Synthesis of Novel Crosslinked Chitosans with a Higher Fatty Diacid Diglycidyl and Their Adsorption Abilities Towards Acid Dyes

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ABSTRACT: Novel chitosan-based adsorbent materials with a higher fatty diacid diglycidyl as the crosslinking agent were synthesized and the adsorption abilities of the resulting polymers evaluated towards typical acid dyes. The successful formation of a crosslinked structure was confirmed via infrared spectroscopic measurements and the solubility of the polymer towards 10% aqueous solutions of acetic and formic acids determined. At higher dye concentrations, the adsorption abilities of the crosslinked chitosan towards hydrophilic CI Acid Orange 7 and CI Acid Red 1 increased with decreasing degree of substitution. However, at lower dye concentrations, the crosslinked chitosan with the lowest degree of substitution exhibited the lowest adsorption capability. With such hydrophilic acid dyes, the extent of adsorption decreased significantly as the pH of the solution increased. On the other hand, CI Acid Red 138, which contains a dodecyl group in the chemical structure, was adsorbed to a considerable extent even at higher pH values, suggesting hydrophobic interaction between the alkyl group in the dye molecule and the hydrophobic crosslinker.

INTRODUCTION

Chitin is the main component in the exoskeletons of crustaceans and insects. The deacetylation product, chitosan, has been shown to be a polyfunctional polymer with biodegradable and biocompatible features. Taking advantage of these unique characteristics, various chemically modified chitosan materials have been applied in a variety of scientific and industrial fields such as cosmetics, foods, agriculture, medicines and fibres. One of the most important uses of chitosan is as a coagulant material in the wastewater-treatment process, where the chitosan should be crosslinked to improve its stability towards organic acids. Such crosslinking should improve the adsorptive ability and selectivity towards organic molecules significantly (Chitin and Chitosan Handbook 1995). Various types of bifunctional reagents such as diisocyanurate, diepoxy and dialdehyde (Shimizu et al. 1998) have been used as crosslinking reagents and the adsorption properties of these crosslinked chitosans towards amino acids, proteins and metal ions examined (Masri et al. 1978; Koyama and Taniguchi 1986; Kurita et al. 1986; Ohga et al. 1987; Inoue et al. 1988; Gao et al. 2002). In contrast to these extensive studies on the adsorption properties, a limited numbers of reports have been published regarding dye adsorption onto crosslinked chitosan materials. Examples include the adsorption behaviours of acid dyes and direct dyes onto chitin and chitosan (Maghami and Roberts 1988). We have recently synthesized a novel chitosan-based adsorbent with a higher fatty diacid diglycidyl as the crosslinking reagent, and demonstrated

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that the adsorbent exhibits excellent adsorption properties towards Cu\(^{2+}\) ions (Shimizu \textit{et al.} 2003) as well as providing a stationary phase for the separation of aromatic compounds in liquid chromatography (Saito \textit{et al.} 2002).

As an extension of our previous investigations (Shimizu \textit{et al.} 2003; Saito \textit{et al.} 2002), this paper describes the adsorption characteristics of the crosslinked chitosan towards typical acid dyes. The contribution of the long aliphatic chains in the crosslinking structure towards the adsorption behaviour was also investigated.

### EXPERIMENTAL

#### Materials

Chitosan (FM 80: degree of \(N\)-deacetylation = 85.0\%) was provided by the Koyo Chemical Co., Osaka, Japan. The crosslinking reagent, i.e. 7-ethyloctadecane diacid diglycidyl (EODD: epoxy equivalent = 294) was donated by Okamura Oil Mills Ltd., Osaka, Japan. These materials were used without further purification. Four dyes, CI Acid Orange 7, CI Acid Red 1, CI Acid Red 88 and CI Acid Red 138, were employed as typical acid dyes. The names of these dyes have been abbreviated below to Orange 7, Red 1, Red 88 and Red 138, respectively. The Robinson–Mills method was used to purify synthesized Orange 7 and the commercial product of Red 138. The other dyes were used without further purification. The chemical structures of the crosslinking agent and the dyes studied are shown in Schemes 1 and 2, respectively.

#### Crosslinking of chitosan

A known amount of chitosan (2.5 g) was first dissolved in 100 ml of a 5\% acetic acid solution and diluted further by the addition of 100 ml of methanol. A methanol solution containing EODD (100 ml) was then added dropwise to the solution and the resulting mixture stirred for 30 min at a temperature of 65 ± 5°C. This temperature was maintained for 20 h. Crosslinked chitosans having different degrees of crosslinking were obtained by adjusting the amount of EODD employed so that the epoxy/amino functionality ratio was between 0.5 and 2.0, preliminary experiments having indicated the time necessary for complete reaction. After completion of the reaction, the resulting solution was neutralized by the addition of a 5\% aqueous KOH solution and poured into 500 ml of acetone. The precipitate was filtered, sequentially washed with acetone and ether, and finally dried in vacuo for 48 h at 50°C. The resulting yellow or pale brown products were ground to powders with a mean diameter of 200–300 \(\mu\)m.

#### Adsorption of acid dyes on crosslinked chitosans

A known weight (0.015 g) of the crosslinked chitosan was added to 200 ml of a dye solution at the prescribed pH (obtained using a 0.1 mol/l buffered solution as the total electrolyte concentration),

![Scheme 1. Chemical structure of novel crosslinking agent employed.](image-url)
and the mixture stirred continuously either for 96 h at 30°C or 72 h at 40°C until equilibrium had been attained. The time necessary for equilibrium to be established was determined by preliminary experiments. After the adsorption process, a portion of the solution was poured into a test tube and centrifuged for 30 min at room temperature. The dye concentration of the supernatant liquid was determined colorimetrically via measurements made at the appropriate maximum wavelength, $\lambda_{\text{max}}$, in each case, the mean error in such adsorption measurements being ±3%.

RESULTS AND DISCUSSION

Characterization of crosslinked chitosan

The synthetic route to the target polymer is depicted in Scheme 3. The formation of a crosslinked structure was confirmed by measuring the differential IR spectrum between the original chitosan and that subjected to reaction with EODD. A Fourier-transform infrared spectrophotometer (FT-IR; model 430, JASCO, Tokyo, Japan) was used for this purpose. Figure 1 shows the differential IR spectrum thus obtained.

The new peak at 1742 cm$^{-1}$ in this spectrum may be assigned to the ester while the intensity of the peak at 1599 cm$^{-1}$ assigned to the amino group was less than that observed in the spectrum of the original material (not shown), thereby indicating the completion of a successful crosslinking reaction.
Scheme 3. Scheme for the synthesis of crosslinked chitosans.

Figure 1. Differential IR spectrum between CC-2 and the parent chitosan.
In addition, the chitosan treated with EODD was incapable of solution by formic acid or a 10% acetic acid solution, in contrast to the behaviour of the parent material which dissolved easily. These results demonstrate that the chitosan reacted successfully with EODD to form a crosslinked structure.

**Determination of the degree of substitution**

The degree of substitution (%) of the amino groups in chitosan reacting with the epoxy groups in EODD was determined from the C/N ratio, where C and N are the carbon content and nitrogen content, respectively, for the resulting crosslinked material. A Yanaco CHN Corder MT-5 (Yanagimoto Seisakusyo Co., Tokyo, Japan) was employed for such analytical determinations. Table 1 shows the results of these elemental analyses of the crosslinked chitosans and the degrees of substitution thereby determined.

**Adsorption of Orange 7 and Red 88 on crosslinked chitosans**

The adsorption behaviour of Orange 7 on three crosslinked chitosans (CC-1, CC-2 and CC-5) was measured in a buffer solution of pH 5 at either 30°C or 40°C. From the corresponding adsorption isotherms illustrated in Figure 2, it is obvious that the adsorption capabilities of crosslinked chitosans at higher dye concentrations decreased in the order: CC-5 > CC-2 > CC-1.

Decreasing the degree of substitution led to an increase in the adsorption ability. This is because an anionic dye such as Orange 7 binds to the protonated amino groups in the substrate, and hence the lower the degree of substitution of such groups the greater the adsorption of the dye. However, the adsorption capability of CC-5 was least at the lower dye concentration because this substrate contained unreacted amino groups that were fully hydrated. In addition, the molecular chains in the substrate were spread out due to repulsion between positive charges. Under these circumstances, the affinity of Orange 7 towards the substrate was quite low because the dye molecule was very hydrophilic and, hence, easily dissolved in water.

Once a dye molecule binds to a protonated amino group, the charge is neutralized and the molecular chains are capable of becoming closer to each other, thereby leading to different hydration states for the chitosan molecule. On neutralization, greater interaction occurred between the dye molecule and the resulting sorbent leading to a significant increase in the adsorptive power. Similar observations have also been reported for the binding of a water-soluble polymer with other dyes.

**TABLE 1.** Elemental Analysis of Crosslinked Chitosans and their Degrees of Substitution

| Crosslinked chitosan | H (%) | C (%) | N (%) | C/N | D.S. (%)\(^a\) |
|----------------------|-------|-------|-------|-----|---------------|
| CC-1                 | 6.229 | 29.827| 1.904 | 15.665 | 54.3          |
| CC-2                 | 8.252 | 48.795| 3.295 | 14.809 | 49.8          |
| CC-3                 | 7.960 | 48.474| 5.159 | 9.396  | 21.1          |
| CC-4                 | 7.868 | 44.047| 5.067 | 8.694  | 17.4          |
| CC-5                 | 6.231 | 33.881| 4.684 | 7.233  | 9.7           |
| CC-6                 | 7.17  | 40.809| 6.12  | 6.668  | 6.7           |
| Original chitosan    | 7.426 | 40.906| 7.571 | 5.403  | 0             |

\(^a\)D.S. = degree of substitution.
(Shimizu et al. 2002). On the other hand, the adsorption profile of Orange 7 onto the crosslinked chitosan with a higher crosslinking density was Langmuir in type because the hydrophobic part in the crosslinking molecule could contribute to the interaction. The results obtained with CC-5 at different temperatures (Figure 2) also demonstrated that the adsorption was exothermic.

The adsorption of Red 88 by three crosslinked chitosans was measured at 40°C using buffer solutions with pH values of 5 and 6, respectively. The corresponding adsorption isotherms for CC-2 at these two pH values are depicted in Figure 3. The affinity of Red 88 towards the substrates was higher than that of Orange 7 because of its more hydrophobic character, brought about by the presence of an additional benzene ring in the structure. Adsorption of this dye also appeared to be induced by other phenomena such as coagulation and/or aggregation by stacking. This interpretation is supported by the adsorption behaviour observed at pH 6 where small amounts of protonated amino groups were present. Thus, a linear relationship was observed between adsorbed and non-adsorbed dye molecules up to a free dye concentration of $4.0 \times 10^{-6}$ mol/dm$^3$. However, above this threshold concentration value, the number of adsorbed dye molecules increased abruptly.

**Effect of alkyl group in the dye structure on its adsorption**

The effects of dye concentration and pH on the adsorption were investigated for the behaviour of Red 1 and Red 138 onto CC-3. Such measurements were undertaken in appropriate buffer solutions for 72 h at 40°C. The corresponding adsorption isotherms are depicted in Figure 4.
Figure 3. Adsorption isotherms of CI Acid Red 88 by CC-2 in buffer solutions of pH 5 (○) and pH 6 (□) at 40°C.

Figure 4. Adsorption isotherms of CI Acid Red 1 and CI Acid Red 138 by CC-3 in buffer solutions of different pH values at 40°C. Experimental points: ○, CI Acid Red 138; ●, CI Acid Red 1.
As can be seen from this figure, Red 138 was adsorbed to a significantly greater extent than Red 1. The difference in the affinity of these dyes towards the crosslinked chitosan was mainly due to the existence of the alkyl group in the Red 138 molecules. Whereas Red 1 was adsorbed solely via ionic bonding between the sulphonic group in the dye molecule and the protonated amino group in the substrate, Red 138 was also capable of binding via hydrophobic interaction between the alkyl group in the dye molecule and the hydrophobic portion of the substrate.

Because of the decreased number of protonated amino groups at higher pH, a more limited number of binding sites in the crosslinked chitosan substrate were capable of interaction with the dye molecules and, hence, the adsorption of both dyes was restricted. This was particularly so for Red 1 where the dye was adsorbed only slightly in a buffer solution of pH 7, but virtually incapable of adsorption at a pH value of 8. This result may be explained theoretically if it is assumed that the pK_a values of the amino groups were 6.3. This would lead to the amino groups in the crosslinked chitosan being protonated only to a limited extent at pH 8. On the other hand, Red 138 was adsorbed to a considerable extent in buffer solutions with pH values of 8 and 9, with no difference being observed between the experimental data obtained under these two circumstances.

The addition of sodium chloride greatly affected the adsorption of Red 1 at pH 5 (Figure 5), whilst almost no effect was observed on the adsorption of Red 138 at pH 8 (Figure 6). On the basis of the above results, it may be concluded that the adsorption of Red 138 involved only hydrophobic interaction between the alkyl group in the dye and the hydrophobic functionalities in the crosslinked chitosan at pH values higher than 8.

![Figure 5](image-url) Adsorption isotherms of CI Acid Red 1 by CC-3 at 40°C: (○) in the absence of NaCl and (●) in the presence of 0.1 mol/dm³ NaCl.
Effect of the degree of substitution

The adsorption isotherms for three crosslinked chitosans having different degrees of substitution were measured for Red 1 and Red 138 in buffer solution of pH values 5 and 8, respectively, at 40°C over 72 h. The adsorption isotherms thus obtained are shown in Figures 7 and 8, respectively.

At pH 5 for extremely low concentrations of Red 1, the adsorption capacities of the various crosslinked chitosans decreased in the following order: CC-3, CC-4 and CC-6. A similar trend was observed for the adsorption of Orange 7 (Figure 2). Since the adsorption isotherms of Red 1 on CC-3 and CC-4 were Langmuirian in type, we applied the Scatchard equation [equation (1)] to dye adsorption by these substrates:

\[
\frac{r}{C} = K \cdot n - K \cdot r
\]

where \( r \) is the dye uptake (mol/kg), \( C \) is the free dye concentration (mol/dm\(^3\)), \( K \) is the binding constant and \( n \) is the maximum number of sites available on the substrate.

A straight line was obtained when \( r/C \) was plotted against \( r \), allowing the value of \( n \) to be calculated from the intercept on the abscissa. The values of \( n \) thus obtained for the adsorption of Red 1 by CC-3 and CC-4 were 201 \( \cdot \) 10\(^{-2}\) mol/kg and 277 \( \cdot \) 10\(^{-2}\) mol/kg, respectively. In addition, the residual amino group contents of the substrates as calculated from the degree of substitution were 197 \( \cdot \) 10\(^{-2}\) mol/kg and 236 \( \cdot \) 10\(^{-2}\) mol/kg, respectively. The value of \( n \) obtained agreed approximately with the amino group content. This suggests that the Red 1 molecule was bound in a stoichiometric manner to the amino group in the crosslinked chitosan, i.e. one dye molecule bound to one cationic site.
Figure 7. Adsorption isotherms of CI Acid Red 1 at 40°C by three crosslinked chitosans having different degrees of substitution: O, CC-3; Δ, CC-4; and ◊, CC-6.

Figure 8. Adsorption isotherms of CI Acid Red 138 at 40°C by three crosslinked chitosans having different degrees of substitution: O, CC-3; Δ, CC-4; and ◊, CC-6.
On the other hand, the adsorption behaviour of Red 138 was not influenced significantly by the degree of substitution of the substrate. Hence, it may be stated that even with CC-6 (which had the lowest degree of substitution), the percentage of hydrophobicity was adequate for the adsorption of Red 138, with the long methylene group in the substrate contributing quite substantially to the adsorption of this dye molecule.

CONCLUSIONS

Novel crosslinked chitosans were synthesized by the reaction of a higher fatty diacid diglycidyl with chitosan. The adsorption behaviours of several crosslinked chitosans towards typical acid dyes were examined. Because of their excellent adsorption capabilities for the acid dyes, such crosslinked chitosans should be capable of further application as novel adsorbents for various types of anionic dyes. These studies are currently underway in our laboratory and include the introduction of novel crosslinking reagents containing specially designed functional group(s).

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