MNP has more terminal amine groups than G3-MNP, but their maximal biotin binding capacity is less than G3-MNP because one SA molecule reacted very likely with several terminal amines on the particle surface and the binding site of SA was overlaid by dendrimer.

Acknowledgments

This work was supported by National Natural Science Foundation of China (No. 30471599), Shanghai Development Foundation of Science and Technology (No. 03ZR14057) and 863 Hi-tech Research and Development Program of China (No. 2002AA302210).

References

[1] T. Matsunaga, R. Sato, S. Kamiya, et al., J. Magn. Magn. Mater. 194 (1999) 126.
[2] P.P. Macaroff, D.M. Oliveira, Z.G.M. Lacava, et al., IEEE. Trans. Magn. 40 (2004) 3027.
[3] A.C. Tedesco, D.M. Oliveira, Z.G.M. Lacava, et al., J. Magn. Magn. Mater. 272–276 (2004) 2404.
[4] T. Hawkins, US Patent No. 5705628, 1998.
[5] C. Bergemann, D.M. Schulte, J. Oster, et al., J. Magn. Magn. Mater. 194 (1999) 45.
[6] A.C. Tedesco, D.M. Oliveira, J. Appl. Phys. 93 (2003) 6704.
[7] R.M. Crooks, M. Zhao, L. Sun, et al., Acc. Chem. Res. 34 (2001) 181.
[8] D.A. Tomalia, H. Baker, J.R. Dewald, et al., Polym. J. 17 (1985) 117.
[9] L. Dorgan, R. Magnotti, J. Hou, et al., J Magn. Magn. Mater. 194 (1999) 69.
[10] J. Langea, R. Kötitz, A. Haller, et al., J. Magn. Magn. Mater. 252 (2002) 381.
[11] D.L. Graham, H.A. Ferreira, P.P. Freitas, et al., Biosens. Bioelectron. 18 (2003) 483.
[12] E.A. Bayer, H.B. Hur, G. Gitlin, et al., J. Biochem. Biophys. Methods 13 (1986) 103.
[13] R.V. Mehta, S.W. Charles, et al., Biotechnol. Tech. 11 (1997) 493.