Synthesis of Modified Chitosan - Montmorillonite Nanocomposite

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Abstract: Bio-nanoparticle- composites prepared from carboxymethyl chitosan(CMCs) and montmorillonite were investigated using three grades from the biopolymer chitosan. The montmorillonite (MMT) is used as nanofiller and water is used as a medium for dissolving and dispersing CMCs and montmorillonite. Morphology and properties of the nanocomposites have been studied compared with those of pure MMT using XRD, FTIR and TEM. The XRD results indicate the formation of an intercalated and exfoliated nanostructure at low MMT content and an intercalated-and-flocculated nanostructure at high MMT content. The existence of epichlorohydrine as cross-linking agent was examined, it is evident from the TEM results that the composite nanoparticle generated without the use of ECH has good uniform distribution of particle morphology compared to that observed when using a cross–linker agent. Increasing the rate of stirring by homogenizer to 10000 rpm for 10 min gives a nano-particles in the range of 2-6 nm. The adsorption capacity of these bio-nanoparticles were examined for lead and were found to be 92 mg Pb/g resin at pH 6 with grade II nano- composite, nanoparticles CMCs-MMT.

Key words: Carboxymethyl chitosan, montmorillonite, nanoparticles, nanocomposite

INTRODUCTION

Chitosan (CS) is a natural nontoxic biopolymer derived by the deacetylation of chitin. Chitosan and its derivatives have attracted considerable interest due to their antimicrobial and antifungal activity[Kendra, and Hadwiger, 1984; Sudarshan, *et al.*, 1992; Tsai and Su, 1999]. It has been used widely as an adsorbent for transition metal ions like Cu [Bayramoglu, 2003] and acid dyes [Shentu, *et al.*, 2005; Mondal, and Gupta,2006], because the amino (–NH$_2$) and hydroxyl (–OH) groups on chitosan chains can serve as the coordination and reaction sites. Generally, in order to improve the mechanical properties and specific gravity of CS and further enhance the adsorption capacity of CS, the surface of CS is modified by a suitable approach. Various studies were conducted to make derivatives of CS by chemical modification techniques, such as PEG-grafting, sulfonation, quaternarization [Jia, *et al.*, 2001], *N*- and *O*-hydroxylation and carboxymethylation of chitosan (CMCs) [Zhao, *et al.*, 2002]. Among the derivatives, CMCs is an amphoteric ether derivatives, which contains active hydroxyl (–OH), carboxyl (–COOH) and amine (–NH$_2$) groups in the molecule, and makes it possible to not only offer enough adsorption groups for increasing adsorption capacity toward dye but also improve the flocculation capacity of CMCs for dye molecules. Moreover, CMCs shows many unique properties, such as biocompatibility, biodegradation, biological activity, low toxicity and so on. Nevertheless, the cost of CMCs is relatively higher. In previous work[Aly, *et al.*,2005], we examined the crosslinked CMCs in the removal of Ni ions and it was found that the maximum capacity to be 90 mg/g resin which was higher than the commercial resin (Amberlite IR 120 H) with an increase of 8%.

Montmorillonite is a 2:1 type aluminosilicate. Its crystalline structure presents an alumina octahedral between two tetrahedral layers of silica. The isomorphous substitution of Al^{3+} for Si^{4+} in the tetrahedral layer and Mg^{2+} for Al^{3+} in the octahedral layer results in a net negative surface charge on the clay. The imbalance of the negative charges at the surface is compensated by exchangeable cations (typically Na$^+$ and Ca^{2+}). The parallel layers in these structures are linked together by weak electrostatic forces [Grim and Guven, 1978]. Intercalation of the clays via exchange of cations located in their interlayer space with large organic or inorganic cations is an effective way to modify them in order to obtain adsorbents or catalysts. On the inorganic pillaring of montmorillonite, many different hydroxy-metal polycations (Al, Zr, Fe, Cr, Si, Ti and Cu) have been used and reported in the literatures [Han *et al.*, 1999; Maes *et al.*, 1997; Canizares *et al.*, 1999; Valverde *et al.*, 2002; Bouberka *et al.*, 2005; Carriazo *et al.*, 2005; Han and Yamanaka 2006]. However, one of the most intensively studied is the hydroxy-aluminum polycations, which is used for the preparation of Al-pillared montmorillonite and has been well defined for its chemical composition and structure [Salerno *et al.*, 2002].

Recently, nanocomposite from montmorillonite and chitosan or its drevatives are being widely considered as alternative low-cost adsorbents. Furthermore, many reports have been recently showed that the modified clays displayed higher adsorption capacity than the original clay [Wang, *et al.*, 2004; Zcan, 2004]. Therefore, in order to further enhance the adsorption capacity of nanocomposite, the preparation of nanoparticles from *N,O*, CMCs– MMT nanocomposite was carried out, characterized, and the adsorption capacities were studied in this paper. Furthermore, the effects of various parameters (e.g. the molar ratios of *N,O*-CMCs and MMT, initial pH

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value in heavy metal (lead Pb) solution and adsorption temperature) on the adsorption capacities of Pb on the nanocomposite have been also investigated.

2- Materials:

The materials used in the preparation of the nanocomposite are as follows:

*The chitosan (CS) used in this study was purchased from HAS HMRZEL Laboratories LTd (Netherlands) and had a molecular weight of 600 000 and degree of deacetylation 78-85%.

* Monochloro-acetic acid was obtained from Aldrich Company with a purity of 98% and had a molecular weight of 94.5.

* Montmorillonite, $Nax(Al,Mg)2Si_4O_{10}(OH)_2zH_2O$ with a semi-quantitative value of 40.4%, commercially named Bentonite, was kindly provided from, Egypt International Export and Import Co. and used as received.

* All other chemical reagents were analytical grade used as received, and solutions were prepared with distilled water.

3- Experimental Procedure:

To achieve our target, the experimental method can be visualized as being composed from three main experimental steps, as presented in the following:

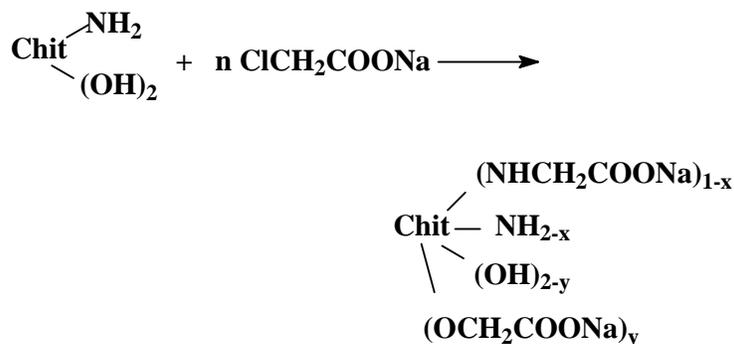### *a)Preparation of Low Molecular Weight Chitosan:*

Low molecular weight chitosan was prepared by hydrolysis of the original chitosan according to Feng *et al* (2003) performed at room temperatures. 2g chitosan suspended in 50 ml distilled water was completely dissolved in 5 ml hydrochloric acid solution of 2% concentration. Afterwards 10 ml of an aqueous hydrogen peroxide solution (15% conc.) was added to the mixture under magnetic stirring. The viscosity of the mixture was measured periodically until it reached the required value relevant to the desired degree of hydrolysis. At that point, the solution was precipitated with NaOH solution followed by filtration. The collected solids (water insoluble chitosan) was washed with distilled water until reaching pH=7 and then dried under vacuum, yielding the required molecular weight chitosan according to viscosity measurements.

b) Preparation of Carboxymethyl Chitosan:

In this study, O,N-carboxymethyl chitosan was synthesized by the method reported by Hayes (1986):

Carboxymethyl chitosan was prepared by treatment of 20g chitosan suspended in 1000 ml isopropanol with 27g sodium hydroxide in a three-necked flask equipped with a condenser, under stirring at room temperature for one hour. Thereafter, monochloro-acetic acid (30g in 20 ml isopropanol) was added into the reaction mixture in six equal portions over a period of 30 min, followed by raising temperature to 60°C and allowed to continue for 2 hours. Finally, the resultant solution was filtered and the filter cake was rinsed with 80% aqueous methanol and dried over night at room temperature, followed by estimation of nitrogen and carboxylic contents.

The following equation represents the preparation reaction of carboxymethyl chitosan:

$$\text{Chit}\begin{array}{c}\diagup NH_2 \\ \diagdown (OH)_2\end{array} + n\ ClCH_2COONa \longrightarrow$$

$$\begin{array}{c} (NHCH_2COONa)_{1-x} \\ \diagup \\ \text{Chit} — NH_{2-x} \\ \diagdown \\ (OH)_{2-y} \\ (OCH_2COONa)_y \end{array}$$

where x and y represent the fractions of amine and hydroxyl groups respectively, which have been carboxymethylated.

c) Preparation of O,N-CMCs-MMT Nanocomposite Nanoparticles:

In a typical preparation, 1g MMT was first swelled by 100 ml distilled water, O,N-CMCs solution, in amounts corresponding to different weight ratios, was prepared by dissolving it in distilled water followed by pH adjustment to 8.0 with 20%wt sodium hydroxide solution.

Afterwards, the resulting solution was slowly added to MMT suspension under stirring at 60°C for 6 hours to obtain the nanocomposite. At the end of that time, epichlorohydrin (ECH) at pre-determined concentration (as

a cross-linking agent) was added to the previous suspension. The pH was then re-adjusted to 10 by 20% NaOH solution under homogenation, using a homogenizer of the type Wise Mix HG–15D ch Daihan Scientific (2000 – 24000 rpm range) at 10000 rpm for 10 min, followed by moderate stirring at 40°C for 12 additional hours. The formed composite was separated by centrifugation, washed with distilled water until the pH of the washed water became neutral, and then dried at 60°C.

4- Analytical Methods:
d) Transmission Electron Micros Copy (TEM):
The morphology of the composite material was conducted to investigate the nano-composite shapes in addition to the nano-particles size.

e) FT-IR Spectral Analysis:
The infra-red spectrum of the obtained molecular weights as well as the nano- composite N,O-CMCs-MMT was undertaken as previously mentioned above.

f) X-ray Diffraction (XRD):
The crystalline character of the N,O-CMCs intercalated MMT was estimated from powder XRD patterns collected on a D8 Advanced Bruker diffractometer by using a CU Kα (40 KV, 40 mA) radiation, and a secondary beam graphite monochromator. The patterns were recorded in a 2-theta range from 2° to 40° in steps of 0.02^{o} and counting time 2 sec. per step.

5- Identification of Results:
5-1- Preparation of N,O- Carboxymethyl Chitosan (N,O-CMCs)
Three different molecular weights of chitosan were prepared via hydrolysis process degrading the original high molecular weight chitosan. The three products of hydrolysis are classified according to viscosity measurements to the following:

Grade I: High molecular weight (about 80% of the original chitosan molecular weight).
Grade II: Medium molecular weight (about 50% of Grade I molecular weight).
Grade III: Low molecular weight (about 29% and 25% of Grade I and original molecular weights respectively).

Table(1) depicts the analysis of the three prepared carboxymethyl chitosan (CMCs), as described above, in terms of viscosity, nitrogen content, degree of substitution (D.S.) and carboxylic group content.

5-2.Evidence of N,O-CMCS – MMT Composite Formation:
The N,O-CMCs-MMT composite formed from the interaction between CMCs grade I and montmorillonite was subjected to IR and X-ray diffraction analysis as a representative sample.

a) IR analysis of the Nanocomposites:
Figure (1) shows the IR spectra of MMT (a), four nanocomposites with the weight ratios of N,O-CMCs to MMT 1:100 (b), 1:10 (c), 1:5 (d), and 1:1(e) and (f) CMCs.

Compared with the IR spectra of MMT (a), Figure(1) shows the absorption band at 3467 cm-1, corresponding to – OH stretching vibration of H$_2$O of MMT, strengthened and shifted to lower wave number 3445 cm-1 (Fig. 1,b), 3446 cm-1 (Fig. 1,c) and 3441 cm-1 (Fig 1,d) and 3446 cm-1 (Fig. 1,e). This suggests the vibration bands in N,O-CMCs(O-H and N-H stretching 3423 cm-1) overlap with the bands of MMT (-OH stretching of H$_2$O). The characteristic absorption band of the asymmetric stretching vibration of the– CH$_3$ (2931 cm-1) of intercalated N,O-CMCs was observed on the IR spectra of N,O-CMCs-MMT (Fig. 1, b-d) and the intensity of the both absorption bands increased with increasing the weight ratio of N,O-CMCs to MMT. In addition, the absorption band at 1642cm-1, assigned to –OH bending vibration of H$_2$O of the MMT, strengthened and shifted to lower wave number 1635 cm-1 (Fig. 1,d), which indicates the –COO- group asymmetric stretching vibration of intercalated N,O-CMCs (1600 cm-1) overlapping with – OH bending vibration of H$_2$O of the MMT. At the same time, the – COO- group symmetric stretching vibration (1420 cm-1) and the second – OH group stretching vibration (1031 cm-1) of intercalated N,O-CMCS were observed and increased on the IR spectra of N,O-CMCs-MMT (Fig.1, b-e) with increasing the weight ratio of N,O-CMCs to MMT.

b) Nitrogen Content in Nanocomposite Products:
Figure (2) illustrates the percent nitrogen content determined for the three obtained products. The increase in %N with the increase in mass ratio of N,O-CMCs to MMT denotes the intercalation of chitosan into MMT layers, resulting from exchange of cations with the quaternary ammonium salts NH$_3^+$ of CMCs.
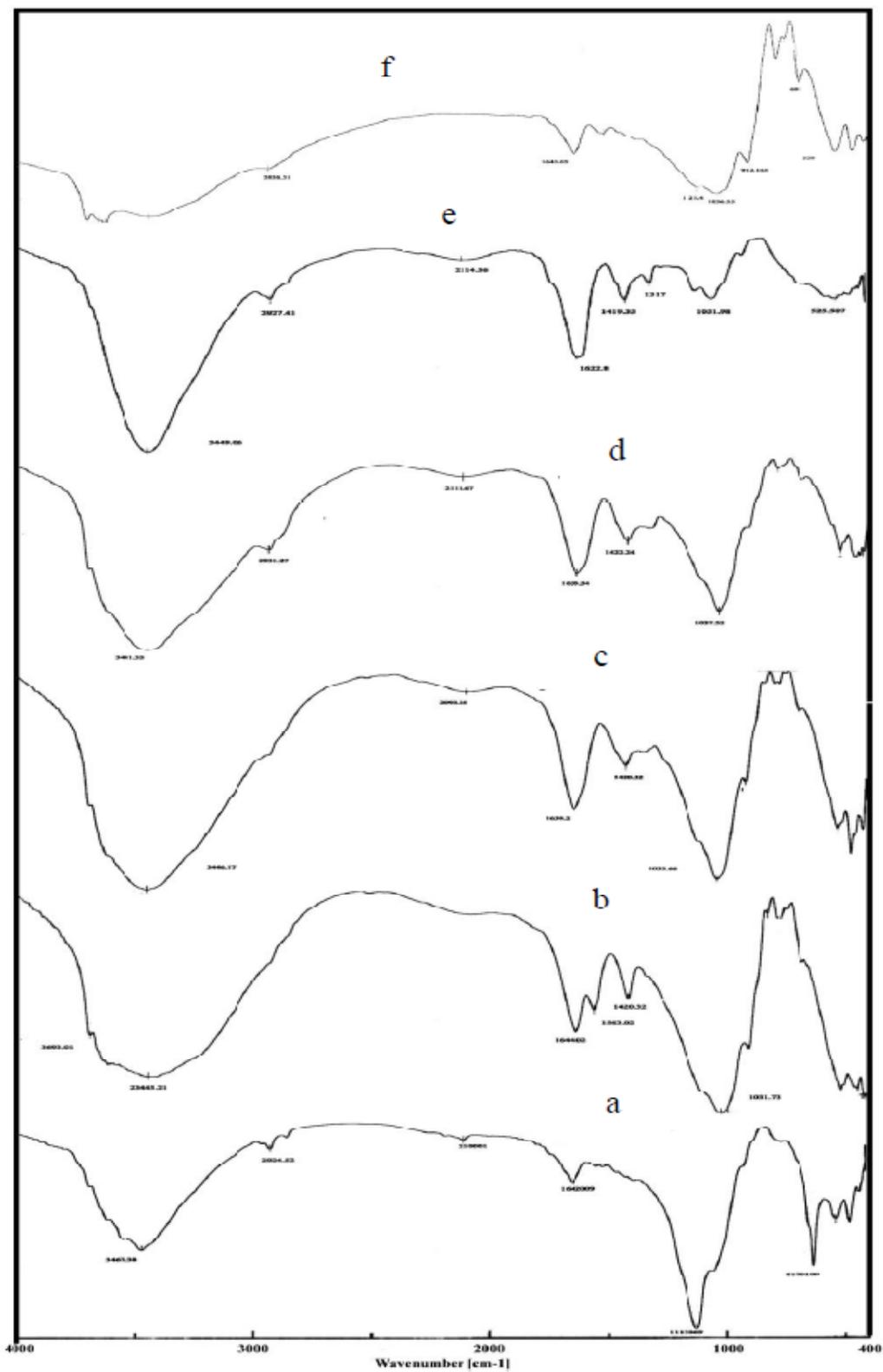
Fig. 1: IR spectra of CMCS\ MMT (grade 1): (a) MMT, (b) weight ratio CMCS to MMT 1: 100, (c) 1:10, (d) 1:5, (e) 1:1& (f) CMCS

Table 1: Analysis Results of CMCS.

Material	Viscosity (C.p.)	% N	D.S.	% COOH
Original Chitosan	68	6.9	–	--
Grade I	55	3.94	0.59	13.06
Grade II	28	4.3	0.46	9.53
Grade III	16	4.1	0.47	10.2
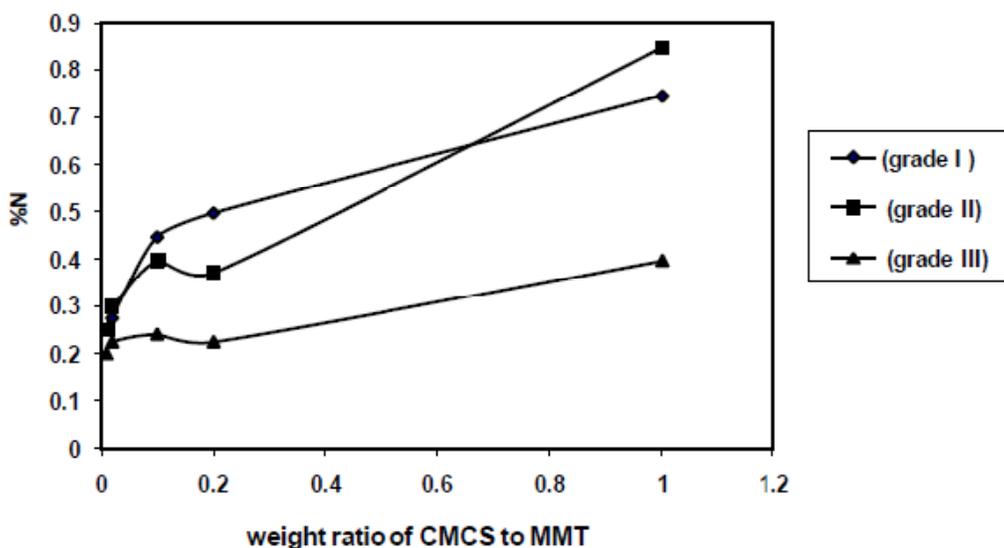
Fig. 2: Effect of weight ratio of CM.CS-MMT nanocomposite on nitrogen percent.

c) X-ray Diffraction Analysis of N,O-CMCS-MMT Nanocomposites:

X-ray diffraction (XRD) is an effective method for the investigation of the intercalation existence of montmorillonite. Figure (3) demonstrates the XRD patterns of MMT (o), and five nanocomposites with the weight ratio of N,O-CMCs to MMT of 1:100 (1); 1:50 (2); 1:10 (3); 1:5 (4) and 1:1 (5). The XRD profile of the MMT shows a typical diffraction peak at 2-theta = 6.99°, corresponding to a basal spacing of d=12.467A°. After intercalation with N,O – CMCs, this peak shifts to lower angle and even disappears. The movement of the typical diffraction peak of MMT to lower angle (6.5°) indicates the formation of intercalated nanostructure with the weight ratio 1:100. The intensity of the peak decreases with increasing of the weight ratios of N,O.-CMCS to MMT and even disappears with weight ratios of 1:5 and 1:1. This indicates most probably that the formation of a disordered intercalated or exfoliated structure in N,O- CMCS-MMT nanocomposite has occurred, where the silicate layers are completely homogenously dispersed in the polymer matrix. These results are in good agreement with previous works concerning the preparation of chitosan/montmorillonite [Wang *et al.*, 2005; Wang and Wang, 2007].

Furthermore, XRD analysis were carried out for the two others prepared grades (II,III) nanocomposites, each at high and lower weight ratios (1:100and 1:5) just to investigate the effect of the N,O-CMCS molecular weight upon the intercalation with MMT. Similar trends as above were obtained for the two others prepared grades (II,III) nanocomposites,

5-3- Effect of Molar Ratio of N,O-CMCs to Epichlorohydrin (ECH) on Nanocomposite Solubility:

Cross-linking with (ECH) has been used to limit the solubility of chitosan in acid solutions. The effect of molar ratio of N,O-CMCs to ECH on the solubility of the prepared resin with high molecular weight chitosan (grade I) as a model nanocomposite, is depicted in Table (2).

The solubility data reveals that there is a negligible and almost constant effect of the cross-linking process on the resin, at different molar ratios. The solubility of the same resin prepared without using the cross-linker was tested and exhibits the same percent as shown in the above table (4% solubility).
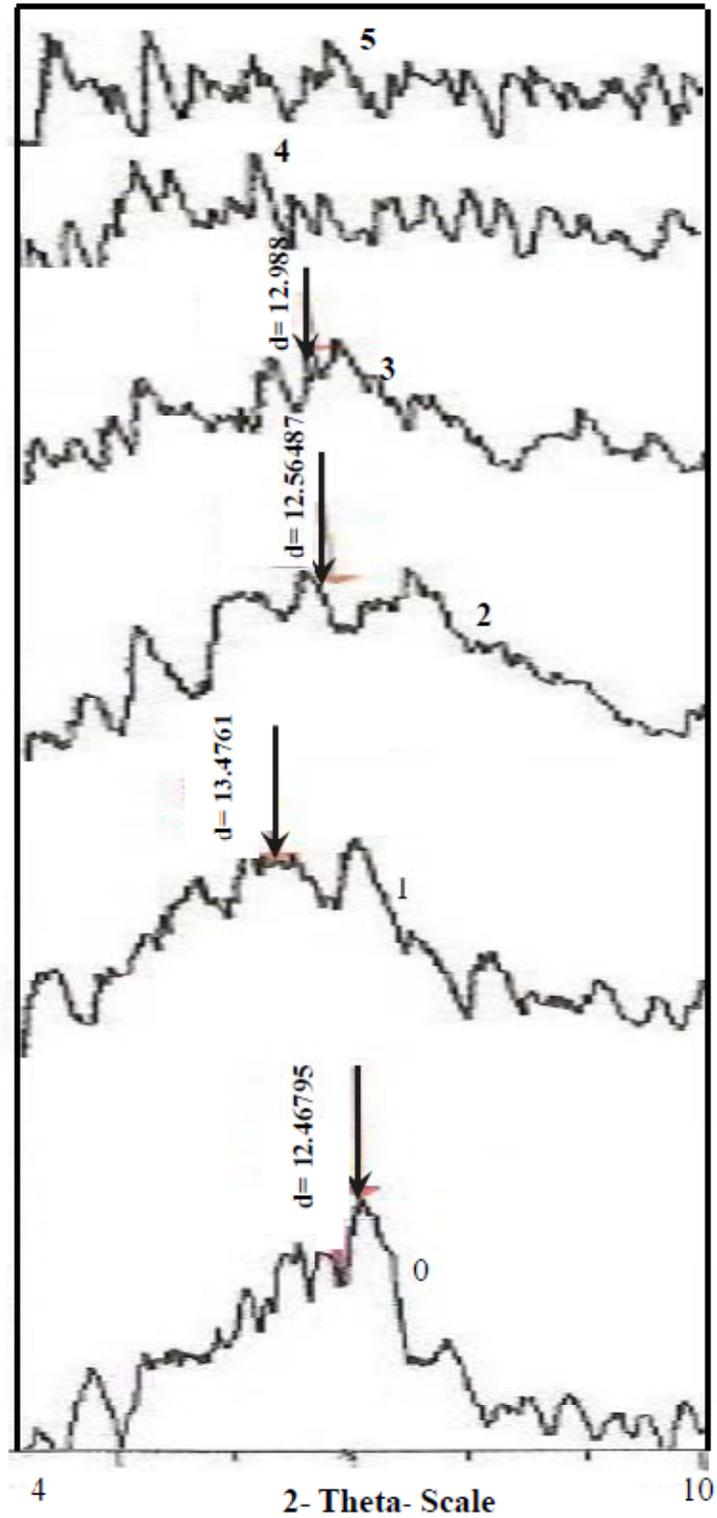
Fig. 3: XRD powder patterns of the MMT (0), the nanocomposite weight ratio of CMCs to MMT of 1:100 (1), 1:50(2), 1:10 (3), 1:5 (4) & 1:1.

Table 2: Effect of ECH on the solubility of nanocomposite, at 80ºC, 120 rpm, and 0.1:1wt. ratio of N,O-CMCS-MMT (Grade I).

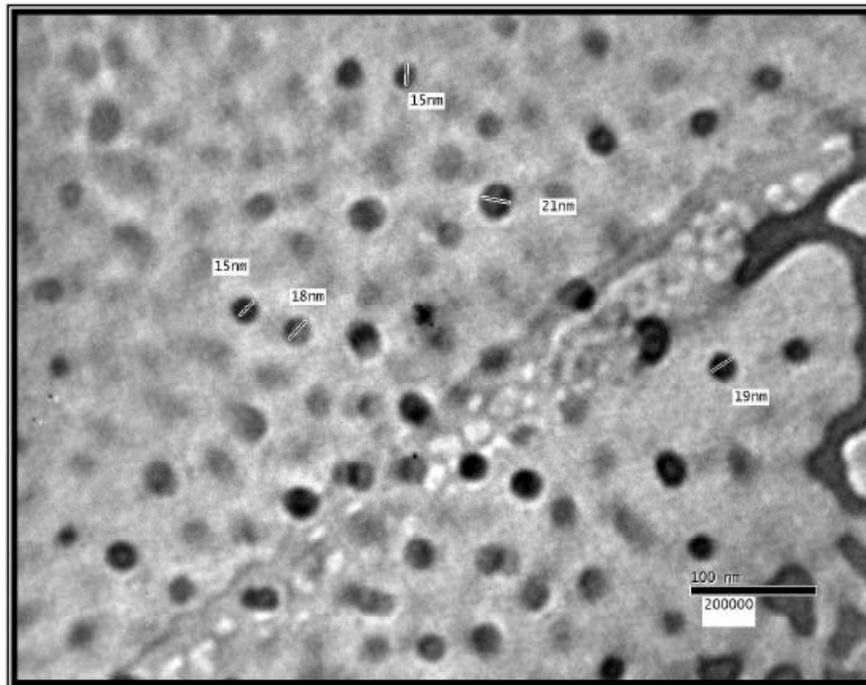| Molar ratio of N,O-CMCS to ECH	%N	% Solubility of resin
10:1	0.348	4.35
5:1	0.2983	3.55
1:1	0.348	3.65
0.334:1	0.2734	4.15
0.1:1	0.2983	3.35

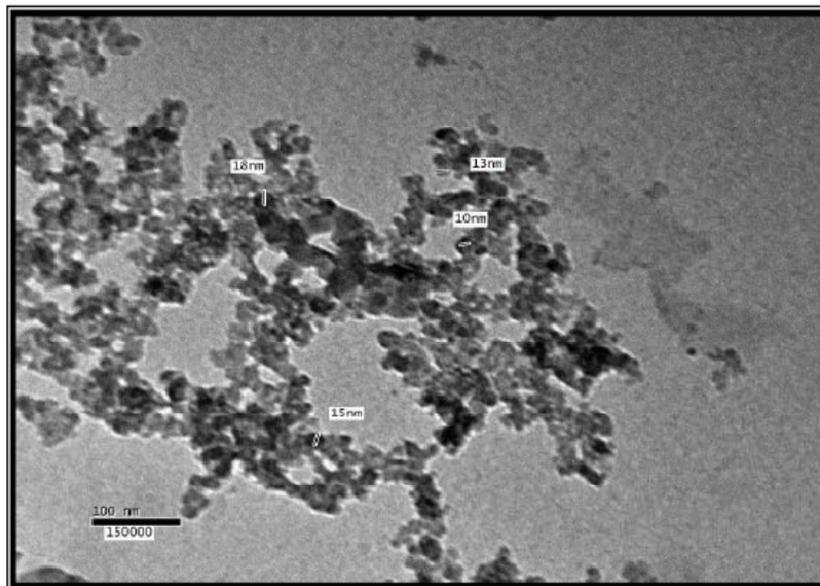**Fig. 4:** TEM CMCS-MMT without cross-linking.

Fig. 5: TEM CMCs-MMT with cross linking.

These results indicate that there are no differences or profit using ECH as cross – linker. The reason may be due to the strength of the ionic bonding existing between N,O-CMCs and MMT containing carboxylic group and the positively charged CMCs which prevent the solubility of chitosan.

Moreover, TEM measurements were performed to investigate the effect of ECH on nanocomposite (displayed in Figures (4 and 5)). Even though, almost same range of nanoparticle size (10 – 21 nm) is obtained for both systems, it is evident that the composite nanoparticles generated without the use of ECH (Fig. 4) have good uniform distribution of particle morphology compared to that observed on using a cross–linker agent (Figure 5) which seems that it caused aggregated particles. Aggregation can be caused by secondary interactions between the individual particles or intermolecular cross – linking reaction (Bodna *et al*., 2006).

To improve the size and shape of the nano-particles formed above, different speeds of the homogenizer (8000, 10000 and 12000 rpm) and different time of agitation (5,10 and 15 min) were examined. The best results were found at 10000 rpm and 10 min agitation with the homogenizer. It's clear from the figure that under these conditions we reached nano-particles in the range of 2-6 nm (Fig.(6)).
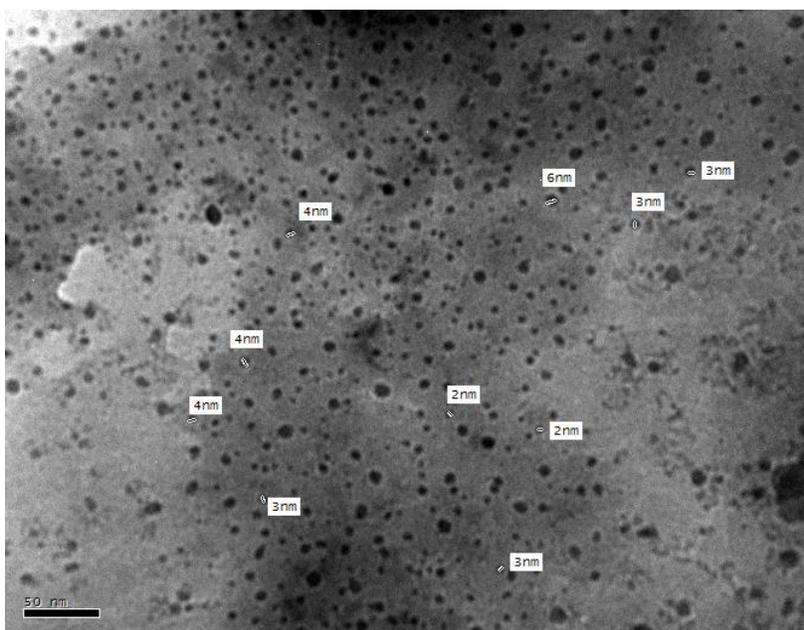

Fig. 6: TEM CMCS-MMT without cross-linking at 10000rpm agitation speed for 10 min.

5-4. Effect of Weight Ratio of N,O-CMCS to MMT on Adsorption Properties:

The information observed from IR spectra and XRD results indicates that the weight ratio of N,O- CMCs to MMT could influence chemical environment of the nano-composites and then may have an influence on its adsorption capabilities. As such, in the present section, the equilibrium resin capacity for heavy metal ions was tested in a batch-mode technique, by the use of aqueous solutions of Pb ions as a model heavy metal; the residual ions were measured by atomic absorption.

The effect of weight ratio of N,O-CMCs to MMT of the three prepared grades nano-composites on their equilibrium capacities for lead removal from aqueous solution is illustrated in Figure (7). The three operated nanocomposite grades exhibit same adsorption behavior: equilibrium capacity increases with the increase of the weight ratio of N,O-CMCs to MMT till reaching a maximum at a specific ratio for each grade, followed by a sharp decrease. The observed increase of equilibrium capacity is mainly attributed to the increased amounts of active hydroxyl (-OH), carboxyl (-COOH) and amine (-NH$_2$) groups with increasing the weight ratios of biosorbent composite, in addition to the absence of internal diffusion resistance up to a certain limit.

On the other hand, results of capacity decrease when the weight ratio of N,O-CMCs to MMT exceeds 0.02:1, 0.1:1 and 0.2:1 for grade I, grade Π and grade Ш respectively, indicate that when the N,O-CMCs increases over these limits, the difficulty of sorbate molecules to reach accessible groups of the resin increases as a result of its encumbrance, and as such, less ion exchange phenomena could be fixed on the biosorbent.

To evaluate the adsorption capacity of each operated grade, as they exhibits almost constant maximum capacity at specific weight ratios, and since the pH is the most important factor affecting the adsorption process, the effect of pH value in the original solution on adsorption capacity of the three prepared grades biosorbent was investigated and shown in Fig (8).
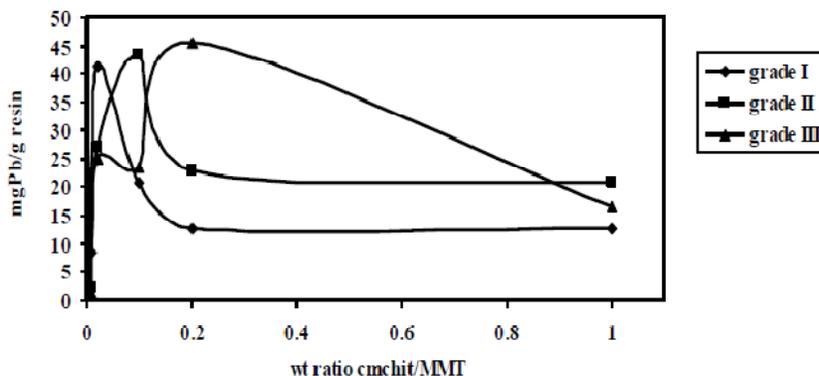
Fig. 7: Effect of weight ratio of CMCS-MMT on the adsorption capacity of the nanocomposite for the three grades(I,II and III);[0.1 g resin, 100ppm Pb(100 ml), 30 ^{o}C].
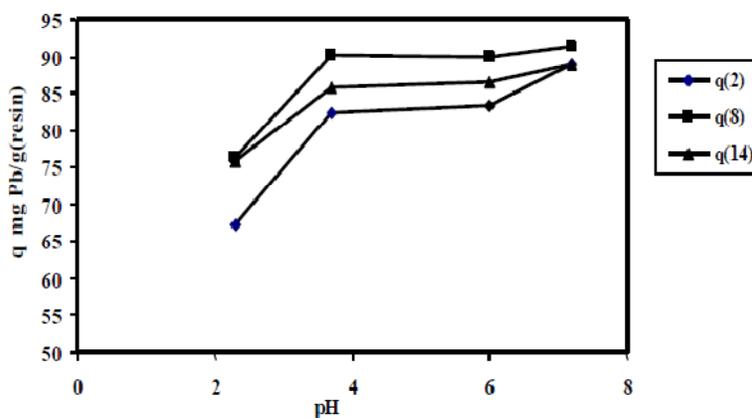

Fig. 8: Effect of pH on the adsorption capacity of the three samples 2,8,14 of the nano composite CM.chitosan-MMT [T = 30°C, 120 rpm, C$_{o}$ =100 ppm Pb].

It can be seen that the adsorption capacities of the three grades nanosorbents increase significantly from pH 2 to pH 4; and with further increase of pH till slightly alkaline, the adsorption capacities of grades I and III hardly increase, while that of grade II reaches a constant value higher than that obtained by the other two grades.

It is well established that in N,O-CMCS there are different types of functional groups (-NH$_2$, -OH, - NH-CH$_2$ COOH, N(CH$_2$ COOH)$_2$ and –O- CH$_2$ COOH) in different proportions. Delben *et al* (1989) found that the approximate relative proportions of the forms were dependent on pH. Accordingly, a fully protonated form (N$^+$H$_2$-CH$_2$-COOH) only exists at pH < ~ 5 and that only the fully dissociated form (-NH-CH$_2$ COO-) exists at pH > ~ 9. Between these two pH values, the polymer exists as a mixture of the mono-dissociated and fully dissociated forms, which seems to be the reason of the consistency of the cation exchanger capacity.

Comparing the results of the present work with some adsorbents used for the removal of lead from wastewater, as shown from Table 3,we found that the capacity of the bio-nanocomposite is very high relative to activated carbon from *Tamarind wood,* microorganism, Saudi Arabian clays, oil shale ash into zeolite, surface modified carbon, surface modified saw dust, However, it has a relatively close capacity to that of the polymerized banana stem and marine algae.

Table 3: Comparison between the capacities of the different adsorbents used for the removal of lead.

Adsorbent	Reference	Capacity of lead mg/g adsorbent
activated carbon from *Tamarind wood*	Jyotikusum Acharyaa, *et al*.(2009)	43.85
microorganism	Bahadir, *et al*.(2007)	2.643
Saudi Arabian clays	A. Al-Jlil, *et al*.(2009)	30
oil shale ash into zeolite	Shawabkeh, *et al*.(2004)	70.58
polymerized banana stem	Noeline, *et al*.(2005)	91.74
surface modified carbon	Rivera-Utrilla, *et al*.(2001)	54.1
marine algae	Jalali, *et al*.(2002)	85.7
surface modified saw dust	Taty-Costodes, *et al*.(2003)	22.2
Our work CM.chit-MMT nanoparticles		92

Conclusion:

A nano-composite , nanoparticles chelating agent prepared from three grades (I,II, and III) of N,O-Carboxymethyl chitosan(N,O-CMCs) , was examined with the montmorillonite. The montmorillonite (MMT) is used as nanofiller and water is used as solvent for dissolving and dispersing N,O-CMCs and montmorillonite. The effect of N.O.CMCs and MMT ratio in nanocomposites has been investigated using XRD and TEM. The XRD results indicate the formation of an intercalated-and-exfoliated nanostructure at low MMT content and an intercalated-and-flocculated nanostructure at high MMT content. The effect of epichlorohydrine as cross-linking agent was examined. It was found that it has no effect on the desolubilization of the resin. From the TEM results, it is evident that the composite nanoparticle generated without the use of ECH have good uniform distribution of particle morphology compared to that observed when using a cross–linking agent and it has particle size in the range of 15-20 nm. Increasing the rate of stirring by homogenizer to 10000 rpm for 10 min gives a nano-particles in the range of 2-6 nm.

The maximal adsorption capacity for lead is found to be 92 mg Pb/g resin at pH 6 with grade II nano-composite, nanoparticles N,O-CMCs-MMT.

ACKNOWLEDGMENT

REFERENCES

Al-Jlil, A.S. and F.D. Alsewailem, 2009. ”Saudi Arabian clays for lead removal in wastewater, Applied Clay Science, 42: 671-674.

Acharyaa, J., J. Sahub, N.C.R. Mohantyc and B.C. Meikapb, 2009. ”Removal of lead(II) from wastewater by activated carbon developed from *Tamarind wood* by zinc chloride activation”, Chemical Engineering Journal, 149: 249-262.

Aly, H.M., M.A. Khedr, A.I. Hafez, H. Abdel-Aziz and A.Waly, 2005. Equilibrium and Kinetic Studies of Heavy Metal Removal By New Synthesized Sorption/Chelating Resins. The transactions of the Egyptian Society of Chemical Engineers., 31(4): 64-76.

Bahadir, T., G. Bakan, L. Altas and H. Buyukgungor, 2007. ”The investigation of lead removal by biosorption:An application at storage battery industry wastewaters” Enzyme and Microbial Technology, 41: 98-102.

Bayramoglu, G., 2003. 'Poly(hydroxyethyl-methacrylate)/chitosan dye and different metal ion immobilised interpenetrating network membranes: preparation and application in metal ion exchange chromatography', J. Appl. Polym. Sci., 88: 1843-1853.

Bodna, M., J.F. Hartman, J. Borbely, 2006. Biomacromolecules, 7: 3030-306.

Bouberka, Z., S. Kacha, M. Kameche, S. Elmaleh, Z. Derriche, 2005. Sorption study of an acid dye from an aqueous solutions using modified clays. J Hazard Mater, B119: 117-24.

Canizares, P., J.L. Valverde, M.R. Sun Kou, C.B. Molina, 1999. 'Synthesis and characterization of PILCs with single and mixed oxide pillars prepared from two different bentonites', Micropor Mesopor Mater, 29: 267-81.

Carriazo, J., E. Guelou, J. Barrault, J.M. Tatibouet, R. Molina, S. Moreno, 2005. Synthesis of pillared clays containing Al, Al–Fe or Al–Ce–Fe from a bentonite: characterization and catalytic activity. Catal Today, 107-108: 126-32.

Delben, F., R.A.A. Muzzarelli, M. Terbojevich, 1989. Carbohydrate Polymers, 11: 205.

Feng, T., L. Yu, H. Keao, Z. Binyuan, 2003. Journal of Materials Science, 38: 4709-4712.

Gemeay, A.H., 2002. 'Adsorption characteristics and the kinetics of cation exchange of rhodamine 6G with Na+-montmorillonite', J. Colloids Interf. Sci., 251: 235-241.

Gemeay, A.H., A.S. EI-Sherbiny, A.B. Zaki, 2002. 'Adsorption and kinetic studies of the intercalation of some organic compounds onto Na+-montmorillonite', J. Colloids Interf. Sci., 245: 116-125.

Grim, R.E., N. Guven, 1978. Bentonites: geology, mineralogy, properties and uses. Amsterdam: Elsevier Scientific.

Han, Y.S., S. Yamanaka, J.H. Choy, 1999. 'Acidic and hydrophobic microporous clays pillared with mixed metal oxide nano-sols'. J Solid State Chem., 144: 45-52.

Han, Y.S., S. Yamanaka, 2006. 'Preparation and characterization of microporous SiO2–ZrO2 pillared montmorillonite'. J Solid State Chem., 179: 1146-53.

Hayes, E.R., 1986. US Patent, 4: 619, 995.

Kendra, D.F., L.A. Hadwiger, 1984. Exp. Mycol., 8: 276-281.

Kumar, V. and T. Yang, 2002. Carbohydrate Polymers, 48(4): 403-412.

Jalali, R., H. Ghafourian, Y. Asef and S.J. Sepehr, 2002. "Removal and recovery of lead using non living biomass of marine algae", J. Hazard. Mater, 92: 253-262.

Jia, Z., D. Shen, W. Xu, 2001. 'Synthesis and antibacterial activities of quaternary ammonium salt of chitosan', Carbohydr. Res., 333: 1-6.

Maes, N., I. Heylen, P. Cool, E.F. Vansant, 1997. The relation between the synthesis of pillared clays and their resulting porosity. Appl Clay Sci., 12: 43-60.

Miyamoto, N., R. Kawai, K. Kuroda, M. Ogawa, 2000. 'Adsorption and aggregation of a cationic cyanime dye on layered clay minerals', Appl. Clay Sci., 16: 161-170.

Mondal, K., M.N. Gupta, 2006. The ion exchange concept in bioseparation: evolving paradigms and expanding range of applications, Biomol. Eng., 23: 59-76.

Noeline, B.F., D.M. Manohar and T.S. Anirudhan, 2005. "Kinetic and equilibrium modelling of lead(II) sorption from water and wastewater by polymerized banana stem in a batch reactor", Separation and Purification Technology, 45: 131-140.

Rivera-Utrilla, J., I. Bautista-Toledo, M.A. Ferro-Gracia and C. Moreno-Castila, 2001. "Activated carbon surface modification by adsorption of bacteria and their effect on aqueous lead adsorption", J. Chem. Technol. Biotechnol., 76: 1209-1215.

Salerno, P., 2002. Mendioroz S. Preparation of Al-pillared montmorillonite from concentrated dispersions. Appl Clay Sci., 22: 115-23.

Shawabkeh, R., A. Al-Harahsheh, M. Hami and A. Khlaifat, 2004. "Conversion of oil shale ash into zeolite for cadmium and lead removal from wastewater" Fuel, 83: 981-985.

Shentu, J., J. Wu, W. Song, Z. Jia, 2005. 'Chitosan microspheres as immobilized dye ion exchange support for catalase adsorption, Int. J. Biol. Macromol., 37: 42-46.

Sudarshan, N.R., D.G. Hoover, D. Knorr, 1992. Food Biotechnol., 6: 257-272.

Standard Test Method for Nitrogen Content (Kjeldah), ASTM D2868 – 07. 53.

Taty-Costodes, V., H. Fauduet, C. Porte and A. Delacroix, 2003. "Removal of Cd(II) and Pb(II) ions, from aqueous solutions, by adsorption onto saw dust of *Pinus sylvestris*", J. Hazard. Mater, B105: 121-142.

Tsai, G.J., W.H. Su, 1999. J. Food. Protect, 62: 239-243.

Valverde, J.L., P. Sanchez, F. Dorado, C.B. Molina, A. Romero, 2002. Influence of the synthesis conditions on the preparation of titanium ethoxide as the pillaring agent. Micropor Mesopor Mater, 54: 155-65.

Wang, C.C., L.C. Juang, T.C. Hsu, C.K. Lee, J.F. Lee, F.C. Huang, 2004. 'Adsorption of basic dyes onto montmorillonite, J. Colloids Interf. Sci., 273: 80-86.

Wang, L. and A. Wang, 2007. Journal of Hazardous Materials, 147: 979-985.

Wang, S.F., L. Shen, Y.J. Tong, L. Chen, I.Y. Phang, P.Q. Lim, T.X. Liu, 2005. Polymer Degradation and Stability, 90: 123-131.

Zcan, A.S.O and A.O. Zcan, 2004. 'Adsorption of acid dyes from aqueous solutions onto acid-activated bentonite', J. Colloids Interf. Sci., 276: 39-46.

Zhao, Z.P., Z. Wang, N. Ye, S.C. Wang, 2002. 'A novel *N,O*-carboxymethyl amphoteric chitosan/poly(ethersulfone) composite MF membrane and its charged characteristics', Desalination, 144: 35-39.