Dye Binding Capacity of Commercial Chitin Products

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Dye binding capacity of different commercial chitins was investigated with two commercial chitin products and two dyes (FD&C Red No. 3 and Yellow No. 5). Dye binding capacity of chitin increased with increasing dye concentrations and was dependent on the chitin products and the specific dyes used. A slight decrease in dye binding capacity was noted with reduction in chitin particle sizes. Within a pH range of 3−9, dye binding capacity was relatively stable. After 24 h of settling, no dye was released from dyed chitin at pH 2 and 3. Above this range, release of dye increased with pH, up to 1.1 and 5.8% of bound red and yellow dye, respectively, at pH 9. Dye release was less noticeable in 1 h of settling.

Keywords: *Chitin; dye; FD&C Red No. 3; FD&C Yellow No. 5*

INTRODUCTION

Chitin, poly-β-(1→4)-*N*-acetyl-D-glucosamine, is a cellulose-like biopolymer distributed throughout nature, especially in marine invertebrates, insects, fungi, and yeasts (Austin et al., 1981). Chitin and its deacetylated form, chitosan, have attracted considerable interest in view of their proposed novel applications in biomedical, food, and various chemical industries (Knorr, 1984; Muzzarelli, 1977; Rha et al., 1984). In studies on functional properties of chitinous polymers, chitin and chitosan have been documented to possess several distinctive properties for use in water and fat uptake, emulsification, surfactant (Knorr, 1982), thickening (Dunn and Farr, 1971), dye binding (Knorr, 1983), and gelation (Vorlop and Klein, 1981). Chitin and chitosan also have been used as supports for enzyme immobilization (Stanley et al., 1978; Synowiecki et al., 1981).

Limited information is available on adsorption of dyes by chitin and chitosan. Earlier, Giles et al. (1958b) studied adsorption of sulfonated azo dyes by chitin and found that the acetamide groups of chitin are the adsorptive groups for sulfonated azo dyes. Dye binding properties of chitin and chitosan using FD&C Red No. 40 have been investigated by Knorr (1983). Dye binding was stable within a pH range of 2.0−7.0, while chitosan formed gels below pH 5.5 and could not be evaluated. More recently, Ahn and Lee (1992) studied dye (Red No. 40) binding capacities of various chitins, chitosans, and microcrystalline chitins prepared from six different crustacean shells and found dissimilarities in binding capacity with products and sources. Byun et al. (1992) examined dye binding capacity of chitin, chitosan, and their derivatives with Blue R-250 and Red No. 2 and suggested that acetylchitin, *N*-acetylchitosan, chitosansulfate, and chitosan are suitable for use as dye adsorbents.

Chitin is insoluble in water and dilute aqueous salt and acid solutions. Conversely, chitosan is soluble in most acid solutions of less than pH 6 (Muzzarelli, 1973), and its dye binding capacity cannot be evaluated below this pH range (Knorr, 1983). To effectively utilize chitins (mostly from crab shell) as a dye carrier, therefore, consistent dye binding capacity of the chitin product is a primary consideration, especially since chitins show considerable variation in chemical and physical properties due to preparation by different methods and from a variety of species (No and Meyers, 1995).

The objective of the present research was to evaluate the dye binding capacity of chitin using two commercial chitin products and two different dyes. The release of dye from treated chitin was examined at various pH levels.

EXPERIMENTAL PROCEDURES

Materials. Two commercial chitins (from crab shells, practical grade), designated chitin S and chitin K, were obtained from Sigma Chemical Co. (St. Louis, MO) and Keumho Chemical Products Co. (Seoul), respectively.

To obtain a uniform size product, both chitin samples were ground separately through a Wiley mill (Model T25, Thomas Scientific, USA); sifted with 40- (0.425 mm), 60- (0.250 mm), 80- (0.180 mm), and 100-mesh (0.150 mm) sieves; placed in opaque plastic bottles; and stored at ambient temperature. Ground chitin of 0.180−0.150 mm size was used throughout this research to obtain reproducible and consistent results, except for the evaluation study of the effect of four different particle sizes of chitin on dye binding capacity.

The dyes used were FD&C Red No. 3 (Erythrosine, disodium salt of 9-(*o*-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3-isoxanthone) and FD&C Yellow No. 5 (Tartrazine, trisodium salt of 3-carboxy-5-hydroxy-1-(4-sulfophenyl)-4-[(*p*-sulfophenyl)-azo]pyrazole). These were designated red and yellow dye, respectively.

Preparation of Dye Solutions. Red or yellow dye solutions were prepared by separately dissolving dyes in deionized water at concentrations of 50, 100, 150, and 200 mg/L. For the standard curve determination, the maximum absorbance of the aqueous dye solutions containing 2.5−20 mg of dye/L was measured with a spectrophotometer (Shimadzu UV-160A, Shimadzu Co., Japan) using deionized water as a blank. The absorbances measured were reduced by the nondye background absorption.

Determination of Reaction Time. To determine the optimum reaction time for sufficient dyeing of chitin, 0.5 g of chitin S was stirred in 50 mL of aqueous dye solution containing 10 mg of red dye at ambient temperature for 0.5, 1, or 2 h. The resulting dye−chitin was filtered using a Whatman No. 4 filter paper and washed thoroughly with deionized water until the filtrate was clear. The dye concentration of the combined filtrate was determined spectrophotometrically. The amount of dye bound to chitin was determined by calculating differences in concentrations between the initial dye solution and the combined filtrate.

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Dyeing of Chitin. Dyeing of chitin was achieved by stirring 0.5 g of chitin and 50 mL of appropriate concentrations (2.5, 5.0, 7.5, or 10.0 mg of dye) of aqueous dye solution at ambient temperature for 30 min. The resulting dye−chitin was filtered and washed, and the amount of dye bound to chitin was quantitatively determined as above. For evaluation of the effect of particle size, four different size ranges (0.425−0.250, 0.250−0.180, 0.180−0.150, and <0.150 mm) of chitin were stirred in 50 mL of aqueous dye solutions containing 2.5 mg of dye for chitin S and 10 mg of dye for chitin K, respectively. In studies of pH effect, the pH of the aqueous dye solutions (containing 2.5 mg of dye for chitin S and 10 mg of dye for chitin K) was adjusted either with 6 and 0.1 N HCl or with 6 and 0.1 N NaOH, to achieve pH levels of 3, 4, 5, 6, 7, 8, and 9.

Effect of pH on Dye Release. The dyed chitin K, previously prepared by stirring 0.5 g of chitin K for 30 min in 50 mL of aqueous dye solution containing 10 mg of dye and washed thoroughly with deionized water, was added to 50 mL of deionized water adjusted to the pH levels of 2−9 and kept for 1 and 24 h at ambient temperature without stirring. The resulting dye−chitin was then filtered and washed, and the dye concentration of the filtrate was determined spectrophotometrically. The percentage of dye released from the dyed chitin was calculated as follows: dye released (%) = (amount of dye released/amount of dye bound to chitin) × 100.

Analyses. Nitrogen was determined using a Buchi auto analyzer (Switzerland). Ash was calculated by standard methods (AOAC, 1990). The degree of deacetylation was established using a IR spectrophotometer (Polaris FT-IR, Mattson Co., USA) as described by Sannan et al. (1978). Percentage of solubility of chitin was determined in *N*,*N*-dimethylacetamide containing 5% lithium chloride (DMAc−5% LiCl), following the procedures of Rutherford and Austin (1978). Water uptake of chitin was calculated using the procedures described by Lin et al. (1974).

All experiments were carried out in duplicate, and average values are reported. The data were analyzed by analysis of variance. Means of the main effects were separated by Duncan's multiple-range test using the SAS software package.

RESULTS AND DISCUSSION

Determination of Reaction Time. Dye binding capacity of chitin with reaction times (0.5, 1, and 2 h) was evaluated to determine the optimum reaction time. Results showed no differences in the amounts of dye bound to chitin S for three reaction times (7.1, 6.9, and 7.2 mg of dye/g of chitin, respectively). Therefore, a reaction time of 30 min was considered sufficient for optimal binding of dye to chitin. The dye appeared to penetrate the chitin fairly evenly, as observed by Giles et al. (1958b).

Effect of Dye Concentration. The effects of dye concentrations on binding capacity of chitin were evaluated with two commercial chitin products and two types of dyes. Results are given in Figures 1 and 2.

For red dye (Figure 1), binding capacity increased with increasing dye concentrations. However, a marked difference was found between chitin K and chitin S, with the former having much higher binding capacity than the latter. Chitin K revealed 100% dye binding capacity at ranges of 5−15 mg of dye concentration/g of chitin, and 95% at 20 mg of dye concentration/g of chitin. In contrast, chitin S showed only a slight increase in dye binding capacity with an increase in concentration.

Increase in binding capacity of chitins also was observed for yellow dye with increasing dye concentrations (Figure 2). Chitin K had higher binding capacity than did chitin S at all concentrations tested; however, the difference was less pronounced compared with that of red dye. Chitin S showed considerably higher binding capacity for yellow dye than for red dye at above 10 mg of dye concentration/g of chitin.

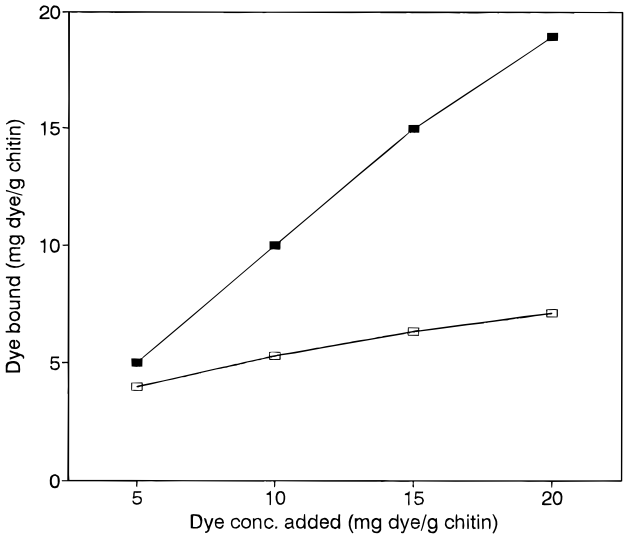

Figure 1. Effect of dye concentrations of FD&C Red No. 3 on dye binding capacity of chitins (■, chitin K; □, chitin S).

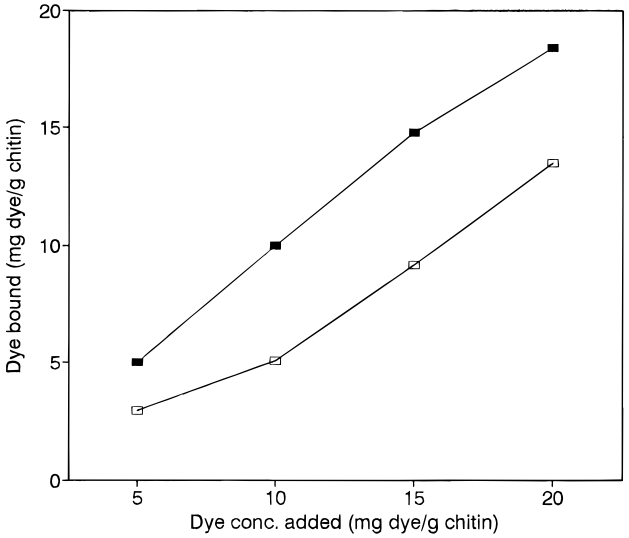

Figure 2. Effect of dye concentrations of FD&C Yellow No. 5 on dye binding capacity of chitins (■, chitin K; □, chitin S).

Table 1. Regression Analysis of Dye Binding Capacity of Chitin on Dye Concentration

dye[a]	chitin[b]	*n*	regression equation[c]	R^2 [d]
red	K	8	$Y = 0.934X + 0.272$	0.997
	S	8	$Y = 0.208X + 1.537$	0.903
yellow	K	8	$Y = 0.899X + 0.400$	0.995
	S	8	$Y = 0.716X - 0.647$	0.977

[a] Red and yellow dye indicate FD&C Red No. 3 and Yellow No. 5, respectively. [b] Chitins K and S indicate commercial crab chitin from Keumho Chemical Products Co. (Seoul) and Sigma Chemical Co. (St. Louis, MO), respectively. [c] Y = dye binding capacity of chitin (mg of dye/g of chitin), X = dye concentration (mg of dye/g of chitin). [d] All $P < 0.01$.

Regression analysis (Table 1) revealed significantly high correlations between dye binding capacity of chitin and dye concentration, irrespective of the chitin products and the types of dyes. Such high correlations also were observed by Knorr (1983) and Nam (1995) who applied dye concentrations ranging from 0.2 to 1.6 mg of dye (FD&C Red No. 40) and from 0.5 to 2.5 mg of dye (FD&C Red No. 5)/g of chitin, respectively.

The present data clearly demonstrate that dye binding capacity of chitin highly correlates with dye concentration, but differs considerably depending on the dye products.

Table 2. Characterization of Chitin K and Chitin S[a]

specification (%)	chitin K	chitin S
nitrogen	6.4	6.6
ash	0.2	3.9
solubility[b]	45.6	—[c]
degree of deacetylation	28	25
water uptake	347	258

[a] Chitins K and S indicate commercial crab chitin from Keumho Chemical Products Co. (Seoul) and Sigma Chemical Co. (St. Louis, MO), respectively. [b] *N*,*N*-Dimethylacetamide containing 5% LiCl. [c] Not dissolved.

Dye binding capacity of chitin may also differ depending on the derivatives or substrate sources of chitin. Recently, Byun et al. (1992) studied the binding capacity of chitin, chitosan, and chitinous derivatives with Blue R-250 and Red No. 2 at constant 1.0 mg of dye concentration/g of sample and found differences in dye binding capacity with both different chitin derivatives and dyes. Ahn and Lee (1992) observed differences in dye binding capacity of chitins, chitosans, and microcrystalline chitins prepared from six different crustacean shells (Antarctic krill, red snow crab, lobster, blue crab, and shrimp *Solenocera prominentis* and *Nephrops thomsoni*), at 1.0 mg of Red No. 40 dye concentration/g of chitin.

As noted above, most workers (Ahn and Lee, 1992; Byun et al., 1992; Knorr, 1983; Nam, 1995) who have investigated dye binding capacity of chitin and its derivatives applied considerably low dye concentrations ranging from 0.2 to 2.5 mg of dye/g of sample. Nevertheless, complete adsorption of dye by chitin could not be achieved at any concentration in contrast to the findings with chitin K reported in this study.

Dye binding capacity of chitin may differ with types of dye. According to Byun et al. (1992), chitin holds 0.506 mg of dye (Blue R-250) and 0.573 mg of dye (Red 2)/g of sample at a constant 1.0 mg of dye concentration. In the present investigation, chitin K revealed almost similar dye binding capacity for both red and yellow dyes, regardless of concentrations. However, chitin S showed higher dye binding capacity for yellow dye (Yellow No. 5, MW = 534.39) than for red dye (Red No. 3, MW = 879.92) at above 10 mg of dye concentration/g of chitin. It is assumed that this difference in adsorption for chitin S probably is related to the different structure or molecular weight of the particular dye. Earlier, Giles et al. (1958b) found that the degree of adsorption of sulfonated azo dyes by chitin was correlated with the dye structures, showing increase in adsorption with size of the aromatic portion of the molecule.

Characterization of Chitin. It is recognized that the physicochemical characteristics of chitin influence its functional properties that differ with crustacean species and preparation methods (No and Meyers, 1995). To establish whether differences in dye binding capacity between two commercial chitins were due to dissimilarities in their physicochemical properties, basic physicochemical properties of the two chitins were determined.

As seen in Table 2, there was no marked difference in nitrogen content and degree of deacetylation. However, the two chitins showed considerable dissimilarities in ash content, solubility, and water uptake. The solubility of chitin K in DMAc−5% LiCl was 45.6%, while chitin S was not dissolved at all. It is possible that some residual ash in chitin S may have affected its solubility and accessibility of dye to acetamide and amino groups, contributing to lower dye binding capacity.

Differences in dye binding capacity between the two chitins may not be due to differences in water uptake since Ahn and Lee (1992) and Byun et al. (1992) found no correlations between water and dye binding capacities of chitin, chitosan, and their derivatives. It is realized that further specific studies such as examination of degree of crystallinity of the products (Giles et al., 1958a) are needed to explain differences in dye binding capacity between two commercial chitin products.

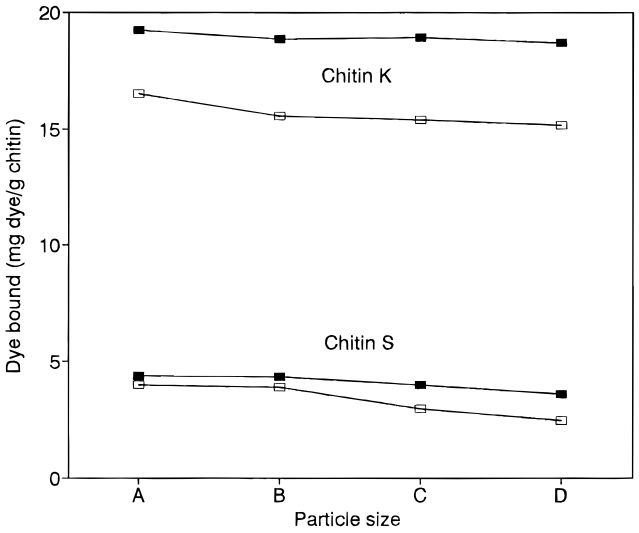

Figure 3. Effect of particle size ranges on dye binding capacity of chitins. Chitin K and chitin S were treated with 10 mg and 2.5 mg of dyes/0.5 g of chitin, respectively. Particle size: A, 0.425−0.250 mm; B, 0.250−0.180 mm; C, 0.180−0.150 mm; D, <0.150 mm (■, red; □, yellow).

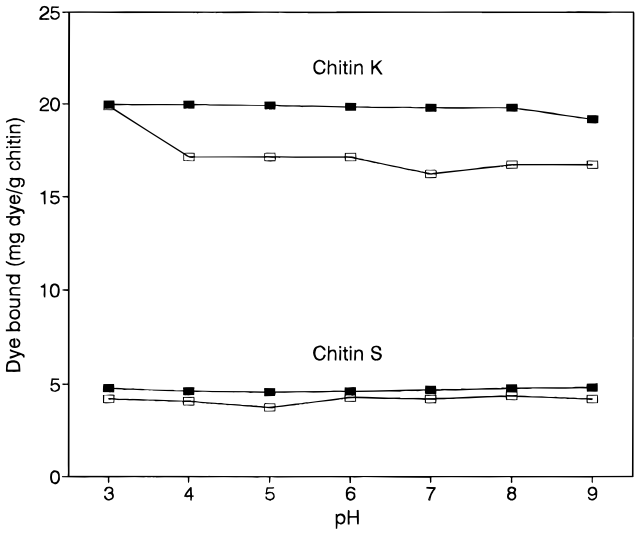

Figure 4. Effect of pH on dye binding capacity of chitins. Chitins K and S were treated with 10 mg and 2.5 mg of dyes/0.5 g of chitin, respectively (■, red; □, yellow).

Effect of Particle Size. Four different particle size ranges of chitins were investigated to compare their dye binding capacity. Chitin K was treated with 10 mg of red or yellow dye concentration, and chitin S with 2.5 mg of red or yellow dye concentration/0.5 g of chitin.

As seen in Figure 3, the chitins showed a slightly reduced dye binding capacity with decreased particle sizes. This small difference may be partially due to dissimilarities in the crystallinity of chitin caused by mechanical action of grinding (Giles et al., 1958a). These data indicate the possible use of various sizes of chitin particle as a dye carrier.

Effect of pH. The effect of pH on dye binding capacity of the two chitins is shown in Figure 4. Chitin K was treated with 10 mg of red or yellow dye concen-

Table 3. Effect of pH and Settling Time on the Release of Dye from Dyed Chitin K[a]

	dye released (% of dye bound)			
	FD&C Red No. 3		FD&C Yellow No. 5	
pH	1 h	24 h	1 h	24 h
2	0	0	0	0
3	0	0	0	0
4	0	0.1	0	0.2
5	0.1	0.2	0.2	0.4
6	0.2	0.3	0.4	0.5
7	0.2	0.4	0.5	1.7
8	0.3	1.0	1.5	3.2
9	0.3	1.1	1.7	5.8

[a] Chitin K indicates commercial crab chitin from Keumho Chemical Products Co. (Seoul).

tration and chitin S with 2.5 mg of red or yellow dye concentration/0.5 g of chitin.

Within a pH range of 3−9, the pH did not noticeably affect binding capacity of chitins for both dyes, with one exception. Yellow dye binding capacity of chitin K significantly increased when treated at pH 3 compared with that at other pH levels. However, Knorr (1983) reported that dye binding capacity of chitin was stable within a pH range of 2.0−7.0, but significantly decreased above pH 7. Compared with present results, the difference in dye binding capacity of chitin above pH 7 may be attributed to differences in the dyes and the chitin products used.

Effect of pH on Dye Release. The effect of pH on the dye release from dyed chitin K was investigated, and results are shown in Table 3. The dyed chitin K contained 18.92 mg of red dye or 15.38 mg of yellow dye/g of chitin.

After 24 h of settling, no effect of pH on dye release between pH 2 and 3 was observed. Above pH 3, the release of dye from dyed chitin increased with increasing pH, particularly for yellow dye. At pH 9, 1.1% of bound red dye and 5.8% of bound yellow dye were released. This release was less affected by pH in 1 h of settling. No dye was released from dyed chitin between pH 2 and 4, while 0.3% of bound red dye and 1.7% of bound yellow dye were released at pH 9.

Earlier, Knorr (1983) found no release of dye from dyed chitin (containing 0.77 mg of FD&C Red No. 40 dye/g of chitin) between pH 2 and 6. Beyond this range, 5.7% of bound dye was released at pH 8.0 and 2.9% at pH 1.0.

It is difficult to directly compare the results of Knorr (1983) with our data due to different chitin products and dyes used. However, it is apparent from both studies that dyed chitin is relatively stable with a small release of dye, particularly above pH 7.

Conclusion. This investigation has provided additional information on the potential of chitin as a dye carrier. Dye binding capacity of chitin was relatively stable within a pH range of 3−9. Dyed chitin also was relatively stable between pH 2 and 9 with a small release of dye. Binding capacity of chitin was demonstrated to be highly correlated with dye concentration. However, different dye binding capacities of two commercial chitin products were observed, even with the same dye. Thus, in order to effectively utilize chitin as a dye carrier, the initial uniform quality of the chitin product is a primary consideration. In projected food application of chitin as a functional ingredient, uniformity and purity are of particular importance. Further investigations with food grade chitin or regenerated chitin, which can be accepted as a food ingredient, are justified and relevant to this entire topic.

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