

Preparation and characterization of magnetite nanoparticles coated by amino silane

Ming Ma^{*}, Yu Zhang, Wei Yu, Hao-ying Shen, Hai-qian Zhang, Ning Gu

National Laboratory of Molecular and Biomolecular Electronics, Southeast University, Nanjing 210096, PR China

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Abstract

Magnetite nanoparticles were prepared by coprecipitation of Fe^{2+} and Fe^{3+} with NH_4OH , and then, amino silane was coated onto the surface of the magnetite nanoparticles. Transmission electronic microscopy shows the average size of 7.5 nm in diameter. Powder X-ray diffraction and electronic diffraction measurements show the spinel structure for the magnetite nanoparticles. FT-IR spectra indicate that amino silane molecules have been bound onto the surface of the magnetite nanoparticles by Fe-O-Si chemical bonds. Energy dispersive X-ray spectroscopy (SEM-EDS) indicates atomic ratio of 96.75:3.25 for Fe:Si, implying a nearly monolayer coating of amino silane on the magnetite particle surface according to a rough calculation. By an enzyme-linked assay, it was proved that the amino silane-coated magnetite nanoparticles could significantly improve the protein immobilization.

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1. Introduction

Magnetic particles (microspheres, nanospheres and ferrofluids) are widely studied for their applications in various fields in biology and medicine such as enzyme and protein immobilization, genes, radiopharmaceuticals, magnetic resonance imaging MRI, diagnostics, immunoassays, RNA and DNA purification, magnetic cell separation and purification, magnetically controlled transport of anti-cancer drugs as well as hyperthermia generation [1–3]. These magnetic beads are

generally of core-shell type: biological species cells, nucleic acids, proteins are connected to the magnetic core through an organic or polymeric shell. The shells are either biocompatible in general (such as dextran, PEG, etc.), or possessing active groups which can be conjugated to biomolecules such as proteins and enzymes. Therefore, the investigation of magnetic nanoparticles with organic coating is of significance for applications.

In this work we prepared magnetite nanoparticles coated with a near monolayer of amino silane, which has active group of $-\text{NH}_2$ that can connect biomolecules, drugs and so on. And the morphology, structure and composition of the coated magnetite nanoparticles were characterized by TEM, ED, XRD, FT-IR and SEM-EDS.

^{*} Corresponding author

E-mail address: maming@seu.edu.cn (M. Ma).

Furthermore, to prove that amina silane-coated magnetite nanoparticles can be conjugated to some biomolecule, an enzyme-linked colorimetric assay was carried out after the enzyme horseradish peroxidase (HRP) of different concentrations was used to interact with the coated and the uncoated magnetite nanoparticles.

2. Experimental

2.1. Synthesis of magnetite nanoparticles

Magnetite was made according to the method of Molday [4]. Typically, a solution of mixture of FeCl_3 (0.01 M) and FeSO_4 (0.006 M) at pH 1.7 was prepared under N_2 protecting. Then, ammonia aqueous solution (1.5 M) was dropped into it with violently stirring until the pH of the solution raised to 9. The obtained magnetite was washed immediately with water for 5 times and ethanol for 2 times by magnetic separation. Finally, part of magnetite nanoparticles were dispersed in ethanol with concentration of 0.0128 M, and the others were dried into powder at room temperature under vacuum.

2.2. APTS-coated magnetite nanoparticles

25 ml magnetite colloid ethanol solution prepared above was diluted to 150 ml by ethanol and 1 ml H_2O . The solution was then treated by ultrasonic wave for 30 min. 35 μl 3-aminopropyltriethoxysilane ($\text{NH}_2(\text{CH}_2)_3\text{Si}(\text{OC}_2\text{H}_5)_3$, APTS) was added into it with rapid stirring for 7 h. The result solution was washed with ethanol for 5 times, and then dried into powder at room temperature under vacuum.

2.3. Characterization

The particle size and morphology of the samples were determined by transmission electronic microscopy (TEM, JEOL, JEM-200CX, 200 kV). Powder X-ray diffraction (XRD, Rigaku, D/Max-RA, Cu K α) and electronic diffraction (ED) were used to determine the crystal structure of the samples. The elemental analysis and APTS loading on

magnetite particles were measured by energy dispersive X-ray spectroscopy (SEM-EDS, EDAX, PV9100). Fourier transform infrared spectroscopy (FT-IR, Nicolet, 750) of the samples were used to study the chemical bonds between Fe_3O_4 and APTS.

2.4. HRP-immobilized and its activity assays

The APTS-coated magnetite nanoparticles and uncoated magnetite nanoparticles as control were all dispersed in phosphate-buffered saline (PBS, 0.01 M, pH 7.4) with identical concentrations of 2 g l^{-1} . Enzyme horseradish peroxidase HRP of different concentrations were added into 200 μl magnetite-PBS solutions. The mixtures were incubated in 37 °C for 1 h, and then retracted in 4 °C refrigerator for 4 h. Then the mixtures were washed carefully by PBS for 4 times and shifted to other vessels to remove the dissociated enzymes. Developed by addition of substrates, namely 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide (TMB- H_2O_2), for 10 min, the reaction was stopped by 2 mol l^{-1} H_2SO_4 . The optical density at 450 nm was read immediately in an automatic plate reader (Stat fax-2100, Beiken Company). All samples were tested in duplicate, arithmetic means and standard deviations of absorbance values were calculated ($x \pm s$).

3. Results and discussion

Fig. 1 is the TEM and ED photography of the magnetite nanoparticles coated with APTS, which shows that most of the particles are quasi-spherical and with an average diameter of 7.5 nm. The distribution of particle diameters is shown in Fig. 2.

According to the ED pattern, the d -spacing can be calculated in the following equation [5],

$$L\lambda = dR \quad (1)$$

where L is the distance between the test sample and the film ($L = 137$ cm), λ is the wavelength of electron beam ($\lambda = 0.0251$ Å), R is the radius of the diffraction ring. The calculation results are shown in Table 1, which accord with the XRD

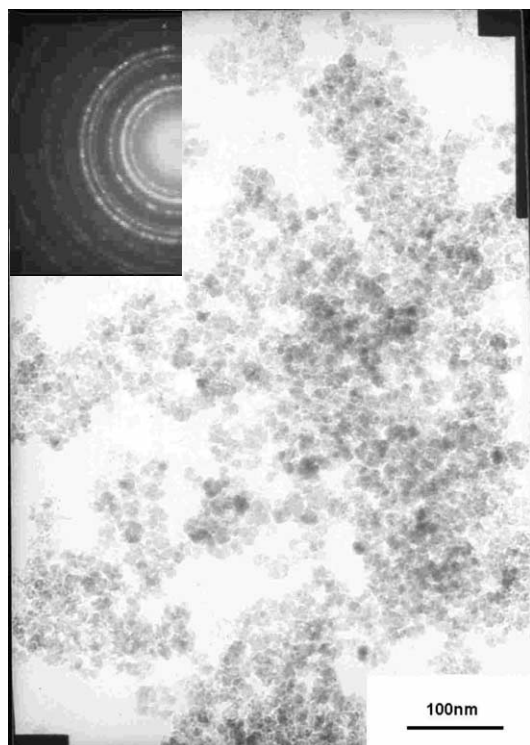


Fig. 1. TEM and ED photograph of the magnetite nanoparticles coated with APTS.

pattern of the sample shown in Fig. 3 and indicate the inverse cubic spinel structure of Fe_3O_4 . Compared with the theoretical values, the reduction of d -spacing obtained experimentally is due to lattice constrictions for nanosized particles [7].

Since there are large surface-to-volume atomic ratio, high surface activity, and amount of dangling bonds on nanoparticle surface, the atoms on the surface are apt to adsorb ions or molecules in solution. For Fe_3O_4 nanoparticles dispersed in a neutral aqueous solution, the bare atoms of Fe and O on the particle surface would adsorb OH^- and H^+ respectively, so that there is OH-rich surface. The $-\text{OH}$ on the surface can react with APTS as the process shown in Fig. 4. Therefore, the magnetite nanoparticles can be coated with APTS molecules by chemical bond. The fact was proven by comparison of FT-IR spectra of the coated and uncoated Fe_3O_4 nanoparticles shown in Fig. 5. It can be seen that, compared with the uncoated sample, the coated Fe_3O_4 nanoparticles

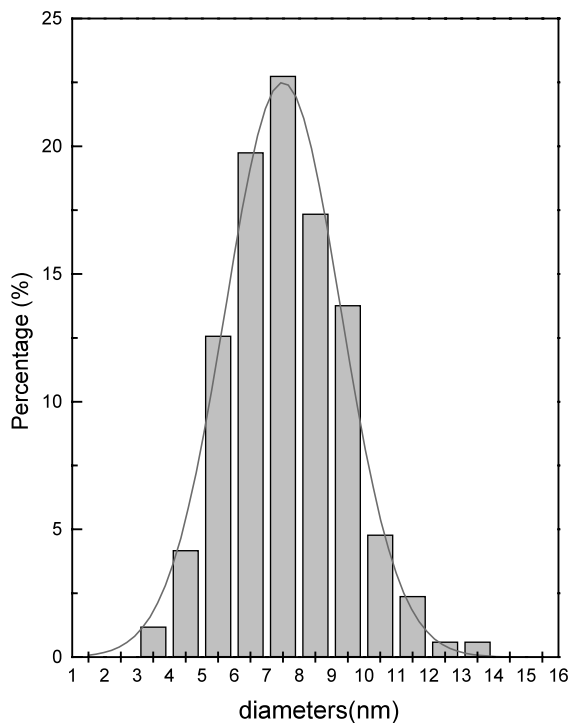


Fig. 2. Diameter distribution of magnetite nanoparticles coated with APTS.

possess absorption bands in 2971.8 and 2925.5 cm^{-1} due to stretching vibration of C–H bond, band in 1091.5 cm^{-1} due to the stretching vibration of C–N bond, band in 1051.0 cm^{-1} due to the stretching vibration of Si–O bond, band in 885.2 cm^{-1} due to the bending vibration of $-\text{NH}_2$ group. All of these reveal the existence of APTS. In addition, in Fig. 5(a) and (b) the absorption bands near 3400 and 1630 cm^{-1} refer to the vibration of remainder H_2O in the samples. And there also exists the contribution of $-\text{NH}_2$ for the band near 3400 cm^{-1} in Fig. 5(a).

Previously, it was reported that the characteristic absorption bands of the Fe–O bond of bulk Fe_3O_4 were in 570 and 375 cm^{-1} [8]. However, in Fig. 5(b) these two bands shift to high wavenumbers of about 600 and 440 cm^{-1} respectively, and the band near 600 cm^{-1} is split into two peaks of 631.4 and 582.9 cm^{-1} . A principal effect of finite size of nanoparticles is the breaking of a large number of bonds for surface atoms, resulting in the rearrangement of inlocalized electrons on the

Table 1
ED and XRD data for the magnetite nanoparticles coated with APTS

	1	2	3	4	5	6	7
<i>R</i> (cm)	0.73	1.19	1.39	1.67	2.04	2.19	2.37
ED results- <i>d</i> (Å)	4.74	2.89	2.47	2.06	1.69	1.57	1.47
XRD results- <i>d</i> (Å)	4.78	2.83	2.50	2.08	1.70	1.60	1.48
Theory values- <i>d</i> (Å)	4.85	2.97	2.53	2.10	1.71	1.62	1.48
Crystalline plane (hkl) [6]	(111)	(220)	(311)	(400)	(422)	(511)	(440)

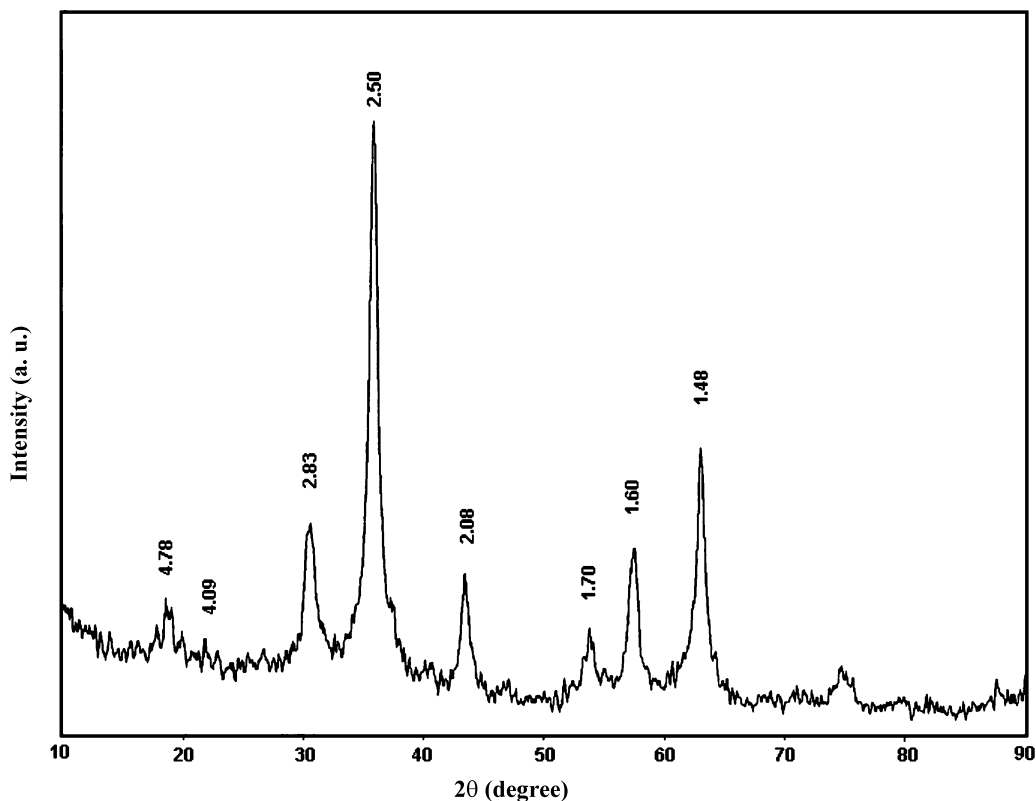


Fig. 3. XRD pattern of the magnetite nanoparticles coated with APTS.

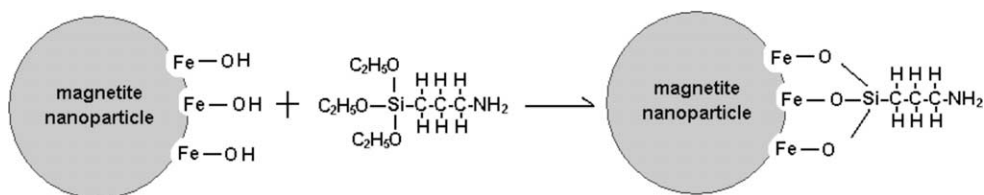


Fig. 4. The procedure of the coating reaction of APTS with magnetite nanoparticles.

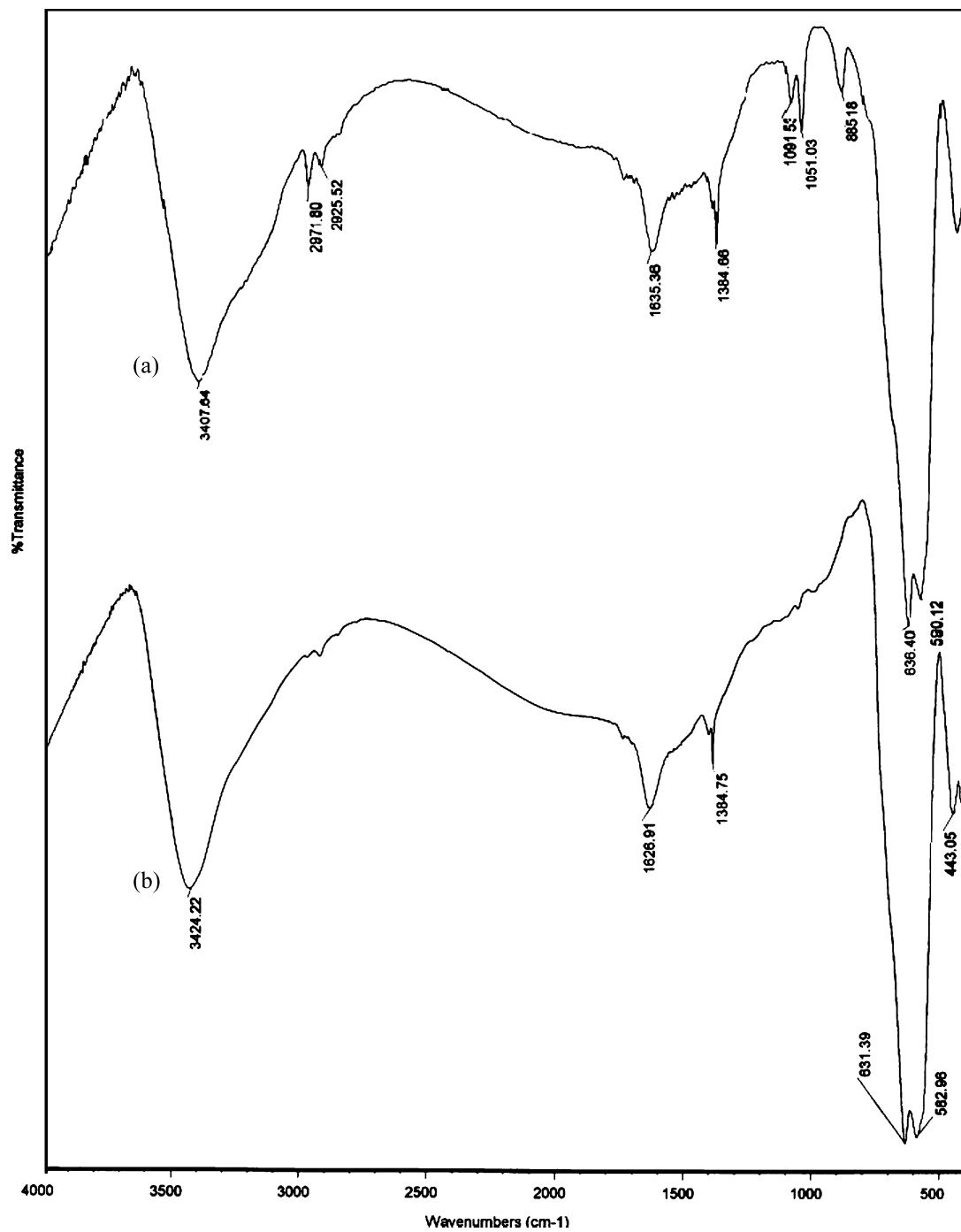


Fig. 5. FT-IR spectra of the coated (a) and uncoated (b) magnetite nanoparticles.

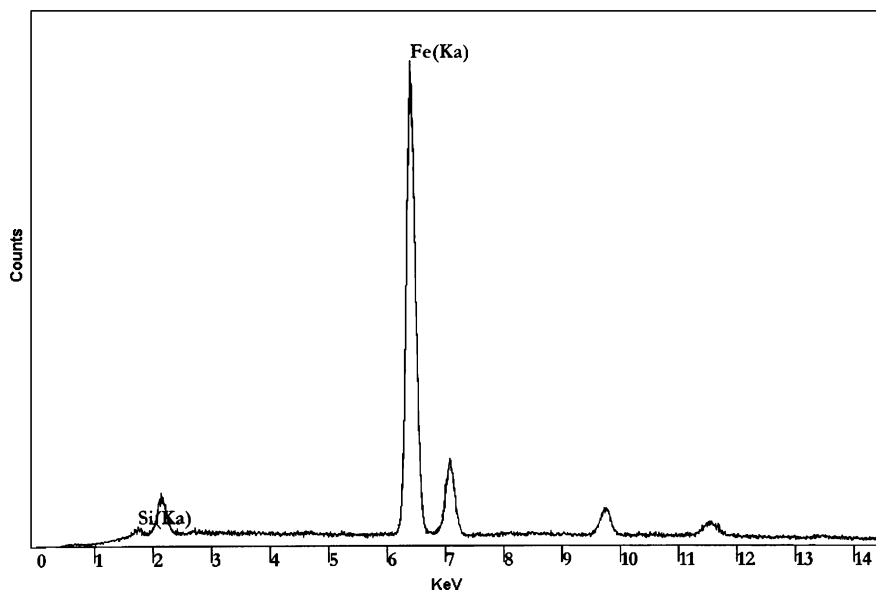


Fig. 6. SEM-EDS elemental analysis of APTS-coated Fe_3O_4 nanoparticles on Au substrate.

particle surface [9]. And the lattice constrictions have been observed as indicated in Table 1. As a result, the surface bond force constant increases as Fe_3O_4 is reduced to nanoscale dimension, so that the absorption bands of IR spectra shift to higher wavenumbers. So the blue-shift of absorption bands of the Fe–O bond of the Fe_3O_4 nanopar-

ticles can be observed. In addition, the split of the bands is attributed to the split of the energy levels of the quantized Fe_3O_4 nanoparticles[10].

It is also found that the characteristic absorption bands of the Fe–O bond of APTS-coated Fe_3O_4 shift to high wavenumbers of 636.4 and 590.1 cm^{-1} compared with that of uncoated

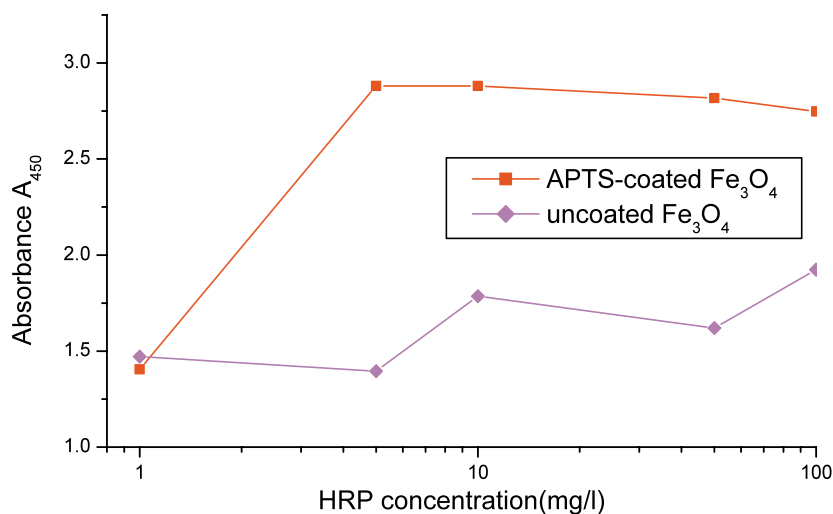


Fig. 7. HRP-linked colorimetric assays of APTS-coated and uncoated Fe_3O_4 nanoparticles.

Fe₃O₄ (in 631.4 and 582.9 cm⁻¹). The phenomenon can be explained according to the formation of Fe–O–Si bonds where Fe–O–H groups on the surface of the Fe₃O₄ particles are replaced by Fe–O–Si(O–)₂–R as shown in Fig. 4. More electro-negativity of –Si(O–)₂– than H leads to the enhancement of bond force constant for Fe–O bonds [11], so that the absorption bands shift to high wavenumbers.

Since we know the mean diameter of the magnetite nanoparticles is 7.5 nm, the number of Fe atoms in every Fe₃O₄ particle can be calculated by means of following formula,

$$N_{\text{Fe}} = \frac{\frac{4}{3} \pi R^3 N_A}{\bar{V}_{\text{Fe}_3\text{O}_4}} \times 3 = 8945 \quad (2)$$

where $\bar{V}_{\text{Fe}_3\text{O}_4}$ refers to the molar volume of bulk Fe₃O₄, R is the mean radius of Fe₃O₄ nanoparticles, N_A is Avogadro's number. If there is a monolayer of APTS molecules coated on the Fe₃O₄ particle, the number of APTS molecules on the surface of every Fe₃O₄ nanoparticle can be calculated by following formula,

$$N_{\text{APTS}} = \frac{S_{\text{Fe}_3\text{O}_4}}{S_{\text{APTS}}} = \frac{4\pi R^2}{S_{\text{APTS}}} = 442 \quad (3)$$

where $S_{\text{Fe}_3\text{O}_4}$ is the surface area of Fe₃O₄ particle, S_{APTS} is the area of surface coverage of about 40 Å² per APTS molecule reported in the literature [12]. So the atomic ratio of Fe/Si is $N_{\text{Fe}}/N_{\text{APTS}} = 20.2$. Fig. 6 shows a typical SEM–EDS elemental analysis of APTS-coated magnetite nanoparticles. From the peak area of Fe and Si, the atomic ratio of Fe/Si is obtained to be 96.75/3.25 = 30. This indicates that the surface APTS coverage ratio of Fe₃O₄ nanoparticles is about 67.3%. Probably, the incompleteness (a near monolayer) of the surface coating is owing to the incompleteness of surface hydroxylation and the existence of the spatial resistance for the surface coating reaction.

Fig. 7 shows the result of the HRP-linked magnetite nanoparticles colorimetric assays. Obviously, the absorbances of HRP-linked APTS-coated magnetite nanoparticles are higher than HRP-linked uncoated magnetite nanoparticles. It is revealed that the amount of adsorbed HRP on

the APTS-coated magnetite nanoparticles is 1.4–2.0 times higher than that of the uncoated magnetite nanoparticles according to the measured absorbance value. The increase of absorption is attributed to the contribution of APTS whose active group of –NH₂ can be conjugated to HRP by chemical band, and the uncoated magnetite nanoparticles connect the HRP by static adsorption only.

4. Conclusions

APTS-coated magnetite nanoparticles with 7.5 nm average diameter were prepared and characterized by TEM, ED, XRD, FT-IR, and SEM–EDS. Especially, FT-IR spectra were utilized to prove the formation of Fe–O–Si chemical bonds. A near monolayer APTS-coating on the particle surface was also indicated according to the comparison of the experimental analysis by SEM–EDS with a simple calculation.

By an enzyme-linked assay, it has been proved that these APTS-coated magnetite nanoparticles could significantly improve the protein immobilization.

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