SYNTHESIS AND CHARACTERIZATION OF CARBOXYMETHYL CHITOSAN-FE₃O₄ NANOPARTICLES

NURUL HIDAYAH AHMAD SAFEE, MD. PAUZI ABDULLAH & MOHAMMED ROZALI OTHMAN

School of Chemistry Science and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor Darul Ehsan.
Tel.: 03-89215447; Fax: 03-89215410; E-mail address: mpauzi@ukm.my

ABSTRACT

In this study, carboxymethyl chitosan bound Fe_3O_4 magnetic nanoparticles was synthesized by the binding of carboxymethyl chitosan (CC) onto the surface of Fe_3O_4 magnetic nanoparticles, which was developed by using coprecipitating method. The CC-Fe_3O_4 nanoparticles was characterized by transmission electron microscopy (TEM), X-ray diffraction patterns (XRD), vibrating sample magnetometer (VSM) and Fourier Transform Infra Red (FTIR), respectively. The CC-Fe_3O_4 nanoparticles was developed as a magnetic nanoadsorbent for the removal of some heavy metal ions especially in drinking water. TEM studies confirmed that the Fe_3O_4 and CC-Fe_3O_4 particles have a range particle size of 9.73-12.30 nm and 9.70-12.50 nm, respectively. The magnetic properties of the Fe_3O_4 particles were verified using VSM showed their saturation magnetization, remanent magnetization and coercivity were 61.0 emu/g, 2.70 emu/g and 38.20 G, respectively. FTIR studies showed that appearance of peaks of 1629 cm^{-1} and 1397 cm^{-1} were characteristic of COOM (M = metal ions) bands, indicating in the formation of the iron carboxylate.

Keywords: Carboxymethyl chitosan , magnetic, nanoparticles, removal and heavy metal.

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 NH_2). So, chitosan and its derivatives have been widely used in many biomedical fields (Ding et. al. 2006).

Carboxymethylated chitosan has been paid 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 (Zhang et. al. 2004, Chen & Park 2003 & Ramesh et. al. 2004). 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 (Chang & Chen 2006).

In this study, carboxymethyl chitosan bound Fe_3O_4 magnetic nanoparticles was synthesized by the binding of carboxymethyl chitosan (CMC) onto the surface of Fe_3O_4 magnetic nanoparticles. These Fe_3O_4 and carboxymethyl chitosan-Fe_3O_4 nanoparticles were characterized by TEM, XRD, VSM, FTIR and ^{1}H-NMR, respectively.

EXPERIMENTAL

Chemicals

Chitosan was used as raw material. $FeCl_3$ and $FeSO_4.7H_2O$ were purchased by BDH Chemicals Ltd . Carbodiimide were supplied by Aldrich. Monochloroacetic acids and all chemicals were analytic grade reagents.

Preparation of Fe_3O_4 Nanoparticles

Superparamagnetic nanosized magnetite was prepared by controlled chemical co-precipitation of Fe^{2+} and Fe^{3+} (1:2 ratio) from ammoniacal medium at 80° C under argon atmosphere. In a typical experiment, 1 mmol of ferrous sulphate and 2 mmol of $FeCl_3$ were dissolved in 10 ml of de-ionized and de-oxygenated water. The resulting solution was vigorously stirred and heated to 80º C under a nitrogen atmosphere. Subsequently about 5 ml of 32% 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 subjected to magnetic decantation 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 re-washed with distilled water (Mohapatra et.al. 2007.

Preparation of Carboxymethyl Chitosan

Carboxymethyl chitosan was prepared by the Sun et.al. 2008 method. Chitosan (10g), sodium hydroxide (10g), isopropanol (50 ml) and water (50 ml) were added into a flask to swell and alkalize at a given temperature 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 (200 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 Fe_3O_4 Bound Carboxymethyl Chitosan

The binding of carboxymethyl chitosan was conducted following Chang and Chen 2005 method. First, 100 mg of Fe_3O_4 nanoparticles was added to 2 ml of buffer A (0.003 M phosphate, pH 6, 0.1 M NaCl). Then, the reaction mixture was sonicated for 10 min after adding 0.5 ml of carbodiimide solution (0.025 gml^{-1} in buffer A). Finally, 2.5 ml of carboxymethyl chitosan solution (50 mg ml^{-1} in buffer A) was added and the reaction mixture was sonicated for 60 min. The chitosan-bound Fe_3O_4 nanoparticles were recovered from the reaction mixture by magnetic bar. The magnetic particles settled within 1–2 min and then were washed with water and ethanol.

Characterization of Samples

The determination on functional group of chitosan, carboxymethyl chitosan (CC), Fe_3O_4 and CC-Fe_3O_4 nanoparticles were measured on Fourier Transform Infra Red (FTIR). Carboxymethyl chitosan (CC) was tested 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 *Kα*, *λ*=0.1542 nm). Magnetic properties of Fe_3O_4 and CC- Fe_3O_4 nanoparticles was carried out by using Vibrating Sample Magnetometer.

RESULT AND DISCUSSION

FTIR Analysis

The IR spectra of chitosan, are shown in Fig. 1a shows the basic characteristic of chitosan at : 3429 cm^{-1} (O-H stretch) and N-H stretch, 2923 cm^{-1} (C–H stretch), 1642 cm^{-1} N–H bend), 1148 cm^{-1} (bridge-O-stretch), and 1078 cm^{-1} (C–O stretch) ([Brugnerotto et al. 2001 and Shigemasa et al. 1996). IR spectrum of sodium carboxymethyl chitosan (Na-CC) in Fig.1b shows peak at 1603 cm^{-1} indicating of appearence of –COONa group. H-form carboxymethyl chitosan (H-CMC) spectrum in Fig. 1c shown appearence of peak at 1725 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 (El-Sherbiny 2009). Fig.1d shows the peak of 575 cm^{-1}, which is typical characteristic of Fe–O group in Fe$_3$O$_4$. Appearance of the peaks at 1629 cm^{-1} and 1397 cm^{-1} in Fig.1e shows that binding of carboxymethyl chitosan with Fe$_3$O$_4$ was occured. These two peaks were characteristic of COOM (M= metal ion) band, indicating that the carboxyl groups in CC reacted with the surface hydroxide groups of Fe$_3$O$_4$ particles, resulting in the formation of the iron carboxylate (Xu et. al. 2004)

3.2 ^{1}H-NMR Analysis

In the 1H NMR spectrum of carboxymethyl chitosan Fig.2 the signal at 2.00 ppm was 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 carboxmethyl (–O–CH$_2$–COOD) of CM-chitosan (Ragnhild et. al. 1997). The hydrogen bonded 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 (de Abreu & Campana-Filho 2009).

TEM Analysis

It can be seen from TEM micrograph Fig.3 that the resulting magnetic Fe$_3$O$_4$ nanoparticles are almost spherical or ellipsoidal. It was clear that the naked Fe$_3$O$_4$ Fig.3a nanoparticles had a mean diameter of 9.73-12.30 nm. After coating with carboxymethyl chitosan Fig.3b the particles remained discrete with a mean diameter 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 (Chang & Chen 2005). But there is little aggregative phenomenon in the Fe$_3$O$_4$ nanoparticles coated with carboxymethyl chitosan.

Magnetic Properties

Magnetization curve for the Fe$_3$O$_4$ and CC-Fe$_3$O$_4$ nanoparticles was shown in Fig.4. The saturated magnetization of CC-Fe$_3$O$_4$ (Ms) was 43.69 emu/g when considering the carboxymethyl chitosan content. While magnetite has saturated magnetization (Ms) about 61.00 emu/g. The remanence (Mr) and coercivity (Hc) of magnetite nanoparticles were 2.70 emu/g and 38.20G, respectively, which was typical for characteristic of superparamagnetic materials.

XRD Pattern

To confirm the presence of crystalline naked Fe_3O_4 and CC- Fe_3O_4 nanoparticles, the structure of the magnetic particles was characterized by XRD and the diffractogram is shown in Fig. 5. There are six diffraction peaks for naked Fe_3O_4 and CC- Fe_3O_4: (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 (Ma et al. 2005). These peaks reveal that the resulting nanoparticles were pure Fe_3O_4 with a spinel structure. Also, the binding process did not result in the phase change of Fe_3O_4. It is explained that the binding process did not result in the phase change of Fe_3O_4.

CONCLUSION

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 for removal of metal ions such as iron and manganese which are the problem of drinking water in Malaysia.

REFERENCES

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.

Chen X.G. & Park H.J., Chemical characteristics of *O*-carboxymethyl chitosans related to the preparation conditions. 2003. *Carbohyd. Polym.* **53**: 355-359.

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.

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.

de Abreu, F.R. & Campana-Filho S.P. Characteristics and properties of carboxymethylchitosan. Carbohydrate Polymers **75**: 214–221.

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.

El-Sherbiny, I.M.2009. Synthesis, characterization and metal uptake capacity of a newcarboxymethyl chitosan derivative. *European Polymer Journal* **45**: 199–210.

Li,G. Y., Jiang, Y.R., Huang,K.L., Ding, P. and Yao, L.L. 2008. Kinetics of adsorption of *Saccharomyces cerevisiae* mandelated dehydrogenase on magnetic Fe_3O_4-chitosan nanoparticles. *Colloids and Surfaces A: Physicochem. Eng. Aspects* **320**: 11–18.

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.

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.

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.

Ramesh H.P., Viswanatha S. & Tharanathan R.N. 2004.Safety evaluation of formulations containing carboxymethyl derivatives of starch and chitosan in albino rats. *Carbohyd. Polym.*, **58**: 435-541.

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.

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

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.

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.

1.0 Figure
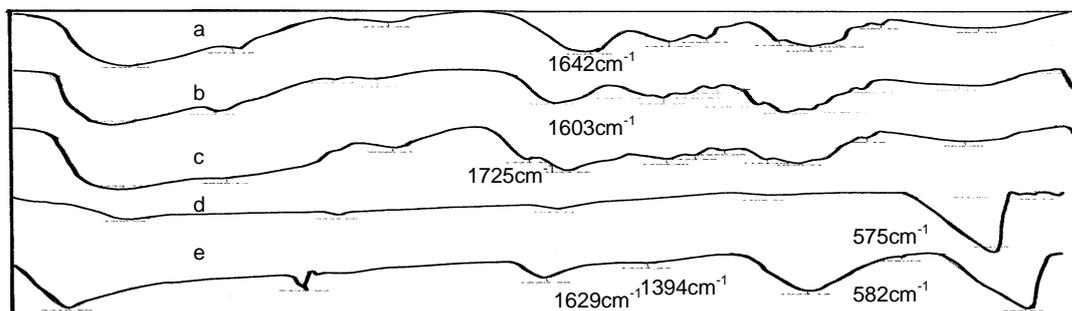
Fig.1 FTIR Spectrum of Chitosan (a), (Na-CC) (b), (H-CC) (c), Fe_3O_4 (d) and CC-Fe_3O_4 (e).
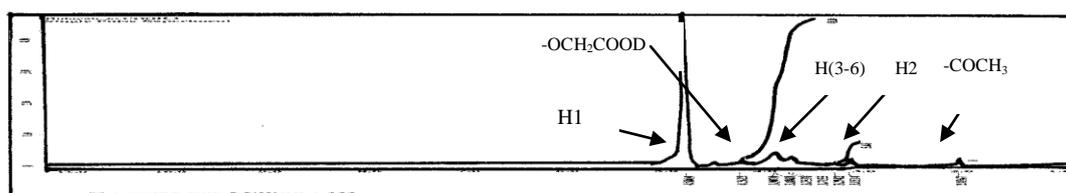
Fig. 2 ^{1}H-NMR Spectrum of carboxymethyl chitosan in D_2O.
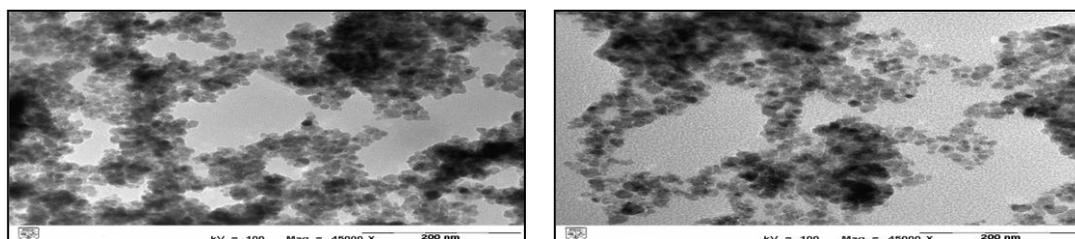
(a)   (b)

Fig.3 TEM micrographs of the Fe_3O_4 (a) and CC- Fe_3O_4 (b) nanoparticles
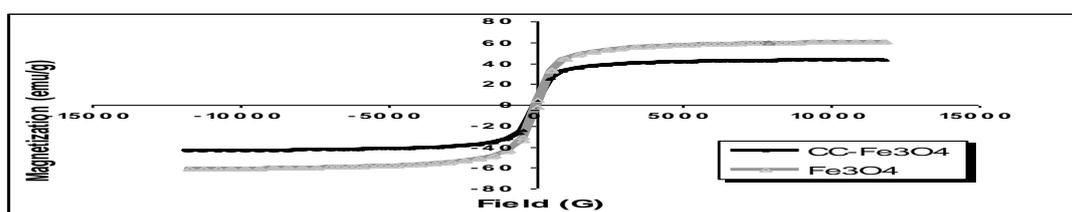
Fig. 4 Magnetization curve for the Fe_3O_4 and CC-Fe_3O_4 nanoparticles.
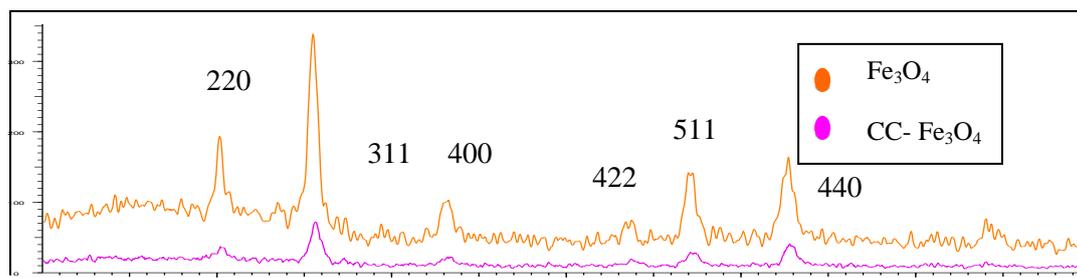
Fig.5 XRD pattern of Fe_3O_4 and CC- Fe_3O_4 nanoparticles.