Nano and micro-hydrogel of chondroitin sulphate: synthesis and characterization

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Abstract

The main idea of this work is to develop methodology for preparing nanospheres of hydrogels of chemically modified CS and to combine the technological benefits of hydrogels to the properties of nanoparticles. It is report here the chemical modification of chodroitin sulpaté (CS) by reaction with glycidyl mehacrylate (GMA), the synthesis of hydrogel and respective characterization. The modification of CS by reaction withy GMA allows to obtain hydrogels by subsequent reaction of modified product using persulfate (as initiator) and TEMED (as catalyst). Micro and nano-particles hydrogel were synthesized by use of emulsion techniques. The raw, modified and hydrogels were characterized by NMR (¹H and ¹³C). The size of hydrogels particles were measured by SEM analysis. The hydrogels synthesized in this work have potential to be applied as micro and nano-spheres devices injected to the body for arthritis treatment joined to anti-inflammatory delivery.

Key-words: nano-hydrogel; chondroitin sulphate; drug delivery; nano-spheres devices.

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1. Introduction

Nowadays, several drugs, peptides, proteins and cytosine are available due to the significant biotechnology developing. However, most of such chemicals can not be administrated directly to body because it can be enzymatic inactivated, for instance by digestive enzymes on the gastrointestinal medium and loose the pharmacology activity. (Peppas et al., 2000).

Very many therapeutic devices have been investigated in last years. For a ground view, such devices have the basic function to protect the drug of degradation, to promote specific release and also to guarantee that the releasing would be controlled. (Griffith, 2000).

Hydrogels are three-dimensional hydrophilic polymer networks able to absorb and to retain large amount of water and biological fluids. Upon contact with water, these materials can exhibit a reversible environmental responsive by external stimuli such as temperature, pH, ionic strength, etc. In addition, the hydrogels are compatible with various drugs and are well-accepted by both the body tissues and the biological fluids. Their characteristics such as biocompatibility, drug protection, site-specific drug delivery and controlled drug release enable hydrogels as a promising system for uses in drug delivery (Vandamme et al. 2002).

The chondroitin sulphate (CS) is a mucopolysaccharide constituted by repeat units of D-glucuronic acid and sulphated-N-acetyl-D-galactosamin connected alternately by tipo $\beta1-4$ e $\beta1-3$ bound (Volpi, 2004). The CS is often used in various pharmaceutical formulations like tablets, capsules (hard and soft), ophthalmic solutions and liquid preparations for medical uses. (Sim, 2005; Liang, 2003; Choi, 2003; Okamoto, 2004). The CS presents high solubility in water and does not form hydrogels. However, if chemically modified, by reaction with glycidyl methacrylate (GMA) for instance, the modified CS may be cross-linked to form tridimensional network or a hydrogels.

The main idea of this work is to develop methodology for preparing nanospheres of hydrogels of chemically modified CS and combine the technological benefits of hydrogels to the properties of nanoparticles. On promised employment using of nanospheres of hydrogels may be in arthritis treatment, mainly the rheumatoid arthritis. This is possible because the micro and nanospheres loaded with one or more drugs can be injected directly in the articulations of patient and the drug target directly to the local of action. Once injected, the delivering of drug might controlled by a specific kinetic proper of the device. Simultaneously, the particles of modified CS, joined to the synovial liquid, can act as lubricant reducing
the articulations friction. So, it is expected that a significant improvement may be achieved in the arthritis treatment by use of micro and nanospheres of chemically modified chondroitin sulphate.

2. Experimental

2.1. Materials

The Chondroitin Sulfate (CS) was kindly supplied from Company-Solabia, Maringá, Brazil. Glycidyl methacrylate (GMA, Acros), sodium persulphate (Sigma), dimethylsulfoxide (DMSO, Labsynt - Brazil), N,N,N’,N’-tetramethylethylenediamine (TEMED, Sigma). Dialysis tubes were purchased from Sigma (D-0530, lot 103H0525).

2.2. Chemical modification of Chondroitin Sulfate (CS)

A mixture of CS, GMA and water solution pH 3.5 was stirred at 50 °C for 24 h. After, ethanol was added to induce the precipitation of methacrylated chondroitin sulphate (MCS). The modified material was separated by filtration and dialyzed for 24 h. The material was lyophilized and analyzed by 1H and 13C NMR.

2.2. Nano-hydrogel synthesis

An aqueous phase of the modified of chondroitin sulphate (MCS) plus sodium persulfate and an organic phase of the benzyl alcohol were mixed and deoxygenated by nitrogen bubbling for 5 min. After a small amount of TEMED was added the mixture and the cross-link reaction started and a respective gelation occurred. At this stage the stirring speed is very important. Using 12,000 rpm for stirring the reaction occurs inside of micro and nano-droplets. So micro and nano-particles of hydrogels are formed. These particles were precipitated by addition of ethanol and purified by cycles of solution-precipitation, in water-ethanol, respectively. After three cycles, the solution was spray-dried and micro and nano-hydrogel were obtained.

2.3. 1H NMR and 13C NMR spectroscopy

The 1H NMR and 13C NMR spectra were obtained by a Varian spectrometer, model Oxford 300 at a frequency of 300 MHz. The 1H NMR and 13C NMR of CS and MCS were obtained using solutions of D2O. The relaxation time length and the angle pulse used were 30 s and 90°, respectively for 1H NMR spectra and 30° and 1 s for the 13C NMR spectra. The 1H NMR and 13C NMR spectra of GMA were obtained in CD3Cl solvent. The 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt was used as intern reference. The chemical shift was given at ppm.
2.4. Scanning electron microscopy for hydrogel morphology

Nano-hydrogels morphology was conducted by scanning electron microscopy images, SEM, (Shimadzu, model SS 550), operating at 12keV. The nano-hydrogels swollen to equilibrium at 37 ℃ were firstly frozen in nitrogen liquid and then lyophilized by a freeze dried for 24 h.

3. Results and Discussion

Chemical modification basically consisted of coupling the chemical group –CO- \((\text{H}_3\text{C})\text{C}=\text{CH}_2\) from the GMA onto polymeric structure of CS. Li et al. (2003), reported that the condroitin sulphate (CS) and glycidyl methacrilate (GMA) react on a protic solvent simultaneously by two pathways: (1) transesterification – fast and reversible; 2) epoxy ring opening – slow and irreversible (Fig. 1). Also, they concluded that in aqueous solution of pH 3 the reaction occurs exclusively by the epoxy ring opening pathway.

![Figure 1 – Possible pathways for reaction of glycidyl methacrylate (GMA).](image)

No important the pathway for that the reaction take place, the chemical groups –CO- \((\text{H}_3\text{C})\text{C}=\text{CH}_2\), from the GMA, are inserted to the structure of CS (Fig. 2). Such modification allows the posterior cross-linking reaction of to form hydrogels.
Figure 2 – Insertion of –CO-(H₃C)C=CH₂ groups on CS structure through epoxy ring opening and transesterification pathways, according to the schemes proposed in the literature by Li et al., (2003) and Hennink et al., 1997.

The chemical modification of CS by reaction with GMA is evidenced by characteristics signals on the ¹H e ¹³C NMR spectra. The signals at δ 6.17, δ 5.57 and δ 1.95 ppm, on ¹H NMR spectrum of modified CS (Fig. 3), characterize the presence of –(H₃C)C=CH₂ groups on CS chemical structure. The signals at δ 6.17 and δ 5.57 ppm were attributed at the vinyl hydrogen atoms (labeled as 2a and 2b in Figure 3) and the signal at δ 1.95 ppm is referred to methylic hydrogen atoms (labeled as 3a, 3b and 3c).

Figure 3 – ¹H NMR spectra of CS and modified CS (MCS). The signals at 6.17, δ 5.57 and δ 1.95 ppm indicate the insertion of –CO-(H₃C)C=CH₂ groups on the CS structure.
On $^{13}$C NMR spectrum of MCS (Fig. 4), the resonance signals of carbons related to the –CO-\((\text{H}_3\text{C})\text{C}=\text{CH}_2\) groups are observed at $\delta$ 172 ppm for the carbon labeled as 4 on the structure inserted in Fig. 4, $\delta$ 138 and $\delta$ 130 ppm for the carbon labeled as 1 e 2, respectively, and $\delta$ 20 ppm for the carbon labeled as 3.

The micro and nano-hydrogel of MCS were obtained by a cross-linking reaction of the MCS in solution sodium persulfate 10 mM. The solid-state CP-MAS $^{13}$C NMR analysis and scanning electron microscopy (SEM) were used for the characterization of nano-hydrogel. For the hydrogel formation, it was found that the vinyl groups, present onto CSM structure, were converted to methyl groups indicative of the cross-linking reaction. The analyses of solid-state CP-MAS $^{13}$C NMR of micro and nano-hydrogels of MCS (Fig. 5) allows to observe the appearing of the signals at $\delta$ 46.02 for the carbon labeled as 1 and $\delta$ 40.61 for carbon labeled as 2 on the structure inserted in Fig. 5. These signals characterize the conversion of vinyl groups and a respective hydrogels formation.

Figure 4 – $^{13}$C NMR spectra for CS and MCS. The signals at $\delta$ 172, $\delta$ 138, $\delta$ 130 and $\delta$ 20 ppm characterize the insertion of –CO-\((\text{H}_3\text{C})\text{C}=\text{CH}_2\) on the CS structure.
Figure 5 - CP/MAS $^{13}$C NMR spectra for CS, MCS and hydrogel of MCS (HMCS). The signals at $\delta$ 46.02 and $\delta$ 40.61 ppm characterize the conversion of vinyl to methyl groups and a respective hydrogel formation.

The particle size of the hydrogels was statistically determined by SEM micrographs. It was observed spherical particles of hydrogel with dimensions up to 20 $\mu$m and up to 500 nm (Fig. 6).

Figure 6 - MEV obtained from spray drier from solution containing the micro and nano-hydrogels of MCS.
4. Conclusions

The CS reacts with glycidyl methacrylate, in water environment. The MCS presents vinyl groups from which hydrogel can be obtained after gelation. In this work these reactions were performed in mild and no-toxic conditions.

The hydrogels synthesized in this work have potential to be applied as micro and nano-spheres devices injected to the body for arthritis treatment joined to anti-inflammatory delivery.

5. References


