

# Preparation and Characterization of Gelatin/Chitosan/Carbodiimide Films

by

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## Abstract

In prior studies, we examined the effects of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and water-dispersible polycarbodiimides (pCDIs) on the properties of gels produced from gelatin and a gelatin/chitosan blend that may be suitable for a role in leather processing. Those studies showed major differences in gel properties as a function of the specific carbodiimide. In this continuing study, the gelable products formed from gelatin or a gelatin/chitosan blend modified with EDC or pCDIs were cast into films. The mechanical properties, hydrothermal stability, solubility, and water absorptivity of the films were examined. The range of values for mechanical properties (tensile strength, % elongation at break, Young's modulus, and toughness) was small enough that it would likely not be a major consideration in the choice of carbodiimide for a particular application. Neither EDC nor the pCDIs at the concentrations tested in this work had any discernible effect on the hydrothermal stability of gelatin or gelatin/chitosan films. The effects of the pCDIs on solubility and water absorption properties of the films trended more closely with the concentration of the pCDI than did the effects of EDC. This preliminary study explores the potential for using the environmentally friendly, water-dispersible pCDIs in gelatin and gelatin/chitosan-based films for use in leather coating or packaging applications.

## Introduction

More than twenty years ago, we demonstrated that high quality technical gelatin could be extracted from solid tannery waste.<sup>1</sup> We proposed the utilization of this inedible gelatin in the production of gels, adhesives, and films for use in the filling and finishing of leather.<sup>2</sup> A comprehensive evaluation of the properties of the isolated gelatin suggested that modification of the protein to introduce new and improved functional properties

might facilitate its utilization in various stages of the leather manufacturing process, to partially replace some petroleum-based materials for filling leather.<sup>3</sup> Our first approach was to explore ways to improve the physical properties of gelatin containing gels by chemical<sup>4-6</sup> and enzymatic<sup>7</sup> methods, using environmentally benign materials. A second gel approach was to augment gelatin with other excess agricultural biopolymers that might provide additional functionality. The choice of agricultural biopolymer has evolved with the availability and market value of the "excess" biopolymer. Early choices were the milk proteins casein<sup>8</sup> and whey protein isolate.<sup>9</sup> Recently, the carbohydrate, chitosan, an abundant waste product derived from crustaceans, has become the secondary biopolymer of choice, with polyphenols or carbodiimides assisting in complex formation.<sup>10-12</sup>

Gelatin is already a major component of biopolymer films, which have uses in food packaging and medical applications as well as leather coating or finishing. Numerous researchers have prepared and stabilized gelatin-based films by chemical or enzymatic crosslinking.<sup>13</sup> One of the difficulties is getting a degree of crosslinking that will stabilize the film while retaining good mechanical properties such as strength and flexibility. Two of the more common agents for stabilization of gelatin-based films are transglutaminase<sup>14-16</sup> and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide commonly known as EDC<sup>17</sup> used alone or in combination. Specifically, Tropini *et al.*<sup>17</sup> used EDC with N-hydroxysuccinimide (NHS) to crosslink wheat gluten films. Several authors have evaluated the effects of crosslinking via EDC or transglutaminase on the physical and mechanical properties of films containing fish gelatin and chitosan.<sup>13,18,19</sup> Their work exploits the ability of the carbodiimide group to crosslink carboxylic acid groups on proteins, peptides or other compounds that contain carboxyl groups. Thus, treatment with EDC of waste proteins, perhaps in conjunction with chitosans or other carbohydrates, might lead to products with interesting functional properties that could prove useful in leather processing.

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Water-dispersible polycarbodiimides (pCDIs) were developed by Hesselmanns *et al.*<sup>20</sup> as environmentally friendly, efficient carboxyl group crosslinkers for use in a variety of coating applications. The Hesselmanns group specifically explored the use of pCDIs as crosslinkers for polyurethane dispersions for leather finishing.<sup>21</sup> The current study builds on our recent publications on the preparation and characterization of gels containing gelatin or a gelatin/chitosan blend stabilized with EDC or commercially available water-dispersible pCDIs.<sup>11,12</sup> Protocols developed for the preparation and evaluation of gelatin/casein films stabilized with transglutaminase mediated crosslinks are used to characterize the films.<sup>14,15</sup>

## Experimental

### Materials

Commercial type B gelatin from bovine skin, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), glycerol, and chitosan (50 – 190 KDa and 75-85% acetylated) were obtained from Sigma-Aldrich Corporation (St. Louis, MO). The water-dispersible hydrophilic polycarbodiimide Picassian® XL-702 (p-CDI-2)<sup>20</sup> and the water-dispersible multifunctional, VOC-free Picassian® XL-725 (p-CDI-3)<sup>20</sup> were generously supplied by Stahl USA (Peabody, MA). All other chemicals were analytical grade and used as received.

### Methods

#### Preparation of Biopolymers

The preparation of carbodiimide-modified gelatin, chitosan, and gelatin/chitosan biopolymer products are detailed by Taylor, *et al.*<sup>11,12</sup> and briefly depicted in Figure 1. Bovine type B gelatin nominally 123g Bloom, was used either with or without chitosan for all film preparations. The biopolymer solutions consisted of 10% gelatin solubilized in water, and a similar solution that also contained 0.35% chitosan (on weight of gelatin) in 0.15 M acetic acid.<sup>11</sup> For film studies, 3% glycerol was added to the solutions prior to the addition of crosslinker. Carbodiimide modified products were prepared by the addition of EDC (containing NHS at a 1:15 ratio) calculated to be 0-5 mM EDC in the final solution or pCDI-2 or pCDI-3 (without NHS) calculated to be 0-25% on the weight of gelatin or gelatin/chitosan in the final solution.

#### Preparation of Films

Films were prepared as described earlier.<sup>14</sup> Approximately 10 g of gelatin, gelatin/chitosan, or carbodiimide modified gelatin or gelatin/chitosan solution was weighed into Petri dishes (100 mm) in triplicate. The dishes were gently rotated to distribute the mixture, heated at 35°C for 1 h and then dried at ambient temperature for 24 h. The films were conditioned in a constant temperature (23°C) and humidity room (50% RH) for at least 48 h.

### Analyses

#### Properties of the Gelable Formulations

All analyses of the gelatin/carbodiimide and gelatin/chitosan/carbodiimide and unmodified control formulations prepared for film casting were performed in triplicate. Gel strength, melting point, and viscosity were determined as described in previous publications.<sup>7</sup> Protein molecular weights were estimated by SDS-PAGE on precast 4-15% gradient gels as previously described.<sup>22</sup>

#### Mechanical Properties of Films

Five strips, 50 mm long and 5 mm wide, were cut and removed from each Petri dish, yielding a total of 15 filmstrips per formulation. The thickness of each strip was measured using a micrometer. Tests of mechanical properties (tensile strength, % elongation at break, Young's modulus and toughness) were performed on the films essentially as described earlier.<sup>15</sup> Briefly, film strips were equilibrated at 23°C and 50% relative humidity for at least 48 h. A MTS Insight 5kN Electromechanical Testing Frame was used for mechanical tests, and all data was collected and processed by TestWorks software, version 4.11C, (MTS Systems Corp., Eden Prairie, MN). The method used was based upon ASTM D882 - 12, standard test method for tensile properties of thin plastic sheeting.<sup>23</sup> The grip separation was set to 25 mm and the test speed was 50 mm/min. Average values and standard deviations were calculated for each property. Tensile strength is defined as the maximum stress. Percent elongation is the ratio of the length at break under strain to the initial length. Young's modulus, a physical quantity representing the stiffness of a material was determined by measuring the slope of a line tangent to the initial stress-strain curve. Toughness (resistance to fracture) was determined from the area under the stress-strain curve.

#### Solubility and Water Absorption Properties of Films

Solubility and water absorption properties of cast films were determined as described earlier.<sup>14</sup> Briefly, for solubility, 2 X 2 cm squares were removed from the Petri dishes and equilibrated at 23°C and 50% relative humidity for at least 48 h. They were then



Figure 1. Flow diagram for preparation of carbodiimide-modified gelatin and gelatin/chitosan biopolymer materials and films.

suspended in 30  $\mu$  of water containing sodium azide (70  $\mu$  of a 1% solution) to prevent bacterial growth, and shaken at 25°C for 24 h. The solutions were filtered, residues recovered, placed in tared weighing bottles, and dried at 100°C for 16 h. Control samples, which had not been subjected to the solubility test, were also dried to determine original moisture content. For water absorption properties, films that did not dissolve in the solubility test were filtered and placed in tared weighing bottles. The samples were weighed, dried overnight at 100°C and then reweighed. The percent water absorbed was calculated.

### Hydrothermal Stability

Hydrothermal stability of carbodiimide-modified gelatin and gelatin/chitosan films and unmodified control samples were

determined on a Multi-Cell Differential Scanning Calorimeter (MC-DSC) from TA Instruments, New Castle, DE, as previously described.<sup>24</sup> Briefly, film samples (100-150 mg) were weighed into ampoules, 500  $\mu$ L of distilled water was added, the ampoules were sealed and placed in the calorimeter. The calorimeter was programmed to record heat flow in  $\mu$ cal/°C while the temperature was increased from 0°C to 120°C at 1.0°C/min with an equilibration period of 600 s at the start. The temperatures at the peaks of this curve,  $T_p$ , were considered to be apparent transition temperatures.

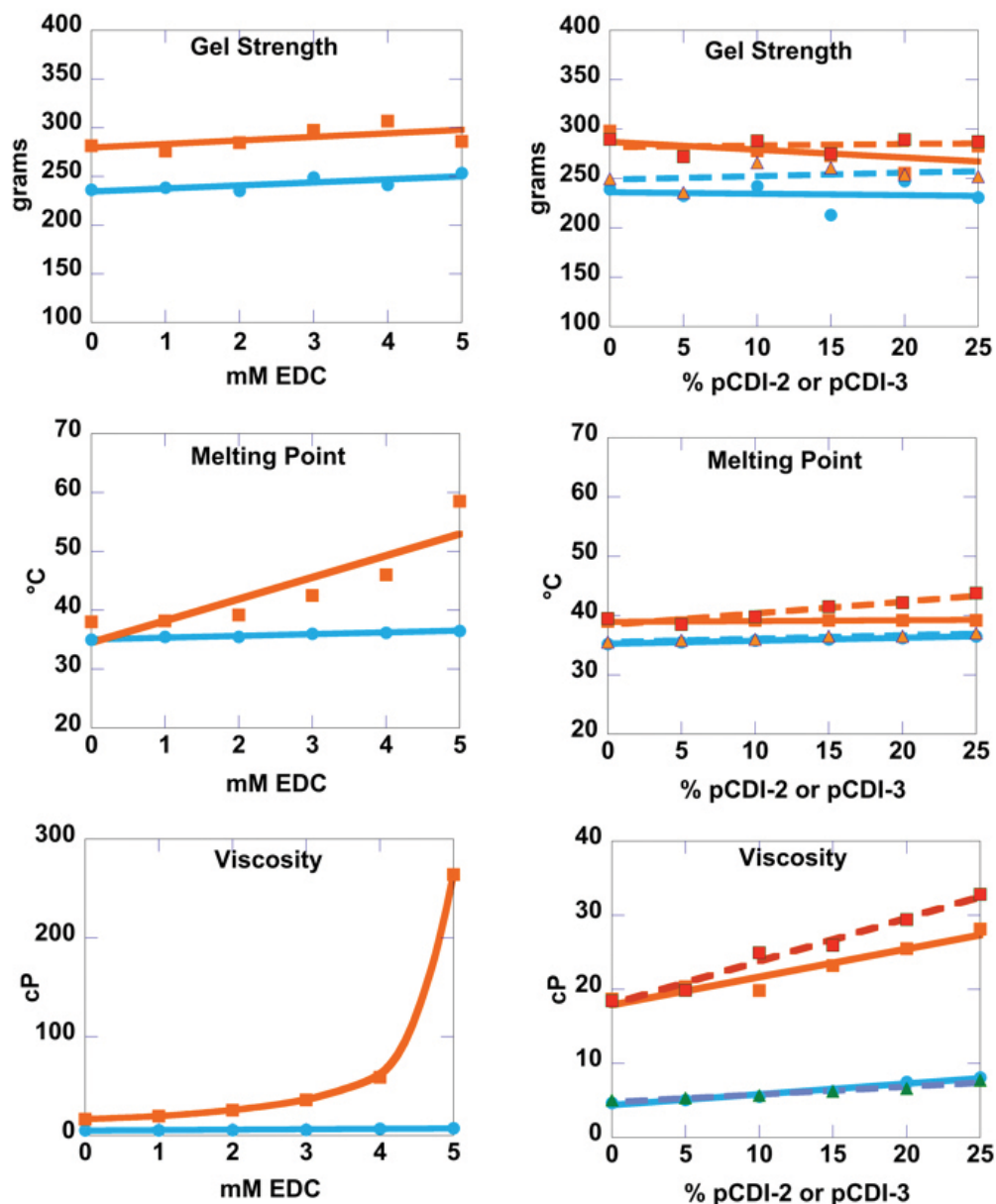


Figure 2. Physical properties of gelatin (blue) and gelatin/chitosan blend (orange) as affected by 0-5 mM EDC or 0-25% pCDI-2 (solid line) and pCDI-3 (dashed line).

## Results and Discussion

### Gel Properties

Gel strength, melting point, and viscosity were determined as previously described<sup>7</sup> on the gelatin/carbodiimide and gelatin/chitosan/carbodiimide-treated biopolymer materials prepared for films. Gel strengths for gels without chitosan or carbodiimides were  $242 \pm 7$  g while for those that contained the gelatin/chitosan mixture the gel strengths were 20% higher at  $290 \pm 8$  g. The addition of EDC (1-5 mM) or either polycarbodiimide (5 - 25%) had little further effect on gel strength (Figure 2). Melting points for gelatin/chitosan blends without carbodiimide were  $38.8 \pm 0.8^\circ\text{C}$ , while those for gelatin alone were lower,  $35.2 \pm 0.2^\circ\text{C}$ . The addition of 5 mM EDC or 25% polycarbodiimide to gelatin formulations increased the melting point by  $1.5^\circ\text{C}$  over that of gelatin alone. Similar small increases in the melting point were observed as the polycarbodiimides were incorporated into gelatin/chitosan blends. The addition of 5 mM EDC to the gelatin/chitosan blend had a much greater effect, up to  $20^\circ\text{C}$  (Figure 2). The viscosity of the gelatin without chitosan or carbodiimides was  $5.0 \pm 0.3$  cP. Small increases (5.0 - 7.7 cP) in viscosity were observed as the carbodiimide concentration was increased in gelatin solutions. The viscosity of gelatin/chitosan blends without carbodiimide was  $17.9 \pm 1.2$  cP. Addition of 25% polycarbodiimide on weight of gelatin/chitosan increased viscosities to  $\sim 30$  cP, while the addition of 5mM EDC dramatically increased the viscosity to  $>200$  cP (Figure 2). The viscosity of a 0.35% chitosan solution without gelatin was unaffected by the addition of EDC or polycarbodiimides. The effects of EDC and polycarbodiimides on the size distribution of gelatin and gelatin/chitosan mixtures was examined by SDS-PAGE and the results (data not shown) agreed closely with those reported earlier.<sup>11,12</sup>

### Mechanical Properties of Films

The thickness of each film strip (a total of 15 strips per formulation) was measured with a micrometer. The thickness of film strips from formulations containing gelatin without chitosan ranged from 0.187 to 0.216 mm, while those formed from gelatin/chitosan mixtures ranged in thickness from 0.191 to 0.259 mm. The average thickness of all film strips was  $0.22 \pm 0.04$  mm. Although the gel strength, melting point and viscosity of the gelatin formulations used in films containing EDC, the hydrophilic pCDI-2, and the multifunctional pCDI-3 were nearly identical, there was greater variation in the mechanical properties of films cast from the unmodified gelatin and gelatin/chitosan blends. One unifying factor was that for each property, all data could be meaningfully represented on a single scale for the parameter axis (Figure 3).

In the EDC series, tensile strengths for unmodified gelatin films were  $34 \pm 4$  MPa. There was no obvious pattern to the tensile strength values obtained as the concentration of EDC was

increased, and the average value in the 1 – 5 mM range decreased to  $31 \pm 4$  MPa. For unmodified gelatin/chitosan films, the tensile strength was higher at  $38 \pm 4$  MPa, and with the addition of EDC, tensile strength increased to  $43 \pm 7$  MPa. Values for Young's modulus, a measure of the stiffness of the film, essentially parallels the values of tensile strength as affected by 1 – 5 mM EDC (Figure 3). Percent elongation at break, a measure of the elasticity and strength of a film, and toughness, a measure of the energy required to fracture the film, are likewise affected similarly by the addition of EDC to the gelatin or gelatin/chitosan formulation.

In the series using the hydrophilic pCDI-2, tensile strengths for unmodified gelatin films were inexplicably high at  $58 \pm 8$  MPa while those for unmodified gelatin/chitosan blends were more typical at  $24 \pm 2$  MPa. When pCDI-2 was included in gelatin films, the tensile strength decreased to 33 MPa at 25% pCDI-2. While the inclusion of pCDI-2 in gelatin/chitosan blend gels resulted in an increase in tensile strength to  $31 \pm 4$  at 15% pCDI-2. Values for Young's modulus as a function of increasing concentration of pCDI-2 were parallel to those of the tensile strength. In other words, the addition of pCDI-2 to these gelatin and gelatin/chitosan formulations tended to modulate the strength and stiffness parameters, sending them to more typical levels. Plots of percent elongation at break and toughness of gelatin and gelatin/chitosan films as affected by increasing concentrations of pCDI-2 were not parallel, but did trend in the same direction (Figure 3).

In contrast, inclusion of the multifunctional pCDI-3 in gelatin or gelatin/chitosan-based films had little effect on tensile strength of either, and only a small decrease in Young's modulus for gelatin-based films. Both % elongation and toughness increased linearly as the pCDI-3 concentration was increased (Figure 3).

In general, the effects of 1 – 5 mM EDC on mechanical properties of gelatin and gelatin/chitosan blend films were greater than the effects of the water-dispersible pCDI-2 or pCDI-3 at 5% - 25% on weight of gelatin or gelatin/chitosan. The effects of the hydrophilic pCDI-2 on mechanical properties were greater than the effects of the multifunctional pCDI-3. The range of values for mechanical properties is small enough that if one of these carbodiimides is appropriate for an application, the others could likely be optimized to work in that application.

### Solubility and Water Absorption Properties of Films

The effects of increasing concentrations of EDC or polycarbodiimides on the solubility of films can be seen in Figure 4. Gelatin films without chitosan or carbodiimides were, as expected, completely soluble in water. The addition of 1 mM EDC to the gelatin film resulted in a decrease in solubility to 65% that did not change as the concentration was raised to 5 mM EDC. In contrast, the addition of 5% of the hydrophilic pCDI-2 decreased the gelatin solubility to 75% and a nearly linear decline

to 50% was observed as the pCDI-2 concentration was increased to 25%. Addition of 5% of the multifunctional pCDI-3 decreased the gelatin solubility to 82% with a nearly linear decline to 55% as the carbodiimide concentration was increased to 25%. Films from the gelatin/chitosan blend were less soluble than those from gelatin alone. Films without carbodiimides were  $70.5 \pm 3.6\%$  soluble. The addition of EDC at 1-2mM had no effect on film solubility, and between 3mM and 5mM the solubility decreased

to 55%. The pCDI-2 had no effect on the solubility of gelatin/chitosan films (Figure 4). The effect of the multifunctional pCDI-3 on the solubility of gelatin/chitosan films was more similar to that of EDC, with film solubility decreasing in the 10 - 25% pCDI-3 range to 57% solubility.

The effects of increasing concentrations of EDC or polycarbodiimides on the ability of the insoluble portion of the

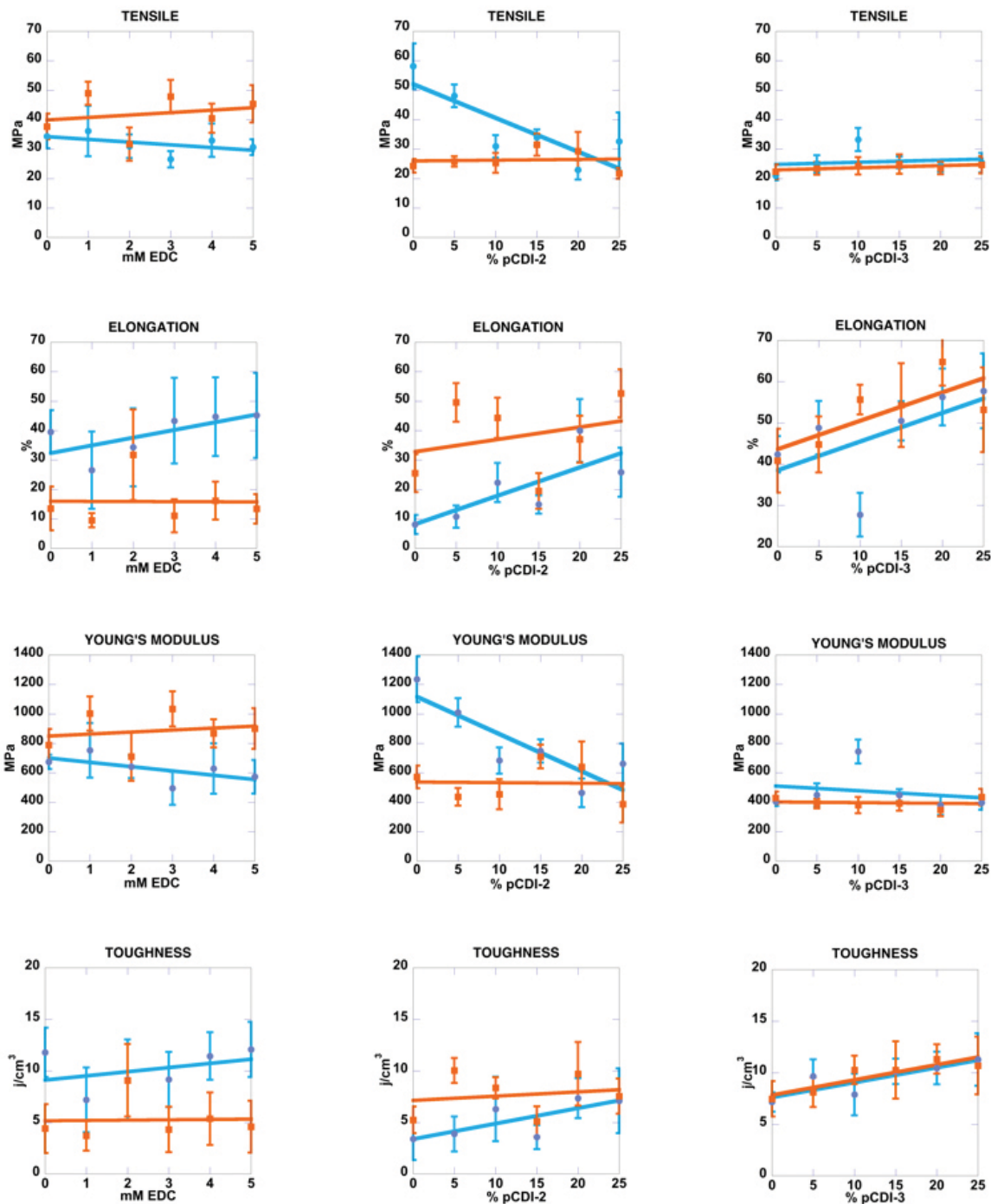


Figure 3. Effects of 0-5mM EDC and pCDI-2 or pCDI-3 on mechanical properties of gelatin based films (blue) and gelatin/chitosan-based films (orange).

films to absorb water can also be seen in Figure 4. Because gelatin films without chitosan or carbodiimides were completely soluble, water absorption could not be determined on them. Gelatin films with 1 mM – 5 mM EDC had good water uptake properties ( $1416 \pm 184\%$  on the weight of the dry film) that were little affected by the EDC concentration. In contrast, gelatin/chitosan films were less able to absorb water than gelatin films. Gelatin/chitosan films containing 1 mM – 5 mM EDC absorbed  $756 \pm 126\%$  of their dry weight in water, about half of the amount of water absorbed by similar films without chitosan. The water absorptivity of gelatin films containing pCDIs decreased linearly by about 40% as the pCDI concentration was increased from

5% to 25%. The water absorptivity of gelatin/chitosan films were not influenced by 5% - 25% pCDI-2, and showed a decrease of 20% at 5% pCDI-3 followed by increases to the level of the carbodiimide-free gelatin/chitosan films at 25% pCDI-3.

The effects of the polycarbodiimides on solubility and water absorption properties of the gelatin and gelatin/chitosan films trend more closely with the concentration of the polycarbodiimide than do the effects of EDC. If these properties were important to the application the pCDI's might give more predictable results.

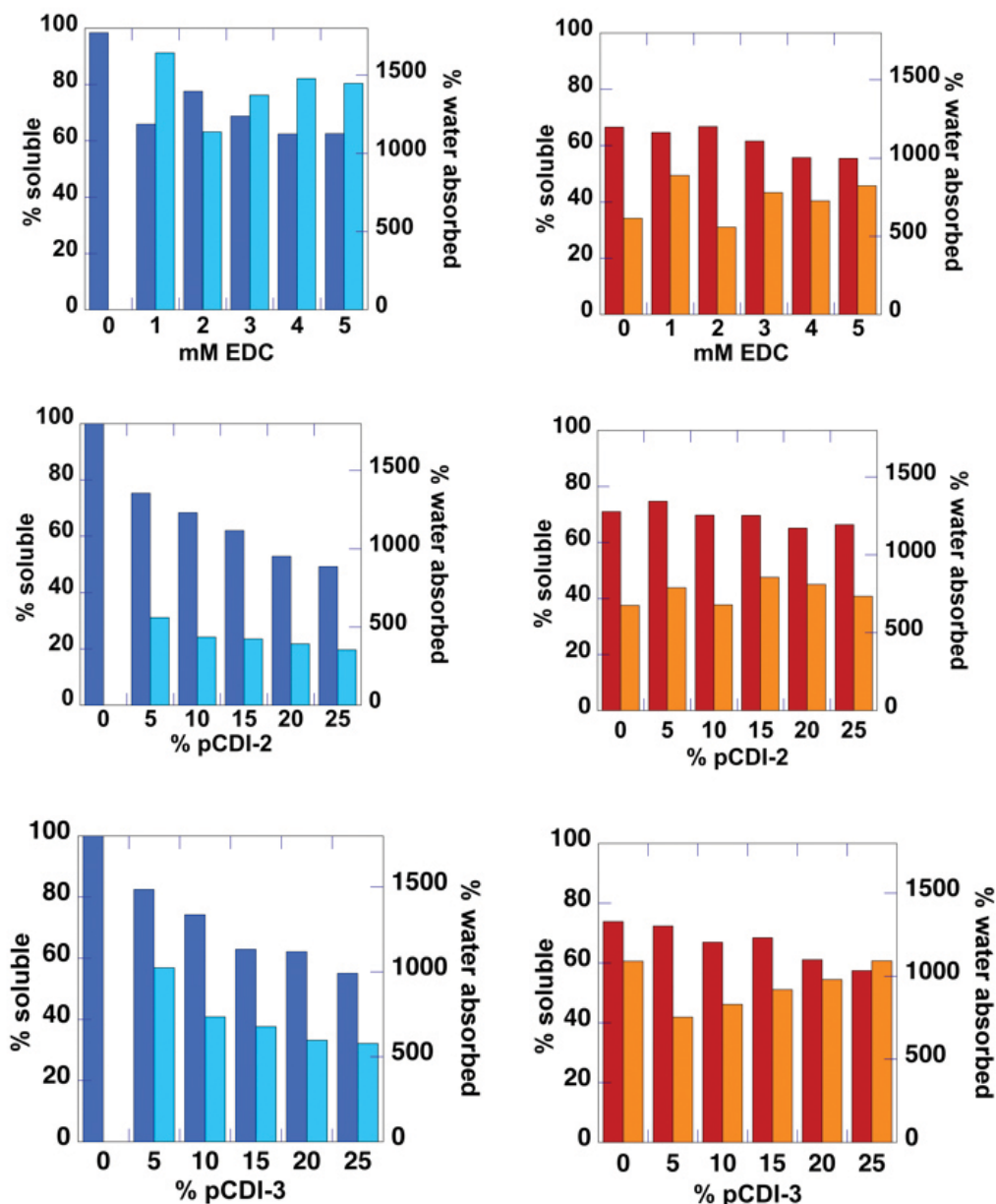


Figure 4. Effects of carbodiimide concentration on water solubility (axis on left side) and water absorption (axis on the right) of films containing carbodiimides and gelatin (blue columns) and films containing a gelatin/chitosan blend (orange columns).

**Table I**  
Effects of Carbodiimides on Hydrothermal Stability of Gelatin and Gelatin/chitosan films.

Sample	Gelatin		Gelatin/chitosan blend	
	Peak 1	Peak 2	Peak 1	Peak 2
0 mM EDC	20.6 ± 1.3	37.6 ± 0.5	21.0 ± 0.6	34.4 ± 0.5
3 mM EDC	20.3 ± 1.7	34.2 ± 1.3	19.4 ± 0.2	33.8 ± 0.5
5 mM EDC	20.6 ± 1.3	37.6 ± 0.5	20.6 ± 0.7	33.8 ± 0.2
0% pCDI-2	20.8 ± 0.5	32.2 ± 0.4	20.9 ± 0.6	33.8 ± 0.0
15% pCDI-2	19.8 ± 0.9	33.7 ± 0.5	20.8 ± 0.4	33.1 ± 0.4
25% pCDI-2	21.1 ± 0.5	34.8 ± 0.3	20.1 ± 0.6	33.0 ± 0.2
0% pCDI-3	21.2 ± 2.2	32.5 ± 0.7	19.3 ± 0.5	33.0 ± 0.2
15% pCDI-3	20.7 ± 0.3	33.0 ± 0.5	20.5 ± 1.2	33.1 ± 0.2
25% pCDI-3	20.3 ± 0.5	33.6 ± 0.3	20.5 ± 0.2	32.9 ± 0.3

### Hydrothermal Stability

When selected films (0, 3 mM, 5 mM EDC and 0, 15%, 25% pCDIs with gelatin and gelatin/chitosan blends) were examined in triplicate by DSC, the resulting traces were remarkably similar. Each trace showed a transition at ~21°C, corresponding to the dissolving of all or part of the film, and a second transition at ~34°C corresponding to the melting or denaturation of gelatin. Neither chitosan nor carbodiimide had any noticeable effect on the temperatures associated with these transitions (Table I). A representative set of traces is shown in Figure 5. Hydrothermal stability of the films was not enhanced by either EDC or the water-dispersible polycarbodiimides. Neither EDC nor the pCDIs tested in this work had any discernible effect on the hydrothermal stability of gelatin or gelatin/chitosan films.

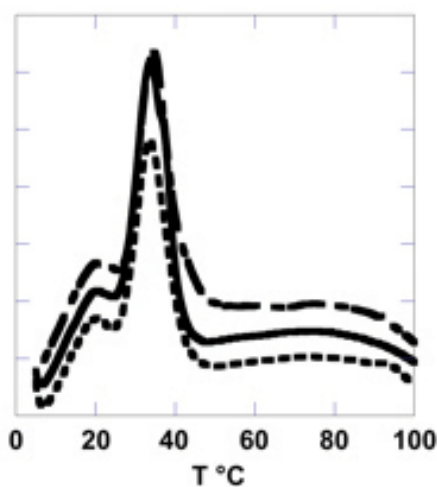


Figure 5. Typical DSC trace, the solid line represents the gelatin or gelatin/chitosan film without carbodiimide, the long dash line represents film containing 3 mM EDC or 15% polycarbodiimide, and the short dash line represents film containing 5 mM EDC or 25% polycarbodiimide.

Numerous published studies have demonstrated the effects of EDC on the properties of gelatin and gelatin/chitosan films.<sup>13,17-19,25</sup> Our earlier research showed that EDC and the polycarbodiimides had similar effects on gelatin and gelatin/chitosan gels.<sup>11,12</sup> The potential for using water-dispersible polycarbodiimides as crosslinkers for acrylic resins and polyurethanes in films for aqueous leather finishes was demonstrated several years ago.<sup>21,26</sup> This is the first study to compare the effects of water-dispersible polycarbodiimides with those of EDC on the properties of gelatin and gelatin/chitosan films, and to show no major differences. The correspondence of 1 - 5 mM EDC with 5 - 25% pCDI is not well defined, future research might develop a better estimate of carbodiimide level in the various pCDIs, and thus a better correspondence.

### Conclusion

Gelable products formed from gelatin or a gelatin/chitosan blend modified with EDC or polycarbodiimides were cast into films. The mechanical properties, hydrothermal stability, solubility, and water absorptivity of the films were examined. The range of values for mechanical properties (tensile strength, % elongation at break, Young's modulus, and toughness) was small enough that it would likely not be a major consideration in the choice of carbodiimide for a particular application. Neither EDC nor the pCDIs tested in this work had any discernible effect on the hydrothermal stability of gelatin or gelatin/chitosan films. The effects of the pCDIs on solubility and water absorption properties of the films trended more closely with the concentration of the pCDI than did the effects of EDC. This preliminary study explores the potential for using the environmentally friendly, water-dispersible pCDIs in gelatin/chitosan-based films for use in leather coating or packaging applications.

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