Synthesis and characterization of cationic chitosan nanoparticles as coagulant in water treatment

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Abstract:

This work deals with the synthesis of soluble cationic chitosan having a tertiary amino group by the reaction of chitosan with 3-chloro2-hydroxy triethyl ammonium chloride (CTAC). Factors affecting the nitrogen content of the produced aminated chitosan mainly: reaction temperature, time and sodium hydroxide concentration besides the mL added of (CTAC) were studied and discussed briefly. It was concluded that higher nitrogen content of chitosan derivative is obtained at reaction temperature $60^{o}C$ for 90 minute. Moreover increasing the liquor ratio for chitosan and (CTAC) was accompanied by an increase in nitrogen content to a limiting value. The product obtained was characterized by FT-IR, Thermogravimetric analysis TGA, and elemental analysis (C,H,N,S).The structure of product nanoparticles was investigated by transmission electron microscopy(TEM). The adsorption efficiency of chitosan derivative for metal ions in water as Cu, Ni, Fe ,and V was determined using atomic absorption spectrometry (AAS) .It was found that the quantity and the type of incorporated functional groups ,as well as, the type of metal ions and contaminants ,play an important role in the efficiency of aminated chitosan toward water clarification.

Keywords: Aminated chitosan, chitosan nanoparticles, water treatment.

1-INTRODUCTION

Chitosan is fishery by products having an economical value for the industry [*Budiyanto ., 1993.*]. Chitosan is the most biopolymers often found in nature after cellulose [*Patil et al.,*

2000]. This biopolymer is a compound resulting from deacetylation of chitin, composed of units of N-acetyl glucosamine and N-glucosamine. The presence of reactive amino groups at C-2 atom and the hydroxy groups at C-3 and C-6 on chitosan are useful in a wide application in various industries such as pharmaceuticals, biochemistry, biotechnology, cosmetic, biomedical, paper industry, food and textile industries, and others.

 Beside that chitosan can be used as an emulsifier, coagulant, chelating agent, and thickener emulsion. In addition, chitosan can also be used as a substitute for some industries that serves a relatively safe for consumption [*Muzzarelli R.,1985*]. Application of chitin and chitosan in various fields is determined by the characterization of quality including the deacetylation degree, solubility, viscosity, and molecular weight. Quality of chitosan is mainly determined by the degree of deacetylation, where the deacetylation degree is depending on materials and conditions process such as concentration of alkali solution, temperature, and time [*Suhardi , 1993*)].

Because of chitosan hydrophilicity, biodegradability, harmlessness for living things and ease of chemical derivatization, besides, chitosan has many amino and hydroxyl groups that can chelate heavy metal ions, chitosan presents a very promising starting material for chelating resins [*Argun, (2008)., Qiu, and., Zheng.,(2009)., Bedelean et al., (2006). , Shukla et al, (2009). , Hu et al., (2011). ,and M.Monier., (2012)*].

In this investigation, we wish to report on the conversion of chitosan produced from waste materials into economically valuable products which can be used as coagulants in water treatment.

2-EXPERIMENTAL

2-1 -Chemicals:

Chitosan was supplied by Fluka , triethylamine LR (M.W 101.19) was supplied by s.d.fine-chem Ltd POICHA (R)391 340. , Epichlorohydrin (99%) was supplied by MERCK-Schuchardt. Other chemicals of laboratory grade were used.

2-2-Preparation of quaternary ammonium salt:

Quaternary ammonium salt was prepared according to a previously reported procedure [Guo. Naini et.al, *2009*] by using triethyleamine , HCl and epichlorohydrin .

2- 3-Preparation of chitosan derivatives:

Chitosan husks have been grind into a fine powder then sieved in 200μm sieve. 2 g of chitosan powder were dissolved in 200ml acetic acid (1%) and stirred at room temperature for 24 hour. Then 6 portions of 10 ml from the dissolved chitosan were separated. The previously prepared (CTAC) was added in different amounts (1 to 6 ml) to the chitosan solutions drop by drop with continuous stirring in stoppered flasks. The mixture was poured into (10 ml) dilute aqueous NaOH solution with different concentration then heated at different temperature, time on a water-bath . Next, after cooling the reaction mixture to room temperature, the solutions pH was adjusted to 7 using conc. HCl. The resulting substituted chitosan quaternized derivative remained dissolved in the aqueous reaction medium and the reaction solution appeared colorless (in most cases) and clear. The produced chitosan derivative was precipitated by acetone. The precipitated products were then filtered twice, washed with an excess of acetone. The products were dried at 60°C in vacuum oven for 24h.

2-4- Analyses:

2-4-1. Elemental analysis:

Carbon, hydrogen, sulphur and nitrogen contents of chitosan specimens were determined using CE instruments EA 110 CHNS-O Elemental Analyzer.

2-4-2.Thermogravimetric Analysis (TGA):

Thermogravimetric analysis of the chitosan and its derivatives were performed with. Thermogravimetric determenator Leco: Mac-500.ST.Joseph, Michigan-USA. This apparatus provides a continuous measurement of sample weight at a range of temperatures between   ambient   and   600 ^{0}C. The samples were heated in an alumina cell to 600 ^{0}C at heating rate of 10^0C/min with nitrogen as the circulating gas.

2-4 -3.IR measurements:

The spectra of chitosan and chitosan/(CTAC) samples (in the forms of KBr disks) were obtained using an IR Instrument (JASCO FTIR 4110, Japan) with a frequency range of 4000-400 cm^{-1}.

2-4-4. Determination of degree of deacetylation of chitosan by IR spectroscopy:

It was calculated using the equation proposed by Roberts that is given below **:**

$$DD = 100 - [(A\ 1665/A3450) \times 100/1.33].$$

Where: DD = Degree of Deacetylation .

A = Absorbance

2-4-5.Morphology and structure characterization of nanoparticle:

The morphology and size distribution of nanoparticles were examined by TEM (CM 100 TEM) at an accelerating voltage of 40 kV. Samples were prepared by depositing a drop of chitosan nanoparticle suspension on a copper grid fitted with a carbon support film and dried under vacuum.

2-4-6.Turbidity of water:

Turbidity of water was measured in nephelometric turbidity units (NTU) using Helige digital direct reading turbidmiter .

2-4-7.The organic matter in water:

Organic matter expressed was determined by consumption of $KMnO_4$ in acidic solution.

2-4-8. Determination of metal ions in water:

The metal ions in water were quantified using Atomic Absorption Spectrometer, Solaar S-4 S- Series Thermo Electron Corporation UK.

2-4-9. The removal efficiency:

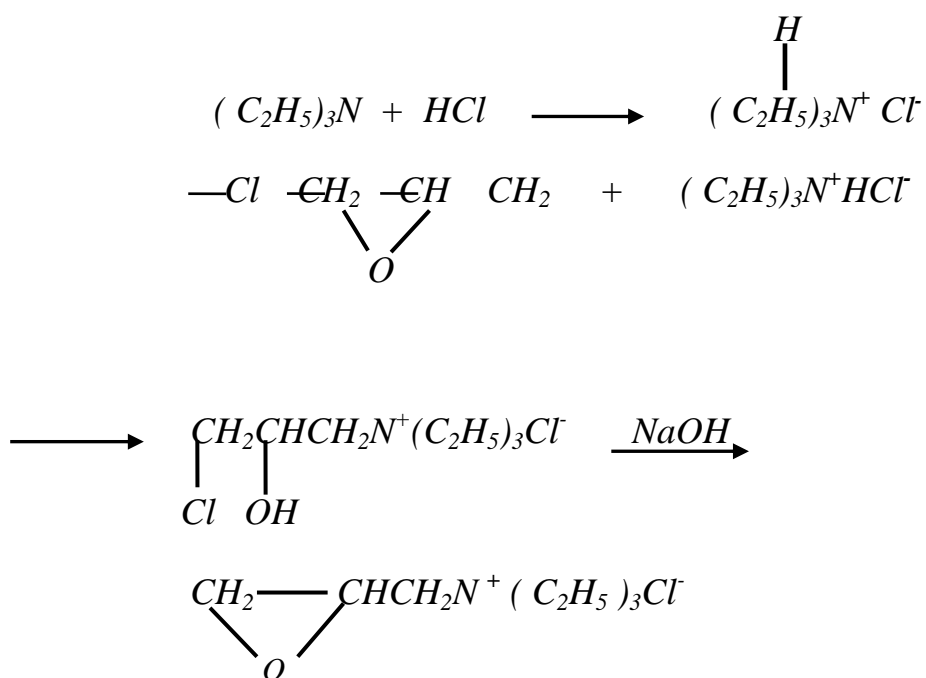% Removal of metal ions, organic matter and turbidity were calculated from the following formula:

$$\%Removal = C_0 \text{-} C / C_0 \times 100$$

Where, C_0 and C = metal ion concentration, organic matter and turbidity of wastewater before and after coagulation treatment by chitosan derivative, respectively.

3- RESULTS AND DISCUSSION

In the last few decades, considerable attention had been devoted to find new water treatment technologies using natural materials. These natural materials are usually nontoxic, environmentally friendly and of low cost. This investigation represents a contribution in this trend. Chitin and chitosan are two abundant, naturally occurring and eco-friendly materials. Thus, a cationic chitosan was prepared by reacting 3-chloro-2-hydroxy triethylammonium chloride (CTAC) with chitosan. The product was prepared as nanoparticles, and its efficiency as coagulants for water clarification and as adsorbent for some heavy metal ions was studied.

The formation of the quaternary ammonium salt and its reaction with chitosan are represented by the following schemes 1, 2, 3 .

Scheme (1)

$$(C_2H_5)_3N \ + \ HCl \longrightarrow (C_2\overset{\overset{\displaystyle H}{|}}{H}_5)_3N^+ \ Cl^-$$

$$-Cl \ -\!\!\!CH_2 \ -\!\!\!CH \ \ CH_2 \ + \ (C_2H_5)_3N^+HCl^-$$
$$\diagdown \diagup$$
$$O$$

$$\longrightarrow \underset{\underset{\displaystyle Cl \ \ OH}{|\quad|}}{CH_2CHCH_2}N^+(C_2H_5)_3Cl^- \xrightarrow{\ NaOH\ }$$

$$CH_2\!\!-\!\!\!-\!\!CHCH_2N^+ (C_2H_5)_3Cl^-$$
$$\diagdown \diagup$$
$$O$$

It is believed that the reaction of CTAC with chitosan might occur by any of the following two pathways (a or b) (Schemes 2 and 3) .
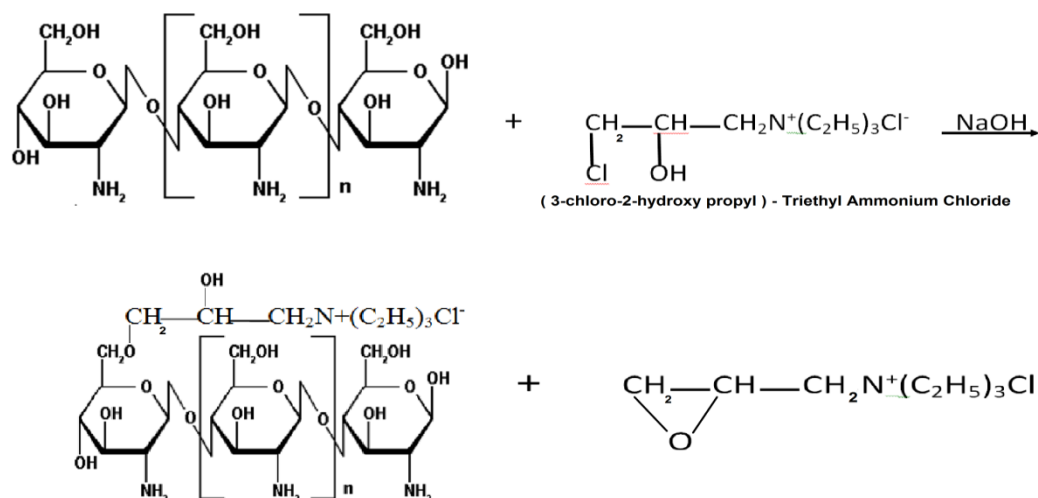
Pathway (a):

Under alkaline condition, (CTAC) readily generates the corresponding epoxide, which reacts with the free primary amino groups of chitosan in nucleophilic addition pathway resulting into quaternization, of the substrate.
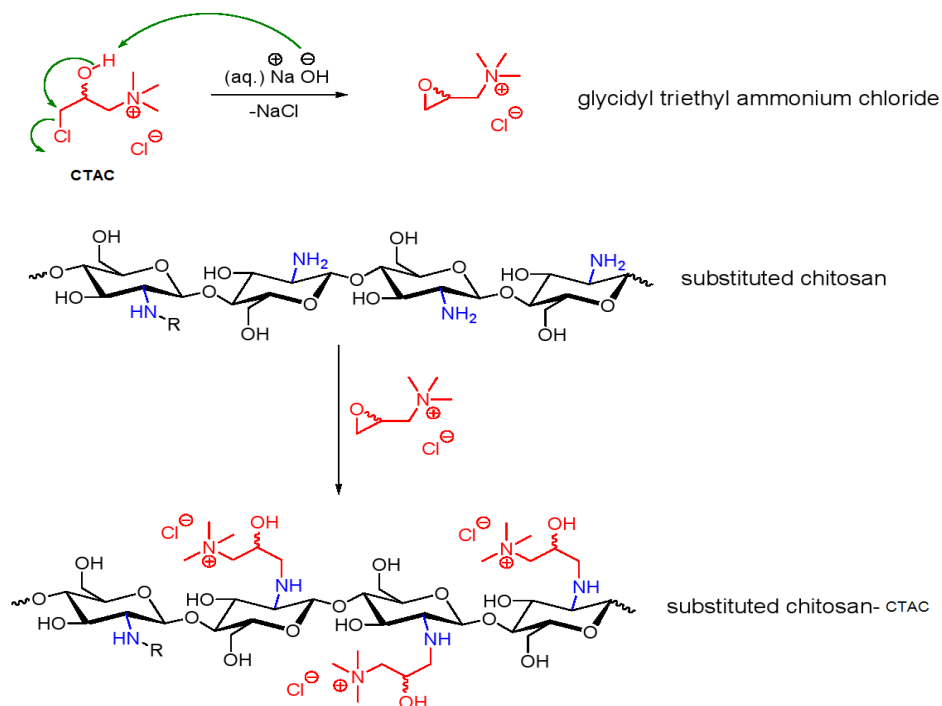
Pathway (b):

Also under the same condition chitosan amino ether can be obtained.

3.1- Degree of deacetylation of chitosan:

The degree of deacetylation of chitosan was determined using the equation proposed by [Roberts 1992] that is given below :

$$DD=100-[(A\ 1665/A3450) \times 100/1.33].$$

Where A1665 and A3450 are the absorbance at $1655cm^{-1}$ of the amide group band as a measure of the N-acetyl group content and $3450cm^{-1}$ of the hydroxyl band as an internal standard for differences in chitosan concentration powder form. The factor '1.33' denotes the value of the ratio of A 1665/A3450 for fully N-acetylated chitosan. It was assumed that the value of this ratio was zero for fully deacetylated chitosan .The degree of deacetylation of the chitosan working materials was calculated by using figure (1) ,according to the above mentioned equation ,the results indicate that, DD = (67.6%).
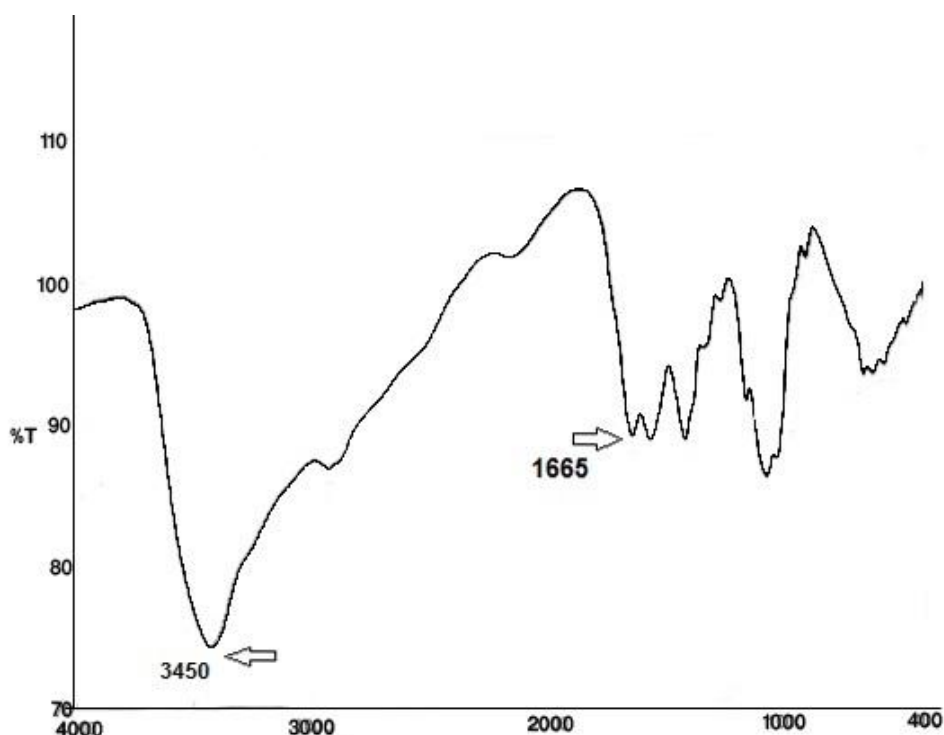
Fig.1- illustrates the FT-IR of chitosan**:**

3-2.Factors affecting reaction of chitosan with (CTAC):

The factors affecting reaction of chitosan with (CTAC) such as: temperature, duration time, sodium hydroxide concentration, as well as, Chitosan/(CTAC) ratio have been studied in order to discover the optimum conditions for reaction yield as nitrogen content .

3.2.1. Effect of temperature:

Figure. (2), shows the effect of temperature on the nitrogen content of the chitosan derivatives at 1ml (CTCA) , 20min and 10% NaOH. It is clear that the nitrogen content increases as the temperature is raised up to 60°C. This may be attributed to the fact that, by raising the temperature the swelling of chitosan granules decreased [*Esam. 2008]*, so that the penetration of the amine through the chitosan granules will be easier. Also the mobility of the ions and the molecules increases by raising the temperature of the solution and, hence, the probability of their collisions increases resulting in an increment in the rate of reaction.

Nevertheless, at a low temperature the viscosity of the solution increases leading to a decrease in the penetration power of the (CTCA) through the chitosan, which becomes more viscous resulting in a decrease in the reaction rate which in turn gives less nitrogen content in the aminated chitosan produced.
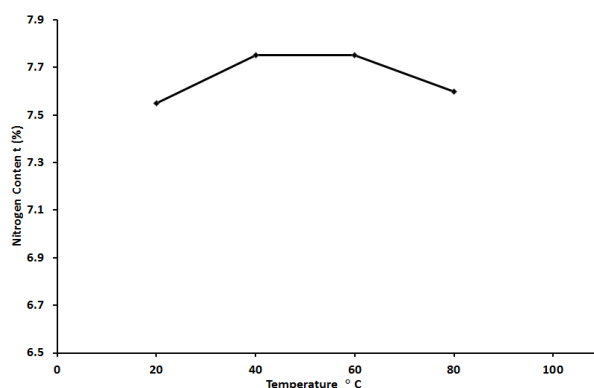

Figure (2): Effect of the temperature on the Nitrogen Content (%) of Chitosan derivative

3.2.2 Effect of Reaction time:

The results represented in Fig. 3 show the effect of the reaction time on the efficiency of the reacting products as nitrogen content at1ml (CTCA), and 10% NaOH . It is found that, at the chosen temperature (60 ^{0}C), the increase of the reaction time from 20 to 120 min is accompanied by an increment in the reaction rate, and that, after 90 min, the increase is so small. It may be concluded that longer reaction time leads to an increase of the reactivity of the ions and molecules in the reacting medium; hence, the activation reaction power is increased leading to greater nitrogen contents in the aminated chitosan obtained.
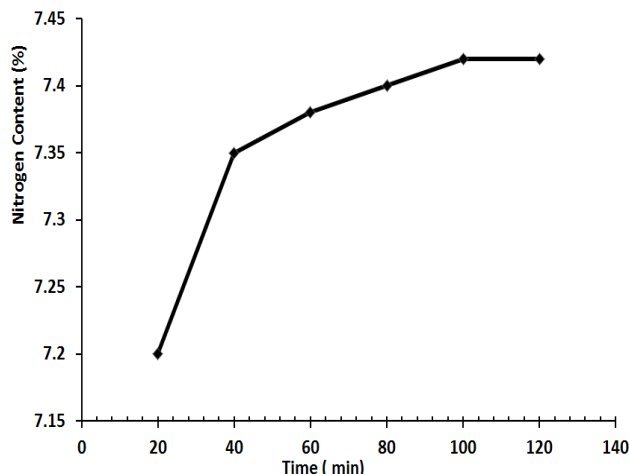
Fig.3- Effect of the Time on the Nitrogen Content (%) of Chitosan derivatives

3.2.3 Effect of sodium hydroxide concentration:

Fig. 4 represents the effect of NaOH concentration on nitrogen content in the product. For the best working conditions of temperature (60°C) , reaction time (100 min) and 1ml (CTCA), different concentrations of NaOH ranging from 10-60% were examined. It is clear that, by increasing the NaOH concentration till 40%, the nitrogen content increases to a maximum value indicating that this concentration is sufficient for complete neutralization of chitosan and amine hydrochloride and swelling of the chitosan granules. Further increase of NaOH concentration caused no increase in the nitrogen content. This may be attributed to the fact that excess of the alkali in the reacting medium caused formation of a gelatinized layer around the chitosan granules, hence, preventing the penetration of the amine to react with it.
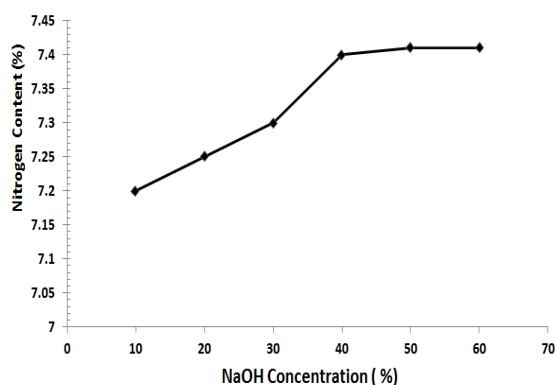
Fig.4- Effect of the NaOH on the Nitrogen Content (%) of Chitosan derivatives

3.2.4 Effect of (CTAC) mL added:

Different milliliters of (CTAC) namely (1, 2, 3, 4, 5, and 6 ml) were examined at the aforementioned chosen conditions. The obtained results are represented in Figure (5) which indicates that by increasing the amount of (CTAC), the reaction product increases till 5 ml.
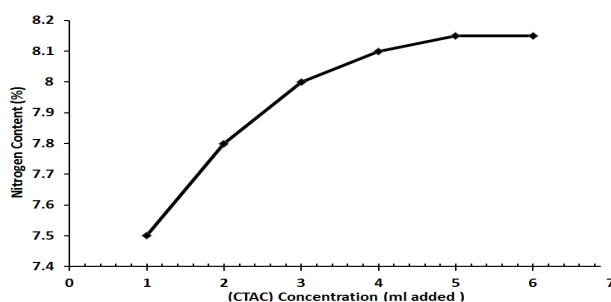
Fig.5- Effect of the CTAC Concentration on the Nitrogen Content of Chitosan/(CTAC).

3-3- Elemental analysis of chitosan with different (CTAC) concentration at optimum condition:

The elemental analysis data of the produced chitosan derivatives are collected in the following table:

Table (1) the elemental analysis of chitosan and its derivatives.

Composition(%)	C	H	N	S	C/N
Chitosan	47.97	8.17	7.21	0.42	6.4
Chitosan/1ml (CTAC)	53.65	9.6	7.52	0.45	6.9
Chitosan/2ml (CTAC)	56.72	9.81	7.85	0.46	7.2
Chitosan/3ml (CTAC)	58.64	9.91	8.00	0.47	7.3
Chitosan/4ml (CTAC)	59.44	9.98	8.1	0.48	7.3
Chitosan/5ml (CTAC)	60.11	10.12	8.15	0.48	7.4
Chitosan/6ml (CTAC)	60.15	10.21	8.15	0.48	7.4

3-4- FT-IR of the produced chitosan/(CTAC):

Figure (6) show the FT-IR spectra of (a) Chitosan blank, (b) Chitosan /1ml(CTAC), (c)Chitosan/3ml(CTAC) and (d) Chitosan/6ml(CTAC). In the spectra of unsubstituted chitosan the absorption band at 1600cm^{-1}is due to characteristic N-H bending vibration for chitosan (*Shin, Y., and Yoo ,ID. 1998*). On the other hand, the IR spectra for each of substituted chitosan show disappearance of N-H band gradually by increasing (CTAC) and with presence of new other characteristic bands. This manifest the proceeding of substitution reaction on the primary amino group of chitosan. The presence of medium band appeared at 1383 cm^{-1} could be attributed to symmetric stretching for C-N group. This absorption band confirms the presence of (CTAC). Nevertheless the absorption broad band at 3430cm^{-1} due to stretched -OH groups and 1060 cm^{-1}for stretched C-O groups were decreased by increasing

(CTAC) this finding indicates that most of substitution occurred at the –OH groups of C-3 or C-6.
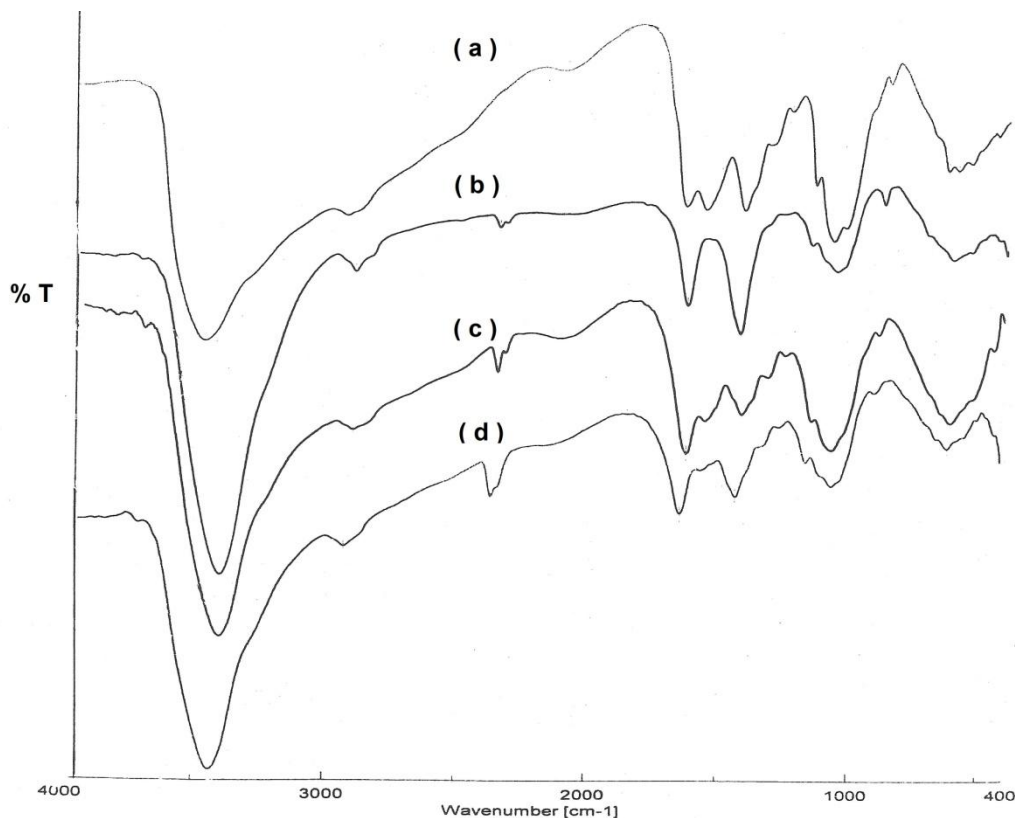

Fig.6- FTIR of (a) chitosan, (b) chitosan/1ml (CTAC),

(c) chitosan/3ml (CTAC), (d) chitosan/5ml (CTAC)

3.2.7-Thermogravimetric analysis (TGA):

TGA is a simple and accurate method for studying the decomposition pattern and the thermal stability of the polymers. Figures (7) show the thermo- gravimetric analysis (TGA) for chitosan (a), chitosan/3mL(CTCA) (b), and chitosan/6mL(CTCA)(c) respectively. As it evident, the spectrum for chitosan(a) figure (7) shows three characteristic stages for decomposition. The first stage starts at 46°C and end at 95°C with a weight loss of 6.3%. This could be recognized as due to the moisture content of chitosan. The second stage which related to the main decomposition of chitosan macromolecules occurs in one step of decomposition start at 246 °C and end at 307 °C with maximum decomposition temperature

of 283 °C and weight loss of 45%. The third stage of decomposition is related to the carbonization process which occurs up to 600 °C with a weight loss of 17.5%.

For the two studied chitosan derivatives (b) and (c) the first stage starts at 46 °C and end at 100 °C with weight loss of 15%. The second stage of decomposition, representing the main decomposition, proceeds in two steps. The first step starts at 210 °C and end at 310 °C. The weight loss in this step for the studied samples was 28 and 33% respectively. This step of degradation simulates and corresponds to main degradation process recorded for chitosan .This step, most probably, could be related to the degradation of chitosan/(CTAC). The third stage of degradation occurs up to 600 °C with weight loss of 9 and16 % for the two samples respectively.
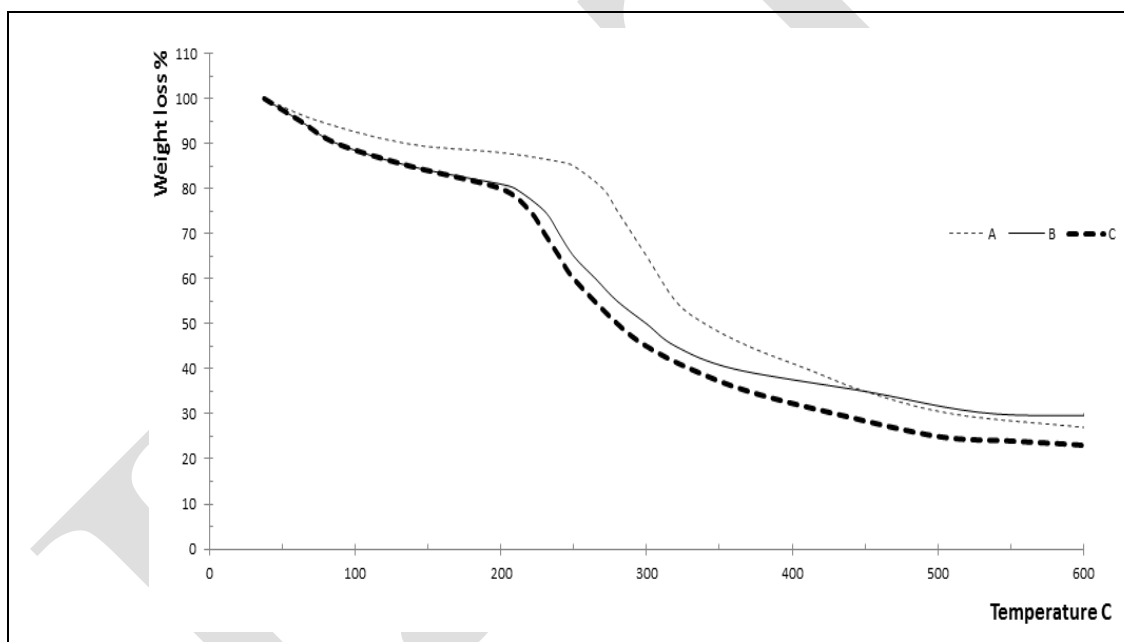

Fig.7- TGA Of (a) Chitosan, (b) Chitosan/3ml (CTAC) , and (c) Chitosan/6ml (CTAC).

3.2.8-Characterization of chitosan nanoparticles

Figure (8) shows an exemplary TEM image of chitosan and chitosan/(CTAC) nanoparticles at two magnifications. TEM images, showed that chitosan derivatives were in the Nano size.
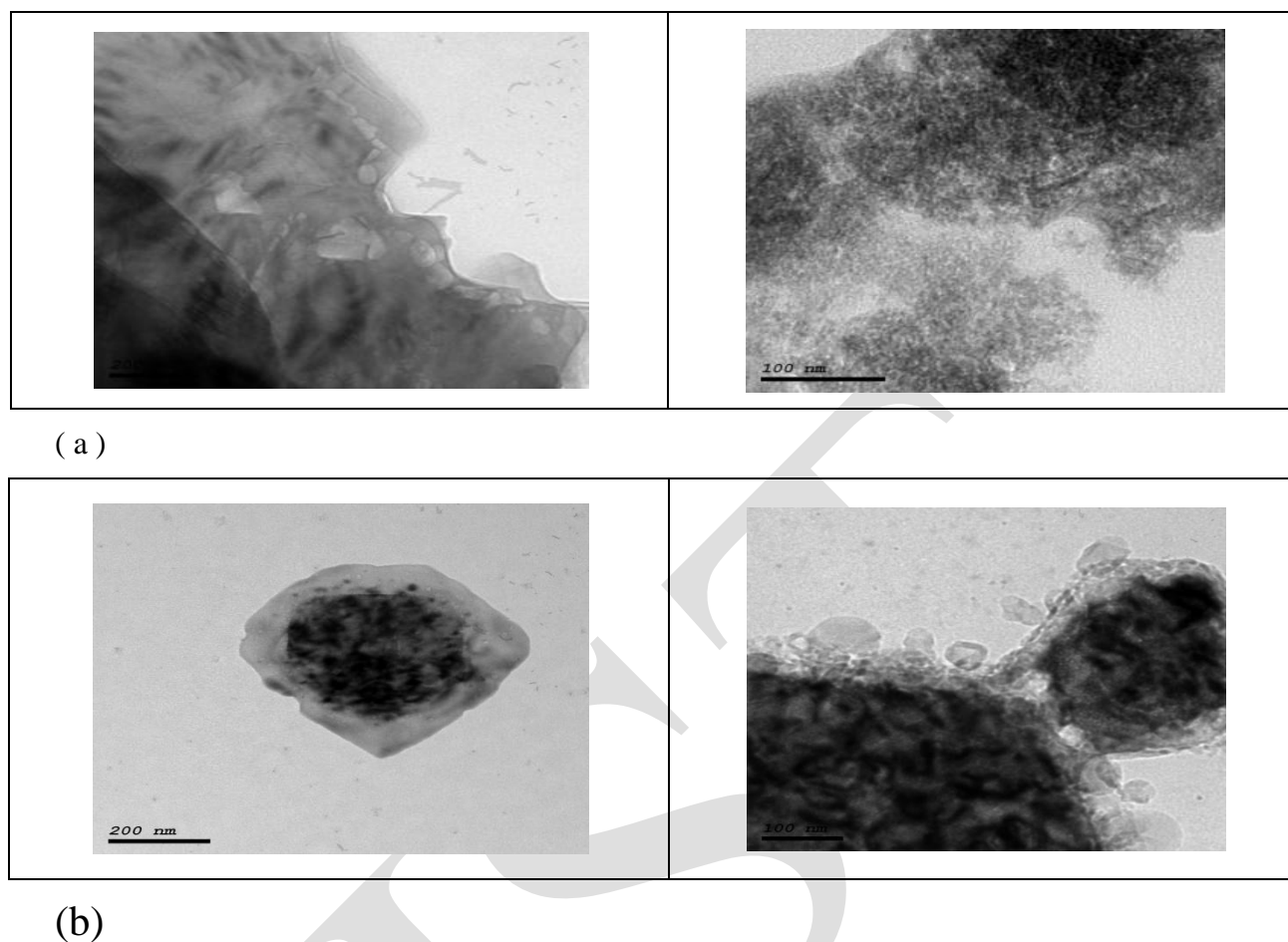
(a)

(b)

Fig.8- Transmission electron micrograph(TEM) in two magnification of

(a) Chitosan and (b) Chitosan derivative nanoparticles.

3-3. Metal ions removal from water by chitosan derivatives:

Industrial effluents may contain a mixture of heavy metal ions, and therefore, testing of an ion exchange is recommended for the obtained chitosan as ion exchanger. The adsorptions of four metal ions (Fe, Ni, Cu and V) by the prepared chitosan derivatives were studied.

To study the efficiency of the prepared chitosan derivative at optimum condition for the removal of metal ions, a sample of water containing each of (Fe, Cu, Ni, and V) ions was prepared . The effect of different chitosan derivatives concentrations (2, 4, 6, 8 ppm) on the

removal of these elements from water was studied. It is evident from figure (9) that chitosan derivative has high efficiency for removal of elements. For instance, as much as 99% of Fe ions and 95% of V ions were removed by using (8 ppm) of chitosan derivative, and 90% removal of Cu ions and 70 % of Ni ions .
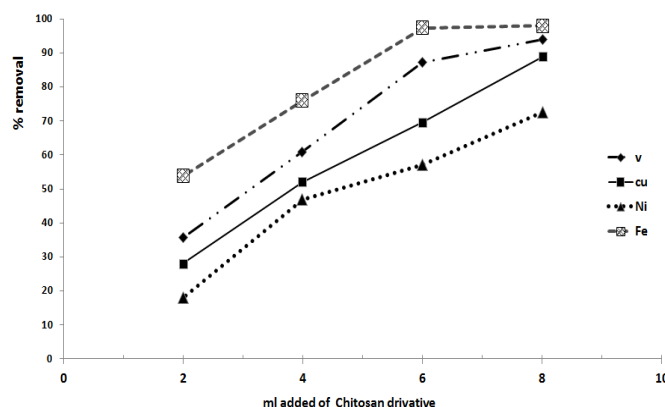

Fig.9-Effect of chitosan derivative as ml added for metal ion removal.

The above finding can be explained on the basis of:

3-3-1.Mechanism of metal ion chelation and coagulation:

It was observed that there is an increase in the pH during the experiments without pH control This may be attributed to swelling of chitosan, followed by the protonation of amino groups with water as proton donor (Argulló et al., 2004) , according to the following reaction :

$$\text{Chitosan–NH}_2 + \text{H}_2\text{O} \longrightarrow \text{'Chitosan–N}^+\text{H} + \text{OH}^-$$

In addition, chitosan acts as a weak base. At low pH, amino groups of chitosan take the protons available in aqueous solution, according to :

$$\text{Chitosan–NH}_2 + \text{H}^+ \longrightarrow \text{'Chitosan–NH}^{3+}$$

This reduces the concentration of H$^+$ ions and raises the pH of the solution .The low adsorption capacity in acidic solution can be explained by the competition between protons and metallic ions for available amino adsorption sites (Wan Ngah et al., 2004), and by

electrostatic repulsion (Jeon and Höll, 2003). The following relation shows the influence of pH on the uptake of metallic ions **:**

$$\text{Chitosan–NH}^{3+} + \text{M}^{2+} \rightleftharpoons \text{'Chitosan–NH}_2 – \text{M}^{2+} + \text{H}^+$$
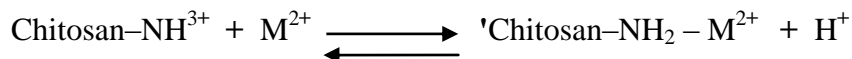
The equilibrium of this reaction is shifted to the left at higher H^+ ion concentrations (lower pH), reducing the number of binding sites for metallic ions. In addition, the protonation of amino groups in acidic solutions induces an electrostatic repulsion of metal cations that reduces the number of binding sites available for metallic ions. On the other hand, at higher pH, there is a decrease in H^+ ions in the solution and both the competition for binding sites and the electrostatic repulsion decrease and the adsorption performance is improved **.** The speciation and distribution of metal–ion species in the solution is an important parameter which determines the effect of pH on sorption capacity. Speciation at a given pH can affect adsorption performance, if this means the existence of species that cannot interact with the adsorbent. At near neutral pH, the cations Cu (II) is found as free ions in aqueous solutions and they can be adsorbed onto chitosan by chelation according to:

$$\text{Chitosan–NH}_2 + \text{M}^{2+} \rightarrow \text{'Chitosan–NH}_2 – \text{M}^{2+}$$
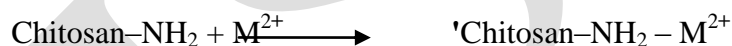
3-3-2- Interaction Mechanisms in Metal Sorption

It is widely known that chitosan can build complexes with certain metal ions such as Cu (II). Amino sites are the main reactive groups for metals ions, although hydroxyl groups (especially in the C-3 position) may contribute to sorption (Guibal, 2004). Adsorption, ion exchange, and chelation have been discussed as the mechanisms responsible for complex formation. The type of interaction depends on the composition of the solution, the pH of the solution, and the speciation of metal ions (Vold et al., 2003 and Rinaudo,2006).

3-3-3 .Chelation:

Most studies of the chelation mechanism have been focused on the adsorption of copper. Two coordination models have been proposed for chitosan-metal complexes. Based on an X-ray study of chitosan complexes with Cu(II) [Ogawa and Oka (1993)] proposed the "Pendant Model" in which the metal ion is attached to an amino group of the chitosan chain like a

pendant. This model was supported by Domard (1986). He suggested that $[Cu\,NH_2(OH)_2]^0$ is the only structure, where the complex is uncharged and has two OH and one NH_2 as ligands, and the fourth site could be occupied by an H_2O molecule or the OH on (C-3) (Figure 10).
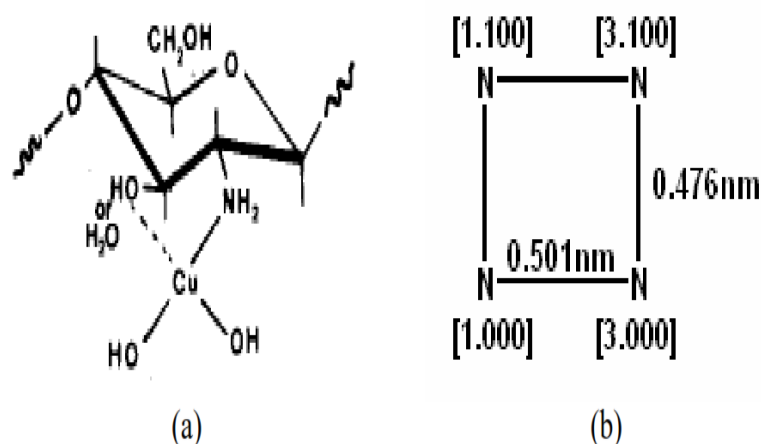

Figure (10) Coordination models of Cu(II)-Chitosan complex: (a) Pendant model and (b) Bridge model. (Schlick, 1985)The "bridge model" proposed by Schlick (1985) suggests that a metal ion such as cupric ion is coordinated with four nitrogen atoms of intra- and inter-chitosan chains forming a square-planar structure.

Figure (10) Proposed structures of Cu-Chitosan complex (a) at pH between 5.3 and 5.8 and (b) at pH > 5.8. (Rhazi et al., 2002) .On the other hand, Rhazi et al. (2002) proposed two types of complex for Cu(II)-chitosan which depend on the pH of the solution. At pH between 5.3–5.8, the complex ($[Cu(–NH_2)]^{2+}$, 2OH-, H_2O) is suggested; whereas at pH>5.8, the complex ($[Cu(–NH_2)_2]^{2+}$, 2OH-) is considered.

3-4. Organic matter and turbidity removal from water by chitosan derivatives:

   The protonation of amino groups in solution makes chitosan positively charged, and thereby very attractive for flocculation and different kinds of binding applications. Since most natural colloidal particles, including organic matter, bacteria and macromolecules, are negatively charged attractive electrostatic interactions may lead to flocculation. Besides chitosan derivatives possess positively charged groups due to the creative tertiary amino group. Therefore the chitosan derivative can remove organic matter and inorganic matter carrying negative charge from water. Using chitosan derivative in a dose of (2-8 ppm), the examined parameters revealed that chitosan derivative is able to remove turbidity, organic

matter and gives better water clarity. Figure (11) showes that the optimum dose that gave best result was (8 ppm).
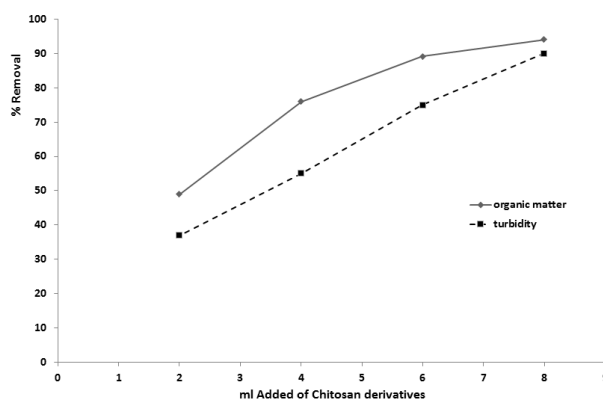

Figure (11) Effect of chitosan derivative doses on the removal of organic mater and turbidity of water

REFERENCES

1- Hu. X.-J., Wang .J.-S., Y.-G.Liu, X.Li, G.-M.Zeng, Z.-L.Bao, X.-X.Zeng, A.-W.Chena, F.Long, J.Hazard. Master. 185(2011)306-314.

2- Monier .M.. Inter.J.Biol.Macromolecules 50 (2012) 773-781.

3- Zouhour, L., Salah, S., Saloua, S. and Amor, E.A. , Extraction and characterization of chitin and chitosan from crustacean by-products- biological and physicochemical properties: Afr.J.Biotechnology, 10 (4):(2010) 640-647.

4- Guo. Naini, Yang. Jianzhou, Yang.Lianl. "Synthesis of triethylamine based epoxychloropropyl triethyl ammonium chloride" J.Text.Res. 30 , No.(2), Feb. 2009

5- Qiu, W., Zheng, Y., J.Chem. Eng. 145(2009) 483-488.

6- Shukla, P.R., Wang, S., Ang, H.M., Tade, M.O., Adv. Powder Technol. 20(2009) 245-250.

7- *Argun, M.E., Hazard, J. Master 150(2008) 587-595.*

8- *Esam.A.E.''Study   of   the   parameter   affecting   the   viscocity   of chitosan''J,Chem.Soc.pak.,.**30** .(4),2008.*

9- *Bedelean, H., Stanca, M., Maicaneanu, A., Burcam, S., Geologia 52(2006) 43-49 .*

10- *Agulló E., R. Mato, C. Tapia, A. Heras, J.San Román, W. Argüelles, F. Goycoolea, A. Mayorga, J. Nakamatsu, and A. Pastor, (2004). "Quitina y Quitosano: Obtención, Caracterización y Aplicaciones", Pontificia Universidad Católica del Perú, Fondo Editorial 2004, pp. 244 – 245.Wan Ngah et al., 2004.*

11- *Guibal, E.. Interactions of metal ions with chitosan-based sorbents: A review. Separation and Purification Technology, 38:(2004) 43-74..*

12- *Jeon and Höll, Chemical modification of chitosan and equilibrium study for mercury ion removal.Water Res. 2003 Nov; 37(19):4770-80.*

13- *Patil R.   S.,   Chormade V.,   Des-phandeM.   V., Chitinolytic   enzymes: An exploration. Enzyme Microb Technol 26: (2000) 473-483.*

14- *Shin, Y., and Yoo ,ID. Journal of Applied Polymer Science.67,1515-1521.1998*

15- *Domard, A., , Some physicochemical and structural basis for applicability of chitin and chitosan, 2nd Asia Pacific chitin Symposium, Bangkok, Nov.9:1-11, 1996 .*

16- *Budiyanto   D.,   1993 Teknologi   Khitin   dan   Khitosan.   Direktorat   Jenderal Perikanan, Balai Bimbingan dan Pengujian Mutu Perikanan, Jakarta.*

17- *Ogawa .K ,   Oka .K ,and Toshifumi. Y; 'X-ray study of chitosan-transition metal complexes' Chem. Mater., 5 (5), (1993)pp 726–728.*

18- *Suhardi Khitin dan Kitosan. Buku Monograf, Pusat Antar Universitas Pangan dan Gizi UGM, Yogyakarta, 1993 ,272-278.*

19- *ROBERTS, George A.F. Chitin Chemistry. London, MacMillan Press, 1992. 350 p. ISBN 0-33-352417-9.*

20- *Muzzarelli R. A. A. , 1985 Chitin. In :The polysaccharides. Aspinall G. O. (ed.), Academic Press, New York pp. 417– 450.*

21- *Schlick, S. "Binding Sites of Cu^{2+} in Chitin and Chitosan. An Electron Spin Resonant Study". Macromolecules,. 19: (1985) 192–195.*