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FULL LENGTH ARTICLE

Extraction of chitosan, characterisation and its use for water purification

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Abstract This study was carried out in the Food Science Department, Agriculture College, Basrah University to investigate the effect of different chitosan concentrations on drinking water quality. The studied parameters were turbidity, TDS, electrical conductivity and pH. The results showed that the turbidity, TDS, electrical conductivity and pH have been decreased with the increase of chitosan concentration. When chitosan concentration increased from 0 to 1 g 100 ml⁻¹, the turbidity, TDS, electrical conductivity and pH were decreased from 1.98 to 0.98 NTU, 5.67 to 4.13 g L⁻¹, 10.18 to 5.27 mS cm⁻¹, 6.1 to 5.71 respectively. The linear equations have represented the relationship between all parameters and chitosan concentration. However, the total bacteria count, total coliform bacteria, *Staphylococci*, Fecal coliform bacteria and *Vibrio* spp. have been eliminated completely by using Chitosan concentration of 0.8, 0.4, 0.8, 0.2 and 0.2 g 100 ml⁻¹ respectively.

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1. Introduction

World Health Organization (WHO) (2007) announced that 1.1 billion people lack access to an improved drinking water supply, 88% of the 4 billion annual cases of diarrheal disease are attributed to unsafe water and inadequate sanitation and hygiene, and 1.8 million people die from diarrhoeal diseases each year (Piyali, 2013). The adsorption process has also received much attention and has become one of the more popular methods for the removal of heavy metal ions and

microbial contaminants from water, because of its competitive and effective process. Numerous adsorbents have been reported for the removal of toxic metal ions, such as chitin, Chitosan, cellulose and Guarana, which are not only eco-friendly and cost effective but are also effective in the remediation of common effluents present in wastewater (Chooaksorn and Nitorisavut, 2015). Water purification plants throughout the world use chitosan to remove oils, grease, heavy metals and the fine particulate matter that cause turbidity in waste water streams (Hennen, 1996).

Chitosan is a biomaterial, primarily produced from the alkaline deacetylation (40–50% NaOH) of chitin where this N-deacetylation is almost never complete. The chitosan is considered as a partially N-deacetylated derivative of chitin. It is an abundant natural biopolymer obtained from the exoskeletons of crustaceans and arthropods which is a non toxic copolymer consisting of β-(1,4)-2-acetamido-2-deoxy-D-glucose and β-(1,4)-2-amino-2-deoxy-D-glucose units. Each

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glucosamine unit contains a free amino group, and these groups can take on a positive charge which gives amazing properties of chitosan, its useful in a wide application in various industries such as pharmaceuticals, biochemistry, biotechnology, cosmetic, biomedical, paper industry, food and textile industries and others (Muzzarelli, 1985). These biopolymers offer a wide range of unique applications including bioconversion for the production of value-added food products, preservation of foods from microbial deterioration, formation of biodegradable films, recovery of waste material from food processing discards, purification of water and clarification and de-acidification of fruit juices (Shahidi et al., 1999; Abd and Niamah, 2012; Luo and Wang, 2013).

Access to safe drinking water is important as a health and development issue at national, regional and local levels; therefore, the present investigation was carried out to study the effectiveness of Chitosan for improving the quality of drinking water by the removal of metal contents and microbial contaminants.

2. Material and method

2.1. Samples collection

The raw water was obtained from the Shatt al-Arab River at Basrah city in Iraq. The shrimp (*Penaeus semisulcatus*) shells were purchased from local markets and used for the isolation of Chitosan.

2.2. Preparation of Chitosan solution

At the preconditioning stage, shrimp shells were washed thoroughly with water and dried to remove excess water. Then dried shells were demineralized using 1N HCL (1:15 w/v) at ambient temperature (approximately 30 °C) for 6 h. The residue was washed with distilled water until pH reached to 6.5–7 then the residue was dried.

After that the demineralized shrimp shells were deproteinized using 3.5% NaOH solution (1:10 w/v) at 65 °C for 2 h and Decoloration was done with NaOCl (0.315%). Then residue was washed thoroughly with water, followed by distilled water until the pH reached in the range of 6.5–7.5. The chitin was dried and ground and screened. The chitin obtained from the above process was deacetylated in 50% NaOH (1:10 w/v) for 5 h at 100 °C. After deacetylation, the chitosan was washed thoroughly with water, followed by distilled water even the pH reached between at 6.5 and 7.5 (Ocloo et al., 2011).

Chitosan powder (0–1 g) was accurately weighed into a glass beaker, mixed with 5 ml of 1% acetic acid solution (in the same of water sample), and kept aside for about 30 min to dissolve. It was then diluted to 100 ml with distilled water and stirred for 1 h at 25 °C. Six samples of 100 ml raw water were placed into six beakers (250 ml), and different concentrations of chitosan (0, 0.2, 0.4, 0.6, 0.8 and 1 g 100 ml⁻¹ water) were added under stirring (100 rpm).

2.3. Chemical analysis of chitosan

The different chemical and functional properties were measured as per the standard methods, Moisture content was determined by the standard method, and Moisture of samples

was determined by drying the samples at 60 °C for 24 h or until the weights were constant. It was then calculated by percentage of weight loss compared to the initial weight of the samples. Yield was determined by comparing weight measurements of the raw material and of the chitosan obtained after treatment. Ash content determination was performed by transferring the samples into a muffle furnace at 550 °C until it turned white and free of carbon. The sample was then removed from the furnace, cooled in a desiccator to a room temperature and reweighed immediately. The weight of the residual ash was then calculated by nitrogen contents (micro Kjeldahl method) and fat (Accurately weighed moisture free sample was taken in a thimble plugged with cotton and extracted with petroleum ether in a Soxhlet apparatus for about 10 h at a condensation rate of 5–6 drops per second.) of chitosan were measured according to a previously described procedure (AOAC, 1990).

2.4. Water quality parameters

2.4.1. Chemical tests

- pH was measured by using pH meter (Sartorius/Germany) with combined glass electrode after calibrated by using buffer solutions of pH 4.0 and pH 7.0 (AOAC, 1990).
- Electrical Conductivity (EC) and Total dissolved solids (TDS) were determined using 4510 conductivity meter (Jenway) (Hp Technical Assistance, 1999).
- Turbidity was measured using Turbidimeter (a Lovibond, TurbiDirect) the sample of which was filled into a sample cell and put into the cell holder for measurement (APHA, 2005).

2.4.2. Microbial tests

- Peptone water: 1 g peptone dissolved in 1000 ml distilled water and used to dilute the water samples (APHA, 2005).
- Alkaline Peptone Water: Alkaline peptone water was used as an enrichment medium in the isolation of *Vibrio* spp. isolation weighing 15 g peptone, 10 g sodium chloride and 20 g sodium citrate dissolved in 1000 ml distilled water, final pH: 8.6 (Lesmana et al., 1985).
- Culture media: Nutrient agar (Hi-media, India) for total aerobic bacteria count. MacConkey agar (Hi-media, India) for total coliform bacteria count. Eosin methylene blue agar (Oxoid, England) for fecal coliform bacteria count, Thiosulfate citrate bile salt agar (LAB, UK) for *Vibrio* spp. count, and Mannitol salt agar (Hi-media, India) for Staphylococci count (Barrow and Feltham, 2003).
- Numbers of bacteria count: Pour plate method was used to number the bacteria count. 1 ml of last dilutions transferred into a Petri dish and culture media poured after that incubation at 35 °C for 24–48 h (Harley and Prescott, 2002).

3. Results and discussion

The chemical composition of prepared chitosan from shrimp shells with yield reached 12.93% which, was lower than that reported by Hossain and Iqbal (2014) who reported 15.40% yield from shrimp waste. This reduction might be due to

depolymerization of the chitosan polymer, loss of sample mass/weight from excessive removal of acetyl groups from the polymer during deacetylation and loss of chitosan particles during washing. However the yields depended on the chitosan extraction method, Table 1 shows that moisture content was 7.84%, and in spite of drying process transaction, presence of moisture attributed to the chitosan has high ability to absorb the moisture. These results have agreed with Alishahi et al. (2011) who stated that the commercial chitosan contains moisture less than 10%, but nitrogen ratio was 6.92%. Presence of this ration was due to covalent bond force that connective of protein with the chitin and chitosan resulting in a constant complex is difficult (Kim, 2004). On the other hand, the ash reached 0.75% that indicates the efficiency of salt removal, as well as ash content in high quality chitosan must not increase on 1% (No and Meyers, 1995). Also, Table 1 shows that the fat ratio was reduced (0.47%) and this is attributed to alkaline treatment to the chitin that led to a reduced fat ratio.

Fig. 1 illustrates the relationship between turbidity (NTU) and chitosan concentration ($\text{g } 100 \text{ ml}^{-1}$), as well as turbidity reduction percentage. The turbidity was decreased with increasing chitosan concentration. When chitosan concentration increased from 0 to $1 \text{ g } 100 \text{ ml}^{-1}$, the turbidity had decreased from 1.98 to 0.98 NTU. This is because the chitosan is a bio multi polymer, has a positive charge and contains free amine groups which give high capability for chemical relevance with molecules that have negative charges such as proteins, fats and mineral ions (Shahidi et al., 1999). The amino groups presented in chitosan also make a good chelating ligand capable of strongly binding to a variety of metal cations, and the lone pairs of electrons on the nitrogen atoms and oxygen atoms are donated to the metal ion to form coordinate bonds, since several amino groups and hydroxyl groups are present on the long polymeric chain, the chain can wrap around the metal ion and adopt configurations such as several amino groups that are bonded to the metal atom at the same time; this type of chelation leads to the formation of very stable metal complexes, and this property makes them useful for concentration of metals removal of radioactive and other harmful heavy metal contaminants (Bassi et al., 2000). On the other hand, the relationship between turbidity and chitosan concentration was linear (first order) and coefficient of determination (R^2) was 0.9569 as illustrated in the following equation:

$$T = -1.0529\varphi + 1.9448 \quad (1)$$

where T is the turbidity (NTU), and φ is the chitosan concentration ($\text{g } 100 \text{ ml}^{-1}$).

Table 1 The chemical composition of prepared chitosan from shrimp shells.

Characteristics	Composition (%)
Moisture	7.84
Total nitrogen	6.92
Ash	0.75
Fat	0.47

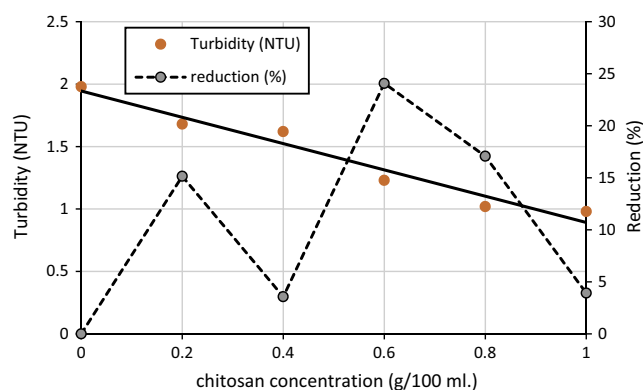


Figure 1 Changing turbidity of water with varied chitosan concentrations.

These results agreed with EAS (1999) who stated that the turbidity of drinking water must be less than 1 NTU. Increasing turbidity leads to increase the water treatment cost which used to drink and food processing (MPCA, 2008). Turbidity reduction percentage has been changed with increasing chitosan concentration as shown in Fig. 1. When chitosan concentration increased from 0 to $0.2 \text{ g } 100 \text{ ml}^{-1}$, the turbidity reduction percentage increased from 0 to 15.15%. The maximum reduction has been reached 24.07% at chitosan concentration of $0.6 \text{ g } 100 \text{ ml}^{-1}$. On the other hand, the total reduction reached 50.5%.

It can be seen from Fig. 2 that TDS (g L^{-1}) was decreased with increase of chitosan concentration ($\text{g } 100 \text{ ml}^{-1}$). When chitosan concentration was 0, 0.2 and $1 \text{ g } 100 \text{ ml}^{-1}$, the TDS reached 5.67, 12.87 and 4.13 g L^{-1} respectively. Moreover, the limit of TDS in drinking water is less than 500 mg L^{-1} (WHO, 2004). The mechanism of sorption of metals with chitosan in suspension is not well defined, but there are many theories as far as the structure of chitosan-metal ion (TDS) complex is concerned. One theory is that, two or more amino groups bind to the same metal ion: the bridge model. According to the bridge model, inter or intramolecular complexation

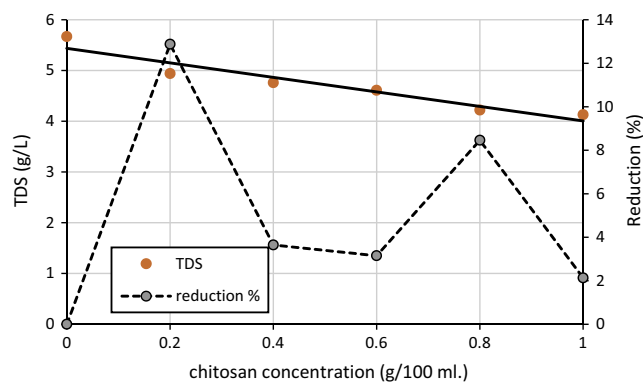


Figure 2 Changing TDS of water with varied chitosan concentrations.

may occur between the metal ion and amine groups from the same or different chains, some other experiments suggest that only one amino group is involved in the binding and the metal ion is bound to the amino group like a pendant; called the pendant model (Vold et al., 2003). Nevertheless, most of the authors agree to consider the formation of a complex between the amino groups of chitosan and the metal ions. However, the relationship between them is linear (first order) with determination coefficient of 0.916 according to Eq. (2):

$$\text{TDS} = -1.43\varphi + 5.4367 \quad (2)$$

where unit of TDS is g L^{-1} .

The TDS reduction percentage has been changed with increasing chitosan concentration. For example, when chitosan concentrations were 0.2, 0.4, 0.6, 0.8 and 1 g 100 ml^{-1} , the TDS reduction percentage values were 12.87%, 3.64%, 3.15%, 8.45% and 2.13% respectively. As a result, the total reduction reached 27.16%.

Influence of varied chitosan concentrations on the electrical conductivity of water, as well as the electrical conductivity reduction percentage is presented in Fig. 3. Results revealed that the electrical conductivity of water has been decreased with increasing chitosan concentrations because of the chelation between chitosan and salts as a result to sedimentation of salts then separate via filtration, this process led to decrease the electrical conductivity. When chitosan concentration was 0, 0.6 and 1 g 100 ml^{-1} , the electrical conductivity of water was 10.18, 7.84 and 5.27 mS cm^{-1} respectively. The empirical equation is of the first order (linear equation) with determination coefficient of 0.964, and the electrical conductivity of water is given by the following empirical formula:

$$\text{EC} = -4.51\varphi + 10.043 \quad (3)$$

where EC is the electrical conductivity (mS cm^{-1}).

When chitosan concentrations were 0.2, 0.4, 0.6, 0.8 and 1 g 100 ml^{-1} , the electrical conductivity reduction percentage values were 14.44%, 5.51%, 4.73%, 17.09% and 18.92% respectively. As a result, the total reduction reached 48.23%. The highest values of conductivity indicate more salinity because of more dissolved solid presence (Samee et al., 2007).

Changing pH of water with varied chitosan concentrations, in addition to pH reduction percentage is given in Fig. 4. pH of water has been decreased with increasing chitosan concentra-

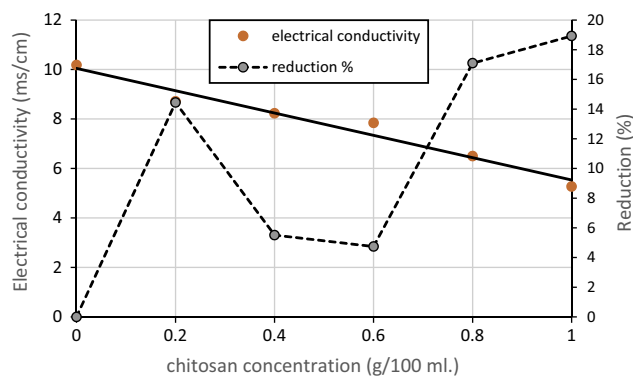


Figure 3 Changing electrical conductivity of water with varied chitosan concentrations.

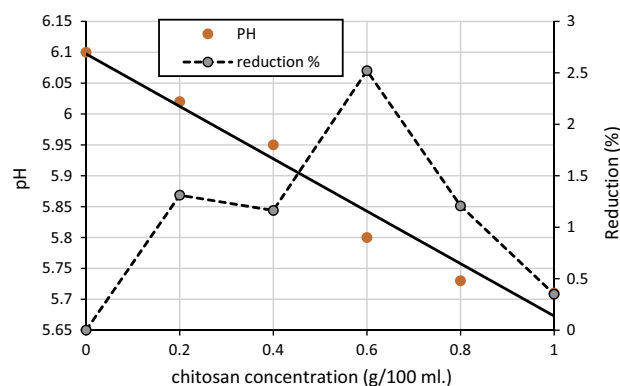


Figure 4 Changing pH of water with varied chitosan concentrations.

tion. When chitosan concentrations were 0.0, 0.2, 0.4, 0.6, 0.8 and 1 g 100 ml^{-1} , the pH of water reached 6.1, 6.02, 5.95, 5.8, 5.73 and 5.71 respectively. pH of water in this study was less than Iraqi standard specification and WHO because the chitosan sediments alkaline salts then separate via filtration as results the pH reduced or may be the reason for this relatively important decrease just related to the initial acid used to solubilize chitosan (Chitosan was found to be soluble in nearly all monovalent and multivalent acids, $\text{pH} < 6.5$). The total reduction percentage of pH reached 27.16%. Eq. (4) describes the relationship between pH of water and chitosan concentration was linear, and the determination coefficient reached 0.9652 as follows:

$$\text{pH} = -6.0971\varphi + 0.4243 \quad (4)$$

The pH range of 6.5–8.5 is acceptable for drinking water, and pH indicates alkalinity or water acidity. Values below 6.5 cause corrosive, while the values above 8.5 give the water of a bitter taste (AHS, 2011).

Table 2 shows the numbers of bacteria in Shatt al-Arab water samples with and without chitosan. The total bacteria count of Shatt al-Arab water sample treated with chitosan was 33×10^5 CFU ml^{-1} , but it decreased after chitosan addition. For example, when adding 0.8 g of chitosan 100 ml^{-1} of water, the numbers of bacteria have been eliminated completely. The fecal coliform bacteria and *Vibrio* spp. were significantly affected by chitosan addition, so the numbers of bacteria did not appear after the addition of chitosan. Chitosan molecule has the ability to interact with bacterial surface and is adsorbed on the surface of the cells and stacks on the microbial cell surface and forming an impervious layer around the cell, leading to the block of the channels (Qin et al., 2006). These results agreed with (Lee et al., 2009; Abd and Niamah, 2012). *Staphylococci* didn't grow after the addition of 0.6 g of chitosan 100 ml^{-1} of water. Tavaría et al. (2012) used chitosan as an antibacterial against *Staphylococci* isolated from normal skin samples. The numbers of Gram negative (G^-) bacteria were most affected compared with Gram positive bacteria (G^+). The chitosan inhibits the bacteria during electrostatic reactions between amine group (NH^+) of chitosan and phosphoryl groups of phospholipids, which are found in the cell walls of bacteria (Bevilacqua et al., 2010). Generally, cell walls of G^- bacteria contain a highest fat ratio than cell walls of G^+ bacteria.

Table 2 Numbers of bacteria in samples of Shatt al-Arab water within chitosan.

Bacterial tests (CFU/ml)	Chitosan (g 100 ml ⁻¹)					
	Shatt al-Arab	0.2	0.4	0.6	0.8	1
Total bacteria count	33 × 10 ⁷	41 × 10 ⁵	69 × 10 ³	35 × 10 ³	Nil	Nil
Total coliform bacteria	43 × 10 ²	31 × 10 ²	Nil	Nil	Nil	Nil
Fecal coliform bacteria	55 × 10	Nil	Nil	Nil	Nil	Nil
<i>Vibrio</i> spp.	46 × 10 ²	Nil	Nil	Nil	Nil	Nil
Staphylococci	32 × 10 ³	99 × 10 ²	63 × 10 ²	12 × 10 ²	Nil	Nil

4. Conclusion

In conclusion, chitosan was successfully synthesized from shrimp shells available in Basrah City. According to the results, increasing concentration of chitosan in drinking water led to decrease turbidity, TDS, electrical conductivity and pH. As well as, the chitosan had sedimented all the salts and improved water quality. On the other hand, the relationship between chitosan concentration and each of turbidity, TDS, electrical conductivity and pH was linear, and the determination coefficient ranged between 0.916 and 0.965. Moreover, the effect of chitosan on the G- bacteria was higher than that on G⁺ bacteria. The unique properties of chitosan made it an exciting and promising agent for using it in the purifying water.

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