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Covalent surface modification of Fe_3O_4 magnetic nanoparticles with alkoxy silanes and amino acids

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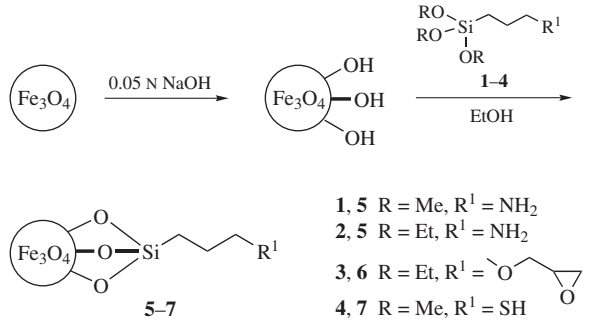
(3-Aminopropyl)silane-modified magnetic nanoparticles containing ε-aminocaproic acid and L-lysine fragments with free functional groups were obtained by the surface modification of Fe_3O_4 with alkoxysilane derivatives.

New materials based on γ-Fe_2O_3 and Fe_3O_4 magnetic nanoparticles (MNPs) with ferromagnetic properties are of interest for targeted drug delivery, magnetic resonance imaging, hyperthermic treatment of tumors and other medical applications.[1,2] The successful implementation of MNPs is associated with the chemical modifications of their surface by coating nanoparticles with functionalized polymers and biopolymers.[3,4] An efficient approach is the covalent linkage of organic molecules to MNPs using alkoxysilane reagents.[5–11] Organic molecules can be attached to MNPs *via* interaction with the surface alkoxysilane functional groups or by using bifunctional linkers, such as ε-aminocaproic acid (ε-ACA)[12] or L-lysine.[13]

The aim of this study was to develop new methods for modification of Fe_3O_4 MNPs (20–40 nm)† with silane derivatives, such as (3-aminopropyl)trimethoxysilane (APTMS) (**1**), (3-aminopropyl)triethoxysilane (APTES) (**2**), (3-glycidyloxypropyl)triethoxysilane (GPTES) (**3**), and (3-mercaptopropyl)trimethoxysilane (MPTMS) (**4**), followed by the modification of the surface layer with ε-ACA or L-lysine as linkers. The surface properties of MNPs obtained by chemical methods from aqueous solutions and those obtained by gas condensation are strongly different. The MNPs obtained from solutions have a hydrophilic surface, which makes it possible to functionalize them with alkoxysilane derivatives. In the latter case, MNPs have a hydrophobic surface, which complicates the surface modification using standard techniques. First, we studied the effects of solvents and the ratio of reactants on the degree of silanization with compounds **1**–**4** (Scheme 1).‡ The immobilization of propylsilanes **1**–**4** on the MNP surface was confirmed by IR spectroscopy (Figure 1) and elemental analysis.§

Anhydrous and 95% aqueous ethanol, benzene containing 1% water, benzene dried over Na and water were used as solvents in the condensation reaction. The best results were obtained using 95% ethanol. An amount of alkoxysilane needed to achieve the maximum degree of MNP modification was 2.5–3.0 mmol of APTMS or APTES per gram of MNPs, while in case of GPTES and MPTMS these values were 1.0–1.5 mmol. These ratios were found as a result of the condensation of MNPs suspensions in



Scheme 1 Functionalization of the MNP surface with alkoxysilanes **1**–**4**.

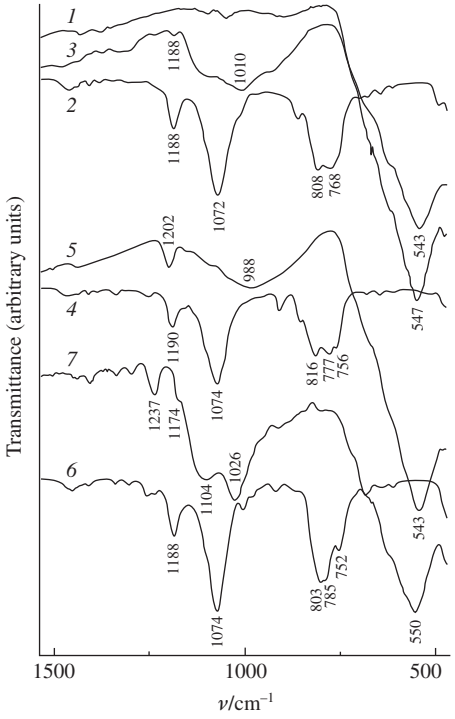


Figure 1 FT-IR spectra of (*1*) the original MNPs, (*2*) APTMS **1**, (*3*) APS-modified MNPs **5**, (*4*) GPTES **3**, (*5*) GPS-modified MNPs **6**, (*6*) MPTMS **4**, and (*7*) MPS-modified MNPs **7**.

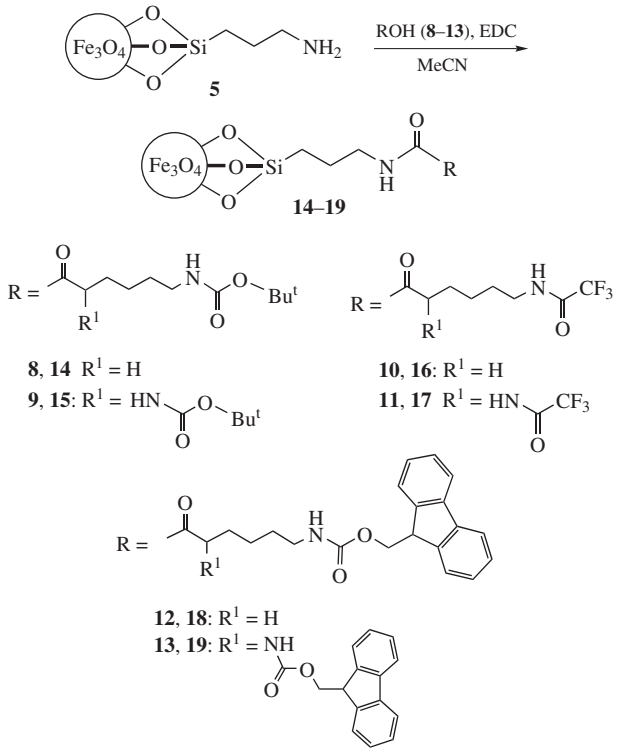
† Fe_3O_4 nanoparticles (20–40 nm) were obtained by gas condensation at the Institute of Metal Physics, Ural Branch of the Russian Academy of Sciences.

‡ After pre-activation (treatment of the original MNPs with 0.05 N NaOH) alkoxysilane reagent **1** (**2**, **3** or **4**) was added to a suspension of MNPs, and the reaction mixture was stirred for 16–20 h.

§ The IR spectra were recorded using a Nicolet 6700 FT-IR spectrometer (Thermo Electron Corporation). Elemental analysis was carried out on a Perkin Elmer PE 2400 (series II CHNS-O EA 1108) instrument.

¶ Morphological studies of particles were performed using a Philips CM30 transmission electron microscope (TEM).

†† The mass fraction of silicon was determined on an iCAP6300 Duo optical emission spectrometer with inductively coupled plasma (ICP-OES) (Thermo Scientific).



Scheme 2 Coupling of N-protected derivatives of ε-ACA and L-lysine to APS-modified MNPs.

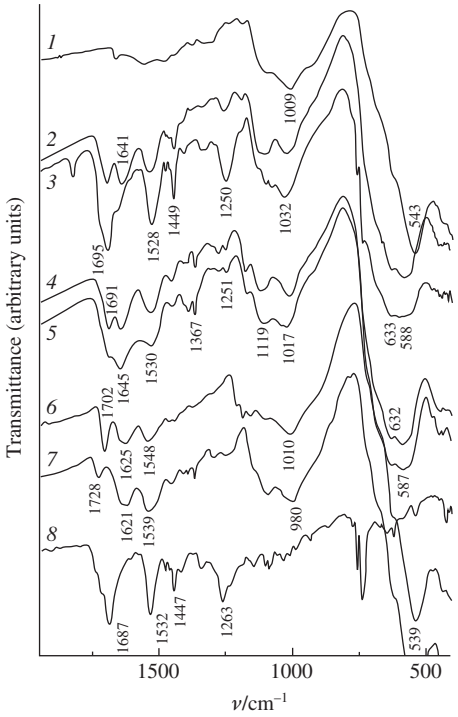


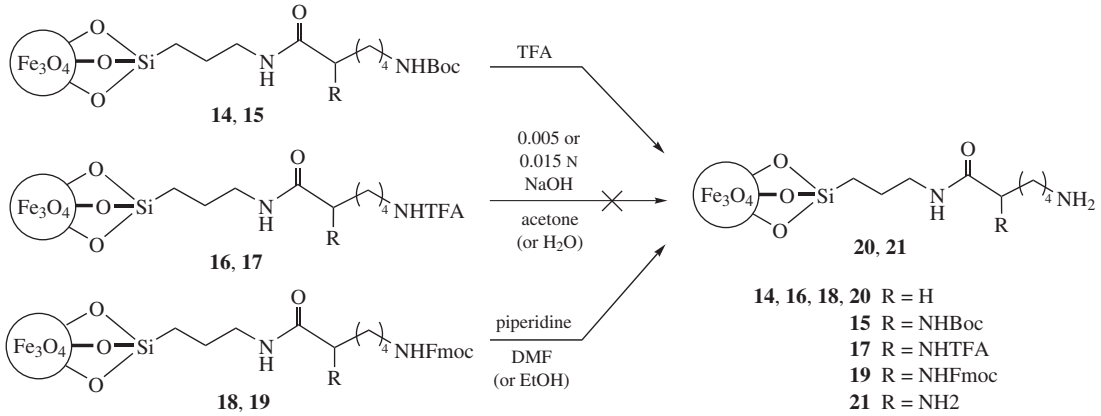
Figure 2 FT-IR spectra of (*1*) APS-modified MNPs **5**, (*2*) MNPs **18** immobilized with *N*-Fmoc-ε-ACA, (*3*) MNPs **19** immobilized with di-Fmoc-L-lysine, (*4*) MNPs **14** immobilized with *N*-Boc-ε-ACA, (*5*) MNPs **15** immobilized with di-Boc-L-lysine, (*6*) MNPs **16** immobilized with *N*-TFA-ε-ACA, (*7*) MNPs **17** immobilized with di-TFA-L-lysine, and (*8*) di-Fmoc-L-lysine **13** (for example).

ethanol (95%) with 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0 and 10 mmol of alkoxysilane per gram of MNPs.

According to the TEM data[¶] obtained for APS- (**5**) and (3-glycidyloxypropyl)silane-modified (GPS-modified) (**6**) MNPs, no difference in the morphology of the original and modified nanoparticles was observed. As for (3-mercaptopropyl)silane-modified (MPS-modified) MNPs (**7**), a substantial thickening of the surface layer was observed due to a profound tendency of mercapto groups towards oxidation into disulfides and also because of a plausible S–Fe bond formation in some cases. Quantitative estimation of the degree of immobilization of silane derivatives on the MNPs surface was carried out by ICP-OES[††] and elemental analysis. The degree of immobilization of APS in cases of APTMS and APTES was 0.7–1.0 mmol g^{-1}. Modified Fe_3O_4 nanocomposites **6** and **7** contained 0.3 and 2.1 mmol g^{-1} of the silane fragments attached to MNPs, respectively.

The presence of amino groups in the structure of APS-modified MNPs allows one to further modify them with amino acids and peptides bearing free carboxylic groups. We carried out the coupling reaction of the APS-modified MNPs with N-protected derivatives of ε-ACA (**8**, **10**, **12**) and L-lysine (**9**, **11**, **13**) (Scheme 2). Three types of N-protecting groups were chosen: *tert*-butyloxycarbonyl (Boc), which can be removed under acidic conditions, trifluoroacetyl (TFA) removable under alkaline conditions, and fluorenylmethyloxycarbonyl (Fmoc), which can be removed in the presence of organic bases. Since a similar reaction with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as a coupling agent proceeds smoothly in acetonitrile solution,[14] we used the same reaction conditions (Scheme 2). The FT-IR spectra of the reaction products exhibit absorption bands that are characteristic of the starting materials; however, these bands are shifted (Figure 2), thus indicating that the covalent binding of amino acids to amino groups on the modified MNPs takes place. The amounts of the immobilized amino acid derivatives were calculated by the carbon mass fraction found from the elemental analysis data for the amino acid-modified MNPs. The best results were obtained using di-Fmoc-L-lysine **19** (to 0.14 mmol g^{-1}), Boc- **14** and Fmoc-ε-ACA **18** (0.15 mmol g^{-1} in both cases).

Another task of this work was to prepare MNPs **20** and **21** modified with ε-ACA and L-lysine with free amino groups



Scheme 3 Removal of N-protecting groups from ε-ACA and L-lysine derivatives immobilized on the APS-modified MNPs.

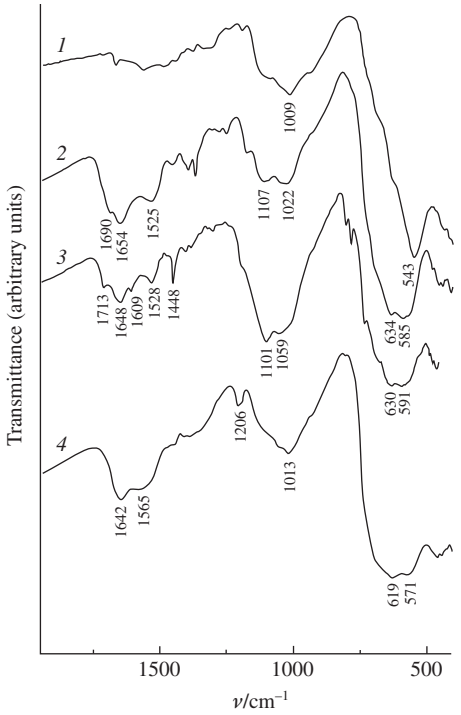


Figure 3 FT-IR spectra of (*1*) the APS-modified MNPs **5**, (*2*) MNPs **19** immobilized with di-Fmoc-L-lysine, (*3*) MNPs **15** immobilized with di-Boc-L-lysine, and (*4*) MNPs **21** immobilized with L-lysine.

(Scheme 3). The nanocomposites are stable under acidic conditions in concentrated trifluoroacetic acid. The treatment of a suspension of MNPs **14** and **15** with concentrated trifluoroacetic acid for 30 min resulted in the complete removal of the Boc protecting group, thus giving compounds **20** and **21**. The removal of Fmoc groups from the amino acid fragments of MNPs **18** and **19** was carried out by reacting with piperidine in ethanol for 24–30 h. We failed to obtain MNPs **20** and **21** on the treatment of MNPs **16** and **17** with a 1.2 molar excess of NaOH (relative to the corresponding amino acid). The alkaline conditions lead to a significant degradation of nanocomposites. It did occur simultaneously with the removal of TFA protection and was accompanied by a marked cleavage of amino acid derivatives from the MNPs surface due to the breakage of the Si–O–Fe bond.

The degree of removal of protecting groups was estimated by FT-IR spectroscopy (Figure 3). In order to evaluate quantitatively the immobilized amino acids, we subtracted the carbon mass fraction of the starting APS-modified MNPs from the carbon mass fraction found from the elemental analysis data for the amino acid-modified MNPs. Based on the obtained carbon mass fraction, we calculated the amount of immobilized amino acid. The removal of Fmoc groups from the L-lysine fragment resulted in the MNPs containing up to 0.10 mmol of amino acid fragments per gram of nanoparticles. After the removal of Boc and Fmoc protections from ε-ACA, we obtained MNPs containing up to 0.14 mmol g^{-1}.

In conclusion, note that the chemical modification of Fe_3O_4 MNPs *via* covalent bond formation with trialkoxysilane derivatives (APTMS, APTES, GPTES and MPTMS) was performed, and an opportunity for further functionalization of the surface of APS-modified MNPs with N-protected derivatives of ε-ACA and L-lysine was shown. The best results were obtained using di-Fmoc-L-lysine, Boc- and Fmoc-ε-ACA. Appropriate conditions for the removal of protecting groups and the preparation of MNPs containing amino acid fragments with free functional groups were found. Both qualitative and quantitative evaluation procedures for the immobilization of propylsilane and amino acid derivatives on the MNPs have been advanced using IR spectroscopy, elemental analysis data and ICP-OES.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2013.01.004.

References

1 Theranostic Nanomedicine Special issue, *Acc. Chem. Res.*, 2011, **44**, 841.
2 C. Sanchez, P. Belleville, M. Popall and L. Nicole, *Chem. Soc. Rev*., 2011, **40**, 696.
3 J. K. Oh and J. M. Park, *Prog. Polym. Sci*., 2011, **36**, 168.
4 O. Veiseh, J.W. Gunn and M. Zhang, *Adv. Drug Delivery Rev*., 2010, **62**, 284.
5 R. A. Bini, R. F. C. Marques, F. J. Santos, J. A. Chaker and M. Jafelicci, Jr., *J. Magn. Magn. Mater.*, 2012, **324**, 534.
6 F. Galeotti, F. Bertini, G. Scavia and A. Bolognesi, *J. Colloid Interface Sci*., 2011, **360**, 540.
7 N. Frickel, R. Messing, T. Gelbrich and A. M. Schmidt, *Langmuir*, 2010, **26**, 2839.
8 C. Zhang, B. Wangler, B. Morgenstern, H. Zentgraf, M. Eisenhut, H. Untenecker, R. Kruger, R. Huss, C. Seliger, W. Semmler and F. Kiessling, *Langmuir*, 2007, **23**, 1427.
9 N. R. Jana, C. Earhart and J. Y. Ying, *Chem. Mater*., 2007, **19**, 5074.
10 A. Campo, T. Sen, J. Lellouche and I. J. Bruce, *J. Magn. Magn. Mater*., 2005, **293**, 33.
11 I. J. Bruce and T. Sen, *Langmuir*, 2005, **21**, 7029.
12 B. Srinivasan and X. Huang, *Chirality*, 2008, **20**, 265.
13 C.-H. Liu, W.-C. Wu, H.-Y. Lai and H.-Y. Hou, *J. Biosci. Bioeng*., 2011, **112**, 219.
14 A. M. Demin, M. A. Uimin, N. N. Shchegoleva, A. E. Yermakov and V. P. Krasnov, *Rossiiskie Nanotekhnologii*, 2012, **7**, 66 (*Nanotechnologies in Russia*, 2012, **7**, 132).

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