

Synthesis and Characterization of Fe₃O₄ Magnetic Nanoparticles Coated with Carboxymethyl Chitosan Grafted Sodium Methacrylate

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Abstract

N-sodium acrylate-O-carboxymethyl chitosan [CMCH-g-PAA(Na)] bound Fe₃O₄ nanoparticles were developed as a novel magnetic nanoparticles with an ionic structure that can be potentially used in many fields. CMCH-g-PAA (Na) was obtained by grafting of sodium polyacrylate on O-carboxymethyl chitosan, which is an amphiphilic polyelectrolyte with the biocompatibility and biodegradability properties. According to the great interest for improving the stability of Fe₃O₄ nanoparticles, CMCH-g-PAA (Na) was used as a stabilizer to prepare a well dispersed suspension of magnetic nanoparticle. According to the results, the presence of CMCH-g-PAA(Na) could eliminate agglomeration of magnetic nanoparticles without destroying the superparamagnetic properties.

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1. Introduction

Magnetic nanoparticles (MNPs) are very promising materials for different applications including cell separation [1], magnetic resonance imaging [2], enzyme immobilization [3,4], drug delivery [5,6] and protein purification [7], because of their ability to be manipulated and detected with an external magnetic field [8]. So several methods have been developed to synthesis of magnetic nanoparticles: (1) co-precipitation of ferrous [Fe²⁺] and ferric [Fe³⁺] aqueous solution in the presence

of a base [5], (2) thermal decomposition of an iron complex [9], (3) by a sonochemical approach [10]. Protection of the synthesized magnetic nanoparticles, is critical to prevent aggregation between particles and also protected magnetic nanoparticles can play a role as building blocks for the fabrication of various functional systems [11]. Consequently suitable protection strategies such as surfactant and polymer coating of magnetic nanoparticles are applied for this reason. Several approaches have been developed for polymer

coating of iron oxide, including in situ coatings and post-synthesis coatings. In the first approach nanoparticles are coated during the synthesis. The poly-synthesis coating method consists of grafting the polymer on the magnetic particles once synthesized and used [12-15]. Chitosan is a polysaccharide formed primarily of repeating unit of β -(1-4)2 amino-D-glucose (glucosamine), being an alkaline, nontoxic, hydrophilic, biocompatible, and biodegradable polymer [16-18]. The active hydroxyl and amino groups on the chitosan molecule favor the development of different chemical-derived modifications with tailored physical and chemical properties that is different from those of chitosan [19]. For example chitosan is soluble in only a few dilute acid solutions which limit its wide application but chitosan derivatives have been shown to improve their water solubility and expand its applications [20]. Another example is high nonspecific protein adsorption in blood, while O-carboxymethyl chitosan, which is one of the chitosan derivatives, has low nonspecific protein adsorption and proved to show good compatibility with blood. For that reason, we have predicted that a chitosan derivative with designed structures and properties will provide the possibility of being used as a novel dispersant to stabilize and functionalize superparamagnetic iron oxide nanoparticles. Among various modification methods of chitosan and chitosan derivatives, graft copolymerization is most attractive because it is a effective technique for modifying the chemical and physical properties of biopolymers. Herein we report our studies on synthesis of Fe_3O_4 nanoparticles with co-precipitation method of Fe(II) and Fe(III) aqueous salt in alkaline medium. In order to obtain well dispersed magnetic nanoparticles with ionic structure we used CMCH-g-PAA(Na) for coating process. The

functional carboxyl groups on CMCH-g-PAA(Na) are supposed to exhibit coordination interaction with Fe_3O_4 and polyelectrolyte properties are supposed to provide the electrostatic stabilization steric hindrance simultaneously to stabilize the suspension of synthesized iron oxide nanoparticles. The aim of this study is performance of CMCH-g-PAA(Na) as an excellent dispersant and a novel layer to prepare well-dispersed superparamagnetic iron oxide nanoparticles with ionic structure. Different analysis methods were applied in order to prove the properties and efficiency of synthesized MNPs.

2. Experimental procedure

Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, >99%), ferrous sulphates tetrahydrate ($\text{FeSO}_4 \cdot 4\text{H}_2\text{O}$, >99%), ammonium hydroxide (25%), acrylic acid (AA), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) and ferrous ammonium sulphates [$\text{Fe}(\text{NH}_4)(\text{SO}_4)$] were purchased from Merck chemical company (Darmstadt, Germany). Sodium acrylate, used in this work was prepared in our laboratory. Chitosan was obtained from New Asia international Co.Ltd. Its molecular weight was $7.9 \times 10^5 \text{ gmol}^{-1}$ and the degree of deacetylation was 92%. Bidistilled water was used as the solvent.

2.1. Preparation of O-carboxymethyl chitosan grafted poly acrylic acid sodium salt

O-carboxymethyl chitosan (CMCH) was prepared according to the method described by Park et al [21]. 0.3 g of CMCH, predetermined amount of sodium acrylate and 15 mL of bidistilled water were charged in a small reactor, then 0.01 g of ferrous ammonium sulfate (FAS) dissolved in 2 mL H_2O_2 was slowly added to the reactor as an initiator. The total volume of the reaction mixture was made up to 20 mL with bidistilled water.

Nitrogen gas was purged for 30 minutes to remove the dissolved oxygen. Reaction was performed in a constant temperature maintained at a 70°C for 2 h. The reaction products were precipitated in an excess of acetone, filtered to eliminate the solvent and then washed with ethanol in order to remove all the no reacted CMCH and ferrous salt. Then the acquired homopolymer was extracted with ethanol for 48 hours and was dried. The first sign of grafting reaction on CMCH is high improvement of water solubility. The reaction mechanism of graft copolymerization is shown in Fig. 1. The grafting percentage and efficiency were calculated according to the equations 1 and 2: [22]

$$(1) \quad (G\%) = \left[\frac{W_1}{W_0} \right] \times 100$$

$$(2) \quad (GE\%) = \left[\frac{W_1}{W_2} \right] \times 100$$

Where W_0 , W_1 and W_2 denote the weight of CMCH, pure graft copolymer and crude graft copolymer, respectively.

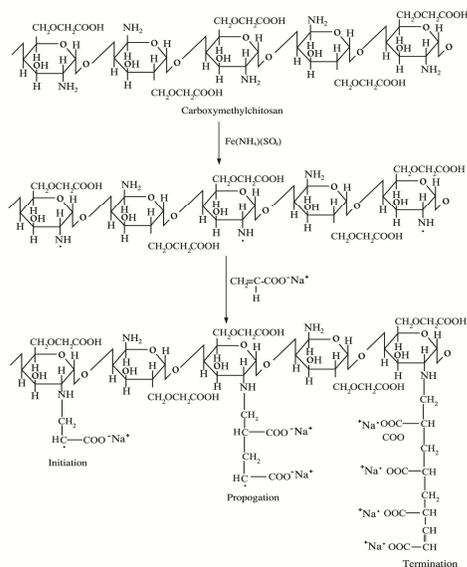
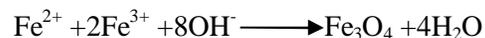


Fig. 1. Reaction scheme of CMCH-g-PAA(Na)

2.2. Synthesis of magnetic nanoparticle

Fe_3O_4 nanoparticles were prepared by co-precipitation of Fe^{3+} and Fe^{2+} ions in the presence of ammonia solution in an autoclave under hydrothermal condition. Ferric chlorides and ferrous sulphates (molar ratio 2:1) were dissolved in bidistilled water at whole concentration of 0.3 mol L^{-1} . Precipitation was obtained at 25°C with vigorous stirring and adding drop wise of NH_4OH solution. The PH of solution was maintained at 10, under hydrothermal treatment at 90°C for 2hours. The prepared nanoparticles were separated by centrifugation and washed several time with water and ethanol and dried in vaccum oven at 50°C. The chemical reaction of Fe_3O_4 formation is written as following equation:



2.3. CMCH-g-PAA(Na) surface – modified Fe_3O_4 nanoparticles

Stabilizing of synthesized magnetic Fe_3O_4 nanoparticles was performed by CMCH-g-PAA(Na) (Na). 20mg of prepared Fe_3O_4 nanoparticles was dispersed into 10mL of CMCH-g-PAA(Na) aqueous solution with known concentration, stirred at 35°C for 24 hours to ensure that the coating process was accomplished. The black dispersion was purified by centrifugation and re-dispersion for several times to remove the free CNCH-g-PAA(Na) molecules. Finally the colloidal solution was dried and CMCH-g-PAA(Na) / Fe_3O_4 were obtained.

3. Results and discussion

The crystalline structure of the synthesized Fe_3O_4 nanoparticles were examined by X-ray diffraction (XRD) [Philips 1140] with aXD - 3A powder diffractometer before and after coating

processes, using nickel – filtered Cu Ka radiation in the range of $5-70^\circ(2\theta)$ at 40kV and 30 mA.

The morphology of modified chitosan and magnetic nanoparticles were monitored by scanning electron microscopy (SEM) [Serontechnology ATS-2100]. The mean size and size distribution of CMCH-g-PAA(Na)–Fe₃O₄ nanoparticles were measured by dynamic laser light scattering apparatus (DLS) [Malvern Mal1001767]. Fourier transfer infrared (FTIR) [Bruker EQ55/5] spectra of Fe₃O₄ nanoparticle, CMCH-g-PAA(Na) and CMCH-g-PAA(Na) – Fe₃O₄ were recorded with KBr discs in the range of 4000-400 Cm⁻¹ on fourier transfer infrared. Thermogravimetric analysis (TGA) [performed by shimadzu 40] was carried out for powder of samples. Samples were heated from 25 to 600°C at a heating rate of 10°C /min in air. A vibrating sample (VSM) [JDM-13D magnetometer] was used to characterize the magnetic properties of CMCH-g-PAA(Na)-Fe₃O₄ nanoparticles. The hysteresis of the magnetization was obtained by changing H between +5000 and -5000 Oe. These measurements were carried out at 25°C. The magnetic CMCH-g-PAA(Na) bound Fe₃O₄ nanoparticles were prepared by two steps ,the first step is the synthesis of Fe₃O₄ and the second one is the binding of the CMCH-g-PAA(Na) to the Fe₃O₄ nanoparticles. Figure 2 shows a schematic representation of this process.

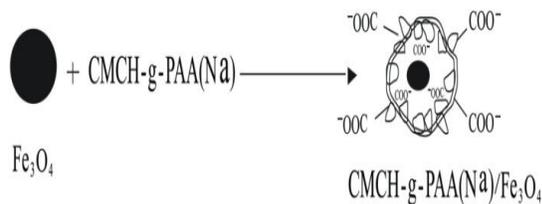


Fig. 2. An illustration for binding of CMCH-g-PAA (Na) to synthesized magnetic Fe₃O₄.

3.1. FTIR data analysis

The FTIR spectra of CMCH-g-PAA (Na) , Fe₃O₄ nanoparticles and CMCH-g-PAA(Na) -Fe₃O₄ nanoparticles are displayed in Figure 3.

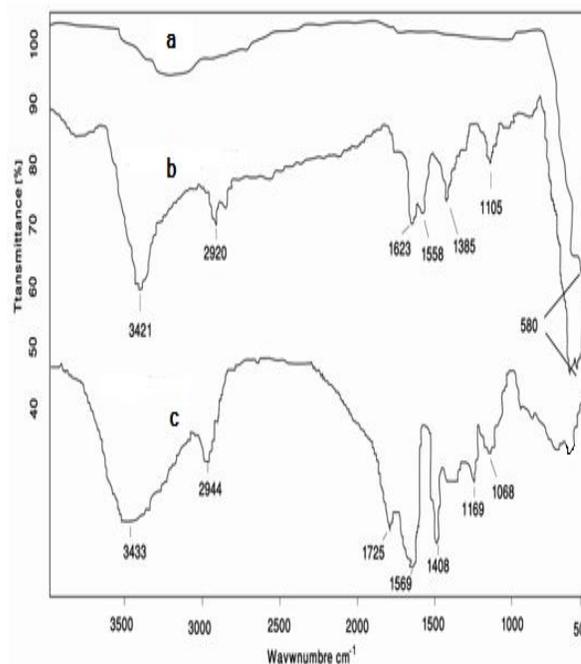
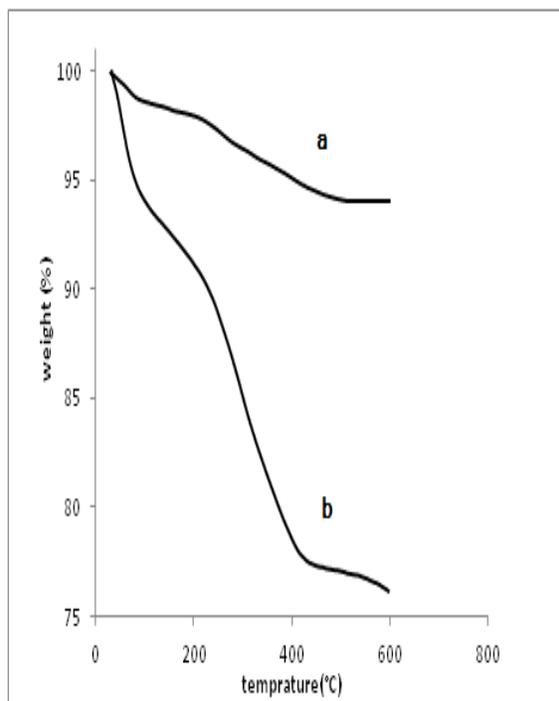


Fig. 3. FTIR of magnetic a) Fe₃O₄ particles, b) Fe₃O₄/CMCH-g-PAA (Na) , c) CMCH-g-PAA (Na).

In the IR spectrum of CMCH-g-PAA (Na), the peak at 1408 cm⁻¹ could be assigned to the symmetrical stretching vibration of COO⁻. The absorption bands at 1569 and 1725 cm⁻¹ are due to N-H bending vibration and C=O stretching vibration. In addition, the absorption peaks at 1169 and 1068 cm⁻¹ were observed, which can be ascribed to C≡N and C=O stretching vibration. For the IR spectrum of Fe₃O₄ the absorption band appeared at 580 cm⁻¹ which can be attributed to Fe₃O₄. Finally in the IR spectrum of modified Fe₃O₄ nanoparticles the collection of the characteristic absorption bands of CMCH-g-PAA (Na) and Fe₃O₄ were appeared. This suggests the successful binding of CMCH-g-PAA (Na) to Fe₃O₄ nanoparticles.

3.2. TGA analysis

The TGA of the naked and CMCH-g-PAA(Na) bound Fe_3O_4 nanoparticles is shown in Figure 4. The TGA curve of naked Fe_3O_4 magnetic nanoparticles shows that the weight loss over the temperature 25 to 300°C is about 5%. This might be due to the loss of residual water in the sample. On the other hand, for CMCH-g-PAA (Na) bound Fe_3O_4 nanoparticles, because of the removal of absorbed physical and chemical water, the weight loss of the entire nanocomposite is quite small below 200°C . Then the principle chains of polymer begin to degrade at about 250°C and the final temperature of decomposition is around 500°C . here, the weight loss in is 12%. At higher temperature (500 to 600°C), there is no significant



change of weight. This implies that there is only iron oxide at this range of temperature.

Fig. 4. Thermogravimetric analysis (TGA) of a) magnetic Fe_3O_4 nanoparticles and b) CMCH-g-PAA(Na) / Fe_3O_4 nanoparticle.

3.3. XRD analysis

Figure 5 (a-c), shows X-ray diffractograms of the powder obtained for CMCH-g-PAA (Na), nanomagnetic Fe_3O_4 and CMCH-g-PAA(Na) bound Fe_3O_4 nanoparticles. Chitosan and carboxymethyl chitosan exhibit crystallinity in their structure as reported in literature [23], but after graft copolymerization process on CMCH, no peak is observed which is shown in Figure 5c.

In fact, the reaction products showed almost amorphous nature. The grafting of PAA (Na) takes place randomly on the carboxymethyl chitosan chain, rising to formation a random copolymer, which destroy the regularity of the packing of original carboxymethyl chitosan chains efficiently. This causes the formation of amorphous copolymer.

Figures 5 (a,b) appeared the XRD patterns for the naked and CMCH-g-PAA(Na)-bound Fe_3O_4 nanoparticles. Six characteristic peaks for Fe_3O_4 ($2\theta=30.1^\circ, 35.5^\circ, 43.1^\circ, 53.4^\circ, 57.0^\circ$ and 62.6°) marked by their indices ((210),(311),(400),(422),(511) and (440)) were observed for both samples.

These peaks are consistent with the database in Joint committee on powder diffraction standards (JCPDS card no. 79-0418) [24] and reveal that the resultant nanoparticles were Fe_3O_4 with cubic spinel phase. It suggests that the functionalization process will not affect the peak positions of the particles.

The size of nanoparticles could be quantitatively evaluated from XRD data using the Debye-Scherrer equation. The diameters of naked Fe_3O_4 and CMCH-g-PAA(Na) bound Fe_3O_4 nanoparticles are calculated 26 and 33 nm respectively.

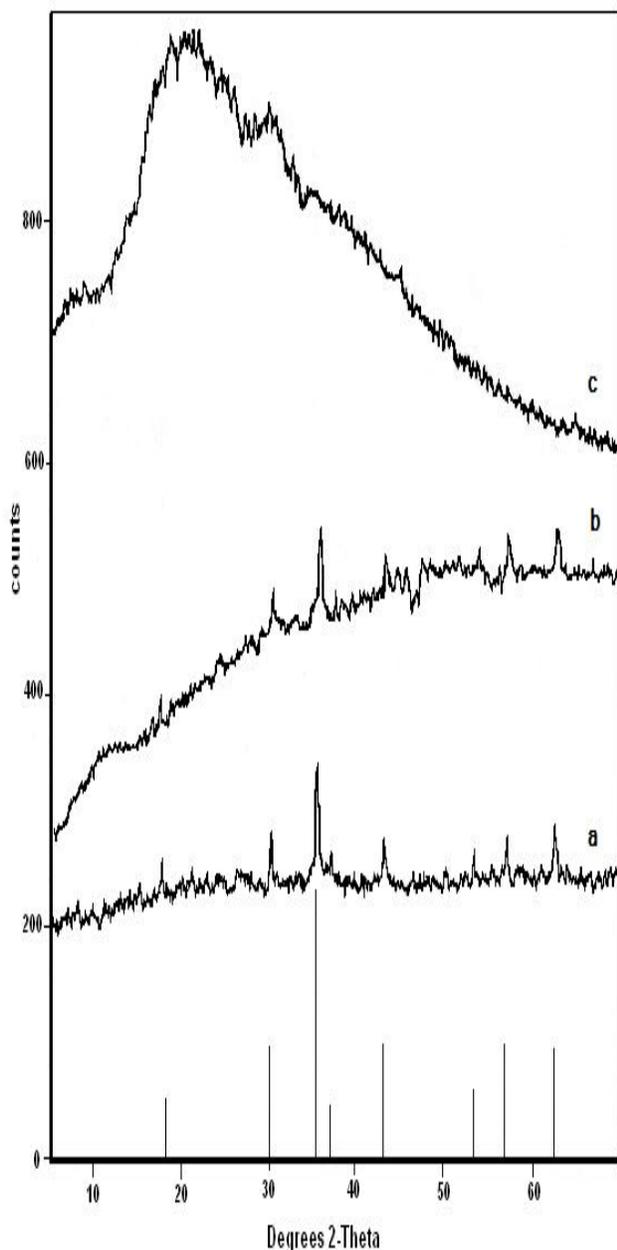


Fig. 5. X-ray powder diffraction patterns of: a) Fe₃O₄ b) CMCH-g-PAA (Na) bound Fe₃O₄ c) CMCH-g-PAA (Na).

3.4.SEM morphology

The Scanning Electron Microscope (SEM) of CMCH, CMCH-g- PAA(Na), and CMCH-g-PAA(Na) bound Fe₃O₄ nanoparticles are shown in Figure 6 (a-c).

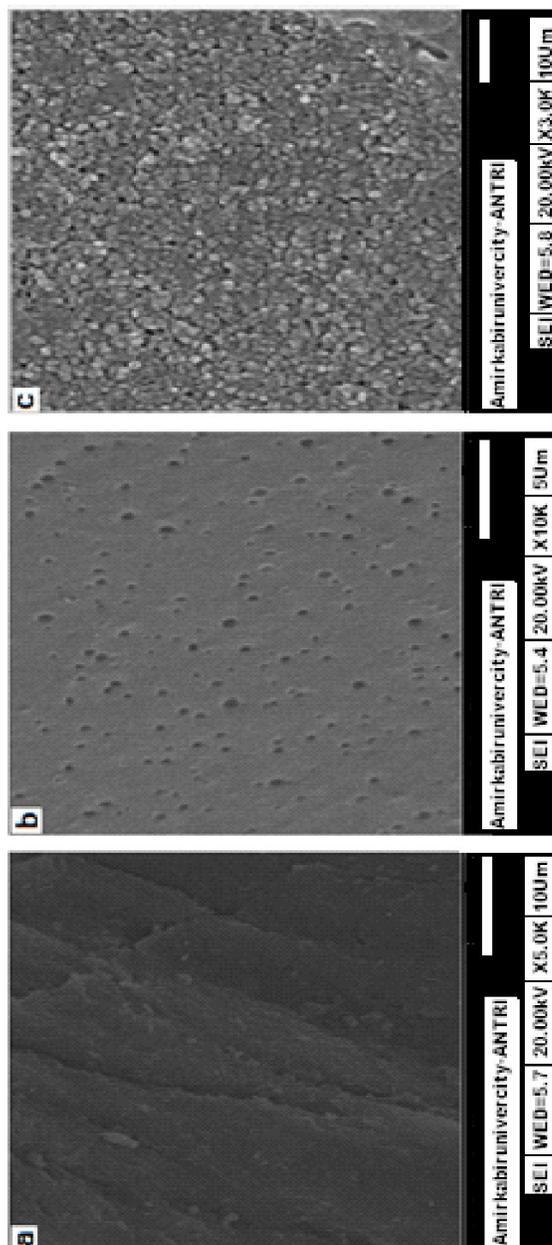


Fig. 6. SEM of a) CMCH, b)CMCH-g-PAA(Na), and c) CMCH-g-PAA(Na) bound Fe₃O₄.

Graft copolymerization of CMCH affected the surface morphology and physical and chemical properties. It is simply seen from Figures 6a and 6b that the flaky and fibrous nature of CMCH is modified in the grafted product. In Figure 6b, CMCH-g-PAA (Na) shows the porous structure and Figure 6c shows the SEM image of functionalized magnetic nanoparticles. Coating the surface of magnetic nanoparticles with suitable and nontoxic compounds has been proven to be one of the most efficient ways for providing stability of the nanoparticles. On the other hand agglomeration of naked Fe_3O_4 nanoparticles due to the van der waals force between the particles reduces after surface modification solution (Figure

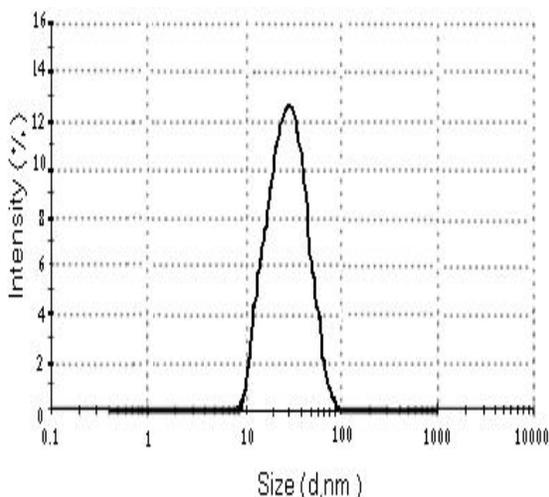


Fig. 7. Particle size distribution of the CMCH-g-PAA(Na) bound Fe_3O_4 nanoparticles.

7).

The size distributions of particles were in the range of 10 to 100 nm. The mean diameter of the nanoparticles was 52 nm, which was bigger than the XRD report which is related to the dry state of the XRD measurement.

3.6. VSM analysis

Fig. 8 showed a typical magnetization curve of naked nanoparticles and CMCH-g-PAA(Na)

bound Fe_3O_4 nanoparticles. The saturation magnetization (M_s), remanent magnetization (M_r), coercivity (H_c), squareness ($S_r = M_r/M_s$) of CMCH-g-PAA(Na) bound Fe_3O_4 nanoparticles at 25°C are 64.1 emu.g^{-1} , 0.92 emu.g^{-1} , 8.5 Oe , and 0.014 , while the M_s and H_c of naked Fe_3O_4 are about 87 emu.g^{-1} , 7.9 Oe . The decrease of the saturation magnetization was most likely attributed to the existence of coated materials on surface of nanoparticles. In addition, several experimental results indicate that the critical size of the nanoparticles for the phase transition from ferromagnetic to superparamagnetic is around 5nm [25,26] So, in this work, the low remanent magnetization and coercivity are understandable and reveal that resultant magnetic nanoparticles are nearly superparamagnetic.

Stability is one of the most important properties of magnetic nanoparticles. The stabilization of Fe_3O_4 nanoparticles is due to the adsorption of CMCH-g-PAA (Na) onto the surface of Fe_3O_4 nanoparticles. As can be seen from the CMCH-g-PAA (Na) structure (Figure 1), there are two carboxyl groups ($-\text{COO}^-$) on each unit of CMCH-g-PAA(Na) so it can be adsorbed on Fe_3O_4 nanoparticles with oxygen atoms present in carboxylate group by symmetrically coordination with the iron atoms of Fe_3O_4 .

For further investigation of the stability of CMCH-g-PAA (Na)/ Fe_3O_4 , the suspension of CMCH-g-PAA(Na)/ Fe_3O_4 were centrifuged at 4000 rpm for 5 min. This suspension exhibits a transparent colloidal appearance which demonstrates the extremely stable colloidal solution. Comparison of this research with other similar work is reported in Table 1.

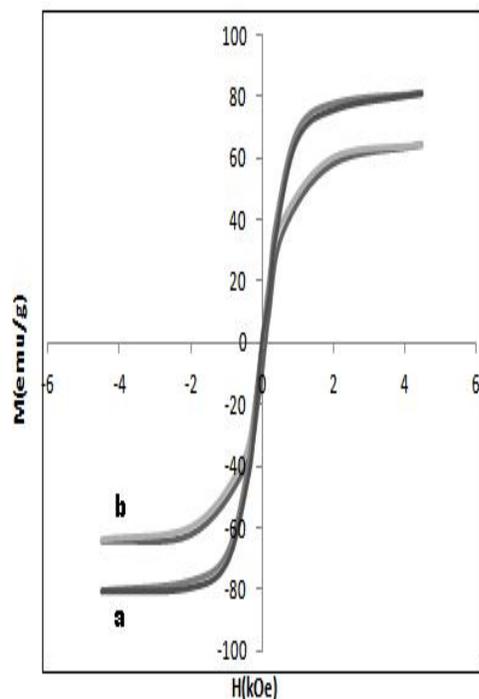


Fig. 8. VSM result of naked Fe_3O_4 (a) and CMCH-g-PAA(Na) bound Fe_3O_4 nanoparticles (b)

4. Conclusion

In the reported research, we have demonstrated a novel approach to synthesis magnetic nanoparticles with ionic structure by using CMCH-g-PAA (Na). The synthesis mechanism of this process was discussed and the obtained nanoparticles were characterized by DLS FT-IR, XRD, VSM, SEM and TGA. The analysis of SEM and XRD indicated the morphology of nanoparticles and binding of CMCH-g-PAA (Na) to the MNPs did not change the spinel structure of Fe_3O_4 . The saturated magnetization of composite nanoparticles could reach 64.1 emu g^{-1} and nanoparticles showed the characteristics of superparamagnetism. Also the results revealed that this approach could eliminate aggregation of nanoparticles, without any effect on the magnetic properties. The synthesized nanoparticles could be used in synthesis of smart polymers, drug delivery systems and other industrial processes.

Table 1. comparison of this research with other work

Title	Highlight
Synthesis of new chitosan coated magnetic nanoparticles with surface modified with long-distanced amino groups as a support for bio ligands binding [27]	1) New $\text{Fe}_3\text{O}_4\text{-CS-(NH}_2\text{)}_3$ nanoparticles have been prepared via co-precipitation method. 2) The chemical modification includes all reactive groups on chitosan structure. 3) The amount of NH_2 groups on the material surface was evaluated as 8.34 mM/g . 4) Prepared nanoparticles were used for bioligands binding (lipase and human serum albumin). 5) The high yield of immobilization was observed (342.3 mg/g for lipase and 76.8 mg/g for HSA)
Different Preparation Methods and Characterization Of magnetic maghemite Coated with Chitosan [28]	1) $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles were synthesized by coprecipitation of Fe^{2+} and Fe^{3+} ions in the presence of ammonium. 2) They were coated with chitosan by the microemulsion process, suspension cross-linking technique, and covalent binding of chitosan on the $\gamma\text{-Fe}_2\text{O}_3$ surface. 3) these magnetic micro- and nanoparticles coated with chitosan may be used as a promising carrier for further diverse biomedical applications.
Synthesis and characterization of Fe_3O_4 magnetic nanoparticles coated with carboxymethyl chitosan grafted sodium methacrylate	1) Fe_3O_4 nanoparticles were synthesized by coprecipitation method. 2) CMCH-g-PAA obtained by grafting of sodium polyacrylate carboxymethyl chitosan. 3) The carboxyl group modified chitosan can adsorb onto the surface of Fe_3O_4 nanoparticles. 4) N-sodium acrylate-Carboxymethyl chitosan [CMCH-g-PAA(Na)] Fe_3O_4 nanoparticles were developed as a novel magnetic nanoparticles with ionic structure. They can be potentially used in many fields such as magnetic resonance imaging (MRI), bioseparation, targeted drug and gene delivery

5. References

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