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Note

Crosslinked chitosan—preparation and characterization

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Abstract—Chitosan undergoes radical-induced depolymerization in the presence of potassium persulfate at 60 °C, leading to extensive crosslinking of the fragmented chains on subsequent cooling at 4 °C. As a result, a possible conformational change leading to higher crystallinity, as evidenced by IR, X-ray and ^{13}C NMR was observed.

Keywords: Chitosan; Persulfate; Free radical; Crosslinking; CP MAS ^{13}C NMR

1. Introduction

Chitosan is an amino-polysaccharide obtained by alkali-induced de-N-acetylation of chitin, which is a byproduct derived from the exoskeleton of invertebrates. Chitosan exhibits innumerable applications in a wide range of fields such as agriculture, packaging, food biotechnology, medicinal and pharmaceutical, etc. For some specific applications, however, low molecular weight chitosans[1,2] or graft (with synthetic monomers) chitosan copolymers are preferred because of their extraordinary physico-chemical characteristics. During such grafting reaction, it was observed that a portion of chitosan undergoes extensive crosslinking to give modified chitosan,[3] whose properties are much different from those of native chitosan. Nevertheless, polymer crosslinking leads to the formation of a permanent covalent network, which may allow the free diffusion of water/bioactive materials and also enhance the mechanical properties of the polymer.[4] Thus, covalently crosslinked chitosans have two main applications, namely as drug delivery systems allowing easy release of bioactive materials by diffusion and as permanent networks used, for example, as scaffolds in cell culture. Chemical crosslinks are formed by irreversible covalent links, as in covalently crosslinked chitosan. Moreover, they do not exhibit characteristics such as the modification of their properties in response to changes in their physiological environment, (pH or temperature) that allow drug delivery to be efficiently controlled.[5]

In crosslinked chitosan, the polymeric chains are interconnected by crosslinkers, leading to the formation of a 3D network. They can be formed by complexation with another polymer, generally ionic, or by aggregation after chitosan grafting.[6] Crosslinkers are molecules of MW much smaller than those of the chains between two consecutive crosslinks.[6] Other components such as additional polymers to form a hybrid polymer networks (HPN) or semi- or full-interpenetrating polymer networks (IPN) can be added during the crosslinking reaction. The biocompatibility of such modified chitosans has not yet been assessed, due to the presence of traces of potentially toxic auxiliary molecules or crosslinkers, whose administration in humans may be problematic.

To date, the most common crosslinkers used with chitosan are dialdehydes such as glyoxal[7,8] and in particular glutaraldehyde.[9,10] However, the main drawback of such reactions is that they are generally considered to be toxic.[11] For example, glutaraldehyde is known to be neurotoxic, its fate in the human body is not fully understood[12] and glyoxal is known to be mutagenic.[13] Therefore, even if products are purified before administration, the presence of free unreacted dialdehydes in the products could not be completely excluded. Besides

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dialdehydes, crosslinkers such as diethyl squarate,[14] oxalic acid[15] or genipin[5] can exhibit direct crosslinking mechanisms, although they remain incompletely elucidated. However, there is a lack of data regarding the biocompatibility of diethyl squarate, while oxalic acid[15] has shown in vitro toxicity in rats.[16] The use of genipin is an interesting alternative to dialdehydes. It is a naturally occurring material, which is commonly used in herbal medicine and as a food dye.[17] The biocompatibility of genipin in humans has not been assessed yet. Another approach is the formation of covalently linked networks, close to HPN, by use of water soluble, biocompatible polymers.[6] Due to their high MW, these polymers do not correspond to the definition of crosslinkers used here. However, they are specially developed to crosslink chitosan and their properties and application potentials are similar to those of covalently crosslinked chitosan hydrogels. Crosslinked chitosan can also be formed by direct interaction between polymeric chains, without the addition of crosslinkers.[18] The present communication deals with the isolation and characterization of such crosslinked chitosan, which was formed as a byproduct of persulfate-induced free radical graft copolymerization.

2. Results and discussion

It is important to optimize the conditions of the crosslinking reaction, since they determine and allow the modulations of the crosslinking density, which is the main parameter influencing the various properties of crosslinked chitosan. Use of potassium persulfate (KPS) was shown to be very effective in degrading chitosan.[18] At 60 °C, KPS undergoes thermal degradation generating anionic persulfate free radicals, which latter attacks the cationic amino groups (NH_3^+) at C-2 of the glucosamine moiety of chitosan. Because of electrostatic attraction, subsequently the radical would attack the C-4 carbon and transfer the radical to the C-4 of the adjacent residue, which weakens and breaks the immediate C–O bond (C-1 carbon of the previous residue as shown in Fig. 1). Consequently, the chitosan chain is degraded into two or many shorter chains.[18,19] After depolymer-

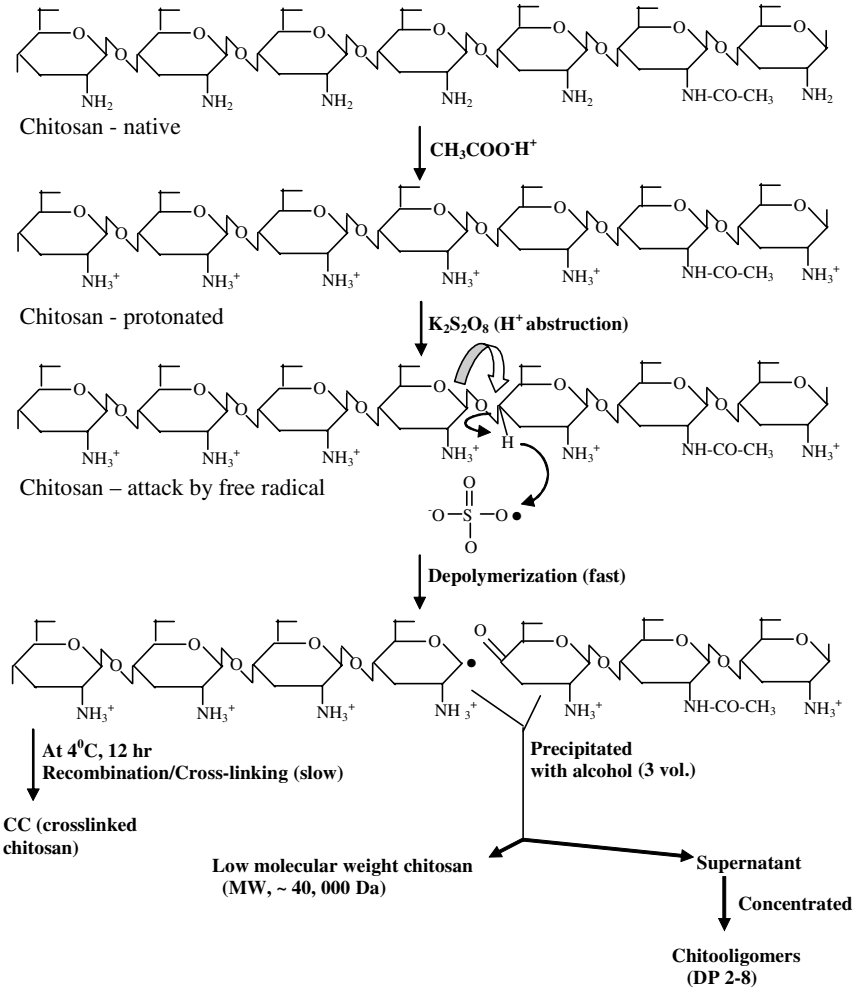

Figure 1. Schematic representation for depolymerization mechanism of chitosan by KPS.

ization, the mixture when kept in the cold (4 °C) for 12 h or at room temperature for 24 h, a sedimented product got separated out (CC). Its IR (Fig. 3a) showed much higher crystallinity with a sharp absorption peak around 618 cm^{-1}. Unexpected sharp intense peaks appeared at 2θ, 11.44° and 18.28° in X-ray diffraction pattern (Fig. 3b). CPMAS ^{13}C NMR spectrum (Fig. 3c) with sharp distinct peaks and much decreased line widths compared to native chitosan indicated a highly crystalline nature of CC and data was supported by SEM (Fig. 4) where fibrous native chitosan[3] (Fig. 4a and b) was modified to crystalline granules (Fig. 4c and d). Further, the spectrum showed all the carbon signals typical of chitosan, that is CH_3—26.3, C-2/C-6—60.5, C-3—74.5, C-5—77.7, C-4—83.2 and C=O—177.5 ppm. Nevertheless, the C-1 was split into two sharp peaks of equal intensities, 100.2 and 103.6 ppm. This may be indicative of recombination/crosslinking reaction taking place, post depolymerization and subsequent cooling, between the free radicals generated, as shown hypothetically in Figure 2. This type of free radical induced crosslinking has been reported in solid-state polypropylene reaction at 60 °C.[20]

Crosslinking may also involve two structural units that may or may not belong to the same depolymerized chitosan chain. Indeed, secondary interactions, such as hydrogen bridges and hydrophobic interactions, between acetylated and non-acetylated units of chitosan lead to a higher degree of crystallinity and as a result inducing conformational changes in the molecule, although there are some examples of products formed by chitosan crosslinked with itself,[6] that exhibit pH sensitive swelling.[21] This report may be the first of its kind in this regard. Insolubility at lower pH 3.5 and solubility at ~pH 10 is in favour of such a crosslinking in CC. Indeed, the numerous interchain interactions formed by crosslinking inhibit swelling, since all the amino groups of chitosan might have reacted during the reaction. Since crosslinking requires mainly deacetylated reactive units, a high degree of deacetylation with high MW of chitosan along with increased temperature and duration is favourable. Such crosslinked chitosans might possibly exhibit specific biofunctionalities.[22,23]

Finally, it should be noted that the crosslinking reaction catalyzed by potassium persulfate could possibly induce a conformational change in chitosan as observed by IR, X-ray diffraction and NMR studies. However, the influence of such a change on the properties of chitosan is not yet clearly understood and requires further investigation.

3. Experimental

3.1. Crosslinked chitosan preparation

Shrimp chitin, procured from CFTRI Regional Center, Mangalore, India, was de-N-acetylated with 40% sodium hydroxide solution at 100 °C for 1 h. After the reaction, the product (MW 96,000 Da, 16% N-acetylated) was washed thoroughly with distilled water and freeze dried.

Chitosan solution (1%, in 0.5% acetic acid), taken in a three-necked flat-bottomed flask, was purged with nitrogen at 60 °C under stirring (200 rpm). Subsequently, potassium persulfate (KPS, 0.8 mM) was added to the solution and the reaction was completed in 2 h. The reaction mixture was cooled to room temperature and later kept at 4 °C for 12 h. The sedimented product (CC) obtained by centrifugation (4000 rpm for 10 min) was thoroughly washed with distilled water and lyophilized.

3.2. Infrared spectroscopy

Infrared spectra were recorded in KBr discs on a Perkin–Elmer 2000 FTIR spectrometer (Norwalk, USA) under dry air at room temperature. The CC (6 mg) was blended with 200 mg of KBr (IR grade) and about 40 mg of the mixture was pelleted for IR spectral measurement.

3.3. X-ray diffractometry

X-ray analysis was done using Sintag XDS-2000 instrument fitted with a θ–θ goniometer and EG-7G solid state germanium liquid nitrogen cooled detector, under the following conditions: 30 kV and 25 mA with $CuK\alpha_1$ radiation at λ 1.54184 Å. The relative intensity was recorded in the scattering range (2θ) of 4–40°.

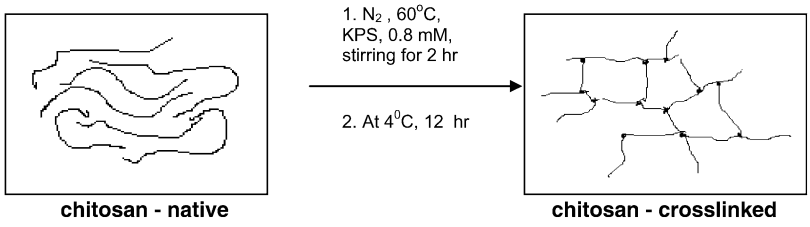

Figure 2. Pictorial representation of the formation of crosslinked chitosan (CC).

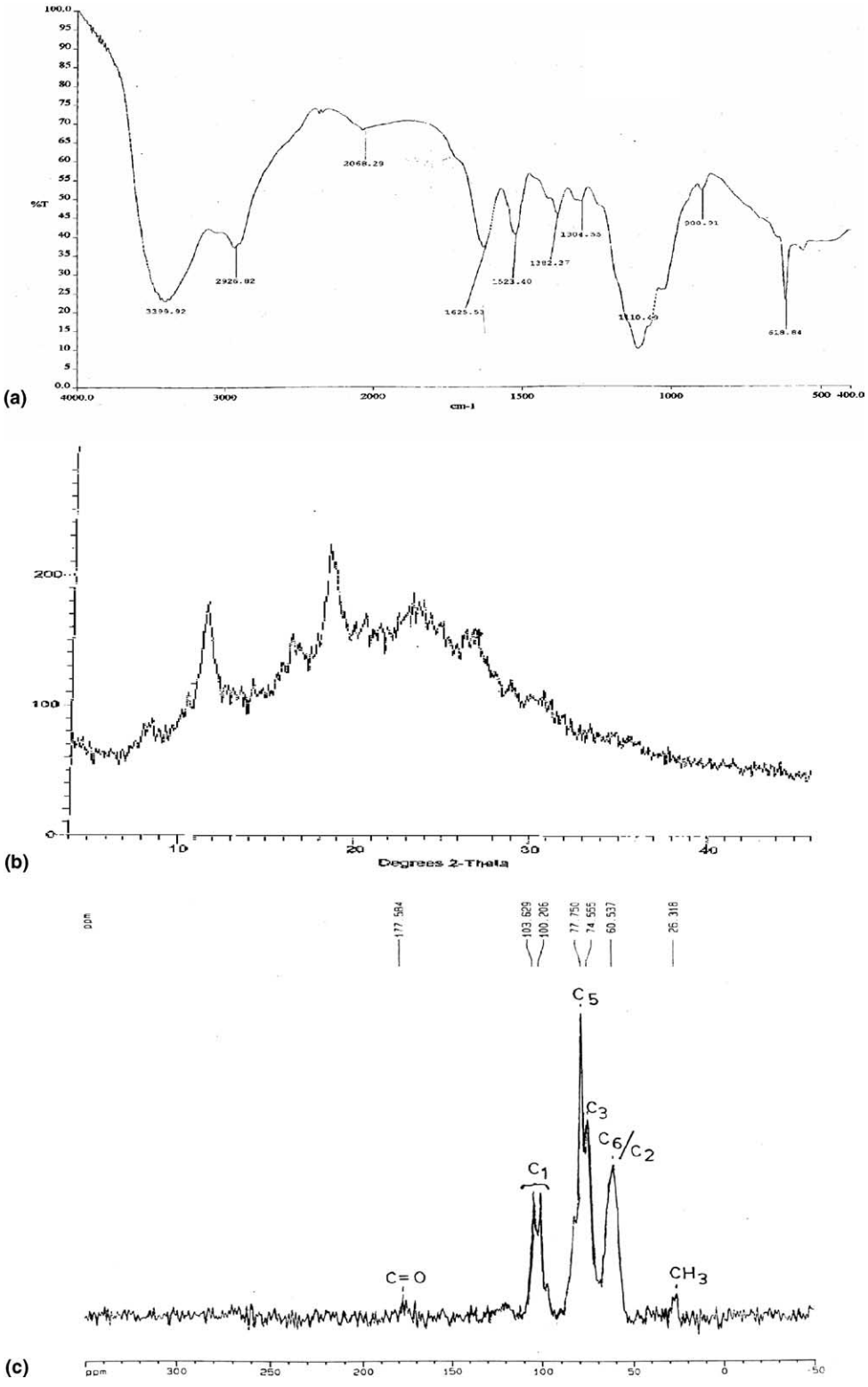

Figure 3. Characterization of CC by (a) IR, (b) X-ray and (c) ^{13}C CPMAS NMR.

3.4. CP-MAS ^{13}C NMR spectroscopy

Solid state NMR measurements were carried out with Bruker DSX 300 spectrometer (Munich, Germany). Spectra were acquired at 75 MHz with crosspolarization magic-angle spinning (CP MAS) technique, which were spun at the magic angle at 5 kHz. A contact time of 1 ms and a pulse repetition time of 5 s were used and more than 3000 scans were accumulated.

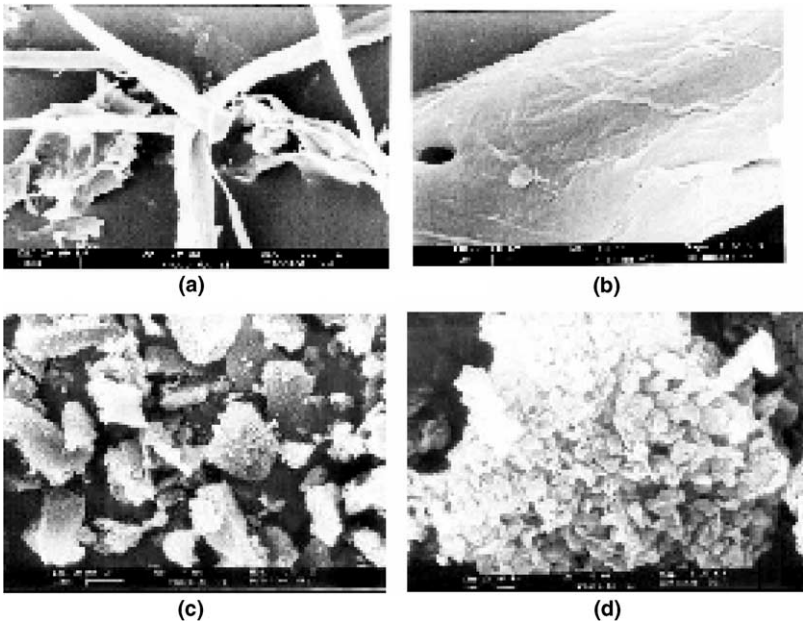

Figure 4. SEM Photographs of native chitosan (a, b) and CC (c, d)—(a, c) low resolution (600×) and (b, d) high resolution (3000×).

3.5. Scanning electron microscopy

The dry sample, spread on a double sided conducting adhesive tape, pasted on a metallic stub, was coated (100 μ) with gold in a sputter coating unit for 2 min and observed in a LEO-435-VP (LEO Electron Microscopy Ltd., Cambridge, UK) electron microscope at 20 kV.

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