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# Carboxymethyl chitosan and its applications

**V.K Mourya[a*], Nazma N. Inamdar[a] and Ashutosh Tiwari[b,c]**

[a]*Government College of Pharmacy, Aurangabad 431 001, India*

[b]*National Institute for Materials Science, Tsukuba, Ibaragi 305 0047, Japan*

[c]*School of Materials Science and Engineering, Jiangsu University, Zhenjiang 212 013, China*

[*]Corresponding author. Tel: (+91) 95240 2346820; Fax: (+91) 95240 2321130; E-mail: vkmourya@gmail.com

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## ABSTRACT

Deacetylation of chitin affords chitosan, a polymer, widely studied for its pharmaceutical and nonpharmaceutical applications. The hurdle in comprehending these applications is its limited solubility. Carboxymethylation of chitosan helps to surmount this hurdle with its improved solubility in water. Though there is ample of research related to carboxymethyl chitosan (CMC) the focused review of the topic is unavailable. Hence an attempt is made in this review to cover the recent findings pertaining to synthesis, characterization of CMC and its applications especially in pharmaceutical field. CMC has been synthesized by ways as direct alkylation, reductive alkylation, Michael addition and characterized by FTIR, NMR spectroscopy, and DSC, titrimetry, viscometry, gel permeation chromatography, X-ray diffraction and capillary zone electrophoresis. The carboxymethyl group can be present at O or N or both the atoms of chitosan molecule. The CMC possess modulated physical and biological properties as chelating, sorption, moisture retention, cell functioning antioxidant, antibacterial, antiapoptotic etc. CMC is used in sustained or controlled release drug delivery, pH responsive drug delivery, DNA delivery as permeation enhancer etc. CMC can be further modified with alkylation, acylation, and grafting. Carboxyalkylation of chitosan yield carboxyethyl, carboxybutyl chitosans. These analogues of CMC may be helpful in substantiating the applications of chitosan. Copyright © 2010 VBRI press.

**Keywords:** Carboxymethyl chitosan; synthesis, characterization; biological properties; drug delivery; DNA delivery; permeation enhancer; modification of carboxymethyl chitosan.

**V. K. Mourya** is presently working as Professor in Pharmaceutics in the Government College of Pharmacy, Aurangabad, India. He is engaged in the development of biomaterials and their application in drug delivery systems especially for the nano confined target. He has contributed in QSAR studies involving molecular modelling. He is pharmacy postgraduate from Dr. H. S. Gaur University, Sagar, India.

**Nazma N. Inamdar** has done her M Pharm from LM College of Pharmacy, Ahemedabad, India. She started her teaching career in NDMVP Samaj College of Pharmacy, Nashik. She is currently working as Assistant Professor at Allana College of Pharmacy, Pune. She teaches medicinal chemistry and analytical chemistry at graduate and post graduate level. She is pursuing PhD.

**Ashutosh Tiwari** is an invited professor of Materials Science and Engineering at the Jiangsu University, China and also works as

foreign researcher at the National Institute of Materials Science, Japan. He received PhD in Chemistry from the University of Allahabad, India, later he joined to the National Physical Laboratory, New Delhi, India as a young scientist. After that he moved to the University of Wisconsin, USA as a post-doctoral researcher. In addition, he obtained JSPS fellowship in Japan and SI fellowship in Sweden. In 2010, he became Marie Curie Incoming Fellow at Cranfield University, UK. In his academic carrier, he has published over 125 publications and patents in the field of materials science and technology. He has also edited five books on the advanced state-of-the-art of materials science for prestigious publishers including Bentham Science, USA, Nova Science, USA, etc. His recent research interest is focus on designing and development of the smart materials for biomedical and engineering values.

## Contents



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## 1. Introduction

Chitosan, a cationic copolymer of glucosamine and N-acetylglucosamine, is a partially deacetylated derivative of a natural polysaccharide - chitin, which is one of the most abundant carbohydrates in nature and is mostly derived from the exoskeleton of crustaceans [**1, 2**]. Chitosan has a unique set of useful characteristics such as biorenewabilty, biodegradability, biocompatibility, bioadhesivity and nontoxicity. These properties make it a polymer of reckon. Chitosan and its derivatives are used in various fields: pharmaceutical, biomedicine [**3-5**] water treatment [**6, 7**], cosmetics [**8, 9**], agriculture [**10**], and food industry [**11-14**]. However the applications of chitosan suffer severe limitations since it is insoluble in neutral or alkaline pH because of its very stable crystalline structure arising from strong hydrogen bonds. The solubility is observed only in acidic aqueous solutions below pH 6.5 (below the pKa of chitosan).The solubility of chitosan can be improved by depolymerization and its chemical modifications [**15**]. Chitosan has reactive amino, primary hydroxyl and secondary hydroxyl groups which can be used for chemical modifications under mild reaction conditions to alter its properties (**Fig. 1**) [**16**]. Many water-soluble derivatives have been prepared by quaternarization [**17, 18**] or by introducing hydrophilic groups like hydroxypropyl, dihydroxyethyl, hydroxyalkylamino [**19-23**]; sulfate [**24**]; phosphate; or carboxyalkyl groups as carboxymethyl, carboxyethyl, carboxybutyl or by grafting water-soluble polymers [**25-29**] in the macromolecular chain of chitosan. Compared with other water-soluble chitosan derivatives, carboxymethyl chitosan (CMC) has been widely studied because of its ease of synthesis, ampholytic character and possibilities of ample of applications.

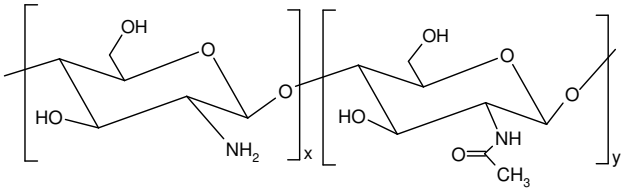


**Fig. 1.** Repeat residues for chitin and chitosan. Chitin is composed predominantly of (y) units and chitosan is composed predominantly of (x) units distributed in random fashion.

## 2. Synthesis of carboxymethyl chitosan

The chitosan derivatives obtained by its carboxymethylation are old in the art with three types: N-carboxymethyl chitosan (NCMC) [**30**], O-carboxymethyl chitosan (OCMC), and N,O-carboxymethyl chitosan (NOCMC). There are three main methods disclosed in the literature for the preparation of N-carboxyalkylchitosan derivatives (**Fig. 2**).

### *2.1. Reductive alkylation*

The $-NH_2$ group of chitosan unit is reacted with the carbonyl group of aldehyde- glyoxylic acid, and then hydrogenated by reaction with $NaBH_4$ or $NaCNBH_3$ to give N-carboxymethyl chitosan. With this method, the carboxymethyl substituent clearly is placed exclusively on the N-atom, with absence of O-substitution. However the reactivity of aldehyde is so high that along with N-carboxymethyl chitosan, partially di-substituted N-carboxymethyl chitosan (N,N-diCMC) is unavoidably produced, even under mild conditions; therefore, the term N-carboxymethyl chitosan should imply that a substantial fraction is di-substituted. The ratio of mono-:di-carboxymethylated units of glucosamine in chitosan depends on the ratio of amine (chitosan) to reagent used and the reaction conditions. Muzzarelli et al., who first reported this method, produced a series of the products from a variety of chitosans differing in molecular sizes, molecular-weight distributions, and degrees of deacetylation by treating them with various amounts of glyoxylic acid [**30**]. The prototype procedure consists of dissolution of chitosan in 1% acetic acid to get approximately 1-1.5 w/v solution, its reaction with solution of glyoxylic acid in molar ratio 1:1 to 1:3 of amine:acid

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followed by reduction with portions of sodium borohydride to obtain pH of 4-5 without precipitation in reaction mixture. The viscous solution is then dialyzed against water and lyophilized to get N-carboxymethyl chitosan. Use of the reductive alkylation process gave a product having approximately 70% N,N-dicarboxymethyl chitosan units [**31**], and this could be raised to 90% by repeating the reaction step a total of four times [**32**]. Dung et al. indicated concentrations as crucial parameters to obtain a fully disubstituted N,N-diCMC. The concentrations suggested are 1% chitosan in 0.9 % w/v acetic acid with molar ratio of amine:glyoxylic acid as 1:9 to get Schiff's base. For subsequent reduction, the ratio by weight of chitosan and sodium borohydride is 1:3. The reductive alkylation reaction requires relatively expensive reagents and is not easy to apply on a large scale. In comparison, the direct alkylation reaction at mildly alkaline pH uses relatively inexpensive reagents and is an easier process to scale up. Hence it should be a route to cheaper products.

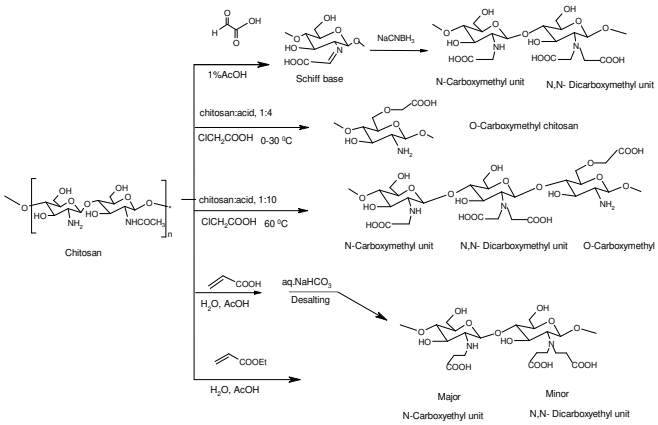


**Fig. 2.** Carboxymethylation and carboxyethylation of chitosan

*2.2. Direct alkylation*

The direct alkylation method utilizes monohalocarboxylic acids as monochloroacetic acid to prepare N-carboxyalkyl and O-carboxyalkyl chitosan derivatives in different reaction conditions. The reaction conditions are responsible to attain the N versus O selectivity of carboxyalkylation and degree of substitution (DS). To proceed with the reaction of carboxymethylation of chitosan in the solvent as water/isopropyl alcohol, chitosan is first activated by soaking it in alkaline solution. During carboxymethylation of chitosan with monochloroacetic acid in the mildly alkaline medium of pH 8–8.5, only the amine groups will be activated and so only N-substitution will take place. Although the chitosan precipitated at this pH will be gradually redissolved as the reaction proceeds and at the end of the reaction all the chitosan molecules will be in solution and will be mono or di-N-substituted. At high alkali concentrations (more than 25% aqueous NaOH) however alkylation with monochloroacetic acid gives mixed N- and O-alkyl chitosan derivatives with substitution at the C6 and C3 OH groups and also some substitution on the C2- $NH_2$ groups. The ease of substitution is in the order OH- 6 > OH-3 > $NH_2$-2 and the figures obtained for one reaction was 0.7 > 0.47 > 0.2 [**31**].

An et al. reacted chitosan 1% w/v swollen in water with monochloroacetic acid in ratio of amine:acid by weight as 1:4 at pH 8-8.5 at temperature of 90°C to get N,N dicarboxymethyl chitosan [**33**]. For synthesis of OCMC and NOCMC, the chitosan is soaked in alkaline solution at freezing or room temperature for 2-24 h. The concentration of chitosan for this purpose commonly reported is 4% -20% w/v in 40-50% w/v solution of NaOH. The activated chitosan is then reacted with monochloroacetic acid in solid or solution form. The concentration of monochloroacetic acid used is 1:1 -1:6 by weight in isopropanol/ethanol and reaction is carried at 0–60°C for 2–24 h [**34-36**]. The product is precipitated by solvent as acetone, ethanol and desalted by pH adjustment or dialysis.

The DS of carboxymethyl groups had no relation with the degree of deacetylation (DD) value of chitosan but is strongly dependent on NaOH concentration used. The concentration of NaOH used determines the DS obtained. Work by Tokura and team demonstrated that the DS value of CMC increased with NaOH concentration changing from 20 to 40% [**37**]. Chen and coworkers increased NaOH concentration from 40 to 50%, when the DS value increased from 0.15 to 0.63 and from 0.43 to 0.68 for chitosan of % DD of 75 and 90 respectively; whereas, further increase of NaOH concentration reduced the carboxymethylation reaction and the DS value dropped to 0.57 and 0.46 for respective chitosans [**35**]. A 50% NaOH solution seemed to provide the optimum alkali concentration in the carboxymethylation process. At lower NaOH concentration, the rigid crystalline structure of chitosan was difficult to disrupt to ensure penetration of the chloroacetic acid into the interlocking polymer chains resulting in lower DS [**38**]. While a high alkali concentration above 60% promoted side reaction between NaOH and chloroacetic acid and the available chloroacetic concentration for the reaction decreased accordingly [**30**]. To obtain DS higher than one, change of the NaOH concentration is insufficient. Repeat procedures increase the DS to 1.08 and 1.28, respectively with 50% NaOH. The employment of such high NaOH concentration is accompanied by deacetylation and depolymerization of the native chitosan.

The ratio of water/isopropyl alcohol is an important element [**36**]. Reaction of chitosan in isopropanol alone gives low yield where as the use of water as lone solvent lowers the yield even more. The reason is the easy swelling of the previously formed carboxymethyl chitosan in water to form jelly which coats the outside of the chitosan particle and inhibits the course of reaction. Quantitative yields can be obtained when the ratios of water/isopropanol is 1:1 and 1:4 The increase of the ratio of water/isopropanol in the reaction solvent decreases the fraction of carboxymethylation and increases the insolubility at higher pHs [**36**]. The ratio of amine: acid is commanding factor in determining the selectivity of the substitution and the degree of the substitution. The ratio of amine: acid used for low DS is 1:1 and for higher DS it is up to 1:4 [**33**]. The higher amine: acid ratio (1:8) was suggested for the optimum reaction of chitosan alkalized for 2 h; pH 13.5; under microwave irradiation conditions like 100°C; microwave power 260 W; reaction time 20 min. The degree of substitution of CMC exceeded 0.85. The increase in


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reaction temperature increases the fraction of carboxymethylation. The employment of other monohalocarboxylic acids like 3-cholorpropionic acid, 4-cholorobutyric acid and 5-chlorovaleric acid provides the higher homologs of CMC like carboxyethyl, carboxypropyl, carboxybutyl chitosan, respectively [**39**].

## 3. **Characterization of carboxymethyl chitosan**

### *3.1. FTIR spectroscopy*

The basic characteristic peaks of chitosan are at 3455 $cm^{-1}$ (O–H stretch), 2923-2867 $cm^{-1}$ (C–H stretch), 1598-1600 $cm^{-1}$ (N–H bend), 1154 $cm^{-1}$ (bridge-O stretch), and 1094 $cm^{-1}$ (C–O stretch) [**40, 41**]. For NOCMC, the spectrum is different from the spectrum of chitosan (**Fig. 3 and Table 1**). The IR spectrum of NOCMC shows the intrinsic peaks of -COOH group at 1741- 1737 $cm^{-1}$. Compared with the peaks of chitosan, the bands at 1597–1650 $cm^{-1}$ and 1414 - 1401 $cm^{-1}$ corresponding to the carboxy group (which overlaps with N–H bend) and -$CH_2COOH$ group, respectively are intense in spectrum of NOCMC indicating carboxymethylation on both the amino and hydroxyl groups of chitosan [**42**]. The peaks at the 1154–1029 $cm^{-1}$ (C -O stretch) also increase. When –COOH becomes –COONa, its absorption peak will shift to 1598 $cm^{-1}$, no bands at 1730 $cm^{-1}$ for –COOH will be observed in the spectrum [**36**]. In the H form of OCMC, the characteristic peaks are 1741 $cm^{-1}$ (-COOH), 1154 –1029$cm^{-1}$ (-C –O -), and 1624 and 1506 $cm^{-1}$ (-$NH_3^+$).

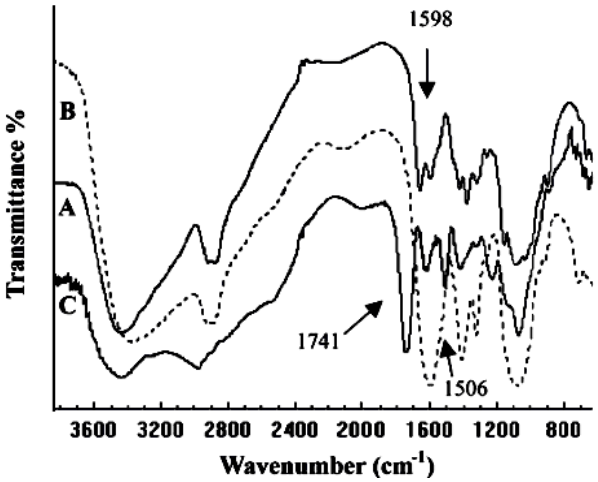


**Fig. 3.** FT-IR spectrum of (A) Chitosan, (B) Na-form CM-chitosan, (C) H-form CMC.

### *3.2. $^1H$ NMR spectroscopy*

The $^1H$ NMR spectrums at 500 MHz are reported for CMC in $D_2O$. (**Fig. 4 and 5**) The basic assignment of the chitosan resonance is that: *a* is the resonance of H–1D (4.8 ppm), *b* is H–1A (4.65 ppm), *h* is the resonance of 3 acetyl-protons (2.0 ppm), *e* is H3–6 protons (3.6–3.9 ppm), *g* is H–2D proton resonance (3.1 ppm). These resonances can be found in the $^1H$ NMR spectrum of chitosan described by Kubota and Eguchi [**43**] and Shigemasa et al. [**42**]. In the region between 4.05 and 4.55 ppm, the resonances are the protons of 3- and 6-substituted carboxymethyl (–O–$CH_2COOD$) of CMC, *d* is the resonance of 3 protons from H-6' (2 protons) and H-3' (1 proton), *c* is the resonance of 1 proton from H-3' [**44**]. The resonance signal of the protons from N–$CH_2COOD$ group can be found at *f* (3.25 ppm). The signal can be found at the $^1H$ NMR spectrum of NCMC, the resonance between 3 and 3.5 ppm, different from the H–2D signal at 3.15 ppm described by Muzzarelli et al. and Hjerde et al. [**44, 45**]. The result shows that the amino groups were partly carboxymethylated along with the hydroxyl groups. An et al have reported a signal at 57.63 ppm for N,N-diCMC [**33**]. The substitution of carboxymethyl groups on O-6, O-3 and N-2 can be determined from the $^1H$ NMR spectrum using the method of Hjerde et al. [**44**]. The calculated equations are as follows (**Eq. 1-4**):

$$f_6 = (1/2)(I_d - I_c)/(I_b + I_g) \quad \text{Eq. 1}$$

$$f_3 = I_c/(I_b + I_g) \quad \text{Eq. 2}$$

$$f_2 = (1/2)I_f/(I_b + I_g) \quad \text{Eq. 3}$$

$$F = f_6 + f_3 + f_2 \quad \text{Eq. 4}$$

where, $I_x$ are intensities of respective peaks as *a, b, c, d* etc. The total value of H-1 can be obtained by summing ($I_a$ + $I_b$) or ($I_b$ + $I_g$).The terms $f_6$; $f_3$ and $f_2$ are the fractions of carboxymethylation at the position O-6, O-3 and N-2, respectively. *F* is the total fraction of carboxymethylation (note that this number can vary between 0 and 2). The carboxymethylation at position 6 is found to be larger than at position 3 since the order of reactivity of the three possible positions is: OH- 6 > OH-3 > NH-2 [**31**].

**Table 1.** FT-IR characteristic bands of chitosan and carboxymethyl chitosan


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| $cm^{-1}$ | Signal |
|---|---|
| 3455-3445 | Axial stretching of O-H and N-H bonds |
| 2923-2867 | Axial stretching of C-H bonds [**36**] |
| 1653 | Axial stretching of C=O bonds |
| 1597-1650 | Angular deformation of the N-H bonds of the amino groups carboxy group (which overlaps with N–H bend) |
| 1414-1401 | Carboxymethyl group |
| 1417-1377 | Coupling of C-N axial stretching and N-H angular deformation [**42**] |
| 1154-1029 | Glycosidic bonds, C-O-C and C-O stretchings |
| 886 | Vibration of ring |
| 1741-1737 | - COOH |

### *3.3. $^{13}C$ NMR Spectroscopy*

The $^{13}C$ NMR spectrums at 500 MHz are reported for CMC in $D_2O$ (**Fig. 6**) [**46**]. The signals for $^{-*}CH_2COOH$ substituted on-OH and -NH are obvious at 173.5 and 170.0 ppm. The three chemical shifts at 70.9, 69.3 and 48.7 ppm are assigned to $-^{-*}CH_2COOH$ groups substituted on O-6, O-3 and N-2 positions [**31**].

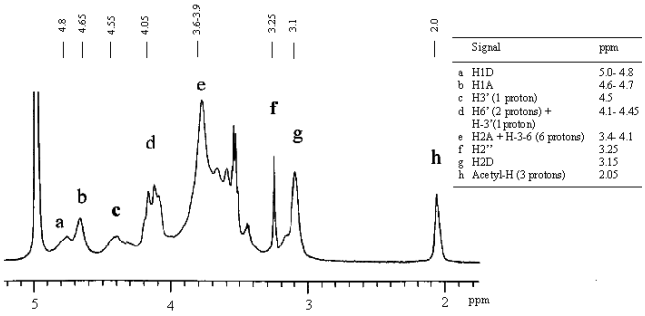


**Fig. 4.** $^1H$ NMR spectrum of CMC in $D_2O$ at 25 °C (500 MHz; 300 K) and spectral data. H′ refers to protons of a carboxymethylated unit. A and D refers to acetylated and deacetylated units.

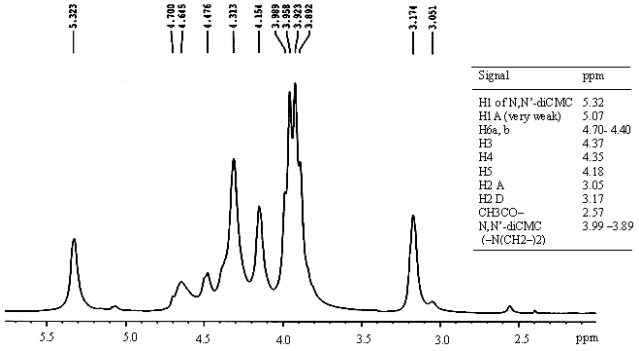


**Fig. 5.** $^1H$ NMR spectrum and spectral data of N,N-diCMC (500 MHz, 353 K, 10 g/L in $D_2O$)

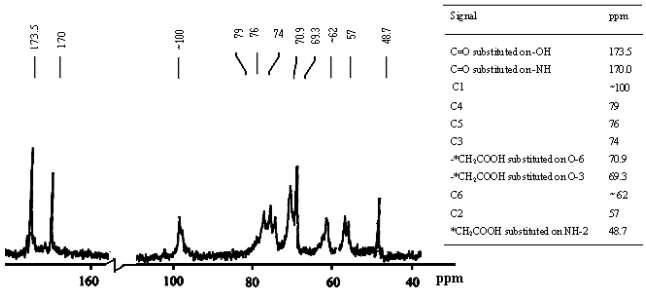


**Fig. 6.** $^{13}C$ NMR spectrum and spectral data of NOCMC (500 MHz, 353 K, 70 g/L in $D_2O$).

### *3.4. Differential scanning calorimetry*

Kittur et al. investigated the use of differential scanning calorimetry (DSC) for the evaluation of the thermal events of chitosan and their O, N-carboxymethyl derivatives [**47**]. The DSC was carried out of CMC in the range of 50-500°C with 5mg of sample under continuous flow of dry nitrogen gas (10 ml/min) at a heating rate of 20°C/min. The thermograms of chitosan and CMC were characterized by two thermal events: the first- endothermic, and the second– exothermic (**Fig. 7**). The endothermic event appeared as a peak centered at 125-150°C with an onset at 90-108°C corresponding to water evaporation. The exothermic event appeared as a peak centered at 270- 330°C with an onset of 180-190°C corresponding to the decomposition of the polymer. In contrast both the peaks for chitosan appeared at lower temperatures (close to 100°C and 300°C respectively) indicating the superior thermal stability of CMC. The thermograms of OCMC and NOCMC were similar except for the decomposition which appeared at lower temperature for NOCMC (OCMC 302°C, NOCMC 283.3°C). Nonetheless, each curve was distinctly and measurably different from the base curve so that their unequivocal identification was assured. The glass transition temperature was not observed in thermogram of CMC despite the presence of substantial amount of amorphous content. The DSC measurements did not showed stepwise increase in specific heat, suggesting that the $T_g$ value of carboxymethyl derivative probably lies at rather higher temperature, where degradation prevents its determination.

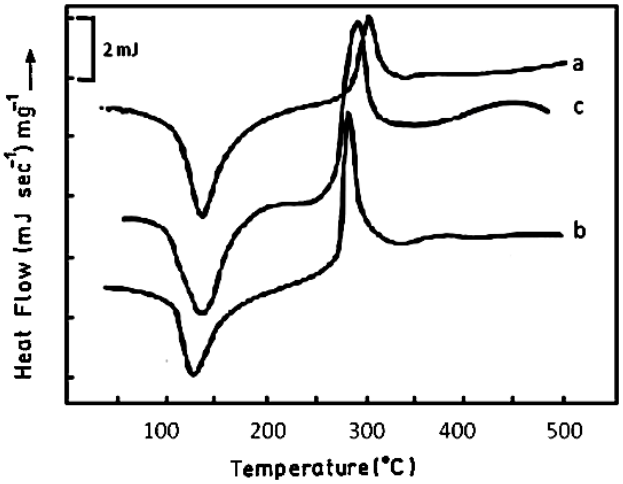



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**Fig. 7.** DSC thermogram of (a) OCMC (b) NOCMC (c) chitosan

The authors observed an increase in water holding capacity with an increase in N-deacetylation and carboxymethylation, which they attributed to newly created hydrophilic centers (amine and carboxymethyl) in the polymer chain and chemical and supramolecular modifications increasing the amorphous region in the derivatives. The authors also suggested the existence of different mechanisms of chitosan decomposition in the presence of carboxymethyl groups. The change in peak temperature and area as a function of primary (acetyl and carboxyl contents) and higher order structures of the polymer was adopted by Kittur et al as theoretical basis to correlate the heat of reaction to the degree of substitution of CMC. A good correlation between peak area/ peak height to degree of substitution was found. Miranda et al. reported an extra thermal event (second thermal degradation peak) for NCMC at 600°C, with an additional weight loss [**48**]. Kinetic thermal analysis preformed in extension showed a slower process of degradation at 100°C for NCMC compared with chitosan, and a 13 times higher activation energy for the former, confirming the higher stability of NCMC.

### *3.5. Titrimetry*

The degree of substitution of CMC was determined by using potentiometric titration [**49**]. For this aqueous solution of weighed amount of CMC is adjusted to pH <2 with hydrochloric acid is titrated with standard aqueous NaOH. The amount of aqueous NaOH is determined by the second order differential method.
The degree of substitution (DS) can be calculated by **Eq. 5**:

$$DS = \frac{161 \times A}{m_{cmc} - 58 \times A} \qquad \text{Eq. 5}$$

$A$ is $A = V_{NaOH} \times C_{NaOH}$ Eq. 6

where, $V_{NaOH}$ and $C_{NaOH}$ are the volume and molarity of aqueous NaOH, respectively, $m_{cmc}$ is the mass of CMC (g), and 161 and 58 are the respective molecular weights of glucosamine (chitosan skeleton unit) and a carboxymethyl group.

### *3.6. Viscometry*

The relative viscosity of CMC solutions in 0.1 M aqueous NaCl at different concentrations was measured with an Ubbelohde viscometer at constant temperature of 30°C and the intrinsic viscosity [$\eta$] was determined by extrapolating to zero concentration of the relative viscosity or by equation of One-Point method (**Eq. 7**) [**50**]. Validity of this equation had been testified in 30 ± 0.1 °C with the concentration range of CMC from 2–5 mg/mL. The viscosity averaged molecular weight $M$ is then calculated according to Mark-Houwink-Sakurada equation (**Eq. 8**) with the values derived from **Eq. 9 and 10**. The accuracy of these equations has shown the deviation less than 5% in proper conditions [**51**].

$$[\eta] = \frac{4\eta_{sp}^{1.02} \ln \eta_r}{C^{1.01}(3\eta_{sp} + \ln \eta_r)} \qquad \text{Eq. 7}$$

$$[\eta] = KM^{\alpha} \qquad \text{Eq. 8}$$

$$\eta_r = t / t_o \qquad \text{Eq. 9}$$

$$\eta_{sp} = \eta_r - 1 \qquad \text{Eq. 10}$$

where $t$ and $t_0$ are the delivery time of CMC solution and the solvent, $c$ is the concentration (g/mL) in 0.1 M aqueous NaCl, $\eta_r$, $\eta sp$ and[$\eta$] are the relative viscosity, specific viscosity and intrinsic viscosity, $M$ is the viscosity average molecular weight of CMC. For CMC values of $K$ and $a$, $K$ = 7.92 x $10^{-2}$ or 7.92 x $10^{-5}$, $a$ = 1.00 [**52**].

### *3.7. Gel Permeation chromatography*

An HPLC instrument equipped with a GPC analytical column (a TSK G5000 PWXL column) of dimensions 7.5 × 300 mm, with differential refractometer as detector was used for GPC analysis of weight-average molecular weight of CMC [**46**]. Each sample was dissolved in 0.1 mol/L aqueous NaCl, which was the eluent too. The flow rate was 0.8 to1.0 mL/min. The weight-average molecular weight ($Mw$) was calculated by the **Eq. 11**:

$$Lg(M_w) = -0.8540V_e + 10.0382 \qquad \text{Eq. 11}$$

Miranda et al. utilized 0.1mol/L sodium nitrite containing 200 ppm of sodium azide as an eluent at a flux of 0.6 mL/min [**48**]. The detection was done by differential refractometer and multiangle light scattering at 632.8 nm. The elution profile showed only one major peak, but it was possible to observe aggregated molecules in the system by refractive index and light scattering at lower elution volume. The degree of polydispersion was 3.4, indicating a high degree of dispersion of the sample, and the average molecular mass (Mw) obtained was 1.45 × $10^6$ g/mol for NCMC of 18.5% DS.

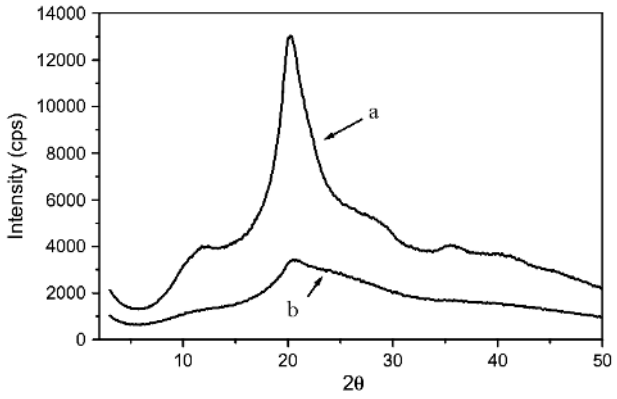


**Fig. 8.** X-ray diffraction patterns of (a) chitosan (b) sodium carboxymethyl chitosan (DS 0.6 determined by conductometry).

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### 3.9. Capillary zone electrophoresis

A direct capillary zone electrophoresis method without any pre-treatment was developed for separation of chitosan and CMC, which proved to be rapid and effective with satisfactory resolution (**Fig. 9**) [**58**]. Investigations for optimization showed that upon employing 50 mM sodium phosphate at pH 2.0 and untreated fused silica capillary, high-molecular weight chitosan and CMC were baseline separated with ultraviolet detector with satisfactory repeatability and excellent linear responses.

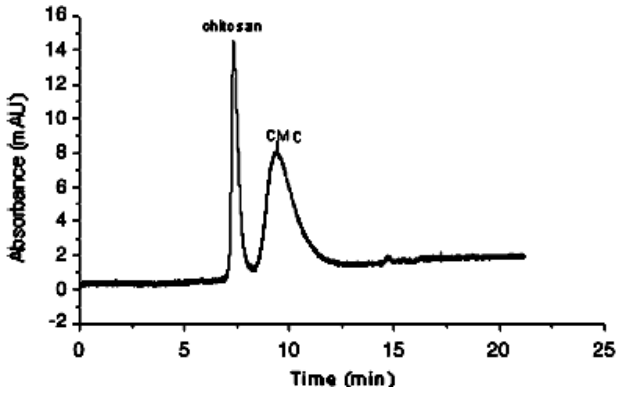


**Fig. 9.** Typical electropherogram of chitosan and CMC.

## 4. Physiochemical properties

### 4.1. Solubility and aggregation

A significant characteristic of CMC is its solubility in water (**Table 2**). Compared with chitosan, the solubility of CMC in aqueous solution is improved remarkably because of the introduction of carboxymethyl group. Hjerde reported that the CMC could be dissolved in acidic, neutral or basic aqueous solution when the DS of carboxymethylation for chitosan is more than 60% [**44**]. Whereas Chen et al. recognized the critical DS value at which CMC becomes soluble in water to be in the range of 0.4 to 0.45 and reported the solubility of CMCs [**46**].

**Table 2.** Solubility and aggregation parameters of CMC.

| | | Molecular weight | | | |
|---|---|---|---|---|---|
| Sample | DS | Mv x $10^4$ | Mw x $10^4$ | d, polydispersity | Insoluble pH range |
| 6-OCMC | 0.82 | 24.3 | 24.8 | 2.2 | 2.3-3.6 |
| 3,6-OCMC | 0.65 | 12.1 | 16.1 | 1.1 | 2.1-6.5 |
| NCMC | 0.50 | 5.6 | 6.9 | 1.0 | 2.5-6.6 |

Recently Chen and Park, with solubility studies of OCMC with varied DS, stated that the water solubility of the CMCs have a close relationships to the modifying conditions and the degree of carboxymethylation [**36**]. The CMCs prepared at temperatures of 0–10°C were soluble in water. But the CMC prepared between 20 and 60°C were insoluble in the water at near-neutral pH. The water insolubility of CMC at various pHs varied with the DS. The increase in reaction temperature increased the fraction of carboxymethylation and increased the insolubility at lower pHs; the increase of the ratio of water/isopropanol in the reaction solvent decreased the fraction of carboxymethylation and increased the insolubility at higher pHs. The insoluble region may be due either to aggregation of highly acetylated chain segments or to amide formation subsequent to thermal drying.

The aggregation behavior is seen in dilute aqueous solution of OCMC [**59**]. The combined driving forces that make OCMCs soluble in water include the H-bonding between water and the polymer and presence of $COO^-$ on the OCMC chain whereas the intermolecular H-bonding of OCMC and the electrostatic repulsion between them are the main driving forces for OCMC aggregation in solution. This aggregation is dominated by interchain aggregation, with the glucose backbones of OCMC forming the hydrophobic domains, and the dissociated carboxylic groups and the hydrophilic groups around the backbone forming the hydrophilic ones. The critical aggregation concentration (CAC) of OCMC was determined to be between 0.042 mg/ml and 0.05 mg/ml. The spherical aggregation is observed in NCMC and confirmed by static and dynamic light scattering (in which the form factor (ρ) was ~1.3 in phosphate buffer pH 7.4) and atomic force microscopy (AFM) images [**60**]. The CAC was determined using pyrene as a hydrophobic fluorescent probe. For NCMC (DS 10-60%), a CAC of 1.0 mg/ mL was observed. Ionic strength was found to have no influence on CMC as well as the parent chitosan in respect of the self-aggregation [**61**].

Thanou et al. also observed that CMC with 87-90% DS has polyampholytic (zwitter ionic) character, which allows the formation of clear gels or solutions depending on the polymer concentration at neutral and alkaline pH values but aggregates under acidic conditions [**62**]. The ionic strength was found to have no influence on aggregation behavior of CMC and chitosan itself [**61**]. All these properties are important for developing CMCs with potential applications in drug delivery systems.

### 4.2. Moisture retention and absorption properties

The moisture retention properties of CMC have received considerable attention for its possible use in cosmetics and clinical medicine. Matsumura et al. reported that CMC appeared to be more suitable than other chitosan derivatives for moisture retention [**63**]. Muzzarelli pointed out that a 0.25% CM-chitosan aqueous solution was comparable to a 20% aqueous solution of propylene glycol in terms of moisture-retention ability, and the viscosity was almost equal to that of hyaluronic acid, a compound with known excellent moisture retention properties [**64**].

Chen et al. studied the moisture-absorption and -retention abilities of CMC with respect to the structural properties of the polymer [**46, 65**]. The moisture-retention ability ($R_h$) was calculated as the percentage of residual water of wet sample prepared by adding 10% water to samples predried over $P_2O_5$ *in vacuo* for 24 h (**Eq. 12**) [**63**].

$$R_h(\%) = 100 \times (H_n / H_o) \qquad \text{Eq. 12}$$

where, $H_0$ and $H_n$ were the weights of water in the sample before and after putting in the saturated $K_2CO_3$ desiccator


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(43% relative humidity) and the silica gel at 20°C after 48 h of the test.

The moisture-absorption ability ($R_a$) was calculated as the percentage of weight increase of the sample dried over $P_2O_5$ *in vacuo* for 24 h by the **Eq. 13.**

$$R_a(\%) = 100 \times (W_n - W_o)/W_o \qquad \text{Eq. 13}$$

where $W_o$ and $W_n$ are the weights of sample before and after putting in the saturated $(NH_4)_2SO_4$ desiccator (81% relative humidity) and the saturated $K_2CO_3$ desiccator (43% relative humidity) at 20°C after 48 h of the test.

The authors found that the moisture-absorption ($R_a$) and -retention ($R_h$) abilities of CMC were closely related to the site of substitution, MW DD, and DS. The 6-carboxymethyl group in the molecular structure of chitin and chitosan was a main active site responsible for moisture retention. Although carboxymethylation at OH-3 and N position was not essential, it contributed to the ability. Moisture-retention ability was also related to molecular weight; that as higher the molecular weight improved was the moisture-retention ability. 6-OCMC (and 6OCM-chitin, i.e., with a DS above 0.8 and molecular weight higher than 24.8 x $10^4$) has the moisture retention ability equivalent to hyaluronic acid. Under conditions of high relative humidity, the maximum $R_a$ and $R_h$ were obtained at DD values of about 50%, and when the DD value deviated from 50%, $R_a$ and $R_h$ decreased. Under dry conditions, when the DD value was 50%, the $R_h$ was the lowest. With the DS value increasing, $R_a$ and $R_h$ increased. However, further increase of the DS value above 1.0 reduced the increasing tendency of $R_a$ and $R_h$, and even some decreases in $R_a$ and $R_h$ were observed. Intermolecular hydrogen bonds play a very important role in moisture-absorption and retention ability of CMC.

### *4.3. Chelating and sorption properties*

The excellent adsorption characteristics of chitosan were considered to be due to: 1). The high hydrophilicity of chitosan owing to large number of hydroxyl groups. 2) The large number of primary amino groups with high activity as adsorption sites. 3) The flexible structure of the polymer chain of chitosan which enables to adopt the suitable configuration for complexation with metal ions [66-68]. Indeed, nitrogen atoms hold free electron doublets that can react with metal cations and uptake metal cations by a chelation mechanism. However, the amine groups are easily protonated in acidic solutions. Hence, the protonation of these amine groups may cause electrostatic attraction of anionic compounds, including metal anions ($CrO_2^{-4}$, $SeO^{-4}$, $VO_3^{-4}$, $SO_2^{-4,}$ $MoO^{-4,}$ $HAsO^{-4}$ etc and anions resulting from metal chelation by chloride, anionic ligands) or anionic materials [**69-72**] including humic acid [**73**], congo red [**74**]. Several contradictory hypotheses have been proposed for the interpretation of uptake mechanisms. They can be generally classified in two groups: (a) the "bridge model" and (b) the "pendant model". In the "bridge model", metal ions are bound with several amine groups from the same chain or from different chains, via inter- or intramolecular complexation [**75-77**] as opposed to the "pendant model", in which the metal ion is bound to an amine group in a pendant fashion [**75-78**]. Whatever be the mechanism and way (chelation versus electrostatic attraction), the sorption of a metal by chitosan depends on fraction of deacetylated units (free amine groups), polymer chain length, crystallinity, molecular weight, conditioning of polymers, physical form of chitosan, solution pH, type and concentration of the acid used for solution, composition of solution, metal ion selectivity, speciation [**79**]. Of the free amino groups only some are necessarily accessible to metal uptake since some of these amine sites are involved in hydrogen bonds (intra- or intermolecular bonds). Moreover, the residual crystallinity of the polymer may control the accessibility to sorption sites. As a chelating agent chitosan is selective for heavy metals and transition metals (Ag, As, Au, Cd, Cu, Co, Cr, Fe, Ga, Hg, Mo, Ni, Pb, Pd, Pt, Se, U, V, Zn) [**80, 81**] and has very limited affinity for alkaline and alkaline-earth metals due to the absence of d and f unsaturated orbitals (unlike transition metals) [**82**]. However presence of $CH_2COOH$ group on N of glucosamine unit of chitosan makes it similar to glycine (hence called as glycine glucan also) and the lone pairs from the N-C-C-O sequence of glycine contribute towards superior chelation properties. NCMC (and /or NOCMC) was reported to enhance Fe [**83**], Co [**84**], Cu [**85, 86**], Ni, Cd, Pb, [**63, 87, 88**], Pt [**89**], Mn [**90**], Au [**91**], and Zn [**92**] ions adsorption capacity or exhibit higher chelating ability compared to chitosan due to this reason. These chitosans carrying carboxyl groups might chelate calcium too [**77, 93, 94**]. These high sorption capacities for metal ions can be of great use for the recovery of valuable metals or the treatment of contaminated effluents, and the loading of the polymer matrix with metal can give interesting complementary properties to the support for the sorption of other organic or inorganic materials, for catalytic applications and for the manufacturing of new optical - electronic devices or for agriculture (plant disease treatment).

## 5. Biological properties

### *5.1. Modulation of cell functioning*

The research has established that the resemblance of chitosan to components of proteoglycans appears to be conducive to cell attachment modulating cell morphology, proliferation, and differentiation [**95, 96**]. The CMCs conserve these properties of native chitosan. OCMC was employed for surface modification of poly(D,L-lactic acid) (PDLLA) film to evaluate its effect on rat osteoblast as well as of poly(lactide-co-glycolide acid) film to evaluate its effect on chondrocyte [**97**]. The modifications resulted in the improvement in adhesion. OCMC improved the hydrophilicity of PDLLA films due to the introduction of some carboxyl groups while it retained the considerable amount of $NH_3^+$ ions on the modified PDLLA surface favorable for the interaction with negatively-charged cell membrane surface [**98**]. The adhesion contact dynamics and morphological changes were investigated by Confocal-reflectance interference contrast microscopy (C-RICM) in conjunction with phase contrast imaging for 3T3 fibroblasts on chitosan and OCMC surface-modified silica coverslips

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[**99**]. The C-RICM results demonstrate that the weak cell contact for OCMC coated surface than chitosan coated surface. 3T3 fibroblasts were found to spread randomly with spindle-like morphology on the chitosan surface, while they exhibit elongated morphology and aligned on the OCMC surface. The altered fibroblast behaviors were correlated with the weak cell contact. The 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) cross-linked CMC films enhanced the spread of Neuro-2a cells and provided a good proliferation substratum for these cells as compared to chitosan films [**100**]. The cross-linking of CMC resulted in beneficial changes as lower hydrophilicity, and elastic modulus.

Chitosan used in tissue engineering serves as a synthetic extracellular matrix that provides signals to the cells and guides new tissue growth. Such use demands that the degradation rate of chitosan should fit the rate of new tissue formation. The degradation rate, however, is very slow and poorly controlled since it occurs via a slow, unpredictable dissolution process likely due to its insolubility in physiological pH. This significant drawback of chitosan is overcome by carboxymethylation [**44,101**]. The degradation rate of covalently crosslinked CMC can be further controlled by utilizing bimodal weight distribution (varying the ratio of high to low molecular weight CMC) [**102**]. The treatments like vacuum heating and γ-irradiation can be of help in controlling the degradation rate [**103**].

The N,N-diCMC–calcium–phosphate chelate favored osteogenesis along with promotion of bone mineralization [**93**]. N,N-diCMC formed self-sustaining gels upon mixing with calcium acetate, as a consequence of calcium chelation but yielded a clear solution when mixed with calcium acetate and disodium hydrogen phosphate in appropriate ratios (molar ratio Ca/ N,N-diCMC close to 2.4). The amorphous N,N-diCMC - calcium-phosphate chelate obtained from this solution on dialysis and freeze-drying was used for the treatment of bone lesions in experimental surgery and in dentistry. Bone tissue regeneration was found to be promoted in sheep, leading to complete healing of otherwise non-healing surgical defects. In human patients undergoing apicectomies and avulsions, radiographic evidence of bone regeneration was observed. This property of N,N-diCMC was adapted for delivery of bone morphogenetic protein to bones [**104**].

Using a surgical sternotomy pericardial adhesion model in rabbit it has been shown that topical application of dialdehyde crosslinked NOCMC hydrogel markedly diminishes postoperative fibrosis and adhesion formation without untoward cardiac side effects [**105**]. The mechanism of NOCMC's activity for such prevention is unclear. NOCMC's function to prevent interactions between the extracellular matrix molecules necessary for adhesion formation can be multifactorial. Since NOCMC is hydrophilic and negatively charged, it is likely to have a low absorption affinity for fibronectin- a protein to which collagen binds forming adhesions [**106**]. It is possible that NOCC may mediate the inflammatory response to tissue injury or simply act as a resorbable barrier that maintains tissue separation. The efficacy of NOCMC to limit adhesion formation has also been demonstrated in rabbit by a cardiac injury model [**107**], an abdominal surgery model [**108**] and in rat by an abdominal aortic anastomosis, a large bowel anastomosis, and an abdominal skin incision [**109**]. The direct use of CMC in vitro promoted wound healing. The actions involved were the significant enhancement in proliferation of the normal skin fibroblast but inhibition of proliferation of keloid fibroblast and decrease in the secretion of collagen type I [**110**].

Along the effect of CMC on various cell behavior, the nontoxicity of CMC has also been established for example in rats with oral administration with parameters of hematology and histology [**111**], in L929 fibroblast-like cell line [**112**], by dermal irritation potential response using the Draize test in rats [**113**] etc. The blood compatibility was enhanced by the use of OCMC for surface modification of Dacron vascular grafts [**114**] and of poly(ethylene terephthalate) films in terms of less platelet adhesion and fibrinogen adsorption [**115**].

### *5.2. Antioxidant activity*

The antioxidant activity of chitosan and its derivatives has indicated that the active hydroxyl and amino groups in the polymer chains may take part in free radical scavenging and contributed to the antioxidant activity. The contents of active hydroxyl, amino, amido groups in their polymer chains as well as molecular weight affect the antioxidant activity of chitosan and derivatives [**116-119**]. The carboxymethyl chitosans (MW< 7.5 x $10^6$ Da) showed molecular weight dependent scavenging activity against superoxide anion [**120**]. The Schiff bases of CMC did not show improvement in the antioxidant activity because of destruction of part of the hydrogen bonds, formation of new hydrogen bonds, and the change of $NH_2$ group to C=N [**121**].

### *5.3. Antimicrobial activity*

Chitosan inhibits the growth of a wide variety of bacteria and fungi [**122, 123**]. Different mechanisms have been proposed for the antimicrobial activity of chitosan and -$NH_2$ is acknowledged as a functional group. According to one mechanism, the polycationic nature of chitosan interferes with the negatively charged residues of macromolecules at the cell surface and alters cell permeability. The other mechanism involves the binding of cationic chitosan with DNA to inhibit RNA synthesis. The antimicrobial activity of chitosan is influenced by its molecular weight, degree of deacetylation, concentration in solution, and pH of the medium. The CMCs defend the antimicrobial activity of chitosan proper with similar trends. The antibacterial activities were found to increase in the order of NOCMC<chitosan< OCMC. The reason is the dependence of polycations' antibacterial action on effective number of -$NH_3^+$ groups. In NOCMC the effective number of -$NH_3^+$ is lowered because of -$CH_2COOH$ substitution leading to decrease in antibacterial action. In OCMC substitution occurred only at –OH, hence its number of -$NH_2$ was not changed. Moreover, its -COOH group may have reacted with the -$NH_2$ group intra- or intermolecularly and charged these -$NH_2$ groups. So, in the same condition, the number of -$NH_3^+$ groups of OCMC was more than that of chitosan. Therefore, the antibacterial activity of OCMC increased.


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With increase in Mw of polymer the -$NH_2$ content of chitosan and OCMC increases. However, the intrinsic flexibility of chain molecule also increases with increasing MW, which in fact makes the available antibacterial groups decrease. The two opposing factors affect the antibacterial activity of the polysaccharides, and the suppositional antibacterial peak appears when the two factors reach a certain balance. This peak appears for chitosan at Mw 9.16 x $10^4$ Da and for OCMC at 2 x $10^5$ Da [**124**]. The antibacterial activity of chitosan increases with Mw from 5000 to 9.16 x $10^4$ Da and decreases with Mw 9.16 x $10^4$ to 1.08 x $10^6$ Da but for OCMC antibacterial activity increases as Mw increases from 2 x $10^4$ to 2 x $10^5$ Da but decreases at Mw above 2 x $10^5$ Da. Alike results (decrease in antifungal activity with declining molecular mass and increasing the masking of the protonated amino groups with carboxymethyl groups) were obtained against *C. albicans*, *C. krusei* and *C. glabrata* [**125**].

### *5.4. Apoptosis inhibitory activity*

Chitosan native seems to possess apoptosis inducing ability probably where its interaction with the cell membrane acts as a trigger. The claims are based on positive observations made in growth inhibiting studies in human bladder tumor cells 5637 for DNA fragmentation, and elevated caspase-3-like activity [**126**]. However with CMC (DS 0.91 and Mw 3.9 × $10^4$ Da) dose dependent protection of rabbit chondrocytes from interleukin-1β-induced apoptosis was detected [**127**]. It was suggested that the apoptosis inhibitory effects of CMC were possibly due to the protection of mitochondrial function, the decline in the levels of nitric oxide and reactive oxygen species. The suggestion was based on other actions observed with CMC in interleukin -1β treated chondrocytes such as partial restoration of the levels of mitochondrial membrane potential and ATP, decrease in nitric oxide production by down-regulation of inducible nitric oxide synthatase mRNA expression, and scavenge of reactive oxygen species. Moreover CMC significantly suppressed the mRNA expressions of matrix metalloproteinase-1, 3 in osteoarthritic cartilage and reduced the severity of cartilage degradation [**128, 129**]. Such actions of CMC portend its use in osteoarthritis because interleukin-1, a cytokine released by synovial cells and invading macrophages in inflamed joints can induce apoptosis as well as enzymes as matrix metalloproteinases and inhibit extracellular matrix synthesis.

## 6. Applications

### *6.1. Modified drug delivery*

As discussed, solubility and aggregation properties of CMC provides interesting opportunities in drug delivery system. For example, the aggregation behavior of OCMC was put to use to increase the solubility of camptothecin and to develop its controlled drug delivery system [**130**]. The solubility of camptothecin was significantly enhanced in OCMC solution compared to that in water up to a concentration of 0.0625 mg/ml (slighltly above CAC 0.05mg/ml). After that the solubility of camptothecin decreased and dropped to a minimum at OCMC concentration of 0.1 mg/ml. At higher OCMC concentrations from 0.1 to 0.3 mg/ml, the solubility of the drug increased again but in a much more moderate way compared to that of OCMC concentrations form 0 to 0.0625 mg/ml. The reasoning for such findings was possible interaction of amphiphilic polymer and lipophilic drug. Below CAC, in absence of aggregation the interactions such as H-bond and hydrophobic interactions could exist between OCMC chains and camptothecin. When the concentration was beyond CAC, the aggregates formed in OCMC solution entrapping and solubilizing comparatively high amount of the drug inside the hydrophobic cores of the aggregates. At concentration equal to or above 0.1 mg/ml, the solubility of camptothecin decreased probably due to formation of much more compact aggregates which can only uptake a smaller amount of drug in comparison with the loosen ones. The most efficient loading of camptothecin occurred at a concentration of 0.0625 mg/ml and the maximum amount of camptothecin loaded in OCMC was 6 times than that of camptothecin in water. In vitro measurements of antiproliferative activity of cancer cell confirmed the slow release behavior of camptothecin from OCMC even when the final concentration of OCMC is far lower than its CAC in the significant dilution.

The hydrophobic drug- gatifloxacin too was entrapped in the OCMC aggregates [**131**]. However here the required concentration of OCMC needed for optimum entrapment was much higher than its CAC with high polymer:drug ratio (5 mg/ml:$10^{-4}$ M) The aggregates of OCMC-gatifloxacin shows the nanosphere morphology (with diameter of 30–70 nm) where as OCMC aggregates formed in distilled water showed very swollen microgel morphology. However, the sustained release behavior of present delivery system is not as significant as the delivery system of camptothecin entrapped into OCMC matrix which is due to the different chemical structure of loaded drug (gatifloxacin) into OCMC matrix. In vitro bacteria antiproliferative activity assay confirmed that the MIC of OCMC-gatifloxacin formulation against Gram-negative bacteria was fourfold lower than the system without OCMC. However, it appeared that OCMC had insignificant effect against Gram-positive bacteria. These results suggested that OCMC matrix had obvious “transmission effect” on Gram-negative bacteria.

Hydrogels of NOCC with human clotting factor IX (hCFIX) released hCFIX slowly in vitro, and when implanted subcutaneously gave prolonged plasma levels over those obtained by bolus intravenous or subcutaneous injection [**132**]. The rate of hCFIX release was dependent on hydrogel composition and on the presence or absence of a membrane filter over the gel surface. Under conditions that allowed swelling and erosion (open gel surface), NOCC gels released most factor IX on the third day of incubation and the level baseline by day 9. With non-swelling conditions (with membrane filters), hCFIX release peaked at day 4 and reached the baseline by day 14. In the NOCC hydrogel formulations, when the mesh size is decreased with addition of cationic polymer such as polylysine, it helped in retarding the release of protein. Under swelling conditions, with NOCC-hCFIX-polylysine hydrogels, hCFIX release was slower, peaked later and

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lasted longer. Under non-swelling conditions the release declined gradually until day 7, and then remained constant through day 14 with only a fraction of protein released within this period.

The gelification or crosslinking of CMC chains too is a prospective method of drug delivery. Nanoparticles as a controlled drug delivery system for delivery of anticancer drug, doxorubicin were designed through ionic gelification of CMC with $Ca^{2+}$ [**133**]. By the way of dynamic light scattering (DLS), transmission electron microscopy (TEM), and AFM; nanoparticles were shown to be around 200–300 nm within a narrow distribution and the polydispersity index (PI) lower than 0.1 was identified. The small PI value indicated a homogeneous dispersion of CMC nanoparticles. The nanoparticles were amorphous since a crystalline peak of CMC at 2θ=19°disappeared in the XRD pattern of the nanoparticles. The formation of nanoparticles was only limited in a narrow concentration range of CMC and $Ca^{2+}$. Minimum size of the particles was obtained at the lowest CMC concentration, and the mean size, and size distribution increased with the increase of either the CMC or the $CaCl_2$ concentration (0.5–2 mg/mL of CMC and $Ca^{2+}$ concentration between 0.5 and 8.0 mg/mL) The Mw and DS of CMC influenced the particle size. The increase of Mw led to larger particle size, since longer molecular chain entangled together giving rise to bigger nanoparticles. The increase of DS decreased the diameter slightly, which may be caused by more compact structure formed between high DS CMC and $Ca^{2+}$. Doxorubicin delivery was affected by the molecular structure of CMC. Increase in Mws of CMC from 4.50 to 38.9 kDa, enhanced doxorubicin entrapment efficiency from 10 to 40% and higher DS slightly improved the load of doxorubicin. The doxorubicin release rate was lowered by CMC with high Mw and DS which showed an initial burst followed by an extended slow release in vitro.

### *6.2. pH responsive drug delivery*

The hydrogels based on CMC of various DD and DS were prepared by crosslinking with glutaraldehyde. When the swelling behavior of CMC gels was studied, swelling was observed at low pH (<2.0) and also in the pH range of pH 4.0–13.0; however, deswelling occurred in the range of pH 2.0–4.0 [**35**]. The reason of such behavior is presence of -$NH_2$ and –COOH groups ionizable to positive and negative charges in pH dependent manner varying the charge state of molecule. The state of swelling of CMC gel in water depends mainly on osmotic pressure difference between inside the gel and the surroundings caused by the redistribution of mobile ions, which is described by Donnan membrane equilibrium. In the case of low pH (<2), the dominant charges in the gel are the protonated amino groups and in the case of high pH (>4), the dominant charges are the unprotonated carboxyl groups. In these pH regions, the CMC is swelled due to the increase of the mobile ions inside the gel, and a large osmotic pressure causes the gel to swell, thereby lowering the concentration of mobile ions inside the gel. While at pH 2.0–4.0, isoelectric point of CMC, the numbers of -$NH_3^+$ and -$COO^-$ groups in the amphoteric gels are equal, intraionic attraction between opposite charges results in seldom residual ionic group. Therefore, the mobile ionic concentration in the gel is the lowest, and osmotic pressure in surrounding bath causes the gel to shrink. However, the maximum degree of swelling was obtained at pH 9.0–10.0, and further increase of pH to 13.0, a decrease in degree of swelling was observed. It is hard to be explained by Donnan equilibrium. A possible explanation for these results may be formation of new crosslinks by hydrogen bondings and hydrophobic interactions in the blend gel. When pH increased from 10.0 to 13.0, the amino groups were completely deprotonated and then contributed to the loss of solubility of the chain segments and also to the formation of new crosslinks by hydrogen bonding [**134**]. Such pH dependent swelling resulted in release of loaded bovine blood proteins much quickly in pH 7.4 buffer than pH 1.2 solutions. The release followed Fickian diffusion in the first 4 h and then steadily increased with the dissolution of the hydrogels.

The crosslinking of CMC can be achieved by metal ions through their chelation by -$COO^-$ ions as indicated by Muzzarelli et al. [**30**]. The resultant reversible ionically-linked hydrogels are significantly less cytotoxic than the hydrogels formed by glutaraldehyde. The ferric ions ($FeCl_3$) were employed for microsphere formation of carboxymethyl chitin (CM-chitin) by ionotropic crosslinking and entrapment of 6-mercaptopurine [**135**]. Drug release rate of the microspheres decreased with increasing concentration of $FeCl_3$ solution and the curing time. Iron elution from the microspheres decreased from pH 1.2 to pH 7.5, especially between pH 4 and pH 3, because of unstability of $Fe^{+3}$ - CM-chitin complex in acid medium. The drug-release patterns of the carboxymethyl chitin microspheres in pH 7.2 seemed not only to be diffusion influenced but also macromolecular relaxation influenced. Whereas, release profiles of the microspheres in pH 1.2 medium seemed to be matrix erosion controlled but are also affected by crosslinking density of the microspheres.

The disadvantages of metal ion crosslinked CMC is the risk of dissolution of the system due to a highly pH-sensitive swelling and the possible lack of mechanical stability [**4**]. The mechanical strength of hydrogels and resiliency of the polymer can be improved by the use of two or more polymers dispersed or mixed at a molecular segmental level to form interpenetrating polymeric network (IPN). If only one polymer of the IPN is cross-linked leaving the other in linear form, the system is termed as a semi-IPN and if both of the two polymers are cross-linked, the system is called a full-IPN [**136**]. Such semi-IPN (with polyurethane) [**137**] and full IPN (with poyacrylamide) [**138**] systems are reported for OCMC. First the semi-IPN in the form of superporous hydrogel (SPH) was synthesized with polymerization of monomers for poly(acrylic acid-co-acrylamide) along with OCMC. It was converted to full IPN by cross-linking OCMC with glutaraldehyde. The characterization of structures of the SPH-IPNs with FTIR, $^{13}$C-NMR, DSC, scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM) and light images revealed the full IPN network, large number of pores with the cross-linked OCMC molecules located on the peripheries of these pores. Due to the cross-linked OCMC network, in vitro mucoadhesive force and mechanical properties and loading capacities of SPH-IPN


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significantly improved and could be modulated by varying the OCMC content, and crosslinking extent. With insulin loaded SPH-IPN, fast release of insulin was obtained where more than 90% of release occurred within 1 h and the remaining drug was almost released within 2 h.

A two-component hydrogel system composed of NOCMC and alginate cross-linked by genipin, glutaraldehyde or $Ca^{2+}$ was investigated for delivery of bovine serum albumin (BSA) as a model protein drug [**139-141**] The hydrogels exhibited pH sensitive swelling behaviors and significantly different drug-release profiles due to their distinct cross-linking structures. At pH 1.2, the swelling ratios of the NOCMC/alginate hydrogels were limited due to formation of hydrogen bonds between NOCMC (-$CH_2$COOH and -OH) and alginate (-COOH and -OH). The lowest swelling ratios of test NOCMC/alginate hydrogels took place at pH 4.0 may be because of the electrostatic interaction of the positively charged amino groups (-$NH_3^+$) on NOCMC and the negatively charged carboxylate ions on alginate (-$COO^-$) or NOCMC (-$CH_2COO^-$) to form polyelectrolyte complexes. At pH 7.4, the carboxylic acid groups on test NOCMC/alginate hydrogels became progressively ionized (-$COO^-$). Therefore, hydrogels swelled significantly due to a large swelling force created by the electrostatic repulsion between the ionized acid groups. The amounts of BSA released from the genipin- and $Ca^{2+}$ cross-linked hydrogels increased in pH dependent manner as swelling increased. The releases from these hydrogels were relatively low (approx. 20%) at pH 1.2 and significantly high (approx. 80%) at pH 7.4. At pH 4.0, there was still significant BSA release from the $Ca^{2+}$-cross-linked hydrogel, while the cumulative BSA released from the genipin-cross-linked hydrogel was limited due to its shrinking behavior. There was barely any BSA released from the glutaraldehyde-cross-linked hydrogel at different levels of pH. This probably was due to covalent binding of nearly all BSA molecules to the hydrogel matrix by glutaraldehyde during loading. The genipin cross-linked NOCMC/alginate hydrogel formed the semi-interpenetrating polymeric network-like (semi-IPN-like) structure (**Fig. 10**). Genipin may react with NOCC via a nucleophilic attack by the primary amino groups of NOCMC on the olefinic carbon atom at C-3 of deoxyloganin aglycone, followed by opening the dihydropyran ring and attacked by the attached secondary amino group on the resulting aldehyde group [**142**]. Dimerization occurs at the second stage, perhaps by radical reaction (**Fig. 11**) [**143, 144**]. The NOCMC/alginate hydrogel cross-linked by glutaraldehyde may produce a covalently cross-linked network (**Fig. 10**). At pH ~ 1.5, the aldehyde groups on glutaraldehyde may react with the hydroxyl groups on NOCMC and alginate to form acetals. Additionally, the amino groups on NOCMC may react with glutaraldehyde.

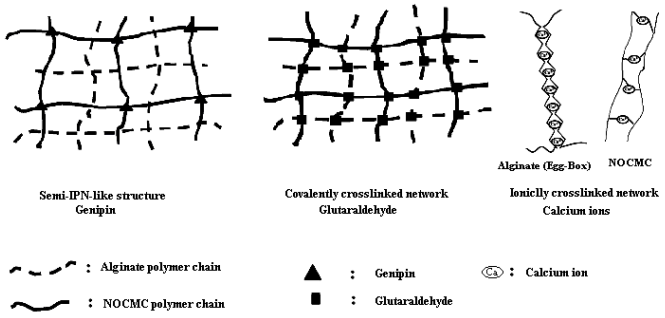


**Fig. 10.** Schematic illustrations of presumed structures of the NOCC/alginate hydrogels cross-linked by genipin, glutaraldehyde or $Ca^{2+}$.

The NOCMC/alginate hydrogel cross-linked by $Ca^{2+}$ involved ionic linking (**Fig. 10**). The interaction between the -$COO^-$ groups of the guluronic acid units on alginate and $Ca^{2+}$ in solution immediately induces a compact 'egg-box' structure [**145**]. Similarly ionic cross-links between the -$COO^-$ on NOCMC can also be established by $Ca^{2+}$. The CMC-$Ca^{2+}$ hydrogels can be strengthened by use of chitosan as second polymer. Liu et al. coated $Ca^{2+}$ crosslinked CMC hydrogel beads with a chitosan for improving entrapment efficiency of BSA as a model protein [**146**]. The beads were prepared by extruding a CMC/BSA solution into a $CaCl_2$/chitosan gelation medium. The entrapment efficiency (73.2%) achieved in the chitosan-coated-CMC beads was much higher compared with that (44.4%) in the bare $Ca^{2+}$-CMC beads. The release studies showed that maximum swelling ratios of the beads at pH 7.4 (17–21) were much higher than those at pH 1.2 (2–2.5). The polyelectrolyte membrane complex membrane formed of CMC –chitosan limited the BSA release, while the final disintegration of beads at pH 7.4 still leaded to a full BSA release.

The CMC-polymer blends show pH dependent swelling. The films of CMC-polyvinyl alcohol blend have been employed for controlled drug release of salicylic acid, theophylline, and ornidazole [**147**]. The drug release followed zero-order kinetics and increased with an increase in the CMC content in the blend, a decrease in the molecular weight of drug, an increase in the pH of medium,

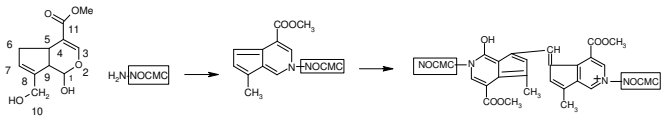


**Fig. 11**. Reaction of crosslinking of NOCMC with genipin.

and a decrease in the film thickness. Such films were used for local drug delivery of ornidazole by subcutaneous implant [**148**]. In the *in vitro* test, the blend films showed pH-responsive swelling behavior and moderate drug release action, and also exhibited a little antimicrobial activity against *E. coli* and *S. aureus* strains. After subcutaneously implanting the blend drug films in Wister rat, the systems kept a good retention at the application site and maintained high drug concentration in long time (5 days) which was longer than the period of drug released in vitro (160 min). The biocompatibility of the blend films was also

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established. Gelatin was used with CMC as other polymer too [**149**]. A two-component pH and ionic sensitive IPN complex system composed of CMC and chitosan cross-linked by glutaraldehyde has been evaluated for entrapment and release of coenzyme A [**150**].

The crosslinking of the CMC chains can be achieved by the use of physical method as electron beam radiation [**151**], microwave radiation [**152**], steam assistance [**153**] or inorganic crosslinking with clay [**154**]. Carboxymethylated chitin/chitosan derivatives irradiated at paste-like conditions were found to introduce crosslinking. When the dosage of irradiation was 20 kGy or more, transparent hydrogels could be produced. In the case of CMC, high DD was found to be negatively correlated to crosslinking even if it has a high DS. The created hydrogels exhibited excellent mechanical properties and good swelling in water with pH-sensitive character in their swelling behavior. The gel of CMC with a high DS (0.91) swelled in acid (pH < 3.5) and alkaline (pH > 6) conditions and deswelled between pH 3.5 and 6.0 due to the ionic composition changes of the gel network.

On the other hand semi-interpenetrating polymer network hydrogel based on linear CMCs and poly (N-isopropylacrylamide) crosslinked by synthetic hectorite clay - "Laponite XLG" ($[Mg_{5.34}Li_{0.66}Si_8O_{20}(OH)_4]$ $Na_{0.66}$, layer size =20–30 nmΦ×1 nm, cation exchange capacity =104 mequiv/100 g) was found to be pH- and temperature-responsive. The hydrogels exhibited a volume phase transition temperature around 33 °C with no significant deviation from the conventional poly (N-isopropylacrylamide) hydrogels. However the hydrogels had much higher response rate than the conventional hydrogels of similar composition and could be elongated to more than 800% and the elongation could be recovered almost completely and instantaneously.

### *6.3. DNA delivery*

It has been recently reported that chitosan reduces the thermotropic enthalpy of dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayer, a major cell membrane constituent and a standard experimental membrane model, during the gel to liquid crystalline transition and induces fusion of small DPPC vesicles to form large lamellar structures [**155, 156**]. OCMC has also emerged as a strong biomembrane perturbant. The results of a study of interaction between OCMC and DPPC with crosspolarization microscopy, DSC and surface pressure–area isotherms techniques proved that in comparison with unmodified chitosan, OCMC is a more effective membrane perturbant not only in neutral but also in acidic and basic conditions [**157**]. OCMC induced the fusion of small DPPC multilamellar vesicles to form large lamellar structures. The concentration needed for such fusion was much lower than chitosan. The physical driving forces for OCMC-induced perturbation of DPPC bilayer in neutral conditions are mainly hydrogen bonding between the amino and carboxyl groups of OCMC with the choline moiety of DPPC and hydrophobic interactions between the acyl chains of DPPC and the hydrophobic domain of OCMCS aggregates In acidic or basic conditions, the physical driving forces are dominated by the electrostatic interactions between ionized functional groups. The strong OCMC–DPPC interaction will potentially increase the effectiveness of OCMCS for gene or drug delivery.

### *6.4. Targeted drug delivery*

$Fe_3O_4$ nanoparticles have attracted scientists due to their multifunctional properties such as small size, superparamagnetism, and low toxicity However, $Fe_3O_4$ nanoparticles tend to aggregate due to strong magnetic dipole–dipole attractions between particles and need stabilization by surfactants, oxides or polymers. A $Fe_3O_4$ nanoparticles coated with polymers are composed of the magnetic cores to ensure a strong magnetic response and a polymeric shell to provide favorable functional groups and features, which have various applications for drug delivery systems, therapeutic regimes, cell/enzyme immobilization, diagnostic magnetic resonance imaging, and so on. Chitosan is one such polymer being used for this purpose. However, chitosan-magnetic composites were either aggregated or unstable due to polymer cross-linking or physisorption, and furthermore chitosan has no suitable functional groups to bind directly onto $Fe_3O_4$ nanoparticles. CMC fulfills this requirement. In addition, using the carboxylic moiety as a binding site, various molecules (e.g., DNA, proteins), and antibodies, could be loaded onto the OCMC/ $Fe_3O_4$ nanoparticles for the magnetically targeted delivery. Moreover, amphiphilic OCMC provides better association with a hydrophobic drug and localizes between the OCMC layer and $Fe_3O_4$ nanocore.

The preparation of $Fe_3O_4$ nanoparticle was reported by Zhu et al. [**158**]. The nanoparticles of $Fe_3O_4$ prepared by the coprecipitation of $Fe^{2+}$ and $Fe^{3+}$ salts in alkaline and anaerobic conditions at ambient temperature were stabilized as a well-dispersed suspension by chitosan and OCMC in a ratio $Fe_3O_4$: chitosan or OCMC as 5:1. The TEM results demonstrated a spherical or ellipsoidal morphology with an average diameter of 14–20 nm. The adsorbed layer of chitosan and OCMC on the magnetite surface was confirmed by FT-IR. The adsorption mechanism of chitosan and OCMC onto the surface of $Fe_3O_4$ nanoparticles is believed to be the electrostatic and coordination interactions of "Fe" of $Fe_3O_4$ and "O" of carboxyl groups in OCMC, respectively along with suppression of intermolecular hydrogen bonding. XRD illustrated that the resulting magnetic nanoparticles had a spinel structure. The zeta potential of suspension for unmodified $Fe_3O_4$ nanoparticles, chitosan/ $Fe_3O_4$ nanoparticles and OCMC/$Fe_3O_4$ nanoparticles was −13.40, +54.20 and −33.45 mV, respectively. The mechanisms of both chitosan and OCMC stabilizing the suspension of $Fe_3O_4$ nanoparticles were supposed electrostatic repulsion. $Fe_3O_4$ nanoparticles were surface-modified by CMC with its covalent binding via carbodiimide activation [**159-161**]. The spraying co-precipitation method is also disclosed for preparation of $Fe_3O_4$ nanoparticles functionalized with carboxymethyl chitosan [**162**].

CMC had been employed in synthesis of gold nanoparticles as well [**163**]. The use of chitosan has been stated as both reducing and stabilizing agents in the synthesis of gold nanoparticles (AuNPs) due to its oxygen-rich structures in hydroxyl and ether groups, which lead to


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a tight binding with metal clusters and nanoparticles via electrostatic interactions [**164**]. However, the solubility of chitosan in acidic solution resulted in the only acidic reducing condition for metal cations. CMC overcomes this problem. Recent study on the laser photolysis of CMC solutions revealed a band at 720 nm in the transient absorption spectra, which was assigned to the hydrated electron ($e^-_{aq}$) produced from the –$NHCOCH_3$ groups of CMC [**165**]. The main reactions are illustrated below in reactions **(1)–(3)**, and $e^-_{aq}$ produced in reaction **(2)** is a good reducing agent for some metal cations such as $Ag^+$ and $Au^{3+}$:

$$CMC \xrightarrow{h\upsilon} (CMC)^* \quad \text{(i)}$$

$$(CMC)^* \longrightarrow CMC^+ + e^-_{aq} \quad \text{(ii)}$$

$$CMC + e^-_{aq} \longrightarrow \bullet CMC^- \quad \text{(iii)}$$

With this explanation, the optimum conditions for nanoparticle formation by photochemical (ultraviolet) reactions of 0.5 mM chloroauric acid ($HAuCl_4$) and 5 mM CMC was found to be pH 12.4 and time 20 minutes. (The reasons for this pH preference might be difficulty in aqueous solubility of CMC due to coiled conformation at an approximate pH of 4.0–7.3, conservation of coiled structure even at higher pH of a few units which will greatly reduce the interaction sites of CMC and $Au^{3+}$cations and a rapid decay of $e^-_{aq}$ in high $H^+$ concentration). The nanoparticles prepared at pH 12.4 were stable for about six months with cubic crystal structure and size of about 6.2–8.2 nm. At this pH the electrostatic repulsive interaction between charged AuNPs and $–COO^-$ of CMC predominated as stabilizing force. Whereas at low pH, improvement in stabilization was not observed since the $–COO^-$ changed into –COOH which formed strong hydrogen bonds with each other leading to aggregation and precipitation of AuNPs [**166**]. The stabilized AuNPs/CMC composite exhibited fluorescence properties suggesting potential applications in biomedicine and bioanalytical fields.

### *6.5. Permeation enhancer*

Chitosan enhances the permeability of intestinal, nasal, buccal and corneal epithelia by opening the tight junctions between cells, thereby favoring paracellular drug transport [**17**]. On carboxymethylation, some of the chitosan monomer residues retained the protonated structure, which is a requisite for enhancing paracellular drug absorption across the epithelial tight junctions. On this basis, verification of similar uses of CMC was persuaded. Thanou et al. tested mono-N-carboxymethyl chitosan (mono-NCMC) on Caco-2 cells for their efficiency to decrease the transepithelial electrical resistance (TEER) and to increase the paracellular permeability of the anionic macromolecular anticoagulant - low molecular weight heparin (LMWH) [**167**]. For in vivo studies, LMWH was administered intraduodenally with or without mono-NCMC to rats. Results showed that mono-NCMC (of two different viscosity grades) managed to significantly decrease the TEER of Caco-2 cell monolayers at pH 7.4 when they were applied apically at concentrations of 3-5% w/v and to increase LMWH permeation substantially as compared to controls. A recent study by DiColo et al. showed that polyanionic NCMC failed to enhance intraocular (pH 2) drug penetration but increased precorneal ofloxacin retention due to its viscosity-increasing effect and mucoadhesive binding to ofloxacin [**168**].

### *6.6. Cosmetics*

Carboxymethyl chitosan and chitin are used more and more widely in cosmetics and as soft tissue augmentation in medicine for their excellent moisture-retention ability [**93, 169**]. NCMC as a 1.0% solution at pH 4.80 is a valuable functional ingredient of cosmetic hydrating creams in view of its durable moisturizing effect on the skin [**170**]. The film-forming ability of NCMC assists in imparting a pleasant feeling of smoothness to the skin and in protecting it from adverse environmental conditions and consequences of the use of detergents. Apart from these, CMC and derivatives have been used for many other purposes in cosmetics (**Table 4**).

### *6.7. Miscellaneous*

*As a template for reactions:* Uniformly globular polyaniline particles with a diameter in the nanometer range were successfully synthesized by the oxidative polymerization approach using glutaraldehyde cross-linked CM-chitin as a template under acidic conditions and the template was removed after the polymerization of aniline was completed [**171**]. The formation mechanism of the polyaniline nanoparticles in the cross-linked CM-chitin template is proposed according to the accumulation and polymerization of the aniline monomer within the pores of the cross-linked CM-chitin network. The electrical conductivities of the compressed polyaniline nanoparticles was significantly higher than that of the conventional ones, attributed to a higher orientation of polyaniline molecules induced by interaction with the cross-linked CM-chitin template.

*As a graft/blend copolymer:* The graft copolymerization of 9'-allyloxyindolinospiro-naphthoxazine onto carboxymethyl chitin resulted in water soluble polymer retaining and stabilizing the photochromic behavior of spirooxazine polymer [**172**]. Blend of CMC with polyvinylpyrrolidone provides hydrogel with improved surface properties for protein adsorption [**173**].

*As a component of membrane:* The composite membranes with coating of NOCMC on base layer of polysulfonates [**174**] and poly(ethersulfone) [**175, 176**] are developed through method of coating and crosslinking for ultrafiltration and pervaporation.

## 7. Modifications

### *7.1. Modifications with acylation*

Owing to distinctive properties and possible applications polymeric amphiphiles are appealing molecules. For example, amphiphilic polymers self-assemble or aggregate to form particles with a hydrophobic core and a hydrophilic shell. Amphiphilic polymer can be generated from CMC, a hydrophilic polymer, with acylation or alkylation of free amino groups.

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For instance, OCMC on acylation with succinic anhydride gives N-succinyl-OCMC (**Fig. 12**) [**177**]. The aggregation behaviors of N-succinyl-OCMC were studied using fluorescence spectroscopy, DLS, and AFM techniques. The critical aggregation concentration of N-succinyl-OCMC in water was determined to be 0.2–0.3 mg/ml. The CAC for N-succinyl-OCMC is 0.2–0.3, which is much higher than that of OCMC (0.05 mg/ml). This is possibly because of added succinyl groups which not only introduces two hydrophobic -$CH_2$-groups but COOH groups also. As a result, there are more dissociated carboxylic acid groups in water solution, which makes N-succinyl-OCMC much less hydrophobic than OCMC, and more prone to solvation and electrostatic repulsive interaction. The hydrophobicity of N-succinyl-OCMC can be further augmented by decorating it with cholesterol moiety [**178**]. This was achieved by reacting N-hydroxy succinimide activated cholesterol succinate with OCMC (**Fig. 12**). The amphiphilic molecules of cholesterol-succinyl-OCMC gave self-aggregated nanoparticles on probe sonication in water. These novel nanoparticles were almost spherical in shape, and their size, ranging from 234.9 to 100.1 nm, could be controlled by DS of cholesterol moiety. The zeta potentials of cholesterol-succinyl-OCMC self-aggregated nanoparticles were negative, and the absolute values decreased with increasing DS of the cholesterol moiety. On comparison, the cholesterol-succinyl-OCMC appeared to be superior with a spherical shape, a smaller size and a more compact structure to nanoparticles of only-cholesterol-modified chitosan. As per polycore model of nanoparticles of hydrophobically modified polysaccharides, the hydrophobic microdomains are formed by the association of hydrophobic groups, and the polysaccharide backbones coil to form the hydrophilic shells outside these hydrophobic microdomains attaining a minimal energy state is attained in aqueous media [**179**].

Introduction of acyl group as hexanoyl at free N of NOCMC by reaction with hexanoyl anhydride in neutral condition affords amphiphatic hexanoyl-NOCMC [**180**]. This modified polymer on crosslinking with genipin provided hydrogel with excellent water-absorption and water retention abilities. The probable reason being the presence of hexanoyl groups which alter the number of water-binding sites by inhibiting the formation of intermolecular hydrogen bonding and retard water mobility during deswelling. When employed as a carrier for delivering amphiphatic agent (ibuprofen), compared with that of pristine chitosan and NOCC, the encapsulation efficiency of ibuprofen was significantly enhanced with hexanoyl-NOCMC.

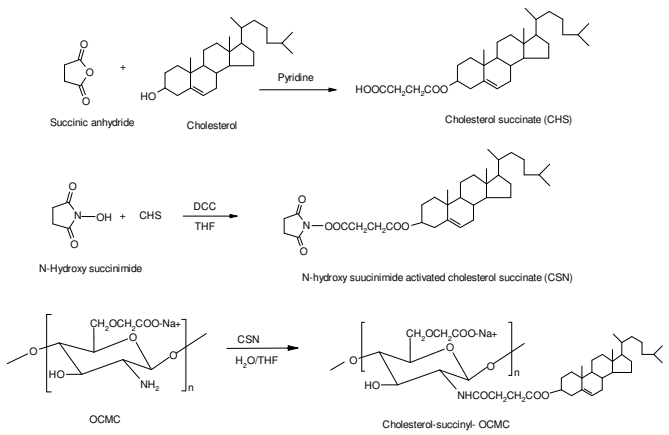


**Fig. 12**. Synthesis of cholesterol-succinyl-OCMC

Acylation of CMC with longer chain as of linoleic acid was carried via EDC-mediated reaction [**181**]. The self assembled nanoparticles formed on sonication exhibited physical entrapment of adriamycin. The drug loading capacity and efficiency increased with increasing concentration of adriamycin. The drug release was slow and could be adjusted by changing the medium pH. Another way to introduce acyl moiety in the CMC backbone is the use of acyl chloride. Sun et al synthesized oleoyl CMC with oleoyl chloride in acetone at low temperature (0-5°C) as used it as coagulation agent [**182**].

### *7.2. Modifications with alkylation*

Alkylation of free amino group of CMC can be achieved by usual reductive alkylation reactions of chitosan by reacting it with aldehyde followed by reduction of formed Schiff's base with $NaBH_4$. Guo et al. reported various alkyl CMC as methyl, ethyl, propyl, butyryl, isobutyryl obtained by this route followed by their quaternization with direct alkylation reaction with methyl iodide/sodium iodide mixture in basic condition [**183**]. The hydroxyl radicals scavenging activity of all quaternized CMC (Degree of quaternization 34.3% to 59.5%) was better than that of CMC as a result of the positive charge of the quaternized chitosan. The alkylation of CMC with longer chains as hexyl, octyl, decyl, myristyl, and lauryl etc are disclosed in the patent by Kawahara for their micelle forming abilities [**184**].

Alkylation (quaternization) of CMC was achieved through the reaction of CMC with 3-chloro-2-hydroxypropyl trimethylammonium chloride (CTA) [**185**] or 2, 3-epoxypropyl trimethylammonium [**186**] as a quaternizing agent in isopropanol medium under basic condition. The synthetic conditions for quternization of CMC were: 40.0% of NaOH aqueous solution as catalyst; reaction temperature - 60.0°C and reaction time - 10.0 h; NaOH/CMC - 0.50; CTA/CMC - 1.50 (mass ratio). The quaternized CMC obtained became a typical amphoteric derivative since it had both carboxymethyl group and quaternary ammonium group into the chitosan molecular chain. This derivative had antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, better than CMC or quaternized chitosan of same DS. Moreover the results of animal experiments with use of quaternized CMC


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complexed with calcium hydroxide as pulp-cap indicated that quaternized CMC can strongly induce reparative dentine formation and showed a better ability in dentin inducing compared with calcium hydroxide [**186**].

Alkylation of CMC with a novel group octadecyl quaternary ammonium is performed by reaction of CMC with glycidyl octadecyl dimethylammonium chloride (**Fig. 13**) [**187**]. Octadecyl quaternized CMC showed a good solubility both in water and organic solvents, which extends its range of applications. The organic solvents tested include dimethyl sulfoxide, dimethyl formamide, tetrahydrofuran, chloroform and acetone. The moisture-absorption and retention abilities of octadecyl quaternized CMC were lower than that of hyaluronic acid and CMC. The carboxymethyl and quaternary ammonium groups did not show a synergistic effect, and the effects of both the molar mass and the hydrophobic side chains of long alkyl moieties were important. Minocycline hydrochloride was successfully incorporated into octadecyl quaternized CMC polymeric micelles with a remarkably high efficiency (10.9%, mass fraction).

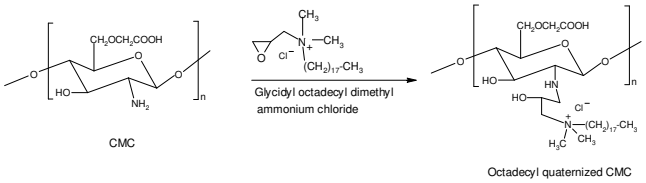


**Fig. 13.** Synthesis route to the chitosan derivatives of octadecyl-quaternized carboxymethyl chitosan.

### *7.3. Modifications of carboxyl group*

The introduced –COOH group provides one more site for chemical modification of CMC. The reaction of amine function at this site is an attractive way. The reaction of CMC with the methyl esters of amino acids arginine and lysine under neutral conditions of solvent benzene with heating directed the amide bond formation [**188**]. The improved positive charge density of arginine, lysine modified CMC facilitated the cholesterol entrapment in vitro. (Arg-CMC 49.62 ± 1.66% Lys-CMC-43.72 ± 1.65 %, chitosan 14.23 ± 0.81% cholesterol retention)

### *7.4. Modification with grafting*

Various modifications can be achieved by use of grafting technique with suitable polymers (**Table 3**) CMC grafted with phosphatidylethanolamine (PEA) groups was synthesized by EDC-mediated coupling reaction (**Fig. 14**) [**189**]. The beads of CMC-g-PEA were prepared with sodium tripolyphosphate ionic-crosslinking. The bead size was in range from 800 to 1200 µm and encapsulation efficiency of hydrophobic drug (ketoprofen) was more than 68%. The drug dissolution kinetics showed longer release times for CMC-g-PEA beads: 20 h (at pH 1.4) and 45 h (at pH 7.4). The amount of the drug release was much higher in acidic solution than in basic solution due to the swelling properties of the matrix at acidic pH.

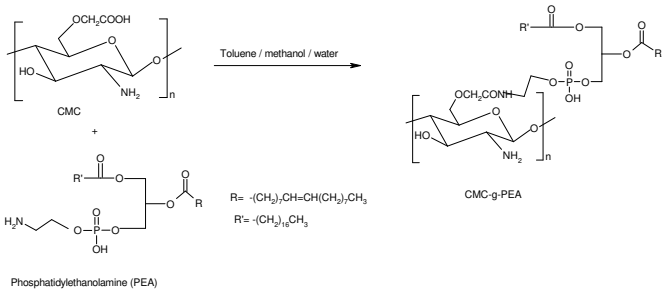


**Fig. 14.** Reaction scheme for the preparation of CMC-g-PEA.

**Table 3.** Grafting of CMC with different monomers

| Monomers grafted on CMC | Comment | Ref |
|---|---|---|
| Methacrylic acid using ammonium persulfate as an initiator | The optimum conditions were: (ammonium persulfate) = 8 mmol/l, (methacrylic acid) = 2.4 mol/l, reaction temperature = 60–70°C, reaction time = 120 min. | [**191**] |
| 2-Hydroxyethylmethacrylate using ceric ammonium nitrate (CAN) as an initiator | The optimum grafting conditions was: OCMC = 2 g; CAN = 0.2 M; and HEMA = 0.384 mol/l; reaction temperature = 40°C; and reaction time = 4.5 h. | [**192**] |
| Poly(ethylene glycol) using reductive alkylation reaction with methylpoly(ethylene glycol) aldehyde | OCMCS-g-MPEGs resolved in water over all pH range and at isoelectric point decreases when DS increases. The hydrodynamic behaviors markedly affected by DS. | [**193**] |
| Polyacrylamide using CAN as an initiator | The optimum grafting conditions were : NCMC = 2 g, CAN = 0.2 M, and Acrylamide = 0.563 mol/L, reaction temperature = 40°C, and reaction time = 4.5 h. | [**194**] |
| Sodium acrylate and 1-vinyl-2-pyrrolidone using azobis(isobutylamine hydrochloride) as the initiator and N,N'-methylene diacrylamide as the crosslinking agent | The optimal conditions to get the polymer with the highest swelling ratio were: reaction time = 5 h, reaction temperature = 60°C, molar ratios of the crosslinking reagent and the initiator to sodium acrylate 0.0208 and 0.0230, respectively. | [**195**] |

A novel thiolated carboxymethyl chitosan-g-β-cyclodextrin (CMC-g-β-CD) drug delivery carrier was reported by Prabhaharan et al. [**190**]. It was synthesized in two steps (**Fig. 15**). First, carboxymethyl β-cyclodextrin (CMC-β-CD) was grafted onto CMC using EDC and N-hydroxysuccinimide as the condensing agents. Next, the resultant product was further grafted with cysteine methyl ester hydrochloride (CMEH). The amorphous thiolated polymer (2θ = 10° and 20°) had higher swelling efficiency and five times greater mucoadhesive properties than that of the unmodified chitosan control. The drug release profile of formulated tablets provided a slower release of the entrapped hydrophobic model drug, ketoprofen, than the chitosan control, and the release behavior was influenced by the amounts of thiol groups present on the polymer chains.

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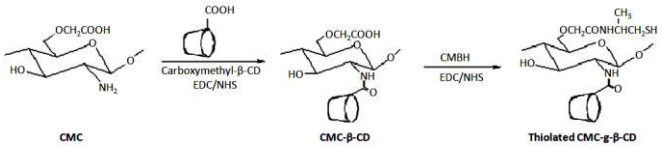


**Fig. 15.** Synthesis of thiolated CMC-g-β-CD.

## 8. Analogous derivatives

### *8.1. Carboxymethyl chitin*

The difference in the solubilities of chitin and chitosan due to difference in degree of deacetylation does not seem to affect the carboxymethylation process. The carboxymethylation of chitin can be achieved by the similar reaction and conditions as that of chitosan carboxymethylation [**152, 196**]. The solvent system can be supplemented with detergent and cosolvent as NaOH/Dimethy formamide/Sodium dodecyl sulfate (**Fig. 16**) [**197**]. CM-chitin dissolves in almost the entire pH range [**198**]. Crosslinking of the microwave-treated CM-chitin films involved mainly the carboxylate and the secondary alcohol groups. This material finds use in biomedicine particularly as a biomaterial for bone augmentation [**199, 200**], flexible bioactive bone-repairing material [**201**], soft issue augmentation [**152**], and in cosmetics, etc.

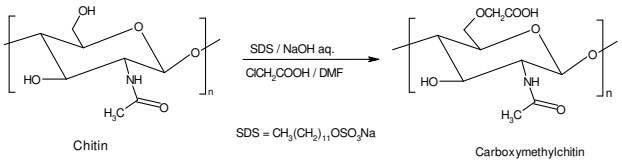


**Fig. 16.** Synthesis of carboxymethyl chitin.

The water solubility of CM-chitin has prompted its employment in drug delivery as well. Watanabe et al. used a gel-broken method to prepare sustained-release BSA or doxorubicin gels [**202**]. For this $FeCl_3$ solution was added into CM-chitin solution, stirred and ground by homogenizer to make uniform gels (10–20 mm). The release of BSA or doxorubicin from the gels was observed to be lysozyme-digestion dependent.

Mi et al. used an in-liquid curing method to prepare a CM-chitin microsphere for sustained-release of 6-mercaptopurine [**203**]. The drug was incorporated into the gel by the ionotropic crosslinking with ferric chloride. Drug release rate of the CM-chitin microspheres decreased with increasing concentration of $FeCl_3$ solution and the curing time. Iron elution from the complex CM-chitin beads decreased from pH 1.2 to pH 7.5, especially between pH 4 and pH 3, because the $Fe^{+3}$- CM-chitin complexes were unstable in acid medium. The drug-release patterns of the CM-chitin microspheres in pH 7.2 seemed not only to be diffusion influenced but also macromolecular relaxation influenced. Whereas, release profiles of the microspheres in pH 1.2 medium seemed to be matrix erosion controlled but were also affected by crosslinking density of the microspheres.

### *8.2. Carboxyethyl chitosan*

The carboxyethylation of chitosan is possible by direct alkylation with 3-halopropionic acid or by Michael addition (**Fig. 2**). Generally, the reaction of chitosan with 3-halopropionic acid allows the introduction of 2-carboxyethyl group at the 3-O, 6-O and N-positions as was observed in the reactions of chitosan with chloroacetic [**204**] or 2-chloropropionic [**205**] acids under strong alkaline conditions. Skorik et al. obtained N-(2-Carboxyethyl)chitosans (NCEC) by reaction of low molecular weight chitosan with a low degree of acetylation and 3-halopropionic acids (halo= chloro, bromo, iodo) under mild alkaline media (pH 8–9, $NaHCO_3$) at 60 °C [**206**]. Under mild alkali conditions that give the possibility to differentiate the reactivity of -OH and -$NH_2$ groups' alkylation occurred exclusively at the amino groups in mono and di substitution pattern. Michael addition with acrylic acid provides carboxyethylation at amine function. Acrylic acid, an α,β-unsaturated carbonyl reagent when is reacted with chitosan, plays two roles - as a proton donor to make chitosan able to dissolve in aqueous medium and as a reagent for reaction with amine groups of chitosan by Michael addition mechanism. The typical reaction procedure consists of reaction of chitosan solution, concentration approximately 0.5 -2 % in water or aqueous acid, and acrylic acid at 50-70°C for 8 h to 6 days, adjusting the pH of the reaction mixture to 8-9 with alkali and desalting it by dialysis or pH adjustment [**207**]. Even with the significant time expenditures the DS of the product obtained remain low. An increase in the concentrations of chitosan and acrylic acid decreases the synthesis duration, but DS remains less than one [**208**]. An increase in the reactivity of acrylic reactant by using it as ethyl ester does not solve the problem, since in this case the DS equal to one is reached only in 240 h (in doing so, the product should be saponified) [**209**]. The common drawback of this procedure is the need to use dilute solutions (due to low concentration of chitosan solutions in aqueous acids and limited solubility of methyl or ethyl acrylate in water), which requires large consumption of solvents (water or aqueous methanol) and decreases the product yield. Pestov et al. claimed to developed new procedure for synthesis of carboxyethyl chitosan in gel [**210**].

The CEC has a number of additional useful characteristics: solubility in water in a wide pH range [**206**], better biodegradability [**208**] and excellent antioxidant characteristics, significant antimutagenic activity [**211**] than chitosan proper. The antioxidant and antimutagenic activities of the NCEC derivatives with three different degrees of substitution have been assayed in vitro in the unicellular flagellate *Euglena gracilis* subjected to the action of genotoxic agents- acridine orange and ofloxacin. The NCEC derivatives exhibited concentration-dependent protective antigenotoxic activity against both mutagens. It was suggested that different mechanisms may be involved in its protective action—antioxidant activity in case of ofloxacin induced DNA damage, as well as possible interaction with the cell membrane that prevents acridine orange from reaching the genetic compartments and subsequent damaging DNA through intercalative binding.


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Dependence of the antimutagenic properties of the studied chitosan derivatives on the degree of substitution was reversed in experiments involving acridine orange and ofloxacin, which also indicated different mechanisms of protection involved in these two cases. CEC turned out to be a suitable mediator for transport of hydrophilic drugs through the skin [**212**] or a substrate for introduction of nitrogen oxide for medical purposes [**213**].

The complexes of CEC with oxidized dextran (formed by Schiff base mediated chemical crosslinking and physical crossslinking) [**214**] and with poly(2-acryloylamido-2-methylpropanesulfonic acid), poly(acrylic acid), or poly(ethylene imine) (formed by polyelectrolyte complexation) are reported. Recently Semi-interpenetrating polymer network hydrogels of NCEC and 2-hydroxyethyl methacrylate prepared by UV irradiation of aqueous solutions in the presence of D-2959 as photoinitiator are reported [**215**].

### *8.3. Carboxybutyl chitosan*

N-carboxybutyl chitosan is an amphoteric chitosan derivative that is soluble under acidic, neutral and basic conditions [**216**]. The bacteriostatic activity and wound healing properties of the N-carboxybutyl chitosan [**217**], together with other favorable properties, such as its viscous action, enhanced film-forming ability, moisturizing effect and emulsion stability, make these novel modified chitosans most suitable as functional cosmetic ingredients [**218**]. It is obtained by the reaction of levulinic acid on chitosan [**93**]. But, depending on the chemical conditions the reaction tends to form N-carboxybutyl chitosan or 5-methylpyrrolidinone chitosan (**Fig. 17**) [**89, 219**]. The solubility range of 5-methylpyrrolidinone chitosan, a cyclic derivative, is restricted to acidic conditions, as is chitosan [**216**].

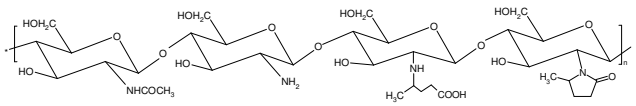

**Fig. 17.** *N*-carboxybutyl chitosan and 5-methylpyrrolidinone chitosan

## 9. Intellectual properties, regulatory issues and commercial exploitation

Commercial exploitation of chitosan native faces significant barriers as difficulties in preparing uniformly reproducible charges in bulk quantities from various marine organisms and the high prices of the polymers. The derivatization as carboxyalkylation further adds to the cost and possible variations in character uniformity. However these hurdles may be overcome by the research and technological advances for which impetus will be provided with growing applications and demand of chitosan along with derivatives. The plethora of applications of CMC is already disclosed in number of patents (**Table 4**).

**Table 4.** Applications of CMC and derivatives disclosed in US Patents

| US Patent | Description | Ref. |
|---|---|---|
| 6039933 | Cosmetic composition pressurized in an aerosol device and the resulting foam | [**220**] |
| 4301067 | Chitin containing poly ion complex | [**221**] |
| 4619995 | N,O-carboxymethyl chitosan and preparative method thereof | [**204**] |
| 4871556 | Inhibition of warmed-over flavor and preserving of uncured meat containing materials | [**222**] |
| 5093319 | Use of derivatives of chitin soluble in aqueous solutions for preventing adhesions | [**223**] |
| 5378472 | Methyl pyrrolidinone chitosan, production process and uses thereof | [**224**] |
| 5382286 | Acoustic gel | [**225**] |
| 5412084 | N,O-carboxymethylchitosonium carboxylate salts | [**226**] |
| 5420197, 6379702 | Gels formed by the interaction of polyvinylpyrrolidone with chitosan derivatives | [**227, 228**] |
| 5460939 | Temporary living skin replacement | [**229**] |
| 5538955 | Process for the preparation of iodinated biopolymers having disinfectant and cicatrizing activity, and the iodinated biopolymers obtainable thereby | [**230**] |
| 5578661 | Gel forming system for use as wound dressings | [**231**] |
| 5621088 | Process for derivatizing polyglucosamines | [**232**] |
| 5679658 | N,O-carboxymethyl chitosan for prevention of surgical adhesions | [**233**] |
| 5684051 | Medical devices with improved elastic response | [**234**] |
| 5718862 | Secondary shaping of ionically crosslinked polymer compositions for medical devices | [**235**] |
| 5720793 | Calcium agent for plants | [**236**] |
| 5773608 | Process for preparing stabilized chitin derivative compounds | [**237**] |
| 5888988 | Covalently linked N,O-carboxymethyl chitosan and uses thereof | [**238**] |
| 5891199 | Polymer dyestuffs and their use for dyeing fibers | [**239**] |
| 5906997 | Bioresorbable compositions of carboxypolysaccharide polyether intermacromolecular complexes and methods for their use in reducing surgical adhesions | [**240**] |
| 5932561 | Dietary composition with lipid binding properties for weight management and serum lipid reduction | [**241**] |
| 6039933 | Cosmetic composition pressurized in an aerosol device and the resulting foam | [**220**] |
| 6060534 | Medical devices comprising ionically and non-ionically crosslinked polymer hydrogels having improved mechanical properties | [**242**] |
| 6080420 | Fibers of cospun alginates | [**243**] |
| 6124273 | Chitin hydrogels, methods of their production and use | [**244**] |
| 6156077 | Hair cosmetic composition comprising an oxyalkylenized xanthan gum | [**245**] |
| 6203845 | Dehydrated hydrogels | [**246**] |
| 6224794 | Methods of microsphere production | [**247**] |
| 6251959 | Chitin derivatives having carboxylated lower alkyl groups as hydrophilic substituents and hydrophobic substituents, and micellar aqueous composition thereof | |
| 6258995 | Wound treatment composition | [**248**] |
| 6261594 | Chitosan-based nitric oxide donor compositions | [**213**] |
| 6274131 | Mascara comprising a mixture of hard waxes and of film-forming polymer | [**249**] |
| 6368356 | Medical devices comprising hydrogel polymers having improved mechanical properties | [**250**] |
| 6372248 | Dehydrated hydrogels | [**251**] |
| 6379702 | Gels formed by the interaction of polyvinylpyrrolidone with chitosan derivatives | [**228**] |
| 6387978 | Medical devices comprising hydrogel polymers having improved mechanical properties | [**252**] |
| 6391292 | Hairstyling composition comprising a polymer with particular characteristics and an ionic film forming polymer | [**253**] |
| 6399050 | Hair cosmetic composition in the form of a water-in-silicone emulsion comprising at least one fixing polymer | [**254**] |
| 6417179 | Ear wax solution | [**255**] |
| 6436151 | Compositions for oxidation dyeing keratin fibers comprising at least one oxidation dye, at least one thickening polymer comprising at least one fatty chain, and at least one fatty alcohol comprising | [**256**] |


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| | | |
|---|---|---|
| | more than twenty carbon atoms and uses thereof | |
| 6451337 | Chitosan-based nitric oxide donor compositions | [257] |
| 6506372 | Cosmetic compositions containing an amphoteric polymer and a fixing/conditioner polymer, and their uses | [258] |
| 6524602 | Polymer delivery system in treatments for parasitic skin diseases | [259] |
| 6534083 | Hydrogels | [260] |
| 6548051 | Hair styling composition comprising adhesive particles | [261] |
| 6602303 | Composition for the oxidation dyeing of keratinous fibers comprising at least one oxidation dye and at least one cationic amphiphilic polymer, and dyeing methods | [262] |
| 6645947 | Adhesive N, O-carboxymethyl chitosan coatings which inhibit attachment of substrate-dependent cells and proteins | [263] |
| 6667378 | Reshapable hair styling composition comprising heterogeneous (meth)acrylic copolymer particles | [264] |
| 6692730 | Washing composition comprising particles of aluminum oxide, at least one conditioning agent and at least one detergent surfactant | [265] |
| 6896878 | Cosmetic composition with a fixing and/or conditionir polymer containing a specific acrylic copolymer | [266] |
| 6936746 | Polyelectrolyte solid system, method for the productio thereof and a wound dressing | [267] |
| 6951053 | Method of manufacturing a prosthesis | [268] |
| 7029661 | Cosmetic composition with a fixing and/or conditionir polymer containing a specific acrylic copolymer | [269] |
| 7048916 | Reshapable hair styling composition comprising heterogeneous (meth)acrylic copolymer particles | [270] |
| 7053068 | Chitosan-thio-amidine conjugates and their cosmetic a well as pharmaceutic use | [271] |
| 7066966 | Oxidation dyeing composition for keratin fibers comprising a cationic poly(vinyllactam) | [272] |
| 7122175 | Reshapable hair styling composition comprising (meth)acrylic copolymers of four or more monomers | [273] |
| 7125967 | Water-soluble chitosan having low endotoxin concentration and methods for making and using the same | [274] |
| 7147672 | Oxidation dyeing composition for keratin fibers comprising a cationic poly(vinyllactam) and at least one C10-C14 fatty acid, methods and devices for oxidation dyeing | [275] |
| 7151079 | Cosmetic compositions containing a fructan, a polysaccharide and a beneficial agent, and uses thereo | [276] |
| 7198649 | Oxidation dyeing composition for keratinous fibers comprising glycerine and a polyol other than glycerine in a specific ratio | [277] |
| 7211108 | Vascular grafts with amphiphilic block copolymer coatings | [278] |
| 7217298 | Ready-to-use bleaching compositions, preparation process and bleaching process | [279] |
| 7238678 | Adhesive N,O-carboxymethyl chitosan coatings which inhibit attachment of substrate-dependent cells and proteins | [280] |
| 7261734 | Resorption-controllable medical implants | [281] |
| 7265097 | Methods of drug delivery using sulphated chitinous polymers | [282] |
| 7288532 | Modified chitosan polymers and enzymatic methods for the production thereof | [283] |
| 7294152 | Dyeing composition comprising at least one fluorindine compound for the dyeing of keratinic fibers, dyeing process comprising the composition and compound | [284] |
| 7307157 | Process for producing chitin derivatives and/or chitosan derivatives having a crosslinked structure | [285] |
| 7309437 | Compositions and methods for removal of toxic metals and radionuclides | [286] |
| 7323015 | Oxidation dyeing composition for keratin fibers comprising a cationic poly(vinyllactam) and at least one C10-C14 fatty alcohol, methods and devices for oxidation dyeing | [287] |
| 7335766 | Method for producing a chitosan containing salt having a function of lowering blood pressure | [288] |

## 10. Conclusion

Chitosan is a fascinating polymer but its appeal is limited because of its inadequate solubility in water and other relevant solvents. This necessitates its derivatization to improve solubility in water. Carboxymethylation is a suitable way to fulfill this necessity. The carboxymethylation can be ensued through reductive alkylation, direct alkylation and Michael addition (carboxyethylation). The substitution can occur at N, or O or both the atoms. The existence of carboxymethyl group in the molecular structure conferred the CMC with improved as viscosity, moisture retention, membrane forming, flocculating, chelating and sorption properties. The CMC shows modifications in biological properties as modulation of cell functioning and activities as antioxidant, antibacterial and antiapoptotic. This derivative finds uses in sustained or controlled release drug delivery, pH responsive drug delivery, DNA delivery and as permeation enhancer too. Many of the uses of CMC including of cosmetic and others have been reported and patented. The CMC can be further modified to get derivatives as amphiphilic, quaternized, or grafted by acylation, alkylation or grafting. Higher homologs of CMC like carboxyethyl, carboxtbutyl chitosan can also emerge as derivatives of worth. These derivatives may become proponent of chitosan applications.

## 11. Abbreviations

**CMC** carboxymethyl chitosan, **NCMC** *N*-carboxymethyl chitosan, **OCMC** *O*-carboxymethyl chitosan, **NOCMC** *N,O*-carboxymethyl chitosan, **mono-NCMC** mono-*N*-carboxymethyl chitosan, ***N,N*-diCMC** *N,N*-dicarboxymethyl chitosan, **DS** degree of substitution, **DD** degree of deacetylation, **DSC** differential scanning calorimetry, **CAC** critical aggregation concentration, **hCFIX** human clotting factor IX, **IPN** interpenetrating polymeric network , **SPH** superporous hydrogel , **SEM** scanning electron microscopy, **CLSM** confocal laser scanning microscopy, **AFM** atomic force microscopy, **BSA** bovine serum albumin, **semi-IPN** semi-interpenetrating polymeric network, **DPPC** dipalmitoyl-sn-glycero-3-phosphocholine, **AuNPs** gold nanoparticles, **TEER** transepithelial electrical resistance, **LMWH** low molecular weight heparin, **DLS** dynamic light scattering, **TEM** transmission electron microscopy, **PEA** phosphatidylethanolamine, **EDC** 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, **CM-chitin** carboxymethyl chitin, **NCEC** *N*-(2-carboxyethyl) chitosan and **CEC** carboxyethyl chitosan.

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