

QUANTITATIVE DETERMINATION OF 3-AMINOPROPYLSILANE ON THE SURFACE OF Fe₃O₄ NANOPARTICLES BY ATTENUATED TOTAL REFLECTION INFRARED SPECTROSCOPY

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A technique for quantitative analysis of 3-aminopropylsilane on the surface of chemically modified Fe₃O₄ magnetic nanoparticles in the concentration range 0.32–3.03 mmol/g was developed using attenuated total reflection infrared spectroscopy. The technique was based on the ratios of band areas corresponding to Fe–O vibrations of the nanoparticles and Si–O vibrations of the coating as a function of the Si mass fraction in the nanocomposite that was determined by inductively coupled plasma atomic-emission spectroscopy.

Keywords: magnetic nanoparticles, infrared spectroscopy, 3-aminopropylsilane, inductively coupled plasma atomic-emission spectroscopy.

Introduction. Magnetic nanoparticles (MNP), including those based on iron and its oxides (γ -Fe₂O₃ and Fe₃O₄), are currently being investigated for medical applications (targeted drug delivery, improved contrast in magnetic-resonance imaging, and hyperthermal treatment of tumors [1–3]). Magnetic materials are used routinely, e.g., in biology for magnetic separation of biomolecules and cells [4] or in chemistry for development of magnetic separation of catalysts [5]. A promising method for modifying iron-oxide nanoparticles (NP) consists of depositing an organic layer on MNP using functionalized alkoxysilanes, in particular, 3-aminopropyltrialkoxysilanes [6–18] that form an organosilicon layer with functional groups on the surface. This enables the NP to be modified further. The ability to conjugate MNP to biomolecules or organic catalysts depends essentially on the surface concentration of functional groups. IR spectroscopy is most often employed for a qualitative assessment of the degree of functionalization. As a rule, elemental analysis, inductively coupled plasma atomic-emission spectroscopy (AES), or thermogravimetric analysis (TGA) is used for a quantitative determination of the number of organic molecules on the MNP surface [6–8, 10–14].

The number of molecules immobilized on the MNP can be determined indirectly using sorption (desorption) or covalent binding of various organic chromophores to the surface groups. Fluorescence or UV/vis spectrometry can determine the number of free molecules remaining in solution [6–9, 15–17]. For example, MNP with surface 3-aminopropylsilane (APS) groups were modified covalently with rhodamine B [15], *N*-difluorenylmethoxycarbonyl-L-lysine [6], or *S*-naproxene [7] (production of amide derivatives) or *p*-nitrobenzaldehyde (Schiff base formation) [16, 17] in order to determine the concentration of surface amines. The degree of functionalization of the starting MNP was determined from the difference between the amounts of reagent added to the reaction mixture and unreacted reagent. Conductometric titration with HCl was used to determine the concentration of amines on the MNP surface [18]. The conductivity of the suspension began to rise rapidly after all amines were neutralized. The amount of consumed HCl and; therefore, the amount of amines could be calculated from this.

The goal of the present work was to develop a technique for measuring the amount of APS from 0.320 to 3.06 mmol/g of MNP on the surface of chemically modified Fe₃O₄ MNP using attenuated total reflection (ATR) IR spectroscopy. The technique was based on the ratio of vibrational band areas of nanomaterial Fe–O and coating Si–O bonds as a function of AES measurements of the Si mass fraction in the nanocomposite.

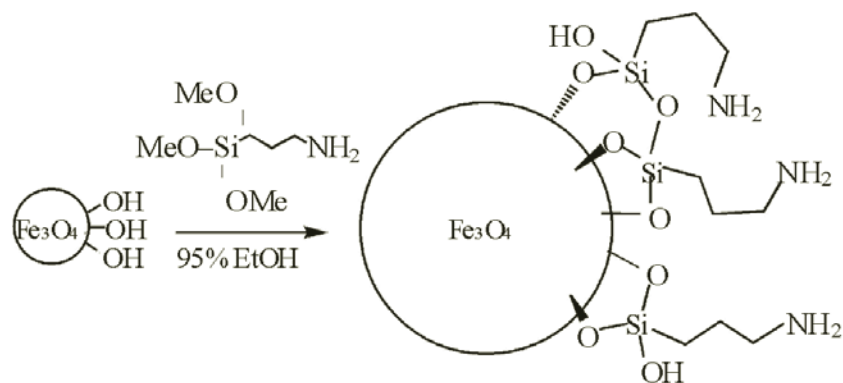
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Experimental. Fe₃O₄ MNP (average diameter 20 nm) were produced by gas-phase synthesis at the Institute of Metal Physics, Ural Branch, RAS [19]. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer (Thermo Scientific) with a diamond crystal at beam-incident angle 45°, an accessory for recording ATR spectra, a DTGS KBr detector, and an XT-KBr beamsplitter. Spectra were recorded in the range 400–4000 cm⁻¹ with 64 scans/min, amplification 8, rate 0.63 cm/s, diaphragm 100, and resolution 4 cm⁻¹. Absorption band areas were calculated using the embedded Omnic 7.3 software.

The amount of APS on the MNP was calculated from the Si mass fraction, which was determined by AES on an iCAP 6300 Duo ICP spectrometer (Thermo Scientific) using the intensity of the most sensitive analytical spectral line at 251.6 nm. The results obtained using the technique developed by us were compared with elemental analysis data. For this, the carbon mass fraction in model samples was determined using a PE automated 2400 Series II CHN analyzer. Then, the amount of APS immobilized on the Fe₃O₄ MNP surface was calculated.

The APS-modified MNP were prepared in analogy with previously described techniques [6–8]. Starting MNP in aqueous EtOH were treated for 6 h with 3-aminopropyltrimethoxy- (APTMS) or triethoxysilane (APTES) calculated for 0.50, 0.75, 1.0, 3.0, and 20.0 mmol of alkoxy silane per gram of MNP. The modified MNP were washed with H₂O and EtOH and dried in vacuo. According to transmission electron microscopy, the MNP did not change size during the modification and remained at ~20 nm. Nine Fe₃O₄ MNP samples containing various amounts of APS were obtained as a result. A weighed portion of the MNP (10 mg) was placed into a jasper mortar and thoroughly ground in order to increase the homogeneity of the analyzed sample. The analytical techniques required at least 5 mg of sample.



Results and Discussion. The Fe₃O₄ MNP (20 nm) surface was modified with APTMS according to the previously developed technique [6–8] and the following scheme:

IR spectroscopy confirmed that APS was immobilized on the surface (Fig. 1a). The principal characteristic bands in spectra of APS-modified MNP were those corresponding to MNP Fe–O and organic coating Si–O vibrations. A band at 548 cm⁻¹ belonging to Fe–O stretching vibrations changed insignificantly. It shifted to 554 cm⁻¹ in ATR spectra of the modified NP. Clearly pronounced bands at 1084 and 816 cm⁻¹ that were due to APTMS Si–O–C stretching vibrations disappeared [20, 21]. Absorption bands at 1103, 1010, and 931 cm⁻¹ appeared in ATR spectra of APS-modified MNP and corresponded to Si–O–Si vibrations. Bands at 1471, 1311, 1233, and 1191 cm⁻¹ were also observed in spectra of modified MNP. These were assigned to CH₂ bending, skeletal CH₂–CH₂, Si–CH₂, and C–N stretching vibrations, respectively. A band at 1568 cm⁻¹ was characteristic of NH₂ bending vibrations. Furthermore, bands for asymmetric (down to 2926 cm⁻¹) and symmetric (up to 2860 cm⁻¹) C–H stretching vibrations in the modified products shifted and diminished compared with the corresponding bands in spectra of starting APTMS (2940 and 2840 cm⁻¹).

Thus, two groups of bands belonging to coating Si–O and MNP Fe–O vibrations were most interesting for a qualitative assessment of the amount of APS on the MNP surface. It was shown that the ATR spectra did not change for nanocomposites containing various amounts of APS. The shape and position of the principal absorption bands remained practically constant. However, the ratio of Si–O and Fe–O absorption band intensities varied smoothly as a function of the MNP surface concentration of organic molecules (Fig. 1b).

A calibration curve (Fig. 2) was constructed using IR spectroscopy and AES data from nine samples of APS-modified MNP in order to quantify the amount of APS on the surface of the chemically modified MNP, which were obtained by treating a suspension of Fe₃O₄ MNP with various amounts of APTMS:

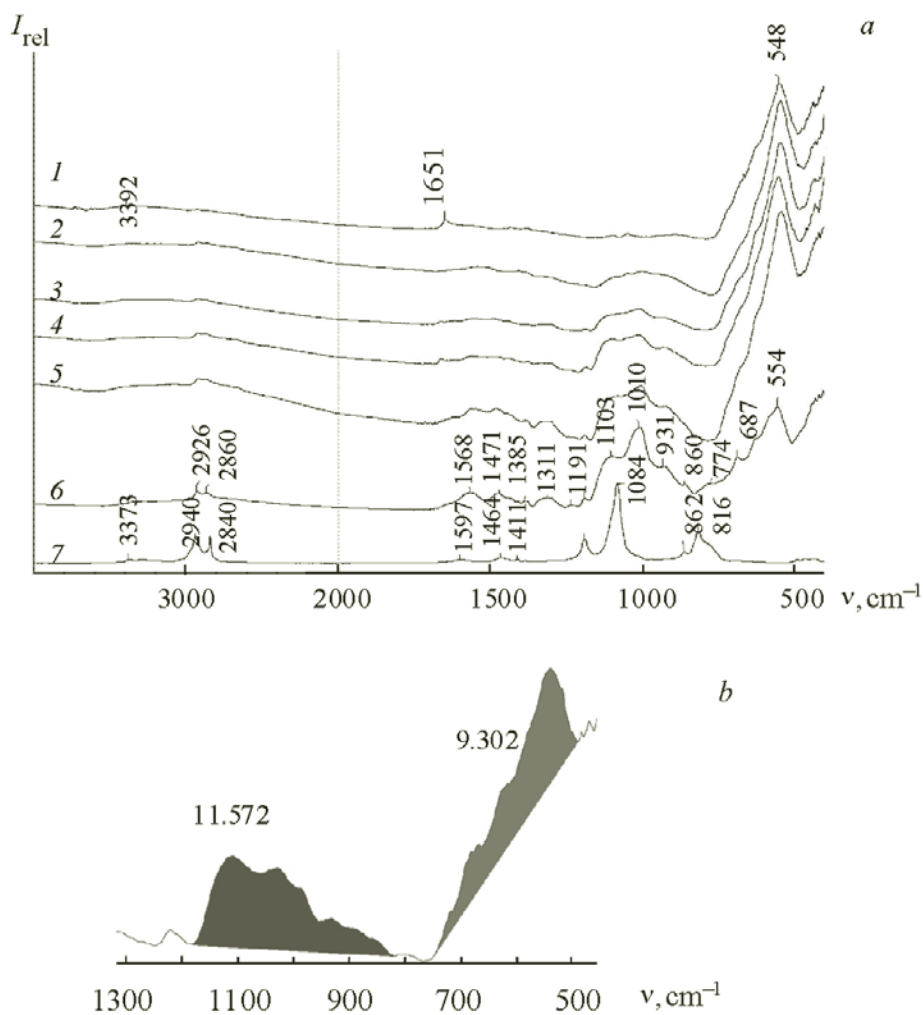


Fig. 1. ATR spectra of starting Fe_3O_4 magnetic nanoparticles (MNP) (1), APS-modified Fe_3O_4 MNP containing 0.32 (2), 0.48 (3), 0.68 (4), 0.84 (5), and 3.06 mmol of APS/g of MNP (6) (calculated from the Si mass fraction determined by AES), and APTMS (7) (a) and an example of baselines relative to which integrated intensities of analytical bands of Si–O and Fe–O vibrations were measured (b).

$$c = 1.038x^{-1.279}, \quad (1)$$

where c is the amount of APS on the Fe_3O_4 NP surface (mol/g of MNP) and x , the ratio of integrated intensities of Fe–O ($S_{\text{Fe-O}}$) (in the range $790\text{--}1180\text{ cm}^{-1}$) and Si–O ($S_{\text{Si-O}}$) (in the range $480\text{--}755\text{ cm}^{-1}$) vibrations in ATR spectra.

ATR spectra of analyzed samples were recorded at least five times ($n = 5$). The average of the ratios of vibrational band integrated intensities x was calculated for each sample (Fig. 1b). Equation (1) was used to calculate the amount of APS (c) on the Fe_3O_4 MNP surface (mol/g of MNP). The calculated x values agreed within uncertainty limits of less than 6%. The systematic error was 3%. The developed technique had 14% relative uncertainty for a calculated characteristic.

This technique for determining the amount of APS was an alternative to elemental analysis or AES for the type of used nanocomposites. It was faster, cheaper, and reproducible. Furthermore, small samples (5 mg) were adequate for analysis by this technique (whereas AES requires ~ 50 mg of MNP). The technique was used to determine the amount of APS in model samples of APS-modified MNP that were synthesized under various conditions [6–8]. The results obtained using the developed technique were comparable to those from elemental analysis of the carbon mass fraction. The relative limit

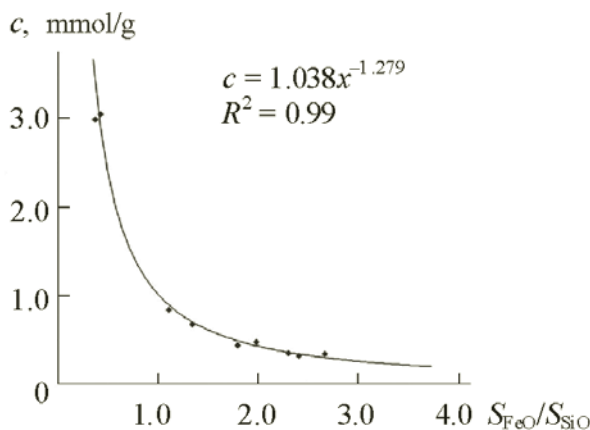


Fig. 2. Calibration curve for quantitative determination of APS immobilized on the MNP surface.

of reproducibility of the results was 11%. Practical application of the technique could be limited by the size of the studied particles (possible application of the technique to particles of different sizes was not studied).

Conclusions. An IR spectroscopic technique for determining the amount of APS on the surface of chemically modified Fe_3O_4 MNP in the concentration range 0.320–3.06 mmol/g of MNP was developed. It was based on the ratios of vibrational band areas of nanomaterial Fe–O and coating Si–O bonds as a function of the Si mass fraction in the sample that was measured using AES. Such a technique could be used to determine the amounts of other organic molecules immobilized on the surface of different NP where individual characteristic absorption bands corresponding to vibrations of the nanocomposite inorganic and organic materials can be identified in IR spectra.

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REFERENCES

1. *Special Issue on Theranostic Nanomedicine, Acc. Chem. Res.*, **44**, No. 10, 841–1134 (2011).
2. C. Sanchez, P. Belleville, and M. Popall, *Chem. Soc. Rev.*, **40**, 696–753 (2011).
3. A. H. Faraji and P. Wipf, *Bioorg. Med. Chem.*, **17**, 2950–2962 (2009).
4. M. A. M. Gijs, F. Lacharme, and U. Lehmann, *Chem. Rev.*, **110**, 1518–1563 (2010).
5. M. B. Gawande, P. S. Branco, and R. S. Varma, *Chem. Soc. Rev.*, **42**, 3371–3393 (2013).
6. A. M. Demin, V. P. Krasnov, and V. N. Charushin, *Mendeleev Commun.*, **23**, 14–16 (2013).
7. A. M. Demin, M. A. Uimin, N. N. Shchegoleva, A. E. Ermakov, and V. P. Krasnov, *Russ. Nanotekhnol.*, **7**, Nos. 3–4, 66–70 (2012) [A. M. Demin, M. A. Uimin, N. N. Shchegoleva, A. E. Yermakov, and V. P. Krasnov, *Nanotechnol. Russ.*, **7**, Nos. 3–4, 132–139 (2012)].
8. A. M. Demin, A. Y. Vigorov, I. A. Nizova, M. A. Uimin, N. N. Shchegoleva, A. E. Ermakov, V. P. Krasnov, and V. N. Charushin, *Mendeleev Commun.*, **24**, 20–22 (2014).
9. R. A. Bini, R. C. Marques, and F. J. Santos, *J. Magn. Magn. Mater.*, **324**, 534–539 (2012).
10. F. Galeotti, F. Bertini, G. Scavia, and A. Bolognesi, *J. Colloid Interface Sci.*, **360**, 540–547 (2011).
11. N. Frickel, R. Messing, and T. Gelbrich, *Langmuir*, **26**, 2839–2846 (2010).
12. N. R. Jana, C. Earhart, and J. Y. Ying, *Chem. Mater.*, **19**, 5074–5082 (2007).
13. B. Feng, R. Y. Hong, L. S. Wang, L. Guo, H. Z. Li, J. Ding, Y. Zheng, and D. G. Wei, *Colloids Surf., A*, **328**, 52–59 (2008).

14. C. Zhang, B. Wangler, and B. Morgenstern, *Langmuir*, **23**, 1427–1434 (2007).
15. B. Srinivasan and X. Huang, *Chirality*, **20**, 265–277 (2008).
16. I. J. Bruce and T. Sen, *Langmuir*, **21**, 7029–7035 (2005).
17. A. del Campo, T. Sen, and I. J. Bruce, *J. Magn. Magn. Mater.*, **293**, 33–40 (2005).
18. S. Kralj, M. Drofenik, and D. Makovec, *J. Nanopart. Res.*, **13**, 2829–2841 (2011).
19. V. A. Sharapova, M. A. Uimin, A. A. Mysik, and A. E. Ermakov, *Phys. Met. Metallogr.*, **110**, No. 1, 5–12 (2010).
20. L. J. Bellamy, *The Infra-Red Spectra of Complex Molecules*, 2nd ed., Methuen & Co., London (1958) [Russian translation pp. 474–485].
21. E. Pretsch, P. Bühlmann, and C. Affolter, *Structure Determination of Organic Compounds*, Springer, Berlin, New York (2000) [Russian translation pp. 310–311].