SYNTHESIS OF Fe_3O_4-COOH NANOPARTICLES AND QUANTIFICATION OF COOH FUNCTIONAL GROUPS ON THE SURFACE OF Fe_3O_4 NANOPARTICLES

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ABSTRACT

For biomedical application, the functionalization of the COOH groups and the concentration evalutation of COOH groups on the Fe_3O_4 nanoparticles play important roles. Fe_3O_4 nanoparticles with carboxyl functional groups have been successfully fabricated by a simple and easy-to-implement chemical method, through the combination of Fe_3O_4 nanoparticles with silane complexes containing COOH radicals. This complex is the product of the reaction between succinic anhydride (SA) and 3-aminopropyltriethoxysilane (APTES). The fabricated Fe_3O_4-COOH nanoparticles have superparamagnetic properties with high technical saturation magnetization (~22 emu/g), and the concentration of carboxyl functional groups on the surface of Fe_3O_4 nanoparticles has been quantified based on the method using methylene blue indicator (MB) through ultraviolet-visible absorption spectroscopy (UV-vis).

Keywords: Fe_3O_4-COOH, Fe_3O_4 nanoparticles, methylene blue, quantification of COOH functional groups.

1. INTRODUCTION

Magnetic nanoparticles attached with biocompatibility functional groups have been widely used in biomedical applications [1] such as separation, cell selection [2], drug delivery [3], magnetic-resonance-imaging contrast enhancement [4], and detection of cells and disease-causing viruses [5]. To increase the biocompatibility, magnetic oxide nanoparticles are often surface-functionalized with biocompatible functional groups such as amine (NH_2), carboxyl (COOH) or biotin (vitamin H) [6]. Among those common active groups on the nanoparticles, carboxyy group is one of the

most popurlar groups for functionalization. Carboxyl groups supply negative Zeta potention, which helps stabilize the nanoparticles and prevents their aggregation [7]. Kraji et al [8] presented a study on functionalization of the COOH functional group on the surface of NH_2-encapsulated SiO_2 coated maghemite nanoparticles by using succinic anhydride (SA) in N,N-Dimethylformamide (DMF) medium. The free COOH functional groups were functionalized onto the surface of the nanoparticles through the formation of amide bonds with the amine groups on the surface of the nanoparticles. However, this synthesis process requires many complicated steps, and the quantification of NH_2 functional group on the surface of nanoparticles before functionalization of COOH functional group has not been investigated. Besides, to be able to apply in biological application, the precise determination of the amount of free COOH functional groups on the nanoparticles is essential for effective binding of biological entities and quality control of the product. For instance, this quantification helps to optimize the productivity and timely adjust the functionalization process. The quantification of the number of free COOH functional groups on the surface of nanoparticles has also been carried out by a number of methods such as using color indicator [9], acid-base titration [10], conductometry [11], gas-phase derivatization [12]. The quantification of functional groups on metal nanoparticles attached to COOH by the method of using Methylene blue indicator (MB) through untraviolet visible absorption spectroscopy [9] is a simple and easy method and suitable for laboratory conditions. In that study, MB was used as an indicator for the reaction with Au-COOH nanoparticles. Then, the ultraviolet-visible absorption spectroscopy of the MB sample was used to evaluate the amount of COOH functional group.

In this study, Fe_3O_4 nanoparticles were simultaneously fabricated and functionalized with COOH functional groups through the intermediate product - silane complex containing free COOH (SCA), which producing of the reaction between succinic anhydride (SA) and 3-aminopropyltriethoxysilane (APTES) to shorten the reaction time and generate products by simple and easy chemical methods. Furthermore, the concentration of COOH functional group on the surface of Fe_3O_4 nanoparticles was quantified by using MB indicator method.

2. EXPERIMENT

2.1. Synthesis of Fe_3O_4 nanoparticles

The Fe_3O_4 nanoparticles were synthesized by chemical coprecipitation [10] using Fe^{2+}/Fe^{3+} with 1:2 molar ratios from the two chloride salts: $FeCl_2$ and $FeCl_3$. The mixed solution was vigorously stirred at 800 cycles/min and kept at 70 °C before NH_4OH 28% was added to initialize a black colored precipitate. The Fe_3O_4 nanoparticles were collected after purification through magneticseparation with

ethanol and distilled water several times to decontaminate the residual chemicals. Then as-prepared Fe_3O_4 nanoparticles were dispersed and stored in ethanol.

2.2. Synthesis of silane complex containing free COOH (SCA)

SCA complex was created by chemical method from precursors of SA and APTES with the ratio 1:1. Dissolve SA in chloroform solvent, stir mechanically and heat to 70 °C. Then the APTES solution was added and further stirred and heated to form a yellow gel. When the chloroform was completely evaporated, the yellow gel was dissolved in absolute ethanol and the SCA product was stored in a refrigerator at 4-8 °C.

2.3. Synthesis of Fe_3O_4- COOH nanoparticles

50 mg of Fe_3O_4 nanoparticles were dispersed in 100 ml of distilled water and stirred. SCA solution was added to the above solution. The mixture was then further stirred and heated to 70 °C for 4 h. During the experiment, N_2 gas was used to control humidity during the reaction. The solution was filtered and washed several times with ethanol to obtain Fe_3O_4-COOH.

2.4. Quantification of the COOH groups

2 mg of Fe_3O_4-COOH nanoparticles were soaked in 20 ml of 1μM MB solution. The solution was shaken well for 10 min. The solution is then magnetized and left overnight (12-16 hours). The concentration of MB before and after soaking with nanoparticles was compared to determine the amount of COOH functional group on the surface of Fe_3O_4 nanoparticles.

The crystallite structure and morphology of the Fe_3O_4 nanoparticles was observed by x-ray diffraction (XRD), recorded by using a SIEMENS D5005 (Bruker-Germany) and transmission electron microscopy (TEM) with a JEOL JEM1010 microscope. To study the formation of chemical bonds in samples Fourier transform infrared (FTIR) spectroscopy was carried out on an 8400S Shimadzu-Japan. The magnetic properties of samples were investigated on a vibrating sample magnetometer (VSM) Digital Measurement Systems 880 (USA). To measure absorbance of MB, an ultraviolet–visible (UV–Vis) spectrometer U2450-PC from Shimadu-Japan was used.

3. RESULTS AND DISCUSSION

3.1. Morphology and structure of the Fe_3O_4 nanoparticles

The right pattern of Figure 1 shows the XRD pattern of the Fe_3O_4 nanoparticles exhibits peaks at 30.1°, 35.5°, 43.3°, 57.1°, and 62.5°corresponding to reflection planes of (220), (311), (400), (511), and (440) respectively, which were indexed in the Fd-3m space group [9,11]. The average lattice parameter of the Fe_3O_4 crystal is calculated as a ~ 8.4

Å, which is in agreement with published results [13], the crystalline size was calculated as D = 10.53 nm from the Debye–Scherrer formula which is almost similar to the average size of Fe_3O_4 nanoparticles of about 10 nm obtained from the TEM image of Fe_3O_4 nanoparticles (the left pattern of Figure 1).

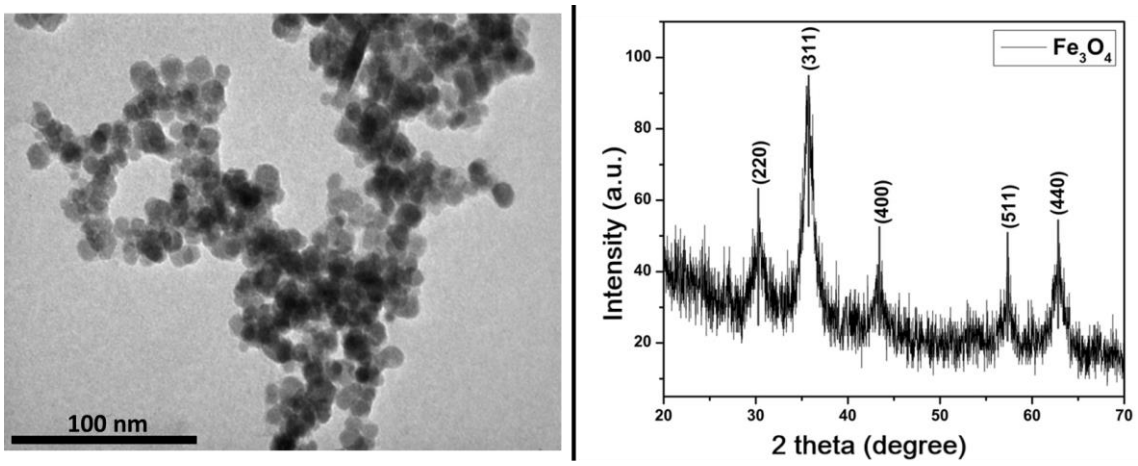

Figure 1. TEM image (the left) and XRD pattern (the right) of the Fe_3O_4 nanoparticles were synthesized by chemical coprecipitation.

3.2. Functional group COOH

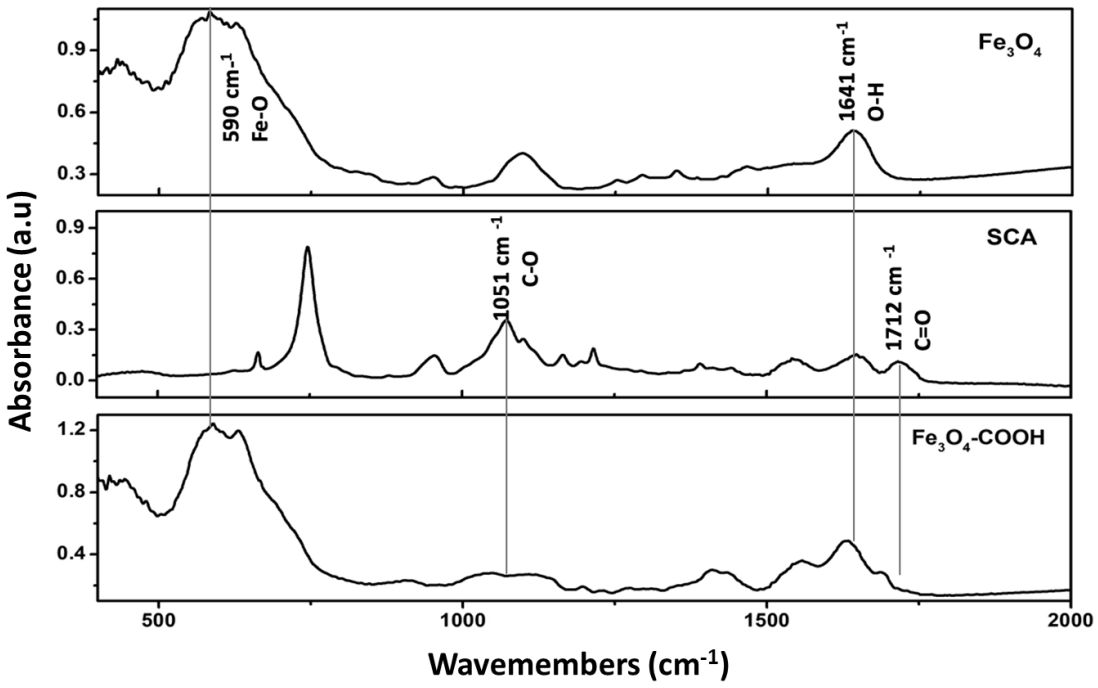

Figure 2. FTIR spectra of the Fe_3O_4 nanoparticles, SCA complex and Fe_3O_4-COOH nanopartilces.

Figure 2 presents the FTIR spectra of Fe_3O_4 nanoparticles, SCA complex and Fe_3O_4-COOH nanoparticles. The FTIR spectrum of Fe_3O_4 shows absorption peaks

characteristic for Fe-O tension vibration at wavelength 590 cm^{-1} in the crystal lattice of Fe_3O_4. Besides, in the FTIR spectrum, there is also another peak at wave number 1641 cm^{-1} which can be assigned to the OH-bending vibration [8]. On the FTIR spectrum of the SCA complex, absorption peaks at around 1712 cm^{-1} appear representing the C=O bond in the free carboxyl group. Further, the absorption peak at 1051 cm^{-1} is characteristic of the C-O bond. In the FTIR spectrum of Fe_3O_4-COOH, to the peak at 590 cm^{-1} appears as the Fe-O tension oscillation [14]. Along with that, the absorption peak at wavelength 1051 cm^{-1} of the CO bond is lost but a new spectral peak appears at the position of wave number near 1700 cm^{-1} simmilar to the C=O bond in the SCA complex [8]. These results demonstrate the successful functionalization of the carboxyl group on the surface of Fe_3O_4 nanoparticles.

3.3. Magnetic properties

The hysteresis curves of Fe_3O_4 nanoparticles and Fe_3O_4-COOH nanoparticles at room temperature are shown in Figure 3. The magnetic hysteresis loops indicates that both nanoparticles are superparamagnetism at room temperature with high saturation magnetization (M_S) (about 58 emu/g and 22 emu/g for the Fe_3O_4 nanoparticles and the Fe_3O_4-COOH nanoparticles at 10 kOe, respectively) with almost zero coercivity (Hc) and zero remanence magnet (M_r) [15]. The cause of the decrease in saturation of nanoparticles after functionalization with the COOH functional group is due to the reduction of the mass ratio of the magnetic material component (Fe_3O_4) in the particle when the non-magnetic COOH group is attached.

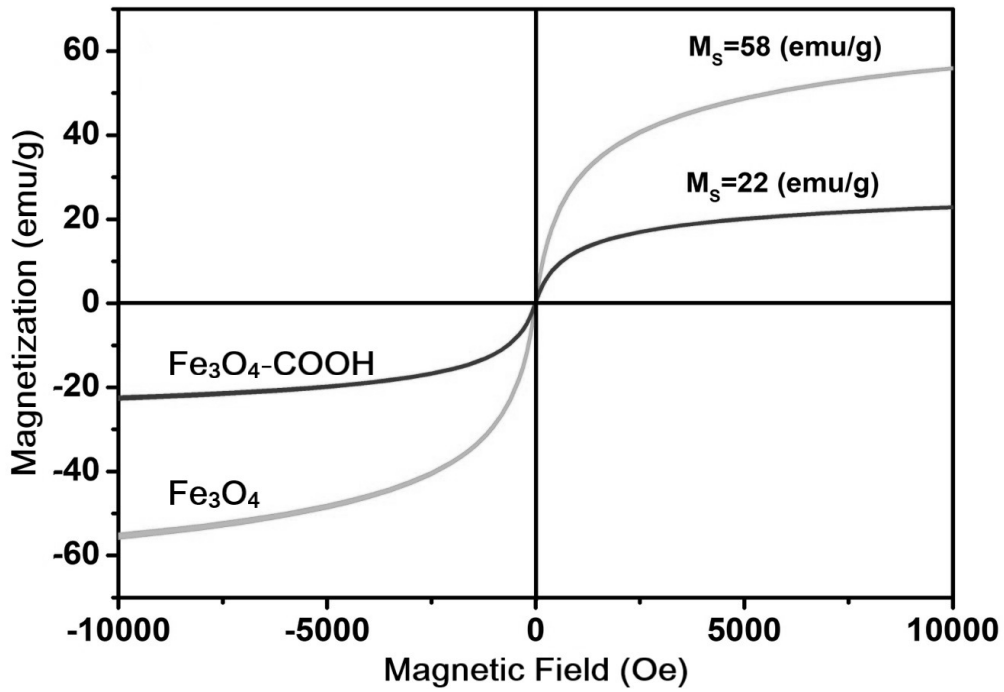

Figure 3. The hysteresis curves of Fe_3O_4 and Fe_3O_4-COOH nanoparticles at room temperature.

3.4. Quantification of the COOH groups

Because the MB molecule reacts with the carboxyl functional group in the ratio 1:1 [9], the number of COOH functional groups on the surface of the Fe_3O_4 nanoparticle is equal to the amount of MB reacted. Since then, the method to determine the amount of COOH is carried out by building a standard curve with different concentrations of MB solution through untraviolet-visible absorption (UV-vis) measurement. Then the attenuation of MB solution was compared to determine the concentration of COOH on the surface of Fe_3O_4 nanoparticles.

The concentration - absorbance calibration curve of MB was built with different MB concentrations, respectively: 0.2 µM; 0.4 µM; 0.6 µM; 1.0 µM; 1.2 µM; 1.4 µM and 1.6 µM. The standard curve of MB is shown in Figure 4. From that, the dependence coefficient K between the concentration of MB and the absorbance of MB is determined based on the formula:

$$C = K\ x\ Abs \tag{1}$$

where, C is the absorption concentration and Abs is the absorption intensity

Figure 4 shows the standard Concentration - Absorption curve of MB and the coefficient K is determined to 11.48 ± 0.11 (µM/Absorption unit). In other words, the absorption and concentration of MB are proportional to each other by the coefficient K = 11.48 ± 0.11 (µM /Abs).

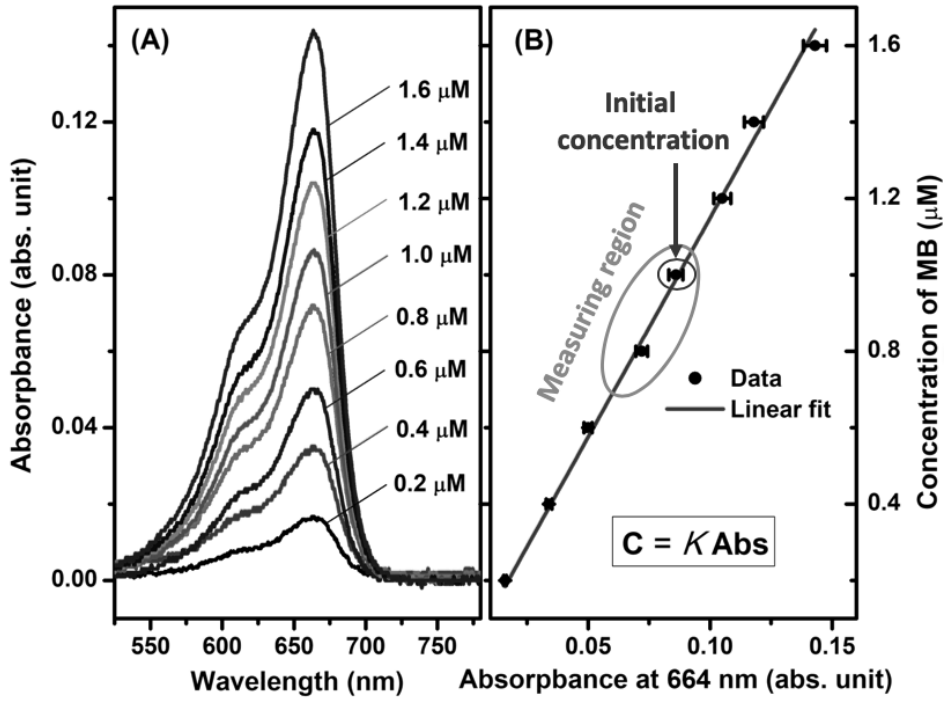

Figure 4. The standard curve Concentration - Absorption of MB.

Based on the UV-vis spectrum of MB before and after reacting with Fe_3O_4-COOH nanoparticles, the absorption intensity (Abs) at 664 nm of MB before reacting with MB (MB-1μM) was 0.86. After reacting with Fe_3O_4-COOH nanoparticles, the absorption intensity at 664 nm of MB decreased to 0.80. According to formula (1), we can calculate the concentration of MB before the reaction with Fe_3O_4-COOH is 9.9 μM ± 0.1 μM and the concentration of MB after the reaction with Fe_3O_4-COOH is 9.2 μM ± 0.1 μM. The reduced concentration of MB is the amount of MB that participated in the reaction and is equal to the concentration of COOH present on the surface of Fe_3O_4-COOH nanoparticles. Thus, the concentration COOH present on the surface of Fe_3O_4-COOH nanoparticles was determined to be 0.07 nmol/mg particles. Figure 5 shows the UV-vis spectrum of MB before (MB-1μM) and after reacting with Fe_3O_4-COOH nanoparticles (MB-FC).

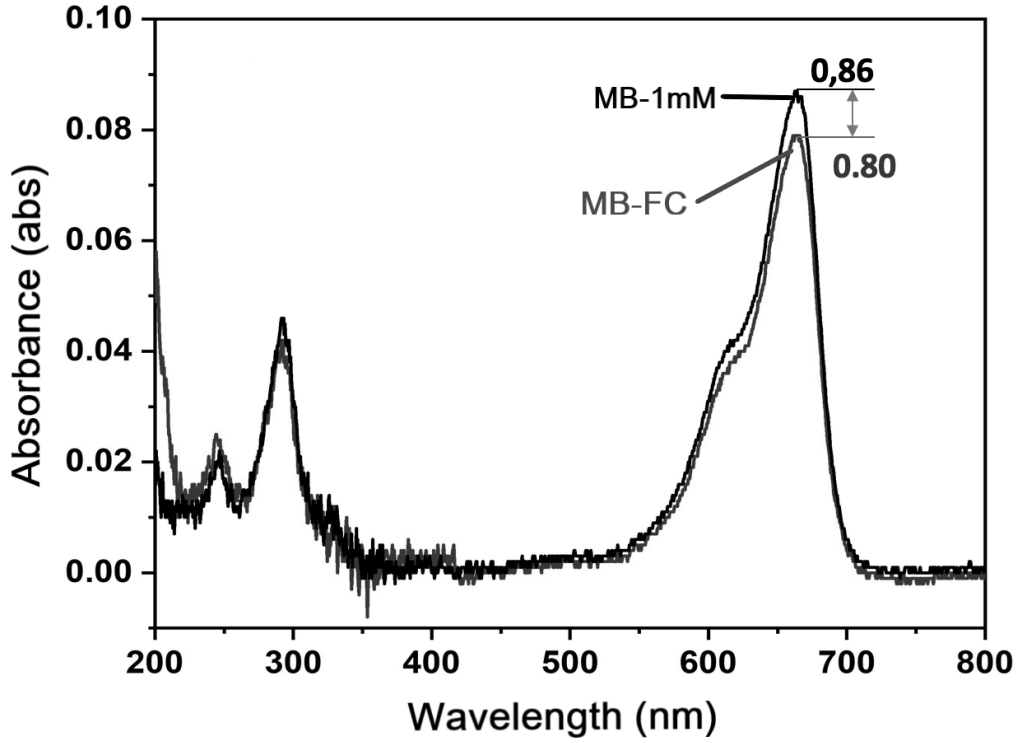

Figure 5. The UV-vis spectrum of MB before (MB-1μM) and after reacting with Fe_3O_4-COOH (MB-FC).

To ensure the authenticity of the results, the experiment was repeated with 3 independent samples of Fe_3O_4-COOH with similar experimental conditions, denoted as FC1, FC2, FC3 and then performed concentration determination concentration of COOH on the all samples. For each Fe_3O_4-COOH sample, the determination of the carboxyl group was performed three times independently. Figure 6 shows the concentration of COOH functional group of 3 samples FC1, FC2 and FC3 with similar

experimental conditions. From that, the average concentration of COOH functional group on 3 series of Fe_3O_4-COOH samples was determined to be 0.68 ± 0.10 nmol/mg.

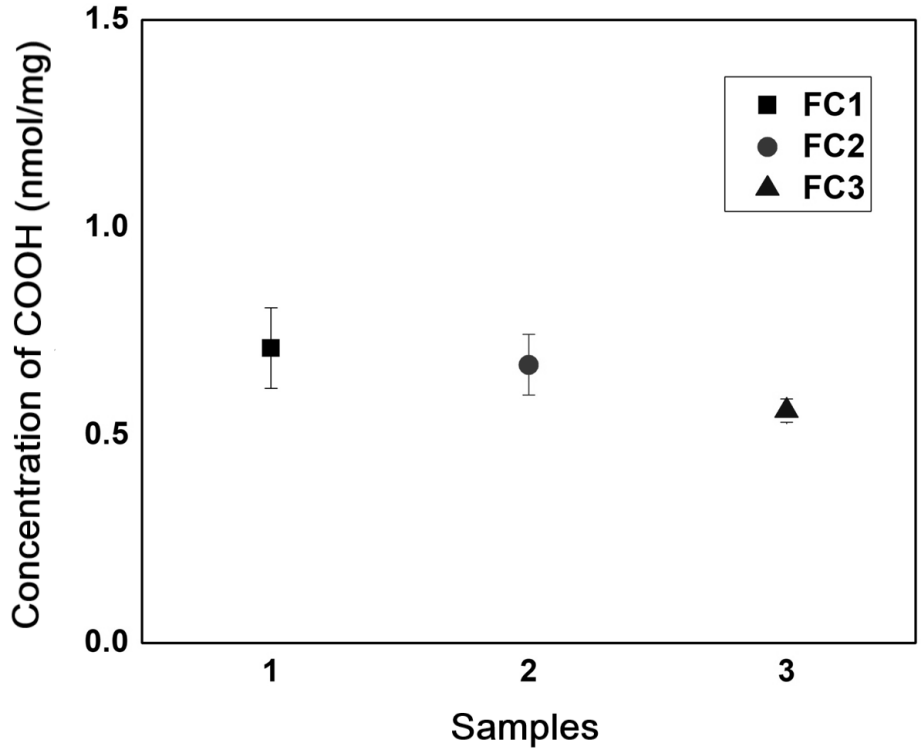

Figure 6. The concentration of COOH functional group of 3 samples FC1, FC2 and FC3 with similar experimental conditions.

4. CONCLUSSION

In conclusion, in this study the carboxyl functional group has been successfully functionalized on Fe_3O_4 nanoparticles and the Fe_3O_4-COOH nanoparticles after functionalization still retain the superparamagnetic properties of ferromagnetic oxide nanoparticles. A new method of functionalization was developed in order to functionalize the carboxyl groups on the surface of magnetic nanoparticles through the successful fabrication of the SCA group containing the COOH functional group. Simultaneously, the concentration of free carboxyl functional groups present on the surface of Fe_3O_4 nanoparticles has been quantified for use in the applications of magnetic nanoparticles as extraction agents and other biomedical applications.

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NGHIÊN CỨU CHẾ TẠO HẠT NANO Fe_3O_4-COOH VÀ ĐỊNH LƯỢNG NHÓM CHỨC COOH TRÊN BỀ MẶT HẠT NANO Fe_3O_4

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TÓM TẮT

Để ứng dụng trong y - sinh học, việc chức năng hóa và định lượng nhóm chức COOH trên bề mặt hạt nano Fe_3O_4 đóng vai trò rất quan trọng. Hạt nano Fe_3O_4 gắn nhóm chức carboxyl đã được chế tạo thành công bằng phương pháp hóa đơn giản và dễ thực hiện, thông qua sự kết hợp của hạt nano Fe_3O_4 với phức chất silane có chứa gốc COOH. Phức chất này là sản phẩm của phản ứng giữa Succinic anhydride (SA) và aminopropy trithoxysilane (APTES). Hạt nano Fe_3O_4-COOH chế tạo được có tính chất siêu thuận từ với từ độ bão hòa kỹ thuật cao (~22 emu/g), đồng thời nồng độ nhóm chức carboxyl trên bề mặt hạt nano Fe_3O_4 đã được định lượng dựa trên phương pháp sử dụng chất chỉ thị xanh Methylene (MB) thông qua phổ hấp thụ hồng ngoại khả kiến (UV-vis).

Từ khóa: Hạt nano Fe_3O_4, Fe_3O_4-COOH, định lượng nhóm chức, xanh Methylene.

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Lĩnh vực nghiên cứu: Vật liệu từ, Vật liệu nano đa chức năng.