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

Alexander M. Demin, Victor P. Krasnov and Valery N. Charushin

MNPs obtained by gas condensation method have hydrophobic surface, which complicates its modification using standard techniques. In order to enhance it, the treatment of the original MNPs with 0.05 N NaOH was performed (Scheme 1).

Surface modification of MNPs with APS. MNPs (0.150 g) were dispersed in 75 ml of 95% of ethanol under sonication. 0.66 ml (2.5 mmol per 1 g MNPs) of APTMS was added dropwise to the suspension and reaction mixture was stirred for 20 h. Obtained modified MNPs were precipitated using an external magnetic field. Solvent was decanted, and MNPs were washed with ethanol (five portions of 20 ml) and redispersed in 75 ml of acetonitrile.

Conjugation of di-Fmoc-L-lysine with APS-modified MNPs. 17.7 mg (1.5 mol excess in case of APS-modified MNPs containing 1.0 mmol APS per 1g MNPs) of di-Fmoc-L-lysine and 7.7 mg of EDC were added to 10 ml suspension of APS-modified MNPs in acetonitrile (2 mg/ml) and reaction mixture was stirred for 20 h. Then, the di-Fmoc-L-lysine-modified MNPs were precipitated using an external magnetic field. The solvent was removed and MNPs were washed with acetonitrile (five portions of 20 ml) and redispersed in 10 ml of 95% ethanol.

Removal of Fmoc-groups. 0.020 ml of piperidine was added to 10 ml suspension of di-Fmoc-L-lysine-modified MNPs in 95% ethanol (2 mg/ml) under stirring. In 48 h MNPs were washed with acetonitrile (20 ml x 5) by magnetic separation. Then MNPs were dried *in vacuo* and 17.9 mg of dark brown powder were obtained.

Immobilization of propylsilanes **1-4**, amino acids and their N-protected derivatives *via* covalent linkage to the MNP surface was confirmed by IR spectroscopy and elemental analysis.

Figure S1 shows the FT-IR spectra of the original MNPs, the propylsilane reagents **1**, **3**, **4**, and the modified MNPs. A broad absorption band at 1010 cm^{-1} corresponding to Si–O–Fe and Si–O–Si stretching vibrations was observed in the FT-IR spectra of the modified MNPs **5**, **7**, **8**. The fact that a strong absorption band at 1072−1074 cm^{-1}, that is characteristic of Si–O stretching vibrations of the starting silanes, is absent in the spectra indicates to the formation of Si–O–Fe and Si–O–Si bonds in the course of the reaction.

Characteristic absorptions for nanoconjugates **14−19** containing amino acid fragments (Figure S2) are bands in the range of 1650−1700 cm^{-1} corresponding to vibrations of the carbonyl group (C=O), and a series of bands in the range of 1550−1650 cm^{-1} corresponding to vibrations of the amide bonds (NH–C=O).

Figure S3 shows the FT-IR spectra of MNPs **21** obtained after the removal of the N-protecting groups, as well as the original MNPs and MNPs **15** and **19** modified with di-Fmoc- and di-Boc-L-lysine. As a result of hydrolysis, the absorption bands corresponding to vibrations of the carbamate C=O bond of Boc and Fmoc groups (in the range of 1700−1650 cm^{-1}) disappeared, while the band corresponding to the C=O amide bond vibrations (1640 cm^{-1}) remained unchanged.

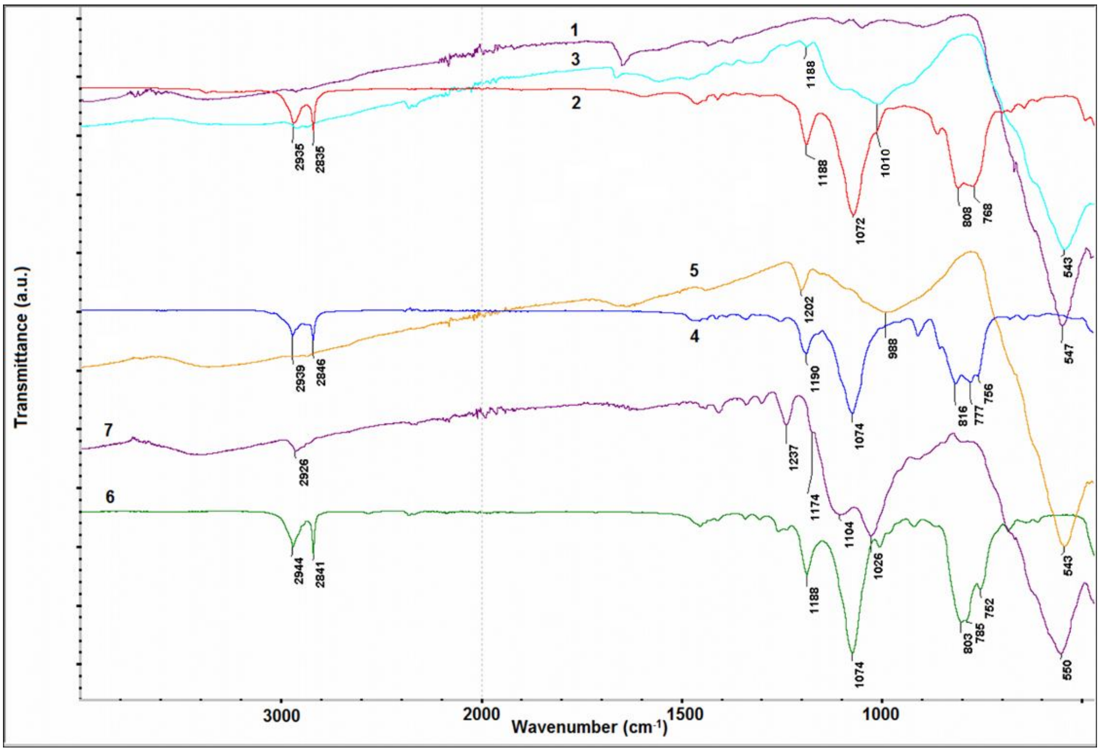

Figure S1 FT-IR spectra of (*1*) the original MNPs, (*2*) APTMS (**1**), (*3*) APS-modified MNPs **5**, (*4*) GPTES (**3**), (*5*) GPS-modified MNPs **7**, (*6*) MPTMS (**4**), and (*7*) MPS-modified MNPs **8**.

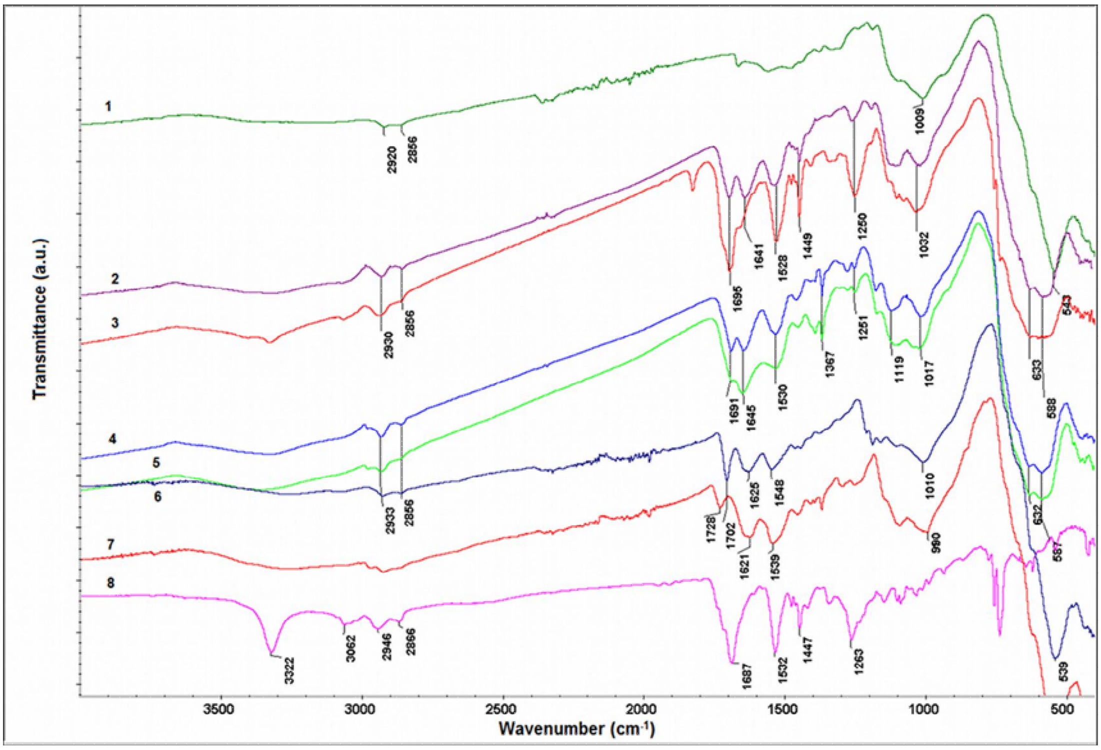

Figure S2 FT-IR spectra of (*1*) APS-modified MNPs **5**, (*2*) MNPs **18** immobilized with *N*-Fmoc-ε-ACA, (*3*) MNPs **19** immobilized with di-Fmoc-L-lysine, (*4*) MNPs **14** immobilized with *N*-Boc-ε-ACA, (*5*) MNPs **15** immobilized with di-Boc-L-lysine, (*6*) MNPs **16** immobilized with *N*-TFA-ε-ACA, (*7*) MNPs **17** immobilized with di-TFA-L-lysine, and (*8*) di-Fmoc-L-lysine **13** (for example).

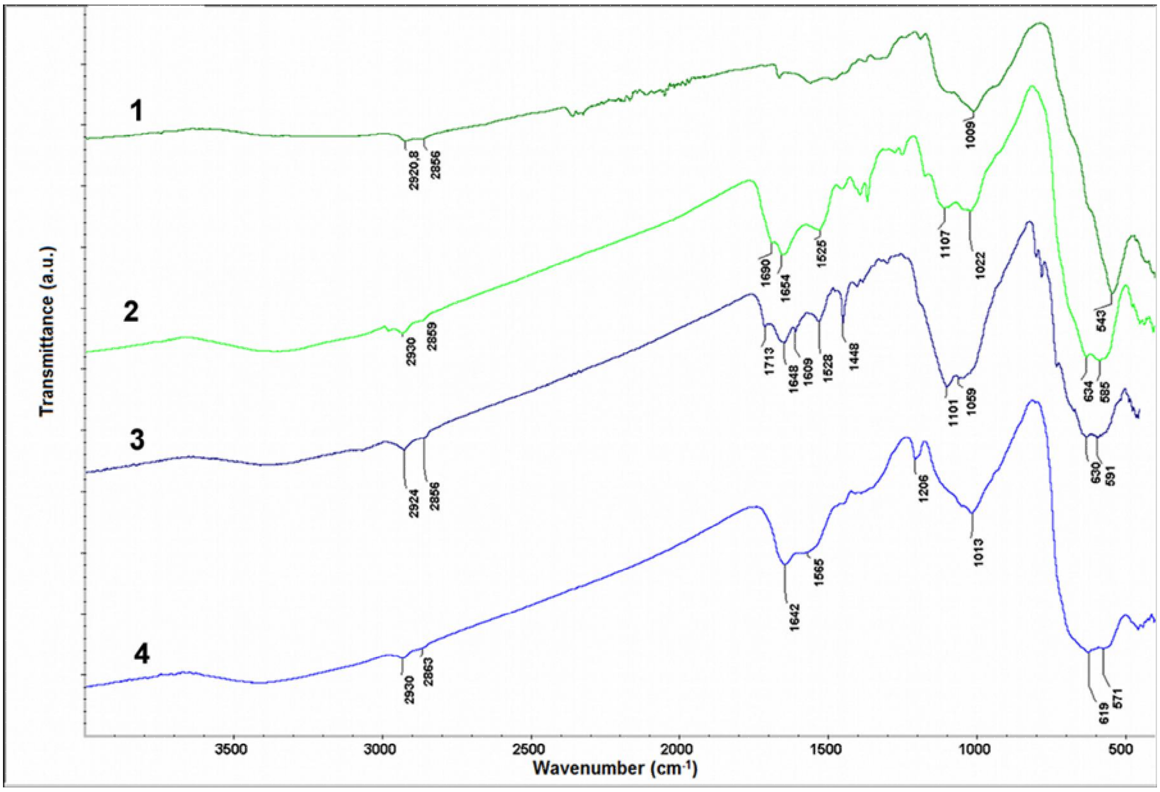

Figure S3 FT-IR spectra of (*1*) the APS-modified MNPs **5**, (*2*) MNPs **19** immobilized with di-Fmoc-L-lysine, *(3)* MNPs **15** immobilized with di-Boc-L-lysine, and *(4)* MNPs **21** immobilized with L-lysine.