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Metal Binding Applications of Chitosan Films Containing Cross-linkers and Plasticizers

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Food Chemistry
ABSTRACT:
Chitosan films are an excellent media for binding metals due to the electrostatic nature of the chitosan molecule. Addition of cross-linking or plasticizing agents can improve binding in fresh films, but their effect on aged films is minimal. Chitosan films were prepared using high-viscosity chitosan in acetic acid. Plasticizing agents include tetraethylene glycol (TEG) and glycerol. Citric acid, ethylene diamine tetra acetic acid (EDTA), and tetraethylene glycol diacrylate (TEGDA) were used as cross-linking agents. The additives were applied in concentrations of 0.5%, 0.25, and 0.1%. Citric acid was additionally tested at 1% concentration. Films were kept at ambient conditions (temperature, humidity) for tests within 1 week (fresh films), 4 weeks, and 8 weeks after casting. Moisture content was then determined using the Karl Fischer method and a volumetric titrator. Metal-binding capacity of the films was determined using a spectrophotometer after 30 minutes of film immersion in 5 mg Cr (VI)/L solution. Puncture strength was tested using the TA.XT.Plus (Texture Technologies Corp., Scarsdale, NY). Cross-linked films showed an increase in rupture strength while plasticized films become more elastic. Acetic acid films prepared with no cross-linking agent bound up to 97% Cr (VI) ions. While chitosan has many applications as a coating in the food industry, it can also be used as metal waste removal system in other industries. Addition of other agents can improve textural qualities of the films as well. Due to the large quantities of waste chitosan produced, it is an ideal binding agent.

Key Words:
Chitosan, Chromium, Film, Binding, Cross-linker
Introduction

Chitosan is a readily available biopolymer found in materials often considered to be waste, such as shellfish and fungal cell walls. Chitosan is derived from the deacetylation of chitin, an oligosaccharide made up of repeating units of N-acetyl glucosamine. It is allowed for food use in Japan and Korea, and though not eaten in America, the US does allow its inclusion in animal feed (Park, Marsh and Rhim 2002).

A readily available and renewable resource, chitosan is a natural polysaccharide with the ability to bind hazardous heavy metals from several media, including organic material and wastewater (Chui and others 1996; Grevatt 1998; Ramnani and Sabharwal 2006; Rojas and others 2005; Sankararamakrishnan and others 2005). The binding ability of chitosan has been attributed to the chelating capacity of the amine groups (Park and others 2002). Because more than one-half of all Superfund hazardous waste sites contain chromium, often as chromate ion (CrO$_4$)$_2^-$, chitosan could be used in remediation of these sites. Chromium (VI) is also a known mutagen and carcinogen; thus, removal of this hazard from the environment is crucial for protection of human health.

The US Department of Health and Human Services Public Health Service reports that chromium is used in many industrial processes such as the production of certain yellow paint pigments, forging and welding of steel, production of wood preservatives, and tanning of leather (1980). The US Army alone generates 13,000,000 lbs/year of chrome-contaminated streams and spends $10 million in disposal costs (Boddu and Smith 2002). Currently, the EPA limits the concentration of total chromium in drinking water to less than 100 µg/L (Kartal and Imamura 2005); however, a concentration of 580 mg Cr(VI)/L detected in late 1980s in drinking water in Hinkley, CA caused a wide range of health problems to more than 650 people (Pellerin and...
Booker 2000). In several instances, health problems (cancers, etc.) have been reported as a result of exposure to hexavalent chromium.

Chitosan has been found to be an ideal metal sorbent because of its ability to bind a wide range of metals at high levels and due to its ability to bind five to six times the amount of metals as chitin (Boddu and Smith 2002). Interestingly, chitosan’s ability to bind metals seems to be limited to the transition metals as alkaline and alkaline-earth metals do not bind well due to the lack of d and f orbitals. This enables chitosan to selectively bind the transition metals over the smaller non-transition metals (Guibal 2004).

It is able to bind both metal cations and metal-containing ions by different methods. Cations can be bound by chelation on the amine groups of the chitosan molecule while anions can be bound in acidic solution by electrostatic attraction to the protonated amine groups (Guibal 2004). This binding is affected by several factors including pH and ionic strength (Vold and others 2003). Removal of the metal ions from the chitosan is thought to be achieved by altering pH of the chitosan once removed from solution or by addition of chelating agents (Guibal 2004; Copello and others 2008).

Polysaccharide films are ideal for use with food due to their tremendous oxygen barrier properties as a result of extensive intermolecular hydrogen bonding between amino and carbonyl groups; however, the water vapor permeability of such films is exceptionally high due to the hydrophilic nature of carbohydrates (Han and others 2006; Yang and Paulson 2000). Polysaccharides previously tested in film applications include starch, cellulose, alginate, carrageenan, gellan, pectin, and chitosan. While each has unique properties individually, the most useful films are those made of a combination of polysaccharides or with the inclusion of
additives. Indeed, use as an edible film or coating may be limited based on the extent of properties given by additives to these films (Kim and others 2006).

The addition of cross-linkers and plasticizers to carbohydrate films has been widely studied as a means of improving textural properties, vapor barrier properties, as well as other chemical and microbiological factors. Water binding has also been extensively studied as a means of food preservation (Godbillot and others 2006). The most-studied plasticizing agent in carbohydrate films has been glycerol. Its efficacy in improving the textural and binding properties of films has been well-documented (Butler and others 1996; Caner, Vergano and Wiles 1998; Yang and Paulson 2000; Kim and others 2006; Han and others 2006). It can be used at relatively low concentrations to achieve significant plasticizing properties. Chitosan films containing 0.25 ml glycerol/g chitosan have been shown to have tensile strength values equal to that of HDPE and LDPE films (Butler and others 1996). Tetraethylene glycol (TEG) was chosen as the second variable plasticizer due to its similar textural properties.

Cross-linking of films is of significance when studying the binding properties and barrier properties of chitosan films. Appearance is also an important parameter if the film is to be used in a food application. For instance, cross-linked films are transparent whereas non-cross-linked films become nearly opaque after aging (Delville and others 2003). Citric acid has been previously studied in carbohydrate films due to its cross-linking ability (Yamaguchi and others 2003; Möller and others 2004). Yamaguchi characterizes this ability by stating that most organic acids are able to complex with the chitosan molecule (2003). EDTA is used in this study, as it is a known chelating agent and cross-linker. Tetraethylene glycol diacrylate (TEGDA) is employed as another treatment due to its similarity to TEG and ability to cross-link chitosan
molecules. Other cross-linking agents tested in past studies include epichlorohydrin and glutaraldehyde (Baroni and others 2008).

The main objective of the study was to create a chitosan film that has the best binding capacity while maintaining favorable textural properties and remaining insoluble in aqueous solution. In addition, it was important to: (1) Understand the importance of chitosan as a wastewater remediation tool, (2) Determine the effects of cross-linkers and plasticizers on the binding properties of chitosan films, and (3) Understand the changes in physical properties exhibited by each type of film.

Unlike many previous studies, this work seeks to implement low concentration chitosan films (1% w/w in film forming solution) whereas other researchers have cast films with a chitosan concentration of 3-5%. This concentration allows for improved textural parameters as well as more efficient processing.

**Materials and Methods**

**Chitosan films** were prepared as 1% w/w chitosan (medium molecular weight, Fluka, Ronkonkoma, NY) in 1% aqueous acetic acid. Chitosan was first heated in DI water until boiling to increase solubility. After cooling, the aqueous acetic acid was added, and the solution was stirred overnight. After the stirring period, film-forming solution was adjusted to the appropriate 1% chitosan, 1% acetic acid concentrations by addition of DI water. The solution was then filtered through Miracloth® (Calbiochem, Santa Barbara, CA) to remove remaining insoluble flakes. Control films were poured in aliquots of approximately 50 grams from this solution. Treated films were formed by mixing film-forming solution with the appropriate concentration of cross-linker (citric acid, TEGDA, EDTA) or plasticizer (TEG, glycerol). EDTA
and citric acid were added in crystalline form; TEGDA, TEG, and glycerol were added in aqueous form. All additives were added at concentrations of 0.1%, 0.25%, and 0.5% w/w. Citric acid was also added at a concentration of 1% to the solution. Solubility of the crystalline and aqueous components was aided by stirring for 2 hours before casting. Films were cast in large (approx. 60 cm² surface area) polystyrene Petri dishes at ambient temperature and relative humidity. Films were analyzed 1, 4, and 8 weeks after casting for five parameters. These include thickness, tensile/puncture strength, water solubility, water content, and metal binding capacity.

**Thickness** was using a micrometer (Mitutoyo, Japan). Measurements were taken by placing the films between the grips and tightening until first resistance. Five measurements were taken, with one in the film’s center, as well as one every 90° around the outer edge. These data points were then average to find the film’s average thickness.

**Tensile and puncture strength** were determined using TA.XTPlus Texture Analyzer (Stable Micro Systems, Surrey, U.K.) equipped with 2 mm-needle probe (TA-52). The probe test speed was 1.0 mm/s. One quarter of a film was placed in the test rig, and the apparatus lid was clamped tightly with the locking nuts before testing.

**Water content** was determined using the Karl Fischer method of volumetric titration using Karl Fischer reagent and methanol. Samples of approximately 0.7 g film were ground in a laboratory mill and were analyzed using the 795 KFT Titrino volumetric titrator (Metrohm, Switzerland) after film storage in a desiccator with 20% RH for 48 hours. The Karl Fischer reagent had a titer of 7.3151 mg/ml, and methanol (<50 ppm H2O) was used as the solvent. A vacuum oven drying method was also utilized. Films were placed in the oven under 20 psi at
95°C for 24 hours. The difference in mass before and after drying was calculated, and loss was understood to be water.

Concentration of hexavalent chromium in solution was analyzed following NIOSH method (NIOSH Manual of Analytical Methods (NMAM), 8/15/94) and previous design by Boddu and Smith (2002). In short, 1 ml sample solution was mixed with 10 ml 0.5 N H2SO4 in 25-ml volumetric flask, 1.0 ml sym-diphenylcarbazide in 50 % acetone was added, and the volume was adjusted with 0.5 N H2SO4 to 25 ml. Absorbance was read at \( \lambda = 540 \) nm using a spectrophotometer (UV-2102PC, Shimadzu, Kyoto, Japan). Samples were analyzed using quartz cuvettes 10 cm in length.

Results and Discussion

THICKNESS

Films containing plasticizing agents showed modest increases in thickness. The control chitosan film had an average thickness of 0.072 mm while 0.5% glycerol chitosan films averaged 0.096 mm, with lower concentrations of glycerol yielding thinner films (Figure 1). TEG added at a concentration of 0.5% produced films with a thickness averaging 0.105 mm. These films also demonstrated a positive correlation between additive concentration and film thickness.

The addition of cross-linking agents tended to increase the film thickness more significantly than those with plasticizing agents at the same concentrations. For instance, films with 0.5% TEGDA had an average thickness of 0.124 mm, and those containing 0.5% EDTA measured 0.114 mm in thickness (Figure 2). Citric acid added at a concentration of 0.5% yielded a film with a thickness consistent with the other cross-linkers, at 0.111 mm.
Without exception, the thickness of the chitosan film was positively correlated with the concentration of additive. Also, cross-linkers added at the same concentration (0.5%) as plasticizers yielded thicker films when compared to the control chitosan film.

TEXTURE

Rupture force of the chitosan films showed significant differences based on the additive used, but the effect of time was not seen in any of the film varieties except for those containing EDTA. As seen in Figures 3 and 4, all of the films containing plasticizing agents had a rupture force measurement in the range of 1.5 to 3.0 kg of force throughout the testing. Control films consistently showed a rupture force of 2.8 kg at each time interval. Those containing cross-linkers showed more variability, though. Fresh films had a measured rupture force ranging from 2.5 to 4.0 kg of force. Films containing citric acid and EDTA; however, continued to increase in strength, and the strongest film—containing 0.5% EDTA required 6.5 kg of force to rupture after eight weeks.

Elasticity of the films was also measured at the indicated time periods. The control chitosan film had a measured elasticity of 1.75 mm throughout the course of the study. Also, the films containing cross-linkers had elasticity values of not more than 2.5 mm throughout the trials. Those containing plasticizers, though, tended to have slightly more elastic properties. When measuring the fresh films (Figure 5), the 0.5% glycerol film demonstrated an elasticity of 5.5 mm; however, this value had decreased to approximately 3.5 mm after four and eight week intervals (Figure 6).

After drying of the films in ambient air temperature and humidity, several films exhibited liquid on their surface. This was most noticeable in those films with the greatest concentrations
of additive, notably those with 0.5% TEGDA. Also, when observed at eight weeks after casting, the films containing EDTA showed crystalline formation within the chitosan matrix (Figure 7). This may have played a factor in the physical and/or chemical measurements of these films. It should also be noted that these formations were not noted upon testing soon after casting. This phenomenon may be able to describe the decrease in utility of the additives to bind chromium after extended periods of time.

WATER CONTENT

The control chitosan and acetic acid film was determined to contain 6.15% moisture by the Karl Fischer method and 16.02% by the oven drying method (Figure 8). This difference in values was typical of the difference between the two methods. On average, plasticized films had greater moisture content than those containing cross-linkers. The plasticized films ranged from 4.33 - 8.33% moisture by the Karl Fischer method (15.21 - 30.67% oven method). The greater concentrations of glycerol and TEG showed the greater moisture contents while 0.1% TEG and 0.1% glycerol contained the least bound water. Similar studies involving plasticized biopolymer films confirm these findings. Gelatin films with plasticizing agents ranged from approximately 10 – 20% moisture, but the plasticizers in this study were added at much greater concentrations (1:5 plasticizer:gelatin) to form the films (Cao, Yang and Fu 2009). A more direct comparison can be drawn from studies which utilize chitosan-glycerol films. One such study reports moisture values for these films at 4.60% ± 0.30 (Bajdik and others 2009); however, the concentration of glycerol used was 1%, twice the amount used here. Another study involving oat starch films with glycerol as a plasticizer also notes a significant trend in which films with lower concentrations of plasticizer absorb less water (Galdeano and others 2009).
Cross-linked films ranged from 0.09 - 4.86% water by the Karl Fischer method and 7.79 - 14.56% by the oven drying method (Figure 9). The most noticeable trend occurred in the films containing TEGDA, which showed the least amount of water at the intermediate (0.25%) concentration of additive. This tendency was exhibited by both moisture content determination methods. The TEGDA 0.1% and 0.5% films contained this greatest amount of moisture among the cross-linked films, each containing 13-15% moisture (oven method) and 3 - 5% moisture by the Karl Fischer titration method.

The discrepancy between the values obtained from the Karl Fischer and oven drying methods may be described by the loss of volatile compounds in the 95°C oven. Acetic acid has a boiling point around 118°C, so the proximity to this point accompanied by error in temperature maintenance of the oven may have caused this difference. This may be tested in the future by determining the pH of dried films to see if acetic acid has indeed been lost.

Film solubility (Figures 10 & 11) differs greatly between fresh films and 8-week aged films. The aged films show a marked decrease in solubility, with the only exception being 0.25% citric acid films. In general, the plasticized films are more soluble with increased plasticizer concentrations, and this is evidenced by the aged films. Cross-linked films with EDTA and TEGDA show a similar trend, but all of the citric acid films show different results. This difference amongst the various citric acid concentrations is consistent with the textural findings.

METAL BINDING

A standard 1% chitosan, 1% acetic acid film bound up to 97% of Cr (VI) after the film was stored for eight weeks, and the film was exposed to the chromium solution for 30 minutes.
This value increases from approximately 70% binding capacity of fresh films. This translates to approximately 0.57 mg of Cr (VI) per gram of film used (Figures 12 & 13). Addition of cross-linkers and plasticizers had varied effects. TEGDA addition causes an increase in the percent bound Cr (VI) of fresh films (90%), but it does not improve the binding capacity of aged films. EDTA addition seemed to have a negative effect on binding, as the only increase in binding was seen at the lowest EDTA concentration (0.1%) in fresh films (Figure 14). This increase was just outside of the margin of error and amounted to an improvement of binding to 80% bound chromium. The lowest concentrations of citric acid also yielded an increase in binding capacity of fresh films, but this increase is negated after the films have aged, and these cross-linkers actually decreased binding capabilities of the films.

As seen in Figure 15, addition of the plasticizers TEG and glycerol yielded similar results. The additives in their lowest concentrations tend to improve the binding capacity of the films when the films are initially tested, but this effect is not seen when the films are measured at four and eight week intervals.

**Conclusion**

Neither cross-linkers nor plasticizers increase the binding capacity of chitosan films after eight weeks of aging, but many of the additives in minute concentrations do improve the binding capacity of freshly-cast films. This effect does not show a trend based on the type of additive, as both plasticizing and cross-linking agents displayed similar results. As a rule, increased additive concentration and binding capabilities showed a negative correlation; the most positive effects were observed in films with 0.1% additive concentration. One of the favorable additives, 0.1% citric acid, effectively improved the binding capacity of the chitosan films, but it caused the films
to lose their favorable texture properties and become fairly soluble when added to fresh films. An additive with comparable metal binding improvement and maintenance of favorable textural properties would be 0.1% TEG, a plasticizer. This additive not only improves binding capacity and tensile strength of the chitosan film, but it also allows the films to maintain their standard level of chromium binding at eight weeks. Thus, TEG improves the binding capacity of fresh films while not hindering the binding capacity of aged films.

Depending on the application of the chitosan film, a cross-linker or a plasticizer may be alternately needed to reach the film’s desired textural properties. As noted, plasticizers increased the flexibility of the films while cross-linkers cause an increase in rupture strength. As a whole, the two groups of compounds were comparable in improving the binding capacity of the chitosan films.

Chitosan films with added compounds have practical uses in wastewater treatment and hazardous waste site remediation. Further studies exploring the possibilities of combining additives to maximize positive attributes of the films should be performed. Some of the additive compounds, such as glycerol and EDTA, are already used to improve food products by altering their chemical or physical properties, and these chemicals have been labeled as GRAS. Use of the other additives, however, would likely require further study if used in a food system application.
References


Figure 1: The thickness of chitosan films can be modestly increased by the addition of plasticizing agents.
Figure 2: Chitosan films containing cross-linkers show a significant increase in thickness when compared to a standard chitosan film.

Figure 3: Rupture force measurements of fresh films containing cross-linking agents are markedly increased from those containing plasticizers.

Figure 4: After 8 weeks, those chitosan films made with cross-linkers continue to increase in rupture strength while those made with plasticizers maintain their initial strength.
Figure 5: Films containing added plasticizers, especially high concentrations of glycerol have a large amount of elasticity.

Figure 6: Elasticity exhibited by fresh chitosan films containing plasticizers decreases after an eight-week period.
Figure 7: EDTA crystallization is observed on the surface of chitosan films containing various concentrations (0.5%-0.1%) of EDTA.
Figure 8: Films containing plasticizers tend to show an increase in moisture as the plasticizer concentration increases.

Figure 9: Cross-linked films show a decrease in moisture as cross-linker concentration increases. TEGDA is an exception to this, as the 0.5% and 0.1% concentrations contain more water than the 0.25% films.
Figure 10: Solubility of aged films increases with the increase in plasticizer concentration.

Figure 11: EDTA and TEGDA cross-linked films follow the same trends as plasticized films, but citric acid films show no trend in solubility.
Figure 12 & 13: A 1% acetic acid, 1% chitosan film can bind 97% of chromium ions when aged for eight weeks. Each gram of film can bind about 57 mg of hexavalent chromium.
Figure 14: Addition of EDTA and other cross-linkers, especially in small concentrations, moderately improves metal-binding capacity of fresh films, but this effect is not seen in aged films.

Figure 15: Addition of plasticizing agents to chitosan films improves metal-binding capacity of the fresh films, but this phenomenon is not observed in aged films.