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Structural characterization of chitosan and oxidized carboxymethyl cellulose based freeze-dried films

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Abstract The conversion of dihydroxyl groups to dialdehyde by periodate oxidation is a useful method widely used in derivatization of cellulose. Periodate oxidation is a highly specific reaction to convert 1,2-dihydroxyl groups to paired aldehyde groups without significant side reactions. This reaction cleaves the C2–C3 bond; the resulting compound is the dialdehyde cellulose (DAC). These aldehyde groups of carboxymethyl cellulose interact with amino group of chitosan and their interaction results in the formation Schiff's base with enhanced properties of both the polymers, i.e., of chitosan and carboxymethyl cellulose. Interaction of chitosan and oxidized carboxymethyl cellulose (OCMC) has been carried out with three combinations, i.e., 5, 10, and 15 wt% OCMC with rest of chitosan in 2% lactic acid solution. This new compound is covalently crosslinked which has been analyzed by various techniques like FTIR, TGA, XRD, and SEM.

Keywords Chitosan · Carboxymethyl cellulose · Dialdehyde cellulose (DAC) · Schiff's base · Covalently crosslink

Introduction

Polymers are playing an important role in various branches of industry today. Many notable advances in technology have followed exploitation of the properties offered by new polymeric materials like blends, composites, etc. Polymer blends are prepared by physical mixing of two or more polymers. The formation of polymeric

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blends constitutes a perspective way of making materials with new properties, especially from natural polymers those are of special importance. The resulting blend system often exhibits properties that are superior to any one of the component polymers [1–5].

Chitin, a natural polymer, is the second most abundant organic resource on the earth next to cellulose [6]. It is an exoskeleton of crustacean, cuticle of insects, and cell wall of fungi.

Deacetylation of chitin yields chitosan, which is relatively reactive and can be produced in numerous forms, such as powder, paste, film, fiber, and more [7, 8]. Several biomedical applications of chitosan have already been reported [6–9]. It has the potential to be used as artificial kidney membrane, absorbable sutures, hypocholesterolemic agents, drug delivery systems, and supports for immobilized enzymes.

Besides, it has been claimed to accelerate the wound-healing process. Chitosan has some advantages due to its nontoxicity and biodegradability without damaging the environment. It is a biocompatible material that breaks down slowly to harmless products (amino sugars) that are absorbed completely in body [10].

Chitosan [β-(1 → 4)-2-amino-2-deoxy-D-glucose] is a unique basic polysaccharide and generally is represented as a homopolymer. However, the deacetylation process is rarely complete and most commercial and laboratory products tend to be a copolymer of *N*-acetylglucosamine (NAG) and *N*-glucosamine repeat units. The ratio of two repeating units depends on the source and preparation of chitosan, but the glucosamine units predominate.

Most commercial polysaccharides (e.g., cellulose, dextran, pectin, alginic acid, agar–agar, agarose, starch, carrageenan, and heparin) are either neutral or acidic, but chitosan is a basic polysaccharide. In neutral or basic pH, chitosan contains free amino groups and is insoluble in water, while in acidic pH, chitosan is soluble in water due to protonation of amino functions. The solubility depends on the distribution of free amino and *N*-acetyl groups [6, 11, 12]. Chitosan is a linear polyelectrolyte at acidic pH. It has high charge density, one charge per glucosamine (Glc-NH_2) [13]. The pH and ionic strength have an important impact on the intrinsic viscosity of polyelectrolytes. Chitosan in aqueous acid solution has been surface reacted with polyanion aqueous solutions (heparin, sodium alginate, carboxymethyl chitin [CM-chitin], polyacrylic acid) to give polyelectrolyte complexes [14].

Cellulose is the most extensively used natural polymer and represents a renewable natural source for organic materials. The main reactions acting on the structure of cellulose and causing its alteration are photo-degradation, acid hydrolysis, oxidation, and biodegradation. A complete analysis of them is quite complex, since these phenomena are all related to each other. In order to get better results by interaction of cellulose with chitosan we will consider only the oxidation process.

The conversion of dihydroxyl groups to dialdehyde by periodate oxidation is a useful method widely used in derivatization of cellulose to active the polymer at further reactions [15]. Periodate oxidation is a highly specific reaction to convert 1,2-dihydroxyl (glycol) groups to paired aldehyde groups without significant side reactions and is widely used in structural analysis of carbohydrates [16–22]. When applied to glucose in the carboxymethyl cellulose chain, this reaction cleaves the C2–C3 bond, according to the mechanism of Malaprade reaction [23, 24]. The resulting compound is the dialdehyde cellulose (DAC) [25, 26]. The application or the quantitative understanding of this reaction has been hampered by complication arising from hemiacetal formation of aldehyde and crystalline nature of cellulose [27, 28]. The structure of dialdehyde cellulose, has been suggested to include the hydrated form [$–CH(OH)_2$], the 2,3-hemialdal form [–CH(OH)–O–CH(OH)–], the 2,6- or 3,6-hemiacetal forms [$–CH(OH)–O–CH_2–$], as well as the reactive free aldehyde form. The, former three types correspond to addition of one molecule of water per each aldehyde group, addition of one molecule of water per two aldehyde groups, and rearrangement between an aldehyde group and one of the remaining alcohol groups without addition of water. All forms act as free aldehydes under appropriate conditions. According to a kinetic study, free or hydrated aldehyde groups react about 300 times faster than hemialdal groups, and hemiacetal groups come in intermediate between both forms [29].

The structure of chitosan is similar to that of cellulose, except at carbon-2, where the hydroxyl group of cellulose is replaced by an amino group.

The interest in using cellulose as a interaction polymer for chitosan arises from two facts: (a) cellulose is the most abundant natural biopolymer with relatively strong mechanical strength of up to 1 GN/m^2 (10,000 MPa) and (b) cellulose has similar chemical structures as chitosan, providing the possibility of producing a homogeneous blend that combine the unique properties of chitosan and the good availability of cellulose to make chitosan cellulose hydrogel.

In this article, covalently crosslink freeze-dried films of chitosan and oxidized cellulose are prepared for the first time. Synergistic interactions between the two polysaccharides are expected to improve properties of the resulting compound. Compatibility or interactions between the two polysaccharides were investigated by FTIR, X-ray diffraction, TGA, and SEM.

Experimental

Materials

Chitosan middle viscous (deacetylation degree 83%, moisture content 10%, viscosity 1% in 1% acetic acid at 20 °C, 200–400 mPas), microcrystalline carboxymethyl cellulose powder (particle size 6–10 mm), periodic acid and isopropanol was purchased from Sigma-Aldrich. Lactic acid that used to dissolve chitosan was bought from Merck. Sulfuric acid and sodium bicarbonate were of reagent grade. Distilled water was used throughout the experiments.

Oxidation of carboxymethyl cellulose

Different concentrations of carboxymethyl cellulose were taken into consideration, i.e., from 0.5 to 2 wt%. Finally, 1 wt% was found the most suitable because of its optimum viscosity and good solubility in aqueous medium.

Added 1 g CMC in 100 mL distilled water, stirred it at room temperature for 6 h, after getting the clear solution added 3 mL of 0.45 M periodic acid. The pH of the solution was adjusted to about 3.5 using dilute sulfuric acid to decrease and sodium bicarbonate to increase the pH of the solution. The oxidation reaction conditions were carried out at temperature 45 °C [1]. The reaction kettle was wrapped with several layers of aluminum foil to prevent exposure to light, i.e., to avoid auto oxidation. Oxidation reaction was carried out for 0.5 to 4 h. Here we found 2 h oxidized carboxymethyl cellulose (OCMC) the best one for its interaction with chitosan because 2 h OCMC posses the sufficient number of aldehyde groups which fulfill our requirement for the fabrication of covalently crosslink films of chitosan with OCMC.

After 2 h of oxidation, precipitation of OCMC has been taken out with isopropanol to get a solid form of OCMC for the further experiment of its crosslinking with chitosan.

Dissolution of chitosan in lactic acid

Dissolution of chitosan has been carried out in 2% lactic acid solution with varying amounts of solute and solvent, 0.5 to 2 wt% of chitosan solution were prepared and finally 1 wt% was found to be the appropriate one for fabrication pure chitosan film because of its good solubility and optimum viscosity otherwise it's a very viscous solution. As lactic acid is not harmful for human body it has not been removed from the film it was still remained in the film. Lactic acid was used to solubilize chitosan because it has been proven to be non-irritating relative to other alternatives, such as acetic acid, on rabbit skin and has the ability to improve the flexibility of the film due to a plasticizing action.

Interaction of chitosan with OCMC (in the presence of lactic acid)

First of all chitosan was dissolved in 2% lactic acid solution and stirred solution on magnetic stirrer for at least 4 h to get the clear solution of chitosan then added OCMC and stirred solution for more 6 h for the proper interaction of chitosan with OCMC. 1% solution with different amount of chitosan and OCMC has been prepared (Table 1). The blend film gets swell in aqueous medium but it is insoluble in this medium which shows very good interaction of OCMC with chitosan which make the blend film insoluble in aqueous medium.

Film formation through freeze drying

20 mL of polymeric solution is poured in 12 mm of petri dish and kept undisturbed at room temperature for 1 h then put it in deep freezer at temperature −81 °C for

Table 1 Different ratios of chitosan and oxidized CMC

CS (%)	OCMC (%)
95	5
90	10
85	15

overnight, after deep freezing samples were freeze dried in freeze dryer at temperature −81 °C for 48 h. After freeze drying film was isolated with the help of spatula. It is easily detachable from petri dish because it is completely dry and does not stick on petri dish.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra have been recorded for the determination of changes occurred in various peaks when chitosan is blended with OCMC. Approximately, 1 mg of dry sample was pressed into a pellet with 200 mg of potassium bromide and FTIR spectroscopy studies of the samples were carried out on Perkin Elmer Spectrum-BX FTIR system. FTIR of the samples were recorded in transmittance mode.

Thermogravimetric analysis (TGA)

TGA was carried out to observe the variation in the thermal stability of chitosan when it has been blended with OCMC. TGA studies of the samples have been recorded with Perkin Elmer TGA-7 system. The thermograms were obtained under nitrogen atmosphere at a uniform heating rate of 20 °C/min in the temperature range of 50–600 °C.

X-ray diffraction (XRD)

X-ray diffraction has been applied to mark the crystallinity changes in the structure of chitosan with CS-OCMC blend films. X-ray diffraction of the films was measured by the X-ray diffractometer Xpert PRO PANalytical, Holland. X-ray diffraction pattern were recorded with Cu-$\acute{\alpha}$ radiation ($\lambda = 1.54$ Å). The voltage and current were recorded 40 kV and 30 mA, respectively. Samples were scanned from 5 to 60° (2θ).

Scanning electron microscope (SEM)

Film surface morphology was examined using scanning electron microscopy. The dried film samples were mounted on a metal stub with double-sided adhesive tape. The morphological structures of the films were studied by a JSM-5600 LV scanning electron microscope of JEOL, Tokyo, Japan and the images were taken at accelerating voltage 5 kV and a magnification 100 times of origin specimen size.

Mechanical property

The membranes with different compositions were carefully cut into specimens with rectangular dimensions of 6 mm in width and 30 mm in length, and the exact thickness of each specimen was assessed using a micrometer. The specimens were mounted on mechanical gripping units leaving a gauge length of 25 mm, and then their tensile properties were measured by a universal testing instrument (Instron

5565, Instron Corp., Canton, MA) at a crosshead speed of 2 mm min^{-1}. Five specimens were tested for each condition.

Result and discussion

Periodate oxidation of cellulose proceeds gradually from the amorphous to the crystalline phase, it cause the changes in the physical and chemical properties of carboxymethyl cellulose. In this study, several oxidations with different time period were carried out. Increasing the reaction time samples degrade more extensively. Oxidation has been carried out from 0.5 to 4 h and we found 2 h the best one because it possessed sufficient number of aldehyde groups which interact with the amino group of chitosan, as the oxidation time is increased number of aldehyde groups also increase which crosslink with more amino group of chitosan and bellow 2 h oxidation time of CMC we did not get sufficient aldehyde groups.

We prepared CS-OCMC blend from 5 to 15 wt% of OCMC and 5% CS-OCMC blend was found the best one because of its good miscibility with chitosan, as the oxidation is increased number of aldehyde group increased as well, which crosslink with amino group of chitosan and formation of covalently crosslinked Schiff's base take place which is insoluble in aqueous medium and enhance many of the properties of chitosan and OCMC.

FTIR

Oxidation of carboxymethyl cellulose

In Fig. 1 the three bands, i.e., at 1728, 2928, and 2718 confirm the oxidation. The oxidation leads to the presence of one more characteristic band of OCMC in 880 cm^{-1} regions. The broad band at 880 cm^{-1} can be assigned to the hemiacetal and hydrated form of the dialdehyde cellulose [21–29], the sharp band at 1,728 cm^{-1} is characteristic of carbonyl groups stretching, bands at 2,928 and 2,718 cm^{-1} show the C–H stretching of aldehyde group. FT-IR analysis of both CMC and OCMC is complicated owing to the high affinity of the samples with water. In fact sometimes the band at 1,613 cm^{-1} can be very broad and can hide the bands of the carbonyl groups. Moreover, in the oxidised samples the identification of the functional groups is complicated because aldehyde, keto, and carboxyl groups of oxycellulose absorb in a very narrow region of the spectrum, between 1,720 and 1,780 cm^{-1}. Bands at 1,117 and 1,062 cm^{-1} correspond to the C–O–C stretching vibration of pyranose ring skeleton and bands at 1,117 and 1,062 cm^{-1} correspond to the C–O–C stretching vibration of pyranose ring skeleton. For samples oxidised with periodate the identification of the aldehydic group is more difficult, since dialdehyde carboxymethyl cellulose can exist in partially or completely hydrated form, as hemiacetal or as hemialdal forms, i.e., under forms that do not present a classical bands of the aldehydic carbonyl.

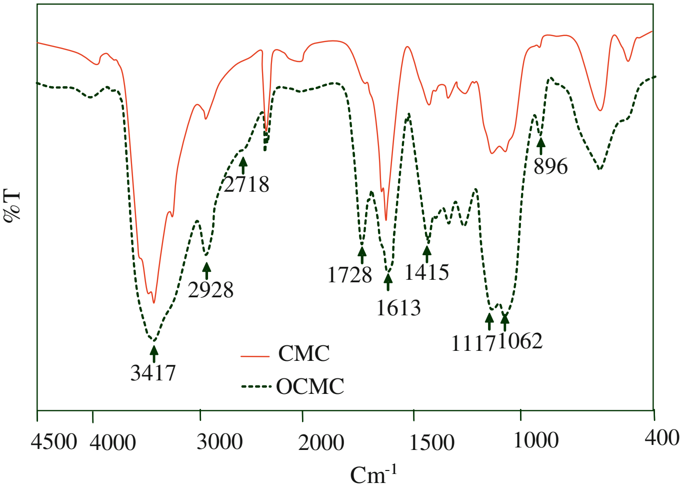

Fig. 1 FTIR spectra of CMC and OCMC

Interaction of OCMC with chitosan

Figure 2 revealed the IR spectra of chitosan at 3,347 cm^{-1} was the OH stretching, which overlaps the NH stretching in the same region [30, 31]. The peak at 2,921 cm^{-1} is typical C–H stretch. A small peak at 1,633 cm^{-1} was due to the C=O stretching (amide I) [32], the band at 1,545 cm^{-1} was NH bending (amide II) [33]. The sharp peaks at 1,420 cm^{-1} correspond to the CH_3 symmetrical deformation mode. The peak at 1,148 cm^{-1} indicates the saccharide structure.

It is reported that when two or more substances are mixed, physical blends versus chemical interactions are reflected by changes in characteristic spectral peaks. The chemical interactions are reflected by changes in the peaks of characteristic spectra after physical interaction of two or more substances. In the spectrum of chitosan/OCMC blend film the OH and/or NH of chitosan, bands shifted and broadened from 3396, 3414, 3421, and 3422 cm^{-1}, respectively. This result indicated that interactions were present between the hydroxyl groups of OCMC and the amino groups of chitosan. Peak at 880 cm^{-1} showing the formation of hemiacetal and hydrated forms of oxidized CMC.

The characteristic absorption band at 1,591 suggesting that Schiff's base (C=N double bond) was formed between aldehyde group of OCMC and amino group of chitosan.

TGA

Oxidation of carboxymethyl cellulose

In thermal degradation studies of OCMC two distinct zones are observed where the weight is being lost Fig. 3. The initial weight loss is due to presence of small

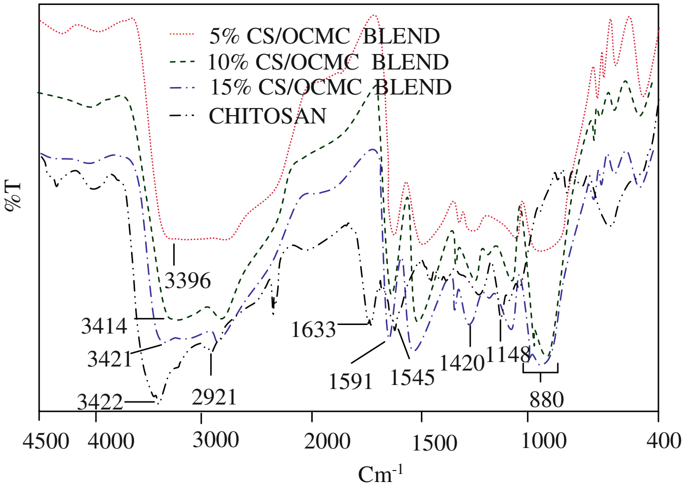

Fig. 2 FTIR spectra of Chitosan, 5, 10, and 15 wt%

amount of moisture in the sample unto 100 °C. The second loss is due to loss of CO_2 from polysaccharide (100–200 °C) while in case of CMC the rapid weight loss occurred at (300–340 °C). It has been observed that in initial stages of weight loss (200–400 °C) oxidized samples started to decompose at lower temperatures (150 °C) than that starting carboxymethyl cellulose (300°), reflecting the reactive nature of OCMC which is due to the ring opening and formation of dialdehyde groups in OCMC.

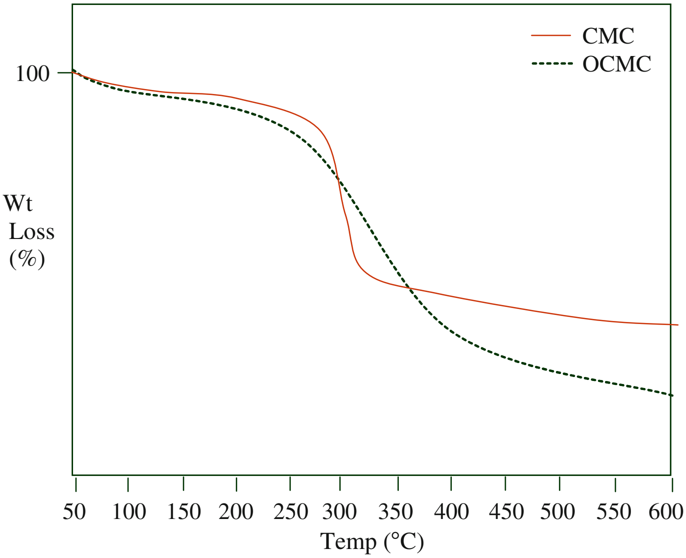

Fig. 3 TGA of CMC and OCMC

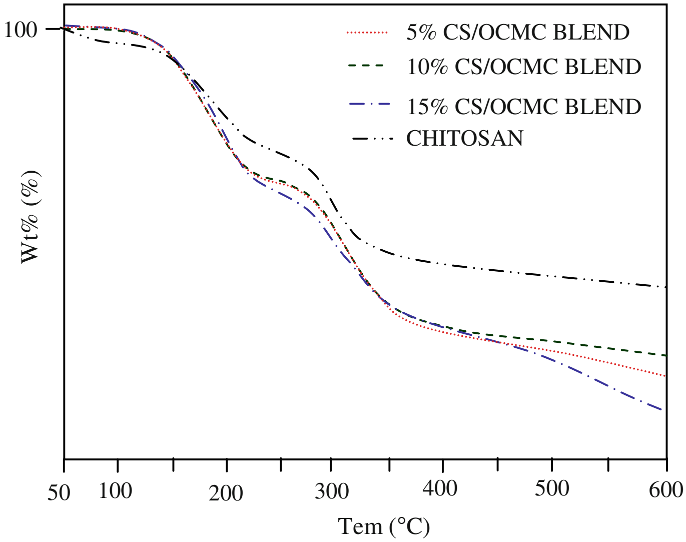

Fig. 4 TGA of Chitosan, 5, 10, and 15 wt%

Interaction of OCMC with chitosan

TG curves of CS/OCMC blend films are shown in Fig. 4. The thermal decomposition of the polymers and their blends under investigation, accompanied by weight losses, takes place in several stages. At the first stage, the weight loss of CS/OCMC blend films during heating at 100 °C is connected with the evaporation of water (6%). Until reaching 250 °C, a slight weight loss occurs due to the changes in amorphous regions. At 250 °C, the weight loss rate increases and attains its maximum at 280 °C, when the degradation of H-bonds in crystal sites of the polymer begins. At 500 °C, the weight loss of OCMC film amounts to 77%.

At the first stage of heating CS film at a temperature of 105 °C, the weight loss is 11.2% corresponding to the evaporation of adsorbed moisture. During heating above 157 °C, the degradation rate sharply increases to reach its maximum at 190 °C. At this temperature the weight loss of the sample is 43%. During further heating, the degradation rate slows down and later on it increases again to reach a new maximum at 349 °C (the weight loss amounts then to 73.5%). Probably at the first stage of degradation, amorphous areas decompose by the dehydroxylation of chitosan units, while at higher temperatures decomposition takes place in the crystal areas of the polymer [34–40]. At a temperature of 500 °C the weight loss amounts to 96%, this corresponds to the evaporation of volatile products of polymer chemical degradation. When some OCMC is added to chitosan, the intensive weight loss begins at a higher temperature as compared with that of 100% chitosan, being characteristic of the thermal degradation of OCMC crystals. The character of curve changes with the increase in the fibroin content. The weight loss rate of blends is lower than that of individual component. The loss of weight decreases with the increase in the content of OCMC. Thus, the addition of OCMC increases the thermal stability of chitosan, slowing down its thermal degradation. The results obtained confirm the presence of strong interaction between diverse macromolecules. Strong contacts between –CHO and –OH groups of OCMC and –NH groups of chitosan that have been formed in solution remain in films after the removal of solvent.

XRD

Oxidation of carboxymethyl cellulose

The well-defined CMC pattern diminished with the increase of oxidation time and the product became almost amorphous. A decrease has been observed in crystalline structure of the polymer.

In X-ray diffractogram of OCMC amorphous nature of oxidized carboxymethyl cellulose has been observed, CMC has a broad peak at 21° of 2θ showing its crystalline structure which has almost been diminished in OCMC. It is then concluded that periodate oxidation make OCMC structure less crystalline as compared to CMC and crystallinity get decrease as the oxidation time is increased. The loss of crystallinity is considered to result from opening of glucopyranose rings and destruction of their ordered packing (Fig. 5).

Interaction of OCMC with chitosan

As shown in Fig. 6, the chitosan-based film was in a semicrystalline state with three main diffraction peaks ($2\theta = 11.28$, 15.6, and 21.98°) present in its X-ray diffraction pattern, whereas an amorphous state was observed in the 5, 10, and 15 wt% blend films.

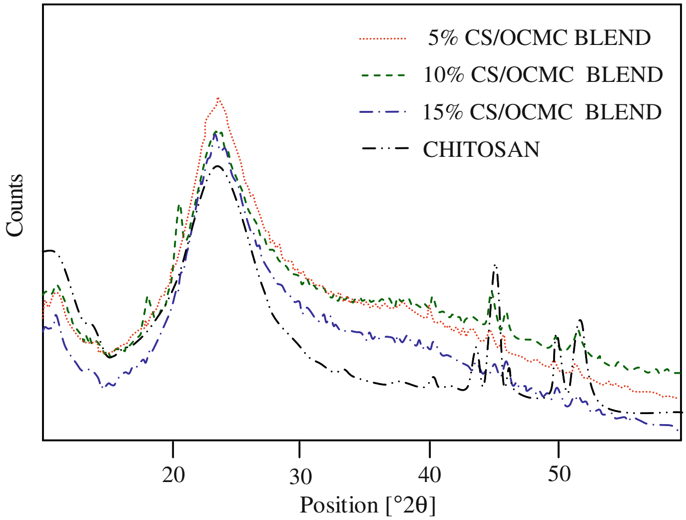

Fig. 5 XRD of Chitosan, 5, 10, and 15 wt%

In these blend films this new broad amorphous peak indicating that there was a molecular miscibility and an interaction among these two components, i.e., in chitosan and OCMC. This interaction was due to the aldehyde group of carboxymethyl cellulose which was formed after oxidation of CMC and amino group of chitosan because these two groups are the reactive sites of these polymers.

Scanning electron microscopy

Figure 7 shows the scanning electron microscopy (SEM) photographs of the surface and cross-section morphology of the macroporous CS/OCMC blend membranes. The pictures clearly indicate that the pores were well distributed and continuous within the membranes. In Fig. 6b, cross-section of 5 wt% CS/OCMC blend, we saw the beautiful three dimensional porous structures. This would meet the requirements of various applications in biomedical field such as drug release, protein separation in future investigations because high permeability could be achieved. This structure can also be used in tissue engineering for tissue growths.

Mechanical property

The mechanical properties of the CS/OCMC films are illustrated in Table 2, and Fig. 7 shows the representative stress–strain curves for CS/OCMC films. The

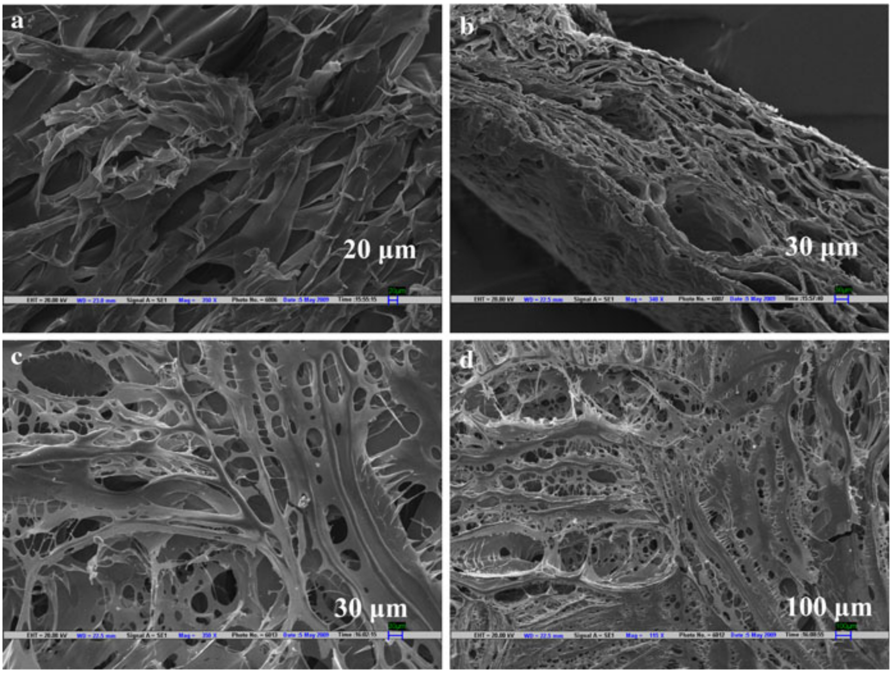

Fig. 6 SEM photographs of a macroporous CS/OCMC blend membrane: **a** 5 wt% surface, **b** 5 wt% cross-section, **c** 10 wt% surface, **d** 15 wt% surface

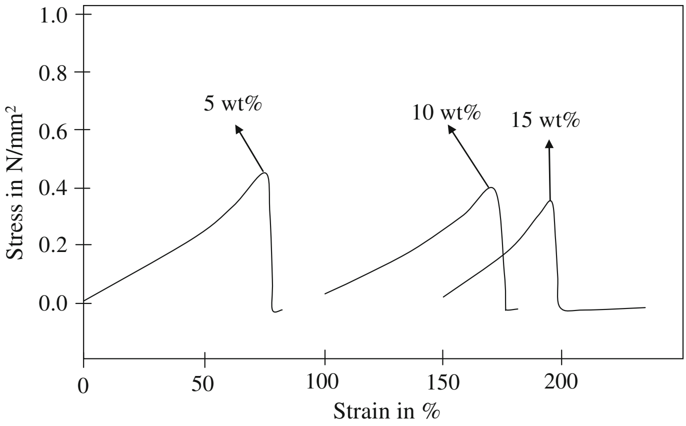

Fig. 7 Representative stress–strain curves of 5, 10, and 15 wt%

Table 2 Mechanical properties of CS/OCMC films

CS/OCMC film %	σM (Max stress) MPa	εtM (Strain at F Max) %	L0 (Gauge length) mm
95/5	0.44	77.00	25
90/10	0.37	71.01	25
85/15	0.34	48.18	25

mechanical properties of the three different concentrations of CS/OCMC films were not greatly different. But the stress and strain of 5 wt% CS/OCMC film is higher than that of the two which is gradually decreasing from 5 to 15 wt%. Although there is not much difference in the mechanical properties of three films but 5 wt% CS/OCMC film shows good mechanical strength over 10 and 15 wt% which make it suitable for various applications in the field of biomedical.

Conclusion

The studies here reported give an insight in understanding the structural changes occurring when oxidized carboxymethyl cellulose is crosslinked with chitosan. FTIR analysis shows interaction between chitosan and OCMC lead to the formation of Schiff's base which is covalently crosslinked compound which is insoluble in aqueous medium, it means it's giving additional properties to the chitosan and OCMC. XRD also showing compatibility in chitosan and OCMC, although crystallinity is not much affected by interaction process through these three ratios, i.e., 5 to 15 wt% but formation of broad amorphous peak showing molecular miscibility and good interaction between chitosan and OCMC. TGA proves that interaction of chitosan with OCMC makes the resulting compound thermally more stable. The weight loss rate of blends is lower than that of individual component. The loss of weight decreases with the increase in the content of OCMC. Thus, the addition of OCMC increases the thermal stability of chitosan, slowing down its thermal degradation. The results obtained confirm the presence of strong interaction between diverse macromolecules. Strong contacts between –CHO and –OH groups of OCMC and –NH groups of chitosan that have been formed in solution remain in films after the removal of solvent. SEM images also describing the compatibility of OCMC with chitosan by its properly distributed pores throughout the film which also showing its three dimensional porous structure which is very useful for its further application in biomedical field as the covalent crosslinking leads to the formation of a permanent network allowing the free diffusion of water and enhancing the mechanical properties of the gel. As a result of these interesting characteristics, covalently crosslinked compound have two main applications, namely as drug delivery systems allowing release of bioactive materials by diffusion and as permanent networks used, for example, as scaffolds in cell culture and whatever the type of structure, networks containing covalently crosslinked chitosan are considered as porous [41–44]. This term is used to describe networks containing free water that can diffuse through the hydrogel. Due to these pores, covalently crosslinked chitosan hydrogels can be used as drug delivery systems from which drugs are released by diffusion [45]. Therefore, finally we can conclude that interaction of chitosan and OCMC make the resulting compound more useful with enhanced properties which is a water insoluble covalently crosslinked compound having a very good flexibility and thermal stability.

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