Adsorption of allura red dye by cross-linked chitosan from shrimp waste
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ABSTRACT
The present study was designed to evaluate the chitosan, which has been obtained by deacetylation of chitin, as a biosorbent. The chitin was isolated from fermented shrimp waste by an important local industrial food biopolymer. The aim of this work was the characterization of chitosan and preparation of cross-linked chitosan- tripolyphosphate (chitosan-TPP) beads for the removal of allura red food dye from aqueous solutions. Conditions of batch adsorption such as pH, time and adsorbent dose were examined. The effectiveness of cross-linked chitosan beads for dye removal was found to be higher for pH 2 (98%, percentage of dye removal) and tends to decrease at pHs of 3 to 11 (up to 49%). The values of percentage removal show that the adsorption capacity increases with time of contact and dosage of chitosan-TPP, but red dye adsorption is mainly influenced by pH level. The cross-linked chitosan-TPP beads can significantly adsorb allura red monoazo dye from aqueous solutions even at acidic pHs unlike raw chitosan beads that tend to dissolve in acidic solutions. Consequently, this modified chitosan has characteristics that allow minimization of environmental pollution and widening the valorization of shrimp waste.

Key words | chitosan, cross-linked, food dye, Red No. 40, removal, wastewater

INTRODUCTION
Synthetic dyestuffs are widely used as colouring agents in a variety of products such as textiles, paper, leather, gasoline and foodstuff. The world manufactures over $7 \times 10^5$ metric tons of approximately 10,000 synthetic dyes and pigments annually, nevertheless 10% of which may be found in wastewater (Cervantes et al. 2001; Wawrzkiewicz & Hubicki 2009). There have been discussions about the toxicity and health risks of synthetic dyes. These risks are usually associated with handling techniques and time of exposure to the dyes. Most of these questions have been associated with the azo colours with respect to hypersensitive reactions as well as with the natural and toxicological significance of reactions between colours and food components during processing and storage. In such conditions, azo dyes undergo fading, giving rise to colourless and coloured subsidiary products whose toxic properties may not be known (Martítt et al. 2010).

Allura red belongs to the monoazo class of synthetic food colourants and is used in dyeing sweets, jellies, juices, jams, drugs and cosmetics. Chemically it is identified as Red No. 40 and this compound is disodium 6-hydroxy-5-(2 methoxy-5-methyl-4-sulphophenylazo)-2-naphthalenesulphonate. There are studies that have examined its adverse health effects (Piccin et al. 2009).

The effluents generated by the food industry are capable of altering the physico-chemical and biological characteristics of water sources. It is known that wastewaters containing dyes are very difficult to treat, as the dyes are recalcitrant molecules (particularly azo dyes), are resistant to aerobic digestion, and are stable in oxidizing agents (Crini & Badot 2008; Salem et al. 2009). Due to the toxicity of azo dyes and the carcinogenicity of their degradation products, the removal of colour from wastewater effluents becomes environmentally important. Various physical, chemical and biological treatment methods are available to remove dye from wastewaters. All of these methods have many limitations and involve complicated procedures that are economically unfeasible. To find an effective and ideal adsorbent, researchers have exploited many low cost,
biodegradable substitutes obtained from natural resources (Dos Santos et al. 2005; Gupta & Suhas 2009).

Chitosan is a natural polymer derived from chitin with intrinsic characteristics that make it an effective and low-cost biosorbent for the removal of colour compared with commercial activated carbon. Chitosan is obtained on an industrial scale by deacetylation from chitin isolated from shellfish waste (López-Cervantes et al. 2007). However, raw chitosan tends to present some disadvantages, such as unsatisfactory mechanical properties and poor heat resistance. Another important limitation is the solubility of the raw material in acidic media. One method to overcome these problems is to chemically modify the polymer by a cross-linking method (Crini & Badot 2008).

Chitosan beads can be cross-linked with glutaraldehyde, epichlorohydrin, ethylene glycol diglycidyl ether and sodium tripolyphosphate. However, epichlorohydrin and ethylene glycol diglycidyl ether are physiologically toxic agents, while tripolyphosphate is considered as a non-toxic material. After the cross-linking, these materials maintain their properties and original characteristics, particularly their adsorption capacity (Aksu et al. 2008).

Some studies have been performed to remove food colourants, including allura red, from aqueous solutions using physico-chemical methods that include ion exchange, an electrochemical membrane and advanced oxidation. The biological methods to remove colourants include bacterial and fungal bio-absorption. However, these technologies are usually costly and are not easily adapted (Crini & Badot 2008; Gupta & Suhas 2009). Some studies have been conducted to evaluate the adsorption of dyes in wastewater using low cost adsorbents; however cross-linking chitosan-TPP beads has not been used. Therefore, the objectives of this study were to characterize chitosan isolated from shrimp waste and to evaluate the efficiency of the application of chitosan-tripolyphosphate beads as an adsorbent for the removal of allura red food dye from aqueous solutions. The adsorption of dye by batch experiments was performed and the resulting behaviour in colour removal was measured and expressed as colour removal percentage.

**MATERIALS AND METHODS**

Characterization and production of chitosan

The shrimp industry generates waste in the Gulf of Mexico region that does not have adequate management and accumulates in source waters and the subfloor. To mitigate the environmental pollution that this causes, the waste has been treated by lactic fermentation to obtain chitin (López-Cervantes et al. 2010) and chitosan later. Now, there is an industry plant that processes the shrimp waste in South Sonora, México.

Chitosan production involves the demineralization, deproteinization and deacetylation of chitin following the methods of Tsai et al. (2002) and Beaney et al. (2005); with some modifications. In the demineralization step, chitin and 1 M HCl solution (1:10 w/v) were mechanically stirred for 4 h at room temperature. In the deproteinization step, chitin and a 4.5% NaOH solution (1:15 w/v) were mechanically stirred at 65 °C in an oil bath for 4h. Then in the deacetylation step, chitin and a 45% NaOH solution were mechanically stirred at 110 °C in an oil bath for 90 min. After each step, the samples were washed with distilled water to obtain neutrality.

Chitosan flakes were characterized to check some important aspects concerning purity. Briefly, the degree of deacetylation was determined by the UV spectrophotometry method of Liu et al. (2006). The total quantity of nitrogen was measured by the Kjeldhal method and the viscosity of chitosan was measured with a viscometer (DV-E Brookfield, Middleboro, MA, USA). The observed characteristics of chitosan obtained were compared with commercial standard chitosan (Sigma-Aldrich, Milwaukee, WI, USA). Chitosan flakes were tested for purity concerns.

**Preparation of cross-linking chitosan beads**

In the preparation of cross-linking chitosan tripolyphosphate (chitosan-TPP) beads, the Chiou & Chuang (2006) method was followed with some modifications. 2 g of chitosan flakes were dissolved in 100 mL of an aqueous 1.5% (v/v) of acetic acid solution. The chitosan solution was allowed to stand 24 h and then filtered. The chitosan solution was added drop by drop from a pipette into an aqueous solution of sodium tripolyphosphate (1 wt%), which formed rigid beads. After setting for 12 h, the cross-linked chitosan beads were washed with distilled water and were stored in distilled water.

**Dye**

The adsorption capacity was tested using synthetic food dye, strawberry red E370L from the DEIMAN Company, Mexico. The structure of the dye is displayed in Figure 1 (Wawrzkiewicz & Hubicki 2009). This monoazo dye is commercial grade and was used without purification. An exact
amount of dye volume was diluted in distilled water to prepare a dye stock solution (0.04% v/v). The maximum adsorption wavelength for the dye solution was determined by running full range wavelength scans using a GENESYS 10uv Scanning spectrophotometer (Thermo Scientific, Madison, Wisconsin, USA).

**Experiments of allura red dye adsorption**

Adsorption studies were performed with the batch method. The pH level, dosage of cross-linked chitosan, and contact time were varied to observe influences on the adsorption capacity.

**pH variation**

Duplicate tests were conducted to observe the influence of pH on the adsorption of allura red by wet chitosan-TPP beads. The pH values ranged from 2–11. Coloured stock solution was placed in 50 mL flasks and the required pH was adjusted with diluted or concentrated HCl and NaOH solution. An equal mass of chitosan-TPP was added to each flask and mechanically stirred for 15 min.

At the end of the predetermined time interval, supernatant aliquots were separated by decantation. The dye adsorption was determined by spectrophotometry at the respective $\lambda_{max}$ value, which is 498 nm. After measuring the dye adsorption, the data obtained from the tests were used to calculate the percentage of residual colour. $R$ (%) was calculated by comparing the adsorption ($A$) of the treated sample with reference to the sample without adsorbent:

$$R = \frac{A(\text{sample})}{A(\text{reference})} \times 100$$  \hspace{1cm} (1)

The percentage of dye removal, Rem (%), was calculated as follows:

$$\text{Rem} = 100 - R \ (%)$$  \hspace{1cm} (2)

**Amount of cross-linked chitosan beads**

Several samples of wet chitosan-TPP beads equivalent to 0.1, 0.3, 0.5, 0.7, 1.0 g of dry beads were shaken mechanically with 50 mL of corresponding dye solution for 15 min at room temperature. Dye solutions of five different pH values (3, 5, 7, 9 and 11) were used. At the end of the predetermined time interval, the data obtained from the procedure were used to calculate the Rem (%).

**Contact time**

Coloured solution stock was placed in 50 mL flasks and the required pH was adjusted. A constant mass of chitosan-TPP was added to each flask and mechanically stirred for 15–120 min. At the end of the 15 min time interval, the data obtained from the test were used to calculate the Rem (%).

**Statistical analysis**

All data presented are means ± standard deviations. The statistical significance of differences between means was estimated by Microsoft Office Excel program. In the figures, data represent the average from duplicate determinations and error bars indicate standard deviation.

**RESULTS AND DISCUSSION**

There are published studies discussing the biopolymers, such as chitosan, in the adsorption of dyes in aqueous solutions. These biosorbents and their derivatives contain a variety of functional groups that can chelate and form complexes with dyes; the effectiveness of adsorption by chitosan is determined by the structure of the dye. The most common way of obtaining adsorption results of the dye solution is through observation before and after the adsorption experiments. These experimental results indicate affinity with the dye molecules and the adsorbent (Crini & Badot 2008; Piccin et al. 2009). But, hitherto there have been few available studies about the adsorption of allura red dye by derivatives of chitosan as an adsorbent, which was prepared from shrimp waste.

The characteristics of chitosan, obtained by the deacetylation process of chitin, are shown in Table 1. Due to the ease of sample preparation, the analytical method of Liu et al. (2006) was applied for the determination of the degree of deacetylation of chitosan. This method was...
standardized and is used for the routine analysis of chitosan obtained in the laboratory. The degree of deacetylation of chitosan is similar to that reported by Sigma-Aldrich for commercial standard chitosan (deacetylation degree, 84%). These values are considered appropriate to produce beads of chitosan and match those values reported by several researchers: Chiou & Li (2003), Crini & Badot (2008) and Piccin et al. (2009). The degree of deacetylation of chitosan indicates an increase in the proportion of amino groups, which were able to be protonated to improve the adsorption of dyes.

The values of humidity, nitrogen and ash found in the chitosan are similar to those reported by Mármol et al. (2009) and Hidalgo et al. (2009), 6.74% humidity, 6.84% nitrogen and values of less than 1% ash. The average value of the viscosity is within the range reported by Mármol et al. (2006) (311–5,110 cp). But there are other values reported as such 34–661 cp by Youn et al. (2007) and 3.1–372.7 cp by Pochanavanich & Suntornsuk (2002). These differences in the characteristics of chitosan can be attributed to the source (crustaceans or fungi), methods of obtaining used and the concentration of chitosan solution.

Conditions, for cross-linking chitosan bead synthesis, were optimized by using various concentrations of chitosan solution (1, 1.5, 2, 2.5, 3% w/v). The beads were dropped into aqueous solution of sodium tripolyphosphate with a fixed concentration (1 wt%). The 2% chitosan solution (w/v) dropped into tripolyphosphate was found to be the best. The resulting beads showed improved mechanical strength for use in adsorption testing. Similar results were reported by Chiou & Li (2003), Chiou & Chuang (2006) and Ngah & Fatinathan (2006).

The beads of chitosan cross-linked with tripolyphosphate contained 93.2% moisture with an average diameter of 2.5 mm. The wet beads are white and acquire a brown colour when dried. The cross-linking of chitosan chains with groups and non-toxic polyvalent counter ions of tripolyphosphate is applied to improve structural strength and prevent the dissolution in acid. The positively charged amino groups of chitosan and negatively charged counter ions of tripolyphosphate improved the mechanical properties of the cross-linked chitosan beads and the time required to form beads decreased from 24 h with other cross-linking agents to 12 h with sodium tripolyphosphate. This effect was also observed by Mi et al. (1999) and Chiou & Li (2003).

The pH of the dye stock solution had a value of 5.4. However, different pH values (ranging from 2 to 11) were tested to determine how pH affected the dye adsorption capacity of chitosan-TPP. The amount of the beads used in the experiments was 0.1 g dry beads and equivalent to 1.46 g of wet beads. Figure 2 shows an adsorption test with the batch method. The highest colour removal (98%) was from a pH 2 due to ionic attraction between the positive charged dye and the negative charge of the chitosan-TPP complex. The chitosan-TPP beads at pH 8.6 are in a coacervation phase so that the dye molecules may be attracted by the hydroxyl (OH−) groups as tripolyphosphoric ion and the NH2 groups which are present in the solution. The red dye removal decrease for the pH in the range of 3–11 varies from 53 to 49%. Similar results for active carbon adsorption indicate that pH 2.5 to 3.5 was optimal for the discolouration of food dye (Mi et al. 1999; Ozsoy & van Leeuwen 2010).

### Table 1 | Characteristics of chitosan from shrimp chitin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample Mean ± SD</th>
<th>Average Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humidity (%)</td>
<td>5.57 ± 0.05</td>
<td>5.63 ± 0.05</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.24 ± 0.02</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>Viscosity (cp)</td>
<td>1,059 ± 10.69</td>
<td>1,047.33 ± 10.69</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>6.34 ± 0.30</td>
<td>6.20 ± 0.30</td>
</tr>
<tr>
<td>Degree of deacetylation (%)</td>
<td>82.97 ± 1.16</td>
<td>81.68 ± 1.16</td>
</tr>
</tbody>
</table>

Means ± standard deviations, n = 3.
Adsorption tests by the sequential method were carried out with the standard solution coloured with allura red with pH 3, 7, and 11. These preliminary tests were started with batch method conditions, and the treatments were repeated sequentially with new cross-linked chitosan beads. The time for each treatment was 15 min and was repeated four times. The adsorbent beads were removed by decanting between treatments. The initial adsorption and final adsorption of each batch was determined spectrophotometrically. With the sequential method the maximum colour removal was at pH 3 (94%), and for pH 7 and pH 11 was 65 and 63%, respectively. These results indicate that the percentage of colour removal tends to increase with the sequential method compared to the results of removal found in the batch adsorption method. Again, it was documented that acidic pH is more successful.

To study the effect of adsorbent dosage on dye removal, various amounts of wet cross-linked chitosan beads (1.46–14.6 g) equivalent to dry beads (0.1–1.0 g), were placed in a fixed initial dye concentration. Figure 3 shows the adsorption capacity expressed in Rem (%) of allura red at different dosages of chitosan-TPP beads. It is noted that the order of the percentage of dye removal (range 59 to 0.7%) was pH 3 > pH 9 > pH 5 > pH 7 > pH 11 at the lower amount 1.46 g of wet chitosan-TPP beads. From 4.38 to 10.22 g of wet beads, the order of removal (higher removal 68%) was pH 9 > pH 3 > pH 5 > pH 11 > pH 7 and with 14.6 g of wet beads, all pHs showed approximately the same percentage of removal (54%). The results illustrate that an increase in adsorbent dosage increases the removal. Similar behavior was reported by Singh et al. (2009), with an increase in the adsorbent dose, additional sorbent sites become available for adsorption, and hence, an increase the removal of dye molecules from the solution.

Figure 4 shows the effect of time on the adsorption behavior of chitosan-TPP beads, expressed in Rem (%). The order of removal efficiency at all time intervals (15–120 min) was pH 3 > pH 9 > pH 5 > pH 7 > pH 11. Also increased dye removal was noted when increasing contact time. The pH 3 had the highest adsorption rate reaching 98% removal in a contact time of 60 min. However, the samples with a pH of 5, 7, and 9 had a 51 and 68% removal rate, respectively, with a contact time of 120 min; while the pH 11 reached only 23% removal. This finding shows that the adsorption capacity of the food colouring with chitosan-TPP is strongly dependent on the pH of the solution, and the adsorption capacity increases significantly at pH 3. Similar effects of pH have also been observed in the adsorption of allura red (Red No. 40) by raw chitosan. In the experiments, it was observed that the ability of allura red dye to be adsorbed by the chitosan-TPP beads increases in pH 2 and 3 and decreases in pH 5, 7, 9 and 11, similar results were obtained by Chiou & Li (2003), Sakkayawong et al. (2005), Crini & Badot (2008) and Piccin et al. (2009).

CONCLUSIONS

The use of shrimp waste in the production of chitosan reduces the associated environmental pollution and could make the cross-linked chitosan-TPP beads economically attractive for the treatment of coloured effluents, compared to many physical-chemical treatments and the activated carbon. Unlike the raw chitosan that is soluble in acidic solutions, the cross-linked chitosan beads can be used in a wide range of pHs. In addition, the adsorption capacity increases with contact time and dosage of chitosan-TPP.

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