# Chitosan Cross-Linked Films for Drug Delivery Application

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**Summary:** This work evaluated such a cross-linked chitosan based controlled release device to be later used for sustained drug release. Cross linking was required to control chitosan swelling/deswelling rate. Hexamethylene 1,6-Bis (aminocarboxysulfonate), a bisulfite blocked diisocyanate obtained by the reaction of 1,6 Hexamethylene Diisocyanate and Sodium bisulfite, was used as cross linking agent. Two films formulations were tested: 30 and 50% cross-linked, and they were prepared by solvent evaporation technique. Chitosan cross-linked films were characterized for cross linkage by FTIR, for hydrophilicity by Contact Angle and for swelling behavior by Gravimetric method. Cross linking reaction was confirmed by FTIR. Moreover, cross linking increased the hydrophilic character of cross-linked films and suppressed swelling. However, 30% cross-linked film swollen less than the 50% one, while 50% cross-linked film swollen less than chitosan film itself. This behavior was attributed to the hydrophilic character of the cross linking agent and to the polymeric network formation by cross linking.

**Keywords:** chitosan; cross linking; diisocyanate; drug delivery vehicle
HMDI – 1,6 Hexamethylene Diisocyanate; HBACS – Hexamethylene 1,6-Bis (aminocarboxysulfonate).

## Introduction

Many researches involving a large number of materials (natural, semi-synthetic or synthetic) have been developed in the bio-delivery applications.[1,8] However, the major challenge of this area is the material choice, since while some naturally abundant polymeric materials exhibit a limitation in their reactivity; most of synthetic polymers show limited biocompatibility and biodegradability compared to the natural ones.

Chitosan is a polysaccharide derived from chitin that is the second most abundant polysaccharide found in nature. Chitin is largely found in external skeleton of insets and in shell of crustaceans, while chitosan is mainly obtained by deacetylation of chitin. However, it can also be found in the cell wall of some fungi, such as *Mucor rouxii*.[1]

Chitosan is recommended as suitable functional material, because of its excellent biocompatibility, biodegradability, non-toxicity and adsorption properties.

Chitosan interesting biological properties also include bactericidal effect, hemostatic and anti-tumoral activities, besides promote wound healing.[2,3]

Due to its unique properties, chitosan has found wide application in biomedical area, such as: vehicle for drug, protein and gene delivery[4–6]; implants; scaffolds for tissue engineering; skin substitutes and wound dressings.[7–9]

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Controlled release of drugs at the site of infection is a new strategy being employed to treat infections. Localized delivery systems, based on biodegradable polymers are capable of slow and controlled release of drug for a required period of time, with initial burst effect to circumvent the infection.[10,11]

Hydrogels are one of the promising and versatile materials for controlled release systems, since they are capable of delivering drug at constant rate over an extend period of time.[12–14]

The primary factors that influence drug delivery are polymer composition, drug diffusion, osmotic pressure and bioerosion.[15,16] The retention and the release of a biomolecule also depend on the swelling/deswelling of hydrogel, which may vary by several factors including the amount of water in hydrogel.

Chitosan is soluble in acid solutions and can easily form films and membranes by solvent evaporation. However, due to its positive charge in acid medium, caused by amino group protonation, it becomes extremely hygroscopic hydrogel. To increase the time frame and consistency of kinetics of drug delivery, hydrophilic polymers need to be cross-linked.[17]

Several methods are available for the cross linking of chitosan (glutaraldehyde, genipin, sulfuric acid), the most common being the one using glutaraldehyde as the cross-linker.[18]

However, there are concerns over the toxicity of the cross linking agents used, especially the glutaraldehyde, the residual retention of which may compromise the biocompatibility of chitosan delivery system.

Chitosan low water and organic solvents solubility may restrict its cross linking, due to the solubility of the cross-linker. Therefore, it is desirable to use a cross-linker that is soluble in acidic aqueous solutions.

Recently, a novel water soluble, bisulfite blocked diisocyanate has been prepared and used as a cross linking agent for the network formation with chitosan gel.[19,20] In fact, the bisulfite blocked diisocyanate is the analogous Bis-aminocarboxysulfonate, which must be devoid of any toxic effects of the relative diisocyanate. The bisulfite derivatization of a diisocyanate group makes the blocked cross-linker soluble in water to allow for easy processability of cross-linked chitosan gel formations. Besides, the blocked diisocyanate preferentially reacts with amines (by urea linkage) than with alcohols or water, avoiding undesirable reactions. The choice of an aliphatic diisocyanate, rather than a more reactive aromatic one is due to the high stability of aliphatic diisocyanates in the presence of amines and consequential long-term storage.

Thus, 1,6- Hexamethylene diisocyanate, a well studied diisocyanate was selected as cross linking agent and reacted with sodium bisulfite to prepare the analogous blocked diisocyanate: Hexamethylene 1,6-Bis (aminocarboxysulfonate).

Therefore, this research designs a chitosan formulation, cross-linked through the aforesaid water soluble blocked diisocyanate cross-linker, for drug delivery applications. The resultant biomaterial was characterized by FTIR, Contact Angle and Gravimetric method for cross-linkage, hydrophilicity and swelling behavior, respectively.

## Materials and Methods

### Materials

High molecular weight chitosan (Mw $\sim$ 100,000 and >75% deacetylated), acetone, acetic acid, sodium bisulfite, and hexamethylene diisocyanate were purchased from Aldrich Chemical Co., Milwauki, Wisconsin.

### Experimental Part

#### *Cross-Linker Synthesis*

The synthesis of Hexamethylene 1,6-Bis (aminocarboxysulfonate) was carried out according to Lin-Gibson et al.[20]: in a 100 mL round-bottom flask containing a magnetic stir bar, 6.73 g of HMDI was added to 8.36 g $Na_2S_2O_5$ dissolved in 15.53 mL $H_2O$ and stirred for 20 h.

 

The product was precipitated with acetone and dried in vacuum. Insoluble polymeric byproducts were removed by dissolving the product in water (30 mL) followed by filtration. Product was isolated from the filtrate by precipitation in acetone and dried in vacuum, resulting in a white powder.

*Cross Linking Reaction*

Cross linking was performed by a modification in the procedure described.[20] Briefly, in a 250 mL flask, 1.5 g of chitosan were dissolved in 1.0% aqueous acetic acid with vigorous stirring. After total dissolution, the desired amount of Hexamethylene 1,6-Bis (aminocarboxysulfonate) was added and the solution was heated to 40 °C under stirring for 24 h for completely reaction. Two cross linking ratios were tested: 30% and 50% (w/w), based on the $NH_2$ availability on chitosan.

*Preparation of Cross-Linked Films*

Chitosan films were prepared by placing one mL of the resultant solution in each of the circular plastic molds of 16 mm diameter. The contents in the molds were allowed to dry at room temperature to form the circular films.

*Swelling Analysis*

Swelling behavior of the films was assessed by the gravimetric method. Samples films were kept in a vacuum desiccator for 24 h before determining dry mass ($m_D$) by weighing to +/−0.0001 g places on an electronic balance. Then, the samples were immersed for 24 h in 0.1 M PBS maintained at pH 7.4 at 37 °C. The soaked samples were then blotted with filter paper to remove non-absorbed surface water and then weighed again to determine wet mass ($m_W$). Swelling ratio (S) was calculated, using the following equation:

$$S\,(\%) = [(m_W - m_D)/m_D] \times 100$$

*FTIR Analysis*

The confirmation of chitosan cross linking with Hexamethylene1,6-Bis-(aminocarboxysulfonate) was done by Fourier Transform Infrared Spectroscopy – FTIR. FTIR analyses on chitosan film samples were performed on a MIDAC - M series FTIR instrument. The samples were scanned in the range of 500 to 2000 $cm^{-1}$.

*Contact Angle Analysis*

Hydrophilicity is an important parameter on drug delivery applications, since both drug-material and body-material interactions depend on the water affinity. For this reason, the experiments for contact angle measurements were performed to evaluate hydrophilicity of the resultant biomaterials.

The water contact angle with chitosan films was obtained by carefully placing a drop of water on the film surface and then, after 30 seconds, both right and left angles of contact were measured by the contact angle instrument, using a reported method.[21]

## Results and Discussion

### Results

*FTIR - Fourier Transform Infrared*

According to the Figure 1, the development of cross linking was shown by a well resolved peak between 1565 $cm^{-1}$ and 1475 $cm^{-1}$, characteristic of NH deformation in secondary amides (Amide II band). In addition, a peak at about 1600 $cm^{-1}$, representing C=0 stretch in secondary amides (Amide I band), was observed for cross-linked films, and its resolution was greater for the 50% cross-linked sample. A peak around 1700 $cm^{-1}$, characteristic of carbonyl groups presence, was also observed for cross-linked films and its resolution was better for the sample with higher cross linking degree.

*Contact Angle*

The results obtained for the 0% cross-linked chitosan film were Right: 85.7° and Left: 85.2°. However, for both cross-linked films (30% and 50% cross-linked), the contact angles were 0°, since the water drop spread on the films.

 

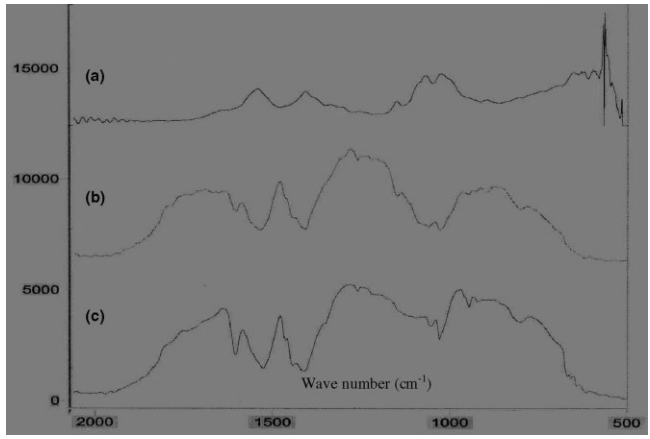


***Figure 1.***
Infrared spectra of chitosan reacted with Hexamethylene 1, 6-Bis (aminocarboxysulfonate). (a) 0% chitosan film, (b) 30% chitosan cross-linked film and (c) 50% chitosan cross-linked film.

Thus, cross linking increased the hydrophilic character of chitosan films.

*Swelling Behavior*

The swelling behavior of chitosan films is presented in Figure 2.

As expected, cross linking suppressed swelling. However, this effect was inversely proportional to the degree of cross linking, which can be attributed to the hydrophilic character of the cross linking agent.

The hypothesis of hydrophilic character of the cross-linker agent can be explained by its chemical composition. HBACS has two amide groups, which are considered hydrophilic. When the cross linking reaction takes place, the NCO group of the cross-linker agent reacts with the $NH_2$ group of chitosan by urea linkage. Then, these hydrophilic groups are incorporated to the polymer chain, increasing its hydrophilicity. On the other hand, after cross linking, the mobility of the polymer chain is decreased by the formation of a polymeric network. For this reason, although cross linking increased the hydrophilic character of chitosan, cross-linked films swollen less than chitosan film itself.

Lin-Gibson et al.[20] also found interesting results for chitosan- Hexamethylene 1, 6-Bis (aminocarboxysulfonate) cross-linked hydrogel. The equilibrium water content did not show a minimal at the stoichiometric ratio, as it was expected assuming full conversion, suggesting that the cross-linker does not react stoichiometrically with the chitosan $NH_2$ groups.

This could be attributed to several factors, such as: side reactions, decreased mobility, reaction time and accessibility of reacting species.

Moreover, further deacetylation of chitosan, which would improve the reactive sites and hydrolysis of the Hexamethylene 1,6-Bis (aminocarboxysulfonate), should

***Table 1.***
Contact angle for chitosan films.

| Sample | Right | Left |
|---|---|---|
| 0% cross-linked chitosan film | 85.7° | 85.2° |
| 30% cross-linked chitosan film | 0° | 0° |
| 50% cross-linked chitosan film | 0° | 0° |

 

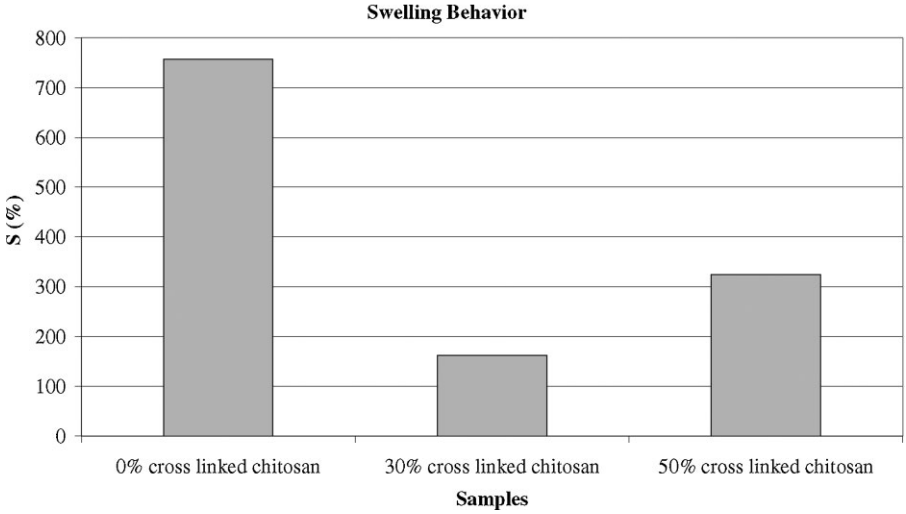


***Figure 2.***
Swelling behavior of Chitosan and Hexamethylene1, 6-Bis- (aminocarboxysulfonate) cross-linked samples.

also be considered to the reaction stoichiometry.

## Discussion

Drug delivery systems such as poly-methylmethacrylate beads, poly (acrylic acid) hydrogel; ceramics and inorganic cements loaded have been developed for controlled release.[22,23] In addition, several researches involving natural, semi-synthetic and synthetic materials have been developed in order to find out an ideal material, since most of synthetic polymers show limited biocompatibility and biodegradability and many naturally abundant polymeric materials exhibit a limitation in their reactivity, biodegradability and processability.[24,25] But chitosan is recommended as suitable functional material, because of its excellent biological properties. Besides being biodegradable and biocompatible, chitosan can also accelerate wound healing and has shown hemostatic and bacteriostatic activities.[2,24]

Topical formulations of chitosan for wound healing and related applications and their effects on wound related components are being intensely investigated.[9]

The use of chitosan for the controlled delivery of biomolecules is on rise recently.[5,6] However, chitosan forms extremely hygroscopic hydrogels due to the protonation of its amino groups at low pH.

Cross linking chitosan is an appropriate methodology for controlling its swelling rate and drug release rates, besides its mechanical properties.

Thus, the controlled retention and release of a biomolecule from chitosan could be achieved by an appropriate biocompatible cross-linker that has physicochemical compatibility with chitosan.

Therefore, this work evaluated such a cross-linked chitosan based controlled release device to be later used for sustained drug release.

## Conclusions

Chitosan – Hexamethylene1,6-Bis-(aminocarboxysulfonate) cross linking was efficient and confirmed by FTIR and hydrophilic behavior analyses.

 

Cross-linked films swell less than chitosan film itself; however, this effect was inversely proportional to cross-linker concentration, due to the hydrophilic character of the cross linking agent.

Moreover, according to contact angle analysis, cross-linked films became more hydrophilic than the chitosan film itself.

Cross-linked films showed satisfactory behavior as drug delivery device. Additional experiments were carried out on gentamicin and silver sulfadiazine sustained releases and will be published in communication later.

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