

CARBOXYMETHYL CHITOSAN-Fe₃O₄ NANOPARTICLES: SYNTHESIS AND CHARACTERIZATION

(Nanzarah Karboksimetil Kitosan-Fe₃O₄: Sintesis dan Pencirian)

Nurul Hidayah Ahmad Safee, Md. Pauzi Abdullah*, Mohamed Rozali Othman

*School of Chemistry Science and Food Technology,
Faculty of Science and Technology,
Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor Darul Ehsan*

**Corresponding author: mpauzi@ukm.my*

Abstract

In this study, carboxymethyl chitosan bound Fe₃O₄ magnetic nanoparticles (CC-Fe₃O₄ NPs) was synthesized by the binding of carboxymethyl chitosan (CC) onto the surface of Fe₃O₄ magnetic nanoparticles, which was prepared by coprecipitating method. The CC-Fe₃O₄ nanoparticles was characterized by transmission electron microscopy (TEM), X-ray diffractometer (XRD), vibrating sample magnetometer (VSM), Nucleus Magnetic Resonance (NMR) and Fourier Transform Infra Red (FTIR). TEM studies confirmed that the Fe₃O₄ and CC-Fe₃O₄ particles have a particle size ranges from 9.73-12.30 nm and 9.70-12.50 nm, respectively. The magnetic properties of the Fe₃O₄ particles were verified using VSM. Their saturation magnetization, remnant magnetization and coercivity were 61.0 emu/g, 2.70 emu/g and 38.20 G, respectively. FTIR studies showed the appearance of peaks at 1629 cm⁻¹ and 1397 cm⁻¹ which were characteristic of COOM (M = metal ions) bands, indicating the formation of the iron carboxylate.

Keywords: Carboxymethyl chitosan, magnetic nanoparticles

Abstrak

Dalam kajian ini, karboksimetil kitosan berikat dengan nanozarah magnetik Fe₃O₄ telah disintesis dengan pengikatan karboksimetil kitosan (CC) terhadap permukaan nanozarah magnetik Fe₃O₄, yang disediakan melalui kaedah kopemendakan. Nanozarah CC-Fe₃O₄ kemudian dicirikan dengan Transmisi Elektron Mikroskopi (TEM), Pembelauan Sinar-X (XRD), Magnetometer Getaran Sampel (VSM), Resonans Magnetik Nukleus (RMN) dan Infra Merah Transformasi Fourier (FTIR). Analisis TEM menunjukkan Fe₃O₄ dan CC-Fe₃O₄ mempunyai julat saiz zarah di antara 9.73-12.30 nm dan 9.70-12.50 nm. Sifat kemagnetan Fe₃O₄ menunjukkan nilai pemagnetan tepu, pemagnetan sisa dan daya paksa sebanyak 61.0 emu/g, 2.70 emu/g dan 38.20 G. Analisis FTIR pula menunjukkan kehadiran puncak pada 1629 cm⁻¹ dan 1397 cm⁻¹ yang merupakan ciri jalur COOM (M = ion logam), menunjukkan pembentukan ferum karboksilat.

Kata Kunci: Karboksimetil kitosan, nanozarah magneti.

Introduction

Chitosan, poly(1→4)-2-amino-2-deoxy-D-glucan, is a polyaminosaccharide with many significant biological (biodegradable, biocompatible, bioactive) and chemical properties (polycationic, hydrogel, reactive groups such as OH and NH₂). All of these properties make chitosan and its derivatives widely used in many biomedical fields [1]. Carboxymethylated chitosan has received more and more attention because of its good water solubility, and it is more convenient to be applied in medicine because it fits the neutral environment of the human body [2,3]. Nano-sized carriers could not be separated easily from the contaminated waste streams by filtration or centrifugation. Magnetic nano-carriers can be easily manipulated by an external magnetic field and hence should be suitable as the support of adsorbents [4]. In this study, carboxymethyl chitosan bound Fe₃O₄ magnetic nanoparticles was synthesized by the binding of carboxymethyl chitosan (CMC) onto the surface of Fe₃O₄ magnetic nanoparticles. These Fe₃O₄ and carboxymethyl chitosan-Fe₃O₄ nanoparticles were characterized by TEM, XRD, VSM, FTIR and ¹H-NMR, respectively.

Experimental

Chemicals

Chitosan used as raw material was supplied by ChitoChem Company. It is of medical grade and has degree of acetylation of more than 90%. FeCl₃ and FeSO₄·7H₂O were purchased from BDH Chemicals Ltd. Carbodiimide were supplied by Aldrich. Monochloroacetic acids were purchased from MERCK-Schuchardt. All chemicals were of analytical grade reagents and used as supplied.

Preparation of Fe₃O₄ Nanoparticles

Nanosized magnetite was prepared by controlled chemical co-precipitation of Fe²⁺ and Fe³⁺ (1:2 ratio) from ammoniacal medium at 80° C under nitrogen atmosphere. In a typical experiment, 0.02 mol of ferrous sulphate and 0.04 mol of FeCl₃ were dissolved in 200 ml of de-ionized and de-oxygenated water. The resulting solution was vigorously stirred and heated to 80° C under nitrogen atmosphere. Subsequently about 12 ml of 25% ammonia solution was injected into the flask and stirring was continued for another 20 minutes to allow the growth of the nanoparticles. The solution was then cooled to room temperature and the resulting particles were centrifuged followed by repeated washing with distilled water. The pH of the suspension was brought to neutral by the addition of dilute HCl, and the particles were rewashed with distilled water [5].

Preparation of Carboxymethyl Chitosan

Carboxymethyl chitosan was prepared by method as suggested by Sun et.al [6]. Chitosan (10g), sodium hydroxide (10g), isopropanol (50 ml) and water (50 ml) were added into a flask to swell and alkalinize at 50°C for 1 h. The monochloroacetic acid (15 g) was dissolved in isopropanol (20 ml), added into the reaction mixture drop-wise for 30 min and reacted for 4 h at the same temperature, then stopped by adding 70% ethyl alcohol (250 ml). The solid was filtered and rinsed in 70-90% ethyl alcohol, and vacuum dried at room temperature. The product was Na salt CC (Na-CC). Na-CC (1 g) was suspended in 80% ethyl alcohol aqueous solution (100 ml), hydrochloric acid (10 ml, 37%) was added and stirred for 30 min. The solid was filtered and rinsed in 70-90% ethyl alcohol to neutral, vacuum dried. The products were the H-form CC (H-CC).

Preparation of Fe₃O₄ Bound Carboxymethyl Chitosan

The binding of carboxymethyl chitosan was conducted following Chang and Chen [7] method but the difference is carboxymethylchitosan used was N,O-carboxymethylchitosan. Approximately, 1 g of Fe₃O₄ nanoparticles was added to 20 ml of buffer A (0.003 M phosphate, pH 6, 0.1 M NaCl). Then, the reaction mixture was shaken for 60 min after adding 5 ml of carbodiimide solution (0.025 gml⁻¹ in buffer A). Finally, 25 ml of carboxymethyl chitosan solution (50 mg ml⁻¹ in buffer A) was added and the reaction mixture was shaken for 2 hours. The chitosan-bound Fe₃O₄ nanoparticles were recovered from the reaction mixture by magnetic bar. The magnetic particles were then washed with water and ethanol.

Characterization of Fe₃O₄ Bound Carboxymethyl Chitosan

The determination on functional group of chitosan, carboxymethyl chitosan (CC), Fe₃O₄ and CC-Fe₃O₄ nanoparticles were performed using FTIR. Carboxymethyl chitosan (CC) was analysed by NMR 400 MHz to determine position of carboxymethylation took placed. The morphology of the magnetic particles was characterized by TEM. TEM analysis was carried out by placing a drop of the ethanol-dispersed magnetic nanoparticle aqueous solution onto a copper grid and allowing the solution to evaporate in air at room temperature. Before sample withdrawal, it was sonicated for 30 min to obtain a better dispersion. XRD measurement was carried out on an X-ray diffractometer (Cu K α , λ =0.1542 nm). Magnetic properties of Fe₃O₄ and CC-Fe₃O₄ nanoparticles was measured by using Vibrating Sample Magnetometer.

Result and Discussion

FTIR Analysis

The IR results of chitosan, were summarised in Table 1, shows the basic characteristic of chitosan at : 3430 cm⁻¹ (O-H stretch) and N-H stretch, 2924 cm⁻¹ (C-H stretch), 1642 cm⁻¹ (C=O stretch), 1148 cm⁻¹ (bridge-O-stretch), and 1079 cm⁻¹ (C-O stretch) [8,9]. Sodium carboxymethyl chitosan (Na-CC) shows peak at 1603 cm⁻¹ indicating of appearance of -COONa group. H-form carboxymethyl chitosan (H-CC) shows appearance of peak at 1726 cm⁻¹ representing the carboxylate C=O asymmetric stretching. The signal at 1394 cm⁻¹ could be assigned to the symmetric stretching vibration of carboxylate C=O [10]. The peak of 577 cm⁻¹ is typical characteristic of Fe-O group in Fe₃O₄. Appearance of the peaks at 1629 cm⁻¹ and 1397 cm⁻¹ shows that binding of carboxymethyl chitosan with Fe₃O₄ has occurred. These two peaks were characteristic of COOM (M= metal ion) band, indicated that carboxyl groups in CC reacted with the surface hydroxide groups of Fe₃O₄ particles, resulting in the formation of the iron carboxylate [11].

¹H-NMR Analysis

In the ¹H NMR spectrum of carboxymethyl chitosan as shown in Fig.1, the signal at 2.00 ppm can be attributed to the hydrogen atoms of the methyl from acetamide groups. The signal at 3.15 ppm corresponds to the

hydrogen bonded to the carbon atom C2 of the glucosamine ring, while the signals between 3.72 and 3.91 ppm correspond to the hydrogen atoms bonded to carbons C3, C4, C5 and C6 of the glucopyranose. The signal between 4.26-4.55 ppm corresponds to the protons of 3- and 6-substituted carboxymethyl ($-O-CH_2-COOD$) of carboxymethyl chitosan [12]. The hydrogen bonding to carbon C1 gives the signals at 4.8 ppm. Moreover, the resonance signal of the protons from $N-CH_2-COOD$ groups could be found at 3.29 ppm. This result indicated that the amino groups were partly carboxymethylated along with hydroxyl groups [13].

Table 1: FTIR results of Chitosan, Na-CC, H-CC and CC- Fe_3O_4

Type of Vibration (cm^{-1}) Type of derivatives	O-H, N-H stretch	C-H stretch	C=O asym stretch	C=O sym stretch	C-O/C-N stretch	Fe-O stretch
Chitosan	3430	2924	1642	-	1148 1079	-
Na-CC	3430	2912	1603	-	1148 1078	-
H-CC	3435	2929	1726	1394	1151 1070	-
CC- Fe_3O_4	3411	2929	1629	1397	1024	577

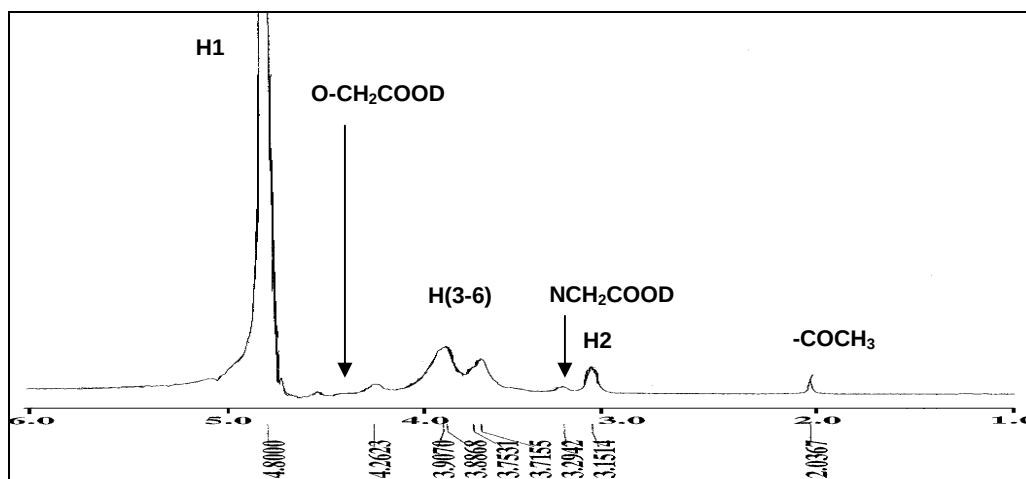


Figure 1: 1H -NMR Spectrum of H-CC in D_2O .

TEM Analysis

It can be seen from TEM micrograph (Fig.2) that the resulting magnetic Fe_3O_4 nanoparticles were almost spherical or ellipsoidal. It was clear that the Fe_3O_4 nanoparticles had a diameter range of 9.73-12.30 nm. After binding with carboxymethyl chitosan Fig.2b the particles remained discrete with a diameter range of about 9.70-12.50 nm. This revealed that the coating process did not significantly result in the agglomeration and the change in size of the particles. This could be attributed to the reaction occurring only on the particle surface [7]. However, there is a little aggregation in the Fe_3O_4 nanoparticles coated with carboxymethyl chitosan.

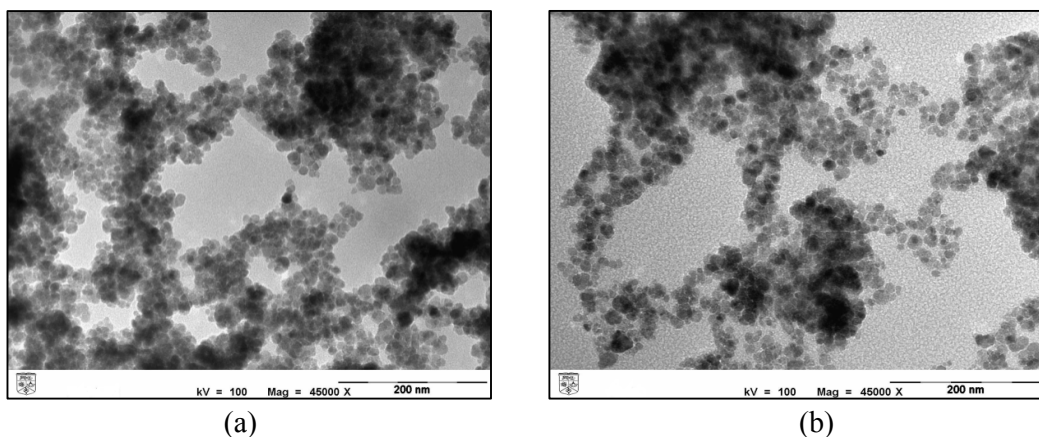


Figure 2: TEM micrographs of the Fe₃O₄ (a) and CC-Fe₃O₄ (b) nanoparticles

Magnetic Properties

The magnetic hysteresis loop at room temperature of the Fe₃O₄ and CC-Fe₃O₄ nanoparticles is shown in Figure 3. Fe₃O₄ has saturated magnetization (*M_s*) about 61.00 emu/g. The remanence (*M_r*) and coercivity (*H_c*) of Fe₃O₄ nanoparticles were 2.70 emu/g and 38.20 G, respectively. The saturated magnetization (*M_s*) of CC-Fe₃O₄ was 43.69 emu/g (51.4 emu/g)[14] when considering the carboxymethyl chitosan content. The significantly decrease *M_s* value of CC-Fe₃O₄ is attributed due to the existence of a magnetically inactive surface layer and some diamagnetic contribution from the carboxymethyl chitosan [15,16].

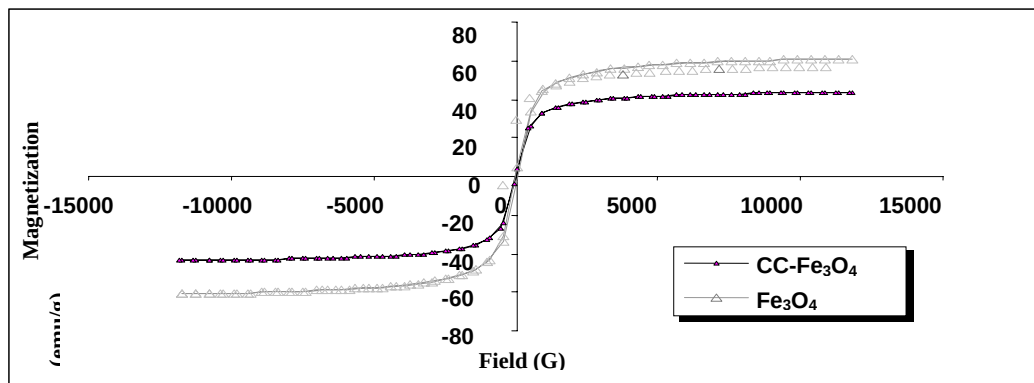


Figure 3: Magnetic hysteresis loop of Fe₃O₄ and CC-Fe₃O₄ nanoparticles.

XRD Pattern

The presence of crystalline structure of Fe₃O₄ and CC-Fe₃O₄ nanoparticles was confirmed by XRD. The diffractogram is shown in Fig. 4. There are six diffraction peaks for Fe₃O₄ and CC-Fe₃O₄: (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1) and (4 4 0), which is the standard pattern for crystalline magnetite with spinel structure

[17]. These peaks reveal that the resulting nanoparticles were pure Fe_3O_4 with a spinel structure. The binding process of CC and Fe_3O_4 did not result in the phase change of Fe_3O_4 .

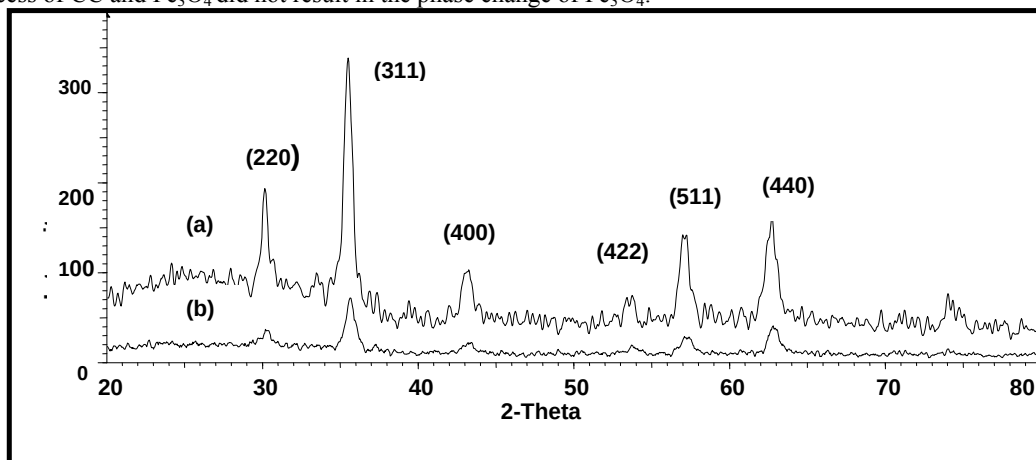


Figure 4: XRD pattern of Fe_3O_4 (a) and CC- Fe_3O_4 (b) nanoparticles.

Conclusion

H-CC bound Fe_3O_4 nanoparticles with diameter 9.70-12.5 nm was prepared in this study by the binding of carboxymethylated chitosan on Fe_3O_4 nanoparticles via carbodiimide activation. The saturated magnetization of CC- Fe_3O_4 nanoparticles could reach 43.69 emu/g. This magnetic chitosan nanoparticles have a good potential to remove metal ions such as iron and manganese which are the problem of drinking water in Malaysia.

Acknowledgement

The authors wish to thank PPSKTM, UKM for the instrumentations and the Ministry of Sciences, Technology and Innovation (MOSTI) for supported this work by its grant 02-01-02 SF0125.

References

1. Ding, P., Huang, K.L. & Li, G.Y. Kinetics of adsorption of Zn(II) ion on chitosan derivatives. 2006. *Int. J. Biol. Macromol.* 39: 222–227.
2. Zhang L., Guo J. & Peng X., Jin Y. Preparation and release behavior of carboxymethylated chitosan/alginate microspheres encapsulating bovine serum albumin. 2004. *J. Appl. Polym. Sci.* 92: 878–882.
3. Chen X.G. & Park H.J., Chemical characteristics of O-carboxymethyl chitosans related to the preparation conditions. 2003. *Carbohydr. Polym.* 53: 355–359.
4. Chang, Y.C., Chang, S.W. & Chen, D.H. 2006. Magnetic chitosan nanoparticles: Studies on chitosan binding and adsorption of Co(II) ions. *Reactive & Functional Polymer* 66: 335–341.
5. Mohapatra, S., Pal, D., Ghosh, S. K. & Pramanik, P. 2007. Design of Superparamagnetic Iron Oxide Nanoparticle for Purification of Recombinant Proteins. *Journal of Nanoscience and Nanotechnology* 7: 1–7.
6. Sun, G., Chen, X., Li, Y., Zheng, B., Gong, Z., Sun, J., Chen, H., Li, J. & Lin, W. 2008. Preparation of H-oleoyl-carboxymethyl-chitosan and the function as a coagulation agent for residual oil in aqueous system. *Front. Mater. Sci. China* 2(1): 105–112.
7. Chang, Y.C. & Chen, D.H. 2005. Preparation and adsorption properties of monodisperse chitosan-bound Fe_3O_4 magnetic nanoparticles for removal of Cu(II) ions. *Journal of Colloid and Interface Science* 283: 446–451.
8. Brugnerotto, J., Lizardi, J., Goycoolea, F.M., Arguelles-Monal, W., Desbrieres, J. and Rinaudo, M. 2001. An infrared investigation in relation with chitin and chitosan characterization. *Polymer* 42: 3569–3580.
9. Shigemasa, Y., Matsuura, H., Sashiwa, H. and Saimoto, H. 1996. Evaluation of different absorbance ratios from infrared spectroscopy for analyzing the degree of deacetylation in chitin. *International Journal of Biological Macromolecules* 18: 237–242.

10. El-Sherbiny, I.M.2009. Synthesis, characterization and metal uptake capacity of a newcarboxymethyl chitosan derivative. *European Polymer Journal* 45: 199–210.
11. Xu, X.Q., Shen, H., Xu, J. R. and Li,X.J.2004. Aqueous-based magnetite magnetic fluids stabilized by surface small micelles of oleolysarcosine. *Applied Surface Science* 221(1-4): 430-436.
12. Ragnhild, J., Hjerde, N., Varum, K.M., Grasdalen, H., Tokura, S. and Smidsrod, O. 1997. Chemical composition of *O*-(carboxymethyl)-chitins in relation to lysozyme degradation rates. *Carbohydrate Polymers* 34:131–139.
13. de Abreu, F.R. & Campana-Filho S.P. Characteristics and properties of carboxymethylchitosan. *Carbohydrate Polymers* 75: 214–221.
14. Zhu, A., Yuan, L., & Liao, T. 2008. Suspension of Fe₃O₄ nanoparticles stabilized by chitosan and *o*-carboxymethylchitosan. *International Journal of Pharmaceutics* 350: 361–368.
15. Morales, M.P., Veintemillas-Verdaguer, S., Montero, M.I., Serna, C.J., Roig, A., Casas, L.I., Martínez, B. & Sandiumenge, F. 1999. Surface and internal spin canting in γ -Fe₂O₃ nanoparticles. *Chemistry of Materials* 11(11): 3058-3064.
16. Iglesias O. & Labarta A. 2001. *Finite-Size and surface effects in maghemite nanoparticles:Monte Carlo simulations*. *Phys. Rev. B* 63, 184416-1/184416-11.
17. Ma, Z.Y., Guan, Y.P., Liu, H.Z., 2005. Synthesis and characterization of micronsized monodisperse superparamagnetic polymer particles with amino groups. *J. Polym. Sci. A: Polym. Chem.* 43: 3433–3439.