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Multifunctional magnetite and silica–magnetite nanoparticles: Synthesis, surface activation and applications in life sciences

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Abstract

A method for the introduction of amine groups onto the surface of magnetite and silica-coated magnetite nanoparticles has been established based on the condensation of aminopropyltriethoxysilane. Amine-modified particles were grafted with an oligonucleotide and used in the capture of a complimentary sequence. The particles' efficiency at capture was observed to correlate directly with amine group surface density.

Keywords: Silica–magnetite nanocomposites; Surface modification; Alkoxysilanes; DNA immobilisation

1. Introduction

Magnetic separation was introduced in the context of biotechnology in the early seventies by Robinson et al. [1] and since then it has become a routine technique for the quick, easy, sensitive and reliable separation of biomolecules [2,3]. The basic procedure in magnetic bioseparations comprises three steps: the selective binding of the biomaterial of interest to a paramagnetic solid phase support, the separation of the support from the surrounding matrix using a magnetic field, and the recovery of the bound molecular species by elution. The paramagnetic materials used in such processes are superparamagnetic, meaning that they respond strongly to magnetic fields, but retain no residual magnetism after the field is removed. The morphology, surface area and the magnetic susceptibility of the support contribute in a major fashion to the efficiency of the separation processes. Additionally, the chemical nature of the support surface can be used to specify the separation process: the adsorption of the molecular species can be driven by the interactions at the molecular level between the surface groups of the paramagnetic particles and those of the target molecules

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(eg. antigens/antibodies or complementarity in the case of nucleic acids).

Magnetite nanoparticles (pure Fe_3O_4) have been synthesised and used as a basis for production of such supports [4–6] and magnetite nanoparticles in the size range of 5–10 nm [7,8] with various morphologies [9,10] have been reported. We have recently developed a scaled-up process for the easy, reproducible and inexpensive bulk synthesis of magnetite nanoparticles (30–100 nm), as well as a method for their surface coating with silica [7]. The surface of these materials can be also modified with polymers [11–15] or other molecules, eg. phospholipids [16], to improve the affinity of the particles for specific target species. In this context, the use of organosilanes has recently been described by Ma et al. [17] and Liu et al. [18], but no detailed study on their surface modification via silanisation in terms of optimisation has been performed so far.

Organosilanes are bifunctional molecules containing a trialkoxy or trichlorosilane group (which can bind covalently to the free –OH groups at the surface of the particles) and an organic head-group functionality that determines the final chemical character of the modified surface (e.g. $–NH_2$, –OH, –SH etc.). Working with alkoxysilanes involves several difficulties. The moisture sensitivity and highly chemically reactive nature of the alkoxysilane moiety can lead to uncontrollable, inhomogeneous and non-reproducible surface coverage if the surface activation reaction is performed under inappropriate experimental conditions. For these reasons, a systematic study of the parameters involved, and their influence on the nature of the surface of the final material is necessary to optimise the efficiency of the method. We report a simple and effective procedure for the surface modification of pure magnetite and silica-coated magnetite with an $–NH_2$ linker using aminopropyltriethoxysilane (APTS) as the surface modification agent. The amine activated surface can be used to covalently link specific biomolecules and thereby generate ''bioactive'' nanoparticles [19–22]. This has been demonstrated by immobilisation of 5′-amino-modified oligonucleotide sequences to surface amine groups which had been converted to aldehyde groups via treatment with glutaraldehyde and which had been used for the specific capture of complementary single stranded DNA in solution.

2. Experimental details

2.1. Materials

All reagents used were available commercially and were of the highest purity grade. The methanolic coupling solution for the colorimetric assay contained 0.8% (v/v) glacial acetic acid in dry methanol. The hydrolysis solution contained 75 ml H_2O, 75 ml MeOH and 0.2 ml glacial acetic acid. $(1\times)$SSC and $(13\times)$SSC buffers were prepared by diluting a stock solution of $(20\times)$SSC buffer (175.3 g NaCl, 88.2 g sodium citrate, 1 l H_2O, pH 7.4) with distilled, deionised water, adjusting to pH 7.4 and were autoclaved before use. Glutaraldehyde solution was prepared immediately before use. Oligonucleotides were purchased from Proligo GmbH (Hamburg, Germany) and APTS from Gelest-ABCR GmbH (Karlsruhe, Germany).

2.2. Characterisation methods

Scanning electron micrographs (SEM) were recorded using either a Philips XL30 or 515 instrument fitted with a field emission gun. The samples were prepared by sprinkling the powder materials onto double-sided sticky carbon films or cellotape and mounted on a microscope stub and coated with a thin carbon or gold film. Transmission electron micrographs (TEM) were recorded on a Phillips 300 instrument. In this case dried solid support was redispersed in water and a drop placed onto a carbon coated copper grid (Lacey Carbon 400 Mesh Cu(50) Grids from Agar Scientific, Cambridge, UK) and dried at 35 °C. Elemental analysis (C, H and N) was performed by REDOX Spa. (Milan, Italy). A Cary Eclipse Fluorescence Spectrophotometer (Varian Ltd., Oxford, UK) was used to measure excitation and emission at wavelengths of 460 and 515 nm, respectively, and UV absorbance was measured with a Cary 100 Scan UV–Vis Spectrophotometer.

2.3. Synthesis of magnetite and silica-coated magnetite nanoparticles

Magnetite nanoparticles were synthesised by precipitation of Fe_3O_4 from a solution of iron sulphate, potassium nitrate and potassium hydroxide [5]. Silica coating was performed by deposition of silica from a supersaturated solution of silicic acid by titration with hydrochloric acid as previously reported [7].

2.4. Synthesis of amine modified magnetite and silica-coated magnetite nanoparticles

Magnetite (or silica-coated magnetite) nanoparticles (150 mg) in suspension were added to a freshly prepared solution of APTS (2% v/v) in water and the final volume adjusted to 15 ml. The mixture was allowed to react with vigorous stirring on a magnetic stirrer at the selected temperature (18, 50 and 70 °C) and 2 ml aliquots removed at increasing time intervals and washed five times (2 ml each) with water using a magnetic separator (Magix Magnetic Rack, Nuclyx Ltd., Hoddeston, UK). The suspension density of each sample was determined by drying 200 μl (× 3) of each sample at 95 °C in vacuum overnight to obtain a dry weight estimation.

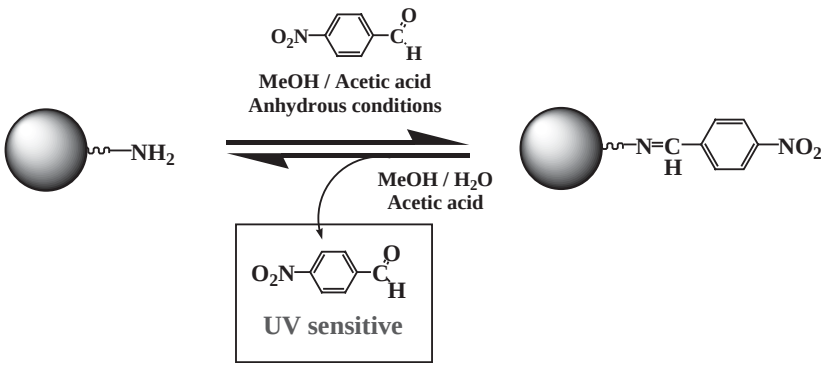
2.5. Colorimetric assay of the amine density

The density of amine groups at the support surface was determined by a colorimetric titration method involving their reaction with a UV sensitive reagent (4-nitrobenzaldehyde). The reaction between amine and aldehyde groups under anhydrous conditions generates an imine group (Scheme 1), which may be hydrolysed back to the precursors in a known volume of water to produce 4-nitrobenzaldehyde whose absorbance can be measured by UV spectroscopy (282 nm). The number of hydrolysed aldehyde molecules is proportional to the number of the imine molecules present. The experimental procedure used was an adaptation of the method by Moon et al. [23] and was performed as follows: 5 mg of nanoparticle were placed in a 1.5 ml microcentrifuge tube and washed four times with 1 ml coupling solution. Subsequently, 1 ml of 4-nitrobenzaldehyde solution (7 mg in 10 ml coupling solution) was added to the particles and the suspension allowed to react for 3 h with end-over-end rotation. After removal of the supernatant and washing (4 × 1 ml coupling solution), 1 ml of hydrolysis solution was added to the particles and the tube shaken for a further hour. The supernatant was then removed from the particles with a magnetic separator and its absorbance measured at 282 nm.

The amount of 4-nitrobenzaldehyde in the hydrolysis solution was calculated by interpolation using a calibration curve constructed from a range of standard solutions of 4-nitrobenzaldehyde prepared separately.

2.6. Covalent coupling of oligonucleotide (or single strand DNA) onto the magnetic particles

Immobilisation of oligonucleotides onto the APTS-modified nanoparticles proceeded via treatment



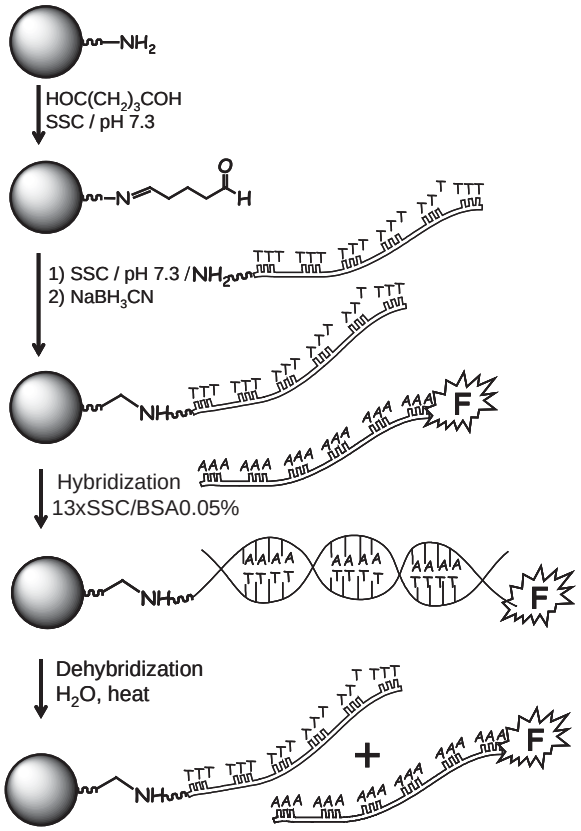
Scheme 1. Reaction of the surface groups of nanoparticles with a UV-sensitive reagent (4-nitrobenzaldehyde).

with glutaraldehyde to yield aldehyde surface groups. These groups can then be reacted with amine-modified oligonucleotides (in this case a 5′-amine modified dC_6dT_{25} oligonucleotide). The reaction between amine and aldehyde groups yields imines, which are reduced to the more stable secondary amine group by treatment with $NaBH_3CN$.

Two milligram of each batch of amine-modified nanoparticles was washed three times with 1 ml coupling buffer (1 × SSC buffer, pH 7.3) for 2 min at 18 °C. After removal of the supernatant, 0.5 ml of a 5% glutaraldehyde solution in coupling buffer were added and the suspension incubated for 3 h with end-over-end rotation at 18 °C. The material was subsequently washed three more times with coupling buffer to remove excess glutaraldehyde. 0.5 ml of a 3.3 μM solution of the amine-modified oligonucleotide were added and the mixture left incubating overnight whilst shaking. The oligo-modified nanoparticles were then washed once with coupling buffer and placed in 0.8 ml of $NaBH_3CN$ solution (0.03% w/v in coupling buffer) for 30 min at 18 °C. The material was finally washed three times with 0.8 ml of coupling buffer and resuspended in 200 μl of the same medium.

2.7. DNA capture experiments

A model system was constructed in which the performance of the oligonucleotide-modified particles in hybrid capture of a complementary fluorescently labelled oligonucleotide could be measured (Scheme 2). The experimental details of the method are as follows: 1 mg of the oligonucleotide-modified particles was washed twice with 0.5 ml of water and heated to 80 °C for 4 min; 200 μl of a micromolar solution of fluorescein-labelled oligonucleotide (HPLC purified 5′-fluorescein modified dA_{25}) in (13 ×)SSC/0.05% BSA was added and the suspension incubated with gentle shaking for 30 min at 18 °C. The supernatant was removed and kept for analysis. After washing three times with 1 ml of (13 ×)SSC, 200 μl of water were added and the nanoparticle suspension heated to 85 °C for 4 min to dehybridise the annealed oligonucleotide. The supernatant was removed and kept for fluorescence analysis. Two control experiments were also performed in order to evaluate non-specific binding: the adsorption of the fluorescently labelled sequence onto aldehyde-modified nanoparticles and the hybrid capture of a non-complementary fluorescently labelled sequence.



Scheme 2. Hybrid capture of a complementary fluorescently labelled oligonucelotides on oligonucleotide-modified nanoparticles.

3. Results and discussion

3.1. Magnetite and silica-coated magnetite nanoparticles

SEM analysis of the nanoparticles indicated that both the pure magnetite and silica–magnetite nanocomposites were approximately spherical in nature (Fig. 1a and b). However, when viewed

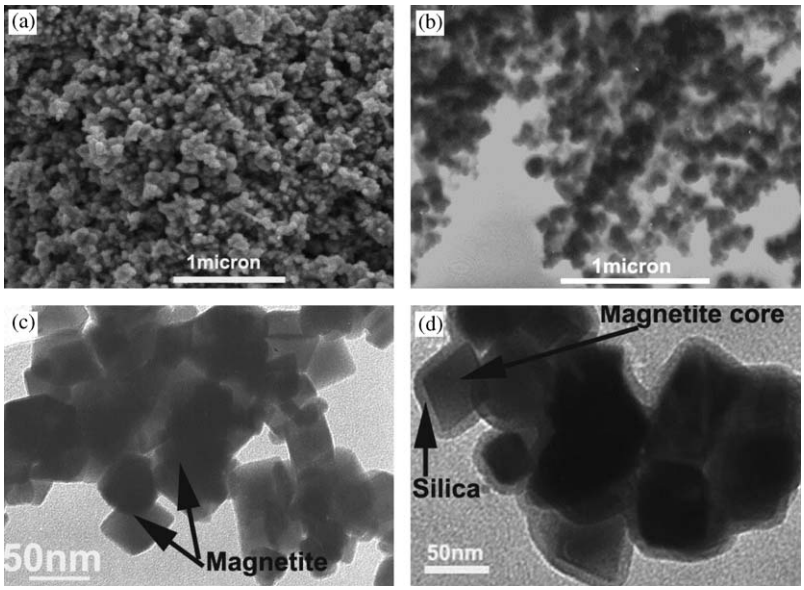


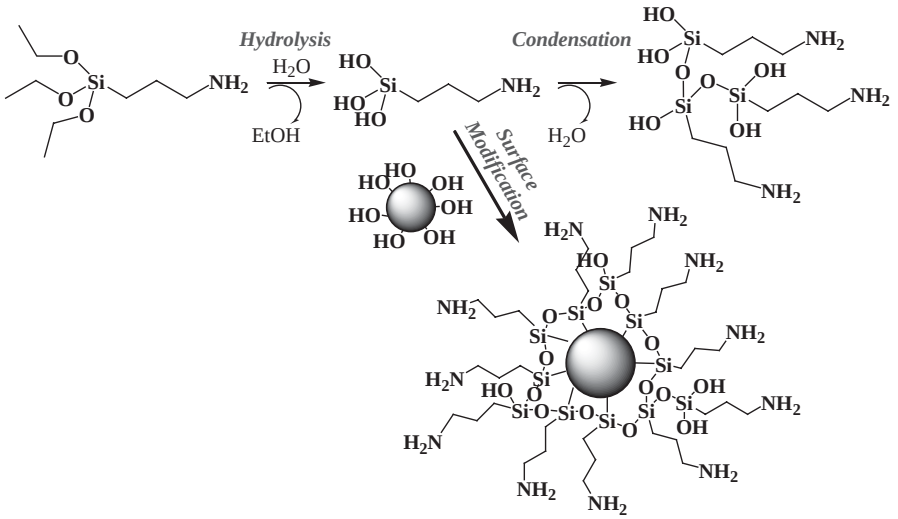
Fig. 1. SEM micrographs: pure magnetite (a), 5 silica-coated magnetite (b), TEM micrographs: pure magnetite (c), 5 silica-coated magnetite (d).

under the transmission electron microscope, with higher magnification and better resolution, the particles exhibited a rhombic structure (Fig. 1c and d) with sizes ranging from 30 to 100 nm. Two regions with different electron densities can be distinguished for the rhombic silica-coated magnetite nanoparticles: an electron dense part (core) that presumably corresponds to magnetite, and a less dense shell region, surrounding each core, that is likely to be the silica coating (Fig. 1d). TEM images suggested that the silica coating was fairly homogeneous and its thickness to be around 2 nm. No crystalline microstructure was observed for the silica phase, which is in agreement with the X-ray results obtained for the materials (data not shown).

3.2. *Amine surface modification of magnetite and silica–magnetite nanoparticles*

Surface modification using alkoxysilanes involves two reactions that take place simultaneously (Scheme 3). In the solution phase, the hydrolysis of the alkoxysilane groups to the highly reactive silanols species occurs whilst on the solid phase their condensation with surface free –OH groups to render stable Si–O–Si bonds takes place. Oligomerisation of the silanols in solution also occurs as a competing reaction with their covalent binding to the surface. The kinetics of these processes depends on the experimental parameters used in the surface modification reaction (reaction time and temperature, solvent, water concentration, presence of catalyst etc.) and, as a consequence, the properties of the final material can also be expected to be dependent on these variables.

Different batches of silane (APTS) surface modified nanoparticles were synthesised by varying reaction time (from 1 to 24 h) and reaction temperature (18, 50 and 70 °C) of the silane-nanoparticles reaction mixture and the effect of reaction variables was quantified in terms of the number of active amine groups present on the surface of the products as described previously (colorimetric assay). In addition, elemental analysis was carried out by a commercial contractor. Table 1 presents the experimental values of N percentage of APTS-modified magnetite nanoparticles measured by elemental analysis. It is clear that the values detected indicate that the organic



Scheme 3. Surface modification of nanoparticles by aminosilane.

Table 1
Results of elemental analysis, colorimetric assay and hybridisation experiments performed with APTS-modified magnetite nanoparticles synthesised at 50 °C

Reaction time (h)	Elemental analysis μmol—NH_2 g^{-1} support	Colorimetric analysis μmol—NH_2 g^{-1} support	Fluorescence analysis μmol hybrid captured oligo g^{-1} support
	N		
0 (Bare magnetite)	—	5	—
1	42	10	0.044
2	50	14	0.051
5	78	17	0.086
8	57	18	0.081
23	71	24	0.145

content of the material is very low. The number of amine groups per gram of nanoparticles was calculated and is also included in the table. For this calculation, the value of N present in the base (non-modified) nanoparticles was subtracted from the value of N detected after modification. These values should correlate directly with the amount of APTS present at the particle surface (since APTS is the only possible source of N in the material). After modification, a higher percentage of N was detected in all nanoparticles. However, the N values (after subtraction) were very low and close to the experimental limit of the technique, limiting their reliability. The amine density calculated by this method lies between 42 to 78 μmol g^{-1} of support depending on the conditions used for synthesis. These numbers can be taken as indicative only, not as absolute values. A more accurate analysis of amine groups can be obtained using the colorimetric assay described previously. Fig. 2 shows the results of colorimetric analyses performed on magnetite and silica-coated magnetite nanoparticles that were modified with APTS under various experimental conditions. It is clear that the reaction conditions strongly influence the number of active amine sites available on the surface. In

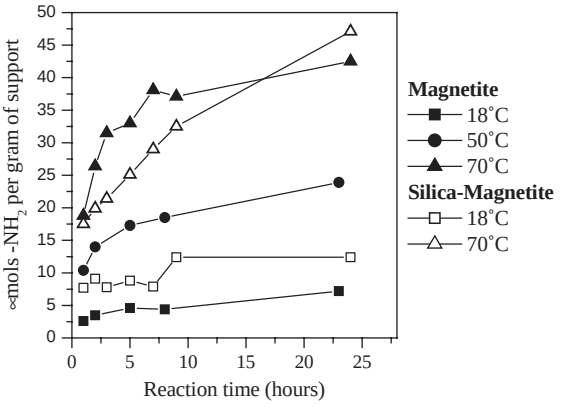


Fig. 2. Variation of the density of active amine groups with the reaction time and temperature in magnetite and silica coated magnetite nanoparticles.

our experiments, increasing the reaction time leads to an increase in amine density on the surface and this observation is more pronounced at higher reaction temperatures. The first and second column in Table 1 shows a comparison between the values of the amine density obtained by elemental analysis and by colorimetric methods. No clear correlation between reaction time and the amine density could be drawn from the elemental analysis values (probably due to detection limit restrictions), but a trend can be clearly deduced from results using the colorimetric assay. The values obtained in the colorimetric assay are lower than those obtained from the elemental analysis, suggesting that not all the amine groups are free to react with the 4-nitrobenzaldehyde at the nanoparticle surface, but may be occluded.

An increase in amine density was also observed with increased reaction temperature. The number of surface amine groups was observed to be enhanced by a factor of up to 10 upon increasing the reaction temperature from 18 to 70 °C. This trend was common to both magnetite and silica surfaces.

3.3. Immobilisation of oligonucleotides and hybrid capture of a complementary sequence

Immobilisation of the oligonucleotide onto the surface of the supports followed the method described previously. The results of the hybrid capture experiments performed with oligonucleotide-modified nanoparticles are presented in the last column of Table 1. The values correspond to particles with differing amine densities. It is clear that it is possible to correlate the efficiency of hybrid capture with the surface amine density of the material. However, the mole ratio of captured oligonucleotides is more than two orders of magnitude smaller than the number of available amine groups detected by colorimetry. There are two possible explanations for this phenomenon. Firstly, the larger relative size (bulk) of the amine-modified oligonucleotide (when compared with the 4-nitrobenzaldehyde molecule used for the colorimetric analysis) causes a steric hindrance effect lowering the yield of the coupling reaction, or some of the immobilised capture sequences may not be present in a favourable conformation for capturing the complementary strand.

Also, it is noteworthy to mention that in the negative control experiment where capture was attempted with a non-complementary oligonucleotide sequence, no sequence capture was observed.

4. Conclusions

Amine-functionalised magnetite and silica–magnetite nanoparticles have been synthesised using APTS as surface modification agent. By variation of the experimental conditions (reaction time and temperature) variants with different surface amine group densities have been obtained and characterised. The performance of these materials in the immobilisation of oligonucleotides and their use in hybrid capture of complementary sequences has been tested where it was observed that efficiency was directly related to the surface density of amine groups. These results indicate the necessity for optimisation of the silanisation surface activating reaction and the need for systematic studies on the effect of reaction variables in the production of high performance materials.

Acknowledgements

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References

[1] P.J. Robinson, P. Dunnill, M.D. Lilly, Biotech. Bioeng. 15 (1973) 603.
[2] W.J. Parak, D. Gerion, T. Pellegrino, et al., Nanotechnology 14 (2003) R15.
[3] C.A. Mirkin, Science 301 (2003) 1884.
[4] T. Sugimoto, E. Matijevic, J. Colloid Interface Sci. 74 (1979) 227.
[5] A.P. Philipse, M.P.B. Vanbruggen, C. Pathmamanoharan, Langmuir 10 (1994) 92.
[6] K. Matsuda, M. Sumida, K. Fujita, S. Mitsuzawa, Bull. Chem. Soc. Japan 60 (1987) 4441.
[7] I.J. Bruce, J. Taylor, M. Todd, et al., J. Magn. Magn. Mater. 284 (2004) 145.
[8] S. Sun, H. Zeng, J. Am. Chem. Soc. 124 (2002) 8204.
[9] H. Itoh, T. Sugimoto, J. Colloid Interface Sci. 265 (2003) 283.
[10] S. Lian, E. Wang, Z. Kang, et al., Solid State Commun. 129 (2004) 485.
[11] P.R. Levison, S.E. Badger, J. Dennis, et al., J. Chromatogr. A 816 (1998) 107.
[12] S.V. Sonti, A.J. Bose, Colloid Interface Sci. 170 (1995) 575.
[13] K. Furusawa, K. Nagashima, C. Anzai, Colloid Polym. Sci. 272 (1994) 1104.
[14] F.C. Meldrum, B.R. Heywood, S. Mann, Science 257 (1992) 522.
[15] J. Lellouche, N. Perlman, A. Joseph, et al., Chem. Commun. (2004) 560.
[16] S. Bucak, D.A. Jones, P.E. Laibinis, T.A. Hatton, Biotechnol. Prog. 19 (2003) 477.
[17] M. Ma, Y. Zhang, W. Yu, et al., Colloids Surface A Physicochem. Eng. Aspects 212 (2003) 219.
[18] X. Liu, Z. Ma, J. Xing, H. Liu, J. Magn. Magn. Mater. 270 (2004) 1.
[19] J. Chua, Scientist 18 (2004) 38.
[20] A. Constans, Scientist 13 (2000) 14.
[21] B. Sinclair, Scientist 12 (1998) 16.
[22] D. White, B. Butler, D. Creswell, C. Smith, Notes from Promega Corp., 1999, pp. 12–14.
[23] J.H. Moon, J.H. Kim, K. Kim, et al., Langmuir 13 (1997) 4305.