

## Synthesis and Characterization of Phosphated Crosslinked Chondroitin Sulfate: Potential Ingredient for Specific Drug Delivery

Oswaldo Albuquerque CAVALCANTI \*<sup>1</sup>; Cassiano Correa da SILVA <sup>1</sup>;  
Edgardo Alfonso Gómez PINEDA <sup>2</sup>; Ana Adelina Winkler HECHENLEITNER <sup>2</sup>

<sup>1</sup> Department of Pharmacy and Pharmacology. <sup>2</sup>Department of Chemistry.  
Universidade Estadual de Maringá (UEM) / Maringá - Paraná.  
Av. Colombo, 5790, Bloco P02, 87020-900 Maringá, PR - Brazil.

**SUMMARY.** Because of its biodegradability several natural polymers, such as polysaccharides, have been proposed as appropriate excipients for the development of controlled drug delivery systems for oral administration. Chondroitin sulfate (ChS), a highly water-soluble mucopolysaccharide, was crosslinked with trisodium trimetaphosphate (TMFS) in order to achieve a reduction of its hydro-solubility. ChS was treated with TMFS in three different proportions, in aqueous dispersion at pH 12 for 2 h at room temperature, and then dried. The products obtained were analysed by their Fourier-transformed infrared (FTIR) spectra and differential scanning calorimetry (DSC) curves, which were compared with the analyses of a natural ChS control sample. The results showed that (a) TMFS has effective crosslinking action over ChS and (b) the crosslinked products have reduced water affinity, compared to the natural polysaccharide. These results lead to the conclusion that modified ChS presents good perspectives for its use on modified release pharmaceutical formulations.

**RESUMEN.** "Síntesis y Caracterización de Sulfato de Condroitina Reticulada: Potencial Excipiente para uso en Sistemas de Liberación Específica". Debido a su biodegradabilidad varios polímeros naturales, tales como los polisacáridos, han sido propuestos como excipientes adecuados para desarrollar sistemas de liberación controlada para la administración oral. El sulfato de condroitina (ChS), un mucopolisacárido altamente soluble en agua, fue reticulado con trimetafosfato de sodio (TMFS) para conseguir una reducción de su hidrosolubilidad. ChS fue tratado con TMFS en tres diferentes proporciones, en dispersión acuosa a pH 12 por 2 h a temperatura ambiente y en seguida secado. Los productos obtenidos fueron analizados por FTIR y DSC y comparados con los correspondientes análisis de ChS. Los resultados mostraron que (a) el TMFS efectivamente retícula al ChS y (b) los productos reticulados presentan una disminución de la absorción de agua, comparado con el polisacárido natural ChS. Estos resultados permiten concluir que el ChS modificado presenta buenas perspectivas para su uso en formulaciones farmacéuticas de liberación modificada.

### INTRODUCTION

Controlled release of active ingredients in specified regions of the digestive tract is a most challenging area in the field of development of the controlled drug delivery systems. Site-specific drug therapy based on polymer matrix and coating systems is a growing field of research and application technology. Several natural polymers, such as those found in the diet, have recently been proposed as appropriate excipients for the development of controlled drug delivery devices for oral administration based on their microbial biodegradability <sup>1,2</sup>. A large number of these polysaccharides and oligosaccharides may form the basis for a suitable bio-

degradable carrier. The administration of drugs directly to the colon, particularly to the proximal portion of the large intestine, has been evaluated as a site for local colonic pathologies but also for systemic drug delivery. Colon targeting still remains one of the most challenging systems to drug delivery. Many diseases of the colon, such as bowel disease, constipation, carcinomas and infections could be benefit from colon specific delivery <sup>2,3</sup>. In these cases, local administration of drugs is advantageous as it promotes reduced exposure of the organism to the drug, as a result of smaller doses employed and reduced systemic absorption, which minimizes the occurrence of side effects related to

**KEY WORDS:** Chondroitin sulfate; Crosslinking; Modified release; Trisodium trimetaphosphate.

**PALABRAS CLAVE:** Liberación modificada; Reticulación; Sulfato de condroitina, Trimetafosfato trisódico.

\* To whom correspondence should be addressed: *E-mail:* oacavalcanti@uem.br

the use of these chemotherapeutic agents <sup>3</sup>. Another use for colon-specific release is shown in the case of drugs degraded on upper portions of the GIT (stomach and small intestine), such as proteins and peptide hormones <sup>3</sup>, successful oral administration of these agents is conditioned to the protection of the pharmaceutical form against gastric and duodenal enzyme attack. Various options for colon-specific delivery have been proposed, including the use of pro-drugs, which release the active drug on the colon, and the production of hydrogels, matrices and coated solid forms, employing biodegradable polymers such as polysaccharides <sup>2-4</sup>.

Polysaccharides are broadly available, non-toxic, water-soluble, biodegradable, highly stable polymers. Also, they form gels and can be chemically and biochemically modified, which enables their use on colon-specific drug delivery <sup>1-5</sup>. Chondroitin sulphate (ChS) is one of these polysaccharides that can potentially be used on the development of new therapeutical systems with high specificity degree. This mucopolysaccharide is found on animal connecting tissues, mainly on cartilages <sup>4</sup>. It is chemically constituted by D-glucuronic acid linked to N-acetyl-D-galactosamide (Fig. 1).

Chondroitin sulphate serves as a substrate to colonic microflora, and in this portion of the GIT it is degraded by anaerobic bacteria, mainly *Bacteriodes thetaiotaomicron* e *B. ovatus* <sup>4</sup>. However, since natural ChS is highly water-soluble <sup>3,4</sup>, it becomes impossible to use it on an oral dosage form, because the polysaccharide would be promptly dissolved in the aqueous content of the digestive tube, completely releasing the drug.

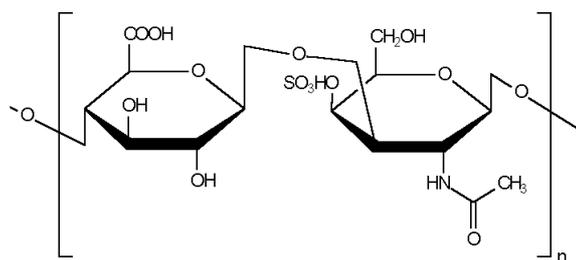


Figure 1. Chondroitin sulphate.

In order to overcome this disability of the natural polysaccharide, modification of the polymer solubility through crosslinking reaction can be employed. This kind of reaction reduces the amount of hydroxyl groups on the polysaccharide chain bonding the chains together, hence decreasing its affinity by water. Chemical modifications applied to polysaccharides can produce new compounds that can be used on innovative therapeutical devices. However, these structural modifications should maintain the potential biodegradability of the polymer by colonic microflora at a specific portion of the GIT.

In this work we propose the use of the methodology applied by Gliko-Kabir *et al.* <sup>5</sup> on guar gum to ChS crosslinking. The crosslinking agent used is sodium trimetaphosphate (TMFS), a non-toxic compound used in food industry to crosslink starch. At alkaline pH a di-polymer phosphate ester complex is formed from ChS and TMFS (Fig. 2).

The obtained crosslinked ChS products were analysed through FTIR <sup>6</sup>, which allows us to identify certain characteristic bonds on the compounds that indicate the formation of new products. In addition, we performed differential scanning calorimetry (DSC) <sup>7</sup> analysis on the products to evaluate the energetic profile of the samples within a specified temperature range.

## EXPERIMENTAL

### Material

Chondroitin sulphate (Solabia, Maringá/PR - Brazil, batch B2L76), Sodium Trimetaphosphate (Sigma, batch 50K02571), Sodium Hydroxide (Henrifarma, São Paulo - Brazil, batch 270401). Other reagents used were of analytical grade.

### Crosslinking of chondroitin sulphate (ChS)

According to the methodology used by Gliko-Kabir *et al.* <sup>5</sup>, a 1% w/v dispersion of ChS was prepared with basified water (pH = 12, with 2M NaOH). The dispersion was stirred for 2 h to allow the polymer to completely dissolve. The following amounts of TMFS were added to three 100 mL portions of the dispersion: 2 mL 15% to the first (sample A), 10 mL 30% to the second (sample B) and 30 mL 30% to the third (sample

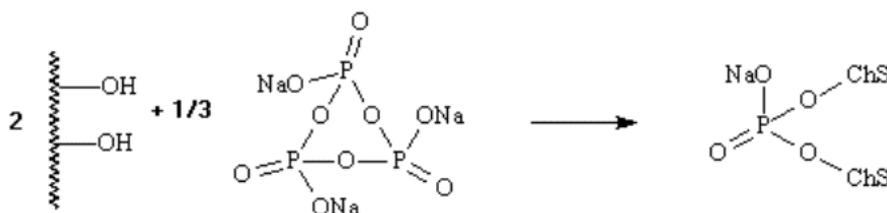


Figure 2. Reaction between TMFS and ChS.

C). The pH was again corrected to 12 with NaOH 2M drops, and the mixture was stirred for another 2 h. Ten mL samples were dried on polytetrafluoroethylene coated polyamide pans under 60 °C for 10 h. The obtained material was kept dry and stored in a desiccator until further analysis.

A dispersion of the natural, unmodified polysaccharide was likewise prepared to be used as reference material.

#### **Fourier-transformed infrared (FTIR) spectra determination**

The FTIR spectra of the sample were carried out on KBr pellets, at the 4000 to 400  $\text{cm}^{-1}$  wave number range, with a Bomem(r) MB-100 Michelson equipment.

#### **Differential scanning calorimetry (DSC)**

Differential scanning calorimetry (DSC) was performed on 6 mg samples with a Shimadzu® DSC-50 Calorimeter, in a nitrogen atmosphere flowing at 10 mL/min. Temperature ranged from room temperature until 350 °C at a rate of 10 °C/min. The instrument was calibrated with an indium and zinc standard.

### **RESULTS AND DISCUSSION**

The evaluation of the FTIR spectra and DSC curves of products A, B and C compared to natural ChS reference, strongly suggests the occurrence of the proposed crosslinking reaction.

#### **FTIR spectroscopy**

Considering that only hydroxyl groups of ChS are involved in the reaction with TMFS, which are converted to O-P linkages in the crosslinked product, a reduction of hydroxyl groups and no change in carbonyl groups content is expected. To evaluate this behaviour, the absorbance at the maximum of the peaks originated by hydroxyl groups (3410  $\text{cm}^{-1}$  - 3470

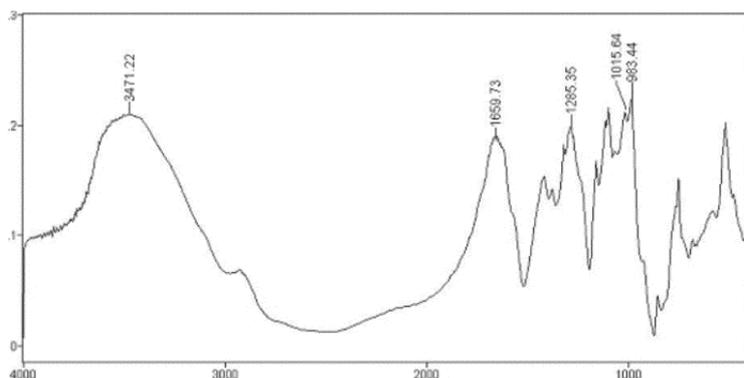
$\text{cm}^{-1}$ ) and by carbonyl group (1644  $\text{cm}^{-1}$  - 1660  $\text{cm}^{-1}$ ) was extracted from the FTIR spectra (Figures 3 and 4). Then, for each sample the quotient of both mentioned absorbances was computed. This parameter {Abs (OH)/Abs (CO)} is 1.71 for ChS and 1.0 for the product A, providing an evidence that the OH/CO ratio varied in the expected way.

In addition, comparing the TMFS spectrum in the 500 to 1500  $\text{cm}^{-1}$  range (Figure 7) with that of products B and C (Figures 5 and 6) in the same wavenumber range, it can be seen that only minimum alterations of the TMFS spectrum occurs in the products spectra. The 1296 and 997  $\text{cm}^{-1}$  (P=O and P-O-P bands, respectively<sup>8,9</sup>) have been shifted to lower energetic levels on the products, such dislocation can be originated by intermolecular interactions between the ChS chains and the TMFS polar molecules. Therefore, these results suggest that in the B and C products much TMFS remains unreacted.

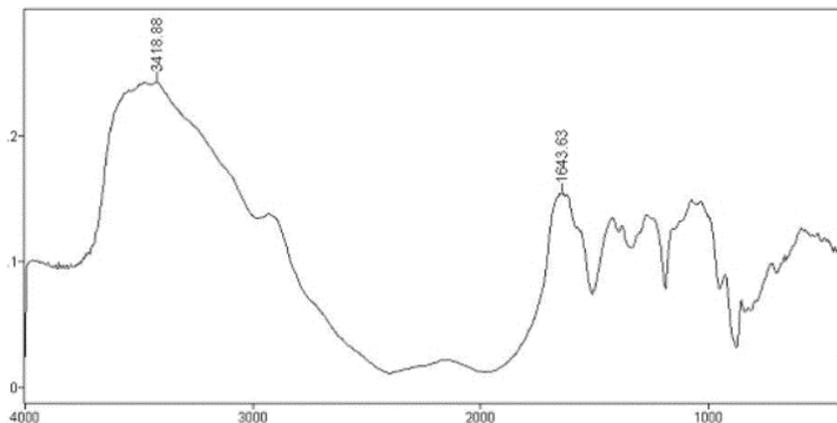
Another observation that can be made is the occurrence of a peak at 1015  $\text{cm}^{-1}$ , related to the formation of P-O-C bonds between the crosslinker and the polysaccharide. This peak is not visible on the crosslinker neither on natural ChS. However, it is visible on sample A, which indicates the formation of the crosslinked product. On samples B and C this peak is superimposed by the P-O-P bond-related peak at 988  $\text{cm}^{-1}$ , probably due to the presence of unreacted TMFS<sup>8</sup>.

#### **Differential scanning calorimetry (DSC)**

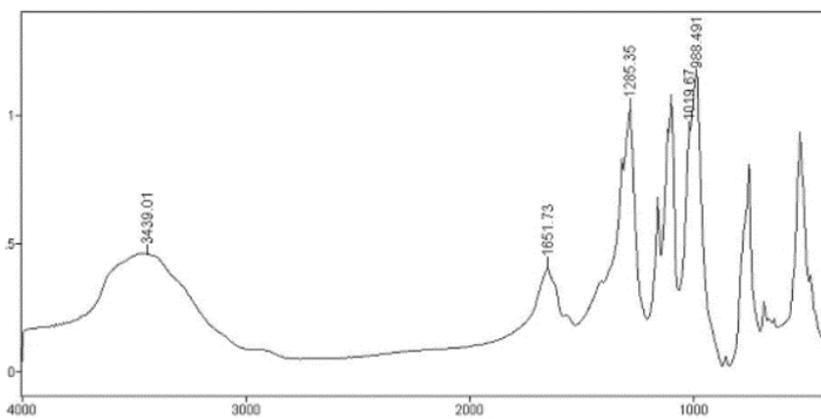
The DSC curves of the products compared to the natural ChS (Figure 8) show that as the crosslinking concentration increases, the endothermic peak related to water evaporation (80-100 °C) decreases and shifts to lower temperature range (close to 60 °C on product C). These results indicate that the crosslinked products have smaller amounts of absorbed water,



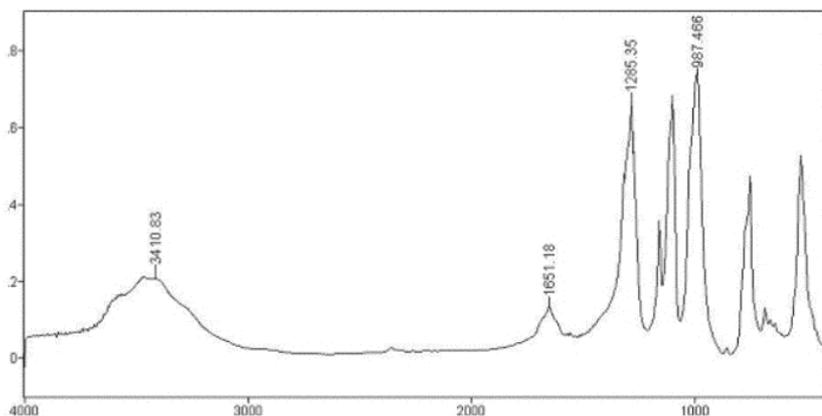
**Figure 3.** FTIR spectrum of natural chondroitin sulfate (ChS).



**Figure 4.**  
FTIR spectrum  
of product A  
(see Experimental  
Part - Crosslinking of ChS).



**Figure 5.**  
FTIR spectrum  
of product B  
(see Experimental  
Part - Crosslinking of ChS).



**Figure 6.**  
FTIR spectrum  
of product C  
(see Experimental  
Part - Crosslinking of ChS).

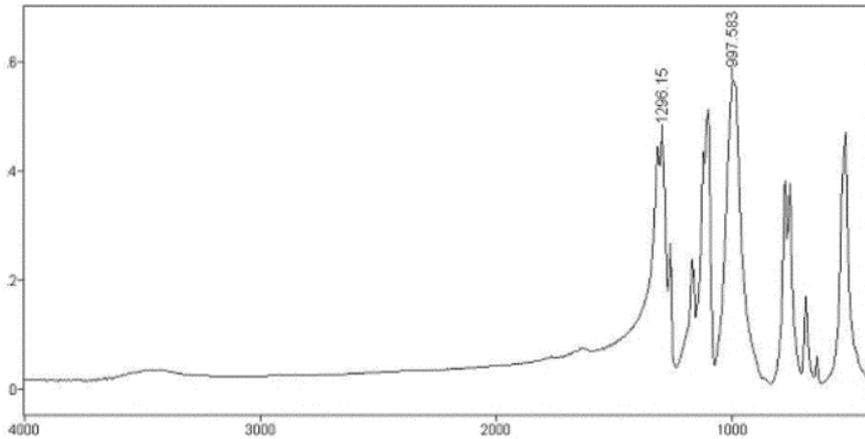
and that this water is weakly bonded to the compound the greater the concentration of the crosslinker.

The absence of a characteristic endothermic fusion peak is observed, which is very frequent among polysaccharides. On the other hand, the exothermic pyrolysis peak that occur on the samples (around 240 °C) is affected by the reticulation showing a dislocation to lower temperature and also a reduction in the peak area (which is directly proportional to the amount of energy liberated by the reaction). Such be-

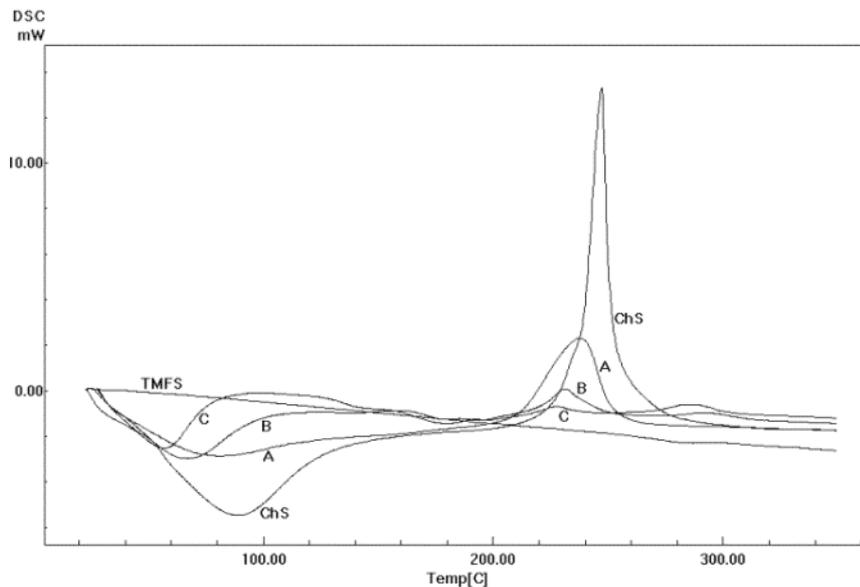
haviour is a common one observed with certain materials such as wood, when a flame retardant additive (typically borates, phosphate or aluminium salts) is incorporated <sup>10</sup>. The presence of that additive provides a mechanism capable to reduce the total energy liberated in the temperature range in which the sample degrades through a highly exothermic reaction.

## CONCLUSION

Solubility of natural polymers remains as a challenge to the use of these compounds as



**Figure 7.** FTIR spectrum of trisodium trimetaphosphate (TMFS).



**Figure 8.** DSC curves of ChS, TMFS and products A, B and C.

pharmaceutical excipients. The results obtained from this investigation lead to the conclusion that TMFS can be used on ChS crosslinking, yielding best results at 0.3 g of crosslinker per gram of polysaccharide ratio. The employed procedure granted structural modification of ChS, decreasing its water affinity. This very promising characteristic allows its possible use on controlled release dosage forms.

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