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Review

Biomedical applications of carboxymethyl chitosans

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ABSTRACT

This review outlines the recent developments on carboxymethyl chitosan-based bio-medical applications. Carboxymethyl chitosan, a water soluble derivative of chitosan, with enhanced biological and physicochemical properties compared to chitosan, has emerged as a promising candidate for different biomedical applications. Introducing small chemical groups like carboxymethyl to the chitosan structure can drastically increase the solubility of chitosan at neutral and alkaline pH values without affecting their characteristic properties. Due to improved biocompatibility, high moisture retention ability more viscosity and enhanced antimicrobial property of carboxymethyl chitosan than chitosan makes it promising candidate for hydrogels and wound healing applications. The biodegradability and biocompatibility of carboxymethyl chitosan has significant interest with application as biomaterial for tissue engineering. Apart from this, the easy of carboxymethyl chitosan can be easily processed into nanoparticles so it has shown promise for drug delivery, bioimaging, biosensors and gene therapy applications. The contribution of carboxymethyl chitosan to green chemistry in the recent years has also been given in detail. This review will focus on preparative methods and physicochemical and biological properties of carboxymethyl chitosan with particular emphasis on biomedical and pharmaceutical applications of this derivative of chitosan.

Contents

Abbreviations: CM-chitin, carboxymethyl chitin; CM-chitosan, carboxymethyl chitosan; CE-chitosan, carboxyethyl chitosan; OCM-chitosan, O-carboxymethyl chitosan; NCM-chitosan, N-carboxymethyl chitosan; NOCM-chitosan, N,O-carboxymethyl chitosan; NCB-chitosan, N-carboxybutyl chitosan; NNDCM-chitosan, N,N-dicarboxymethyl chitosan; NCE-chitosan, N-carboxyethyl chitosan; NCM-chitin, N-carboxymethyl chitin; MW, molecular weight; BMP-2, bone morphogenetic protein-2; DA, degree of acetylation; DD, degree of deacetylation; DS, degree of substitution; HRP, horseradish peroxidase; ROS, reactive oxygen species; IPN, interpenetrating networks; semi-IPN, semi-interpenetrating networks; XPS, X-ray photoelectron spectroscopy; MRI, magnetic resonance imaging; CAC, critical aggregation concentration; CM chitosan-SPIONS, carboxymethyl chitosan modified superparamagnetic iron oxide; FA, folic acid; BSA, bovine serum albumin; GFLX, gatifloxacin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; HEMA, 2-hydroxyethylmethacrylate.

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1. Introduction

A most recent article by Muzzarelli et al. (2012) celebrated the bicentennial of the discovery of chitin, and in the light of our current knowledge, reviewed the major research topics explored by Henry Braconnot, who described for the first time a polysaccharide containing a substantial percent of nitrogen, later to be called chitin. Its importance has been assessed with details on acids isolated from plants, that were later used for the preparation of soluble chitosan derivatives that paved the way for a number of applications. The biocompatibility, biodegradability, antimicrobial activity, absence of toxicity and hydrating properties of chitin and chitosan are today well established. Due to these desirable properties, chitin and chitosan can be easily processed into scaffolds (Duarte, Mano, & Reis, 2010), nanoparticles (Csaba, Hoggard, & Alonso, 2009), microparticles (Lameiro, Lopes, Martins, Alves, & Melo, 2006), beads (Gandhi, Kousalya, Viswanathan, & Meenakshi, 2011), membranes (Beppu, Vieira, Aimoli, & Santana, 2007) and nanofibers (Homayoni, Ravandi, & Valizadeh, 2009) forms. In the recent decades, CM-chitosans have received much attention to their better solubility in water, enhanced antibacterial property (Liu, Guan, Yang, Li, & Yao, 2001), improved biocompatibility (Chen, Wang, Liu, & Park, 2002; Zhu, Chan-Park, Dai, & Li, 2005) and safety for humans (Fu et al., 2011). CM-chitosan is also known to exhibit low toxicity (Tokura, Nishimura, Sakairi, & Nishi, 1996). The carboxymethylation procedure of both chitin and chitosan has been earlier reported (Muzzarelli, 1988). The inspiration behind the development of NCM-chitosan was the functionalization of chitosan in such a way as to have an amino acid moiety in it, actually glycine. The active hydroxyl and amino groups in the polymer chains of CM-chitosan are known to take part in free radical scavenging and hence contribute to its increased antioxidant activity. The enhanced antioxidant property and bile acid binding property of this derivative of chitosan have been well established (Zhao, Huang, Hu, Mao, & Mei, 2011). The influence of MW and the presence of hydroxyl, amino and amido groups in the polymer chain of CM-chitosan on its antioxidant activity have also been described by various researchers (Feng, Du, Li, Hu, & Kennedy, 2008; Sun, Zhou, Xie, & Mao, 2007). Therefore, carboxymethylation of chitosan makes it a promising candidate in a number of biomedical, pharmaceutical, and environmental fields (Chen et al., 2007; Mishra et al., 2011; Wang, Yang, & Niu, 2010; Xu, Mao, Liu, Zhu, & Shen, 2006; Yin, Fei, Cui, Tang, & Yin, 2007). Here we have reviewed the considerable research performed to date on the water-soluble CM-chitosans, particularly with respect to their biomedical applications, but potential applications of this derivative in the other fields will also be discussed.

2. Carboxymethyl chitosan: structural and functional features

2.1. Preparation and characterization of various carboxymethyl chitosans

2.1.1. Preparation of O-carboxmethyl chitosan

OCM-chitosan is an amphiprotic ether derivative which contains —COOH groups and $-NH_2$ groups. The preparation of OCM-chitosan involves suspension of chitosan in the isopropanol + NaOH; then, monochloroacetic acid dissolved in isopropanol is added drop wise within 30 min and reacted for 4 h at 55 °C. The solid is filtered and washed with ethyl alcohol and dried in vacuum. Thus the reaction medium used for OCM-chitosan synthesis is strongly alkaline. The water solubility of OCM-chitosan is governed by the preparation conditions as well as degree of carboxymethylation. While OCM-chitosan prepared between 0 and 10 °C was found to be water soluble, the other prepared between 20 and 60 °C was found to be water insoluble at neutral pH (Chen, Du, & Zeng, 2003). OCM-chitosan has been employed for immobilization of enzymes of clinical significance (Xu, Mao, Liu, Zhu, & Shen, 2006), as well as grafting of polyacrylamide induced by cericammonium onto OCM-chitosan (Joshi & Sinha, 2007). The schematic representation of preparation methods for various carboxymethyl derivatives of chitosan is shown in Fig. 1.

2.1.2. Preparation of N-carboxmethyl chitosan

The preparation of NCM-chitosan involves reacting free amino group of chitosan with glyoxylic acid to give soluble aldimine and then reduction of the latter with sodium borohydride. The choice of chitosan (in terms of DA and MW) and the amount of glyoxylic

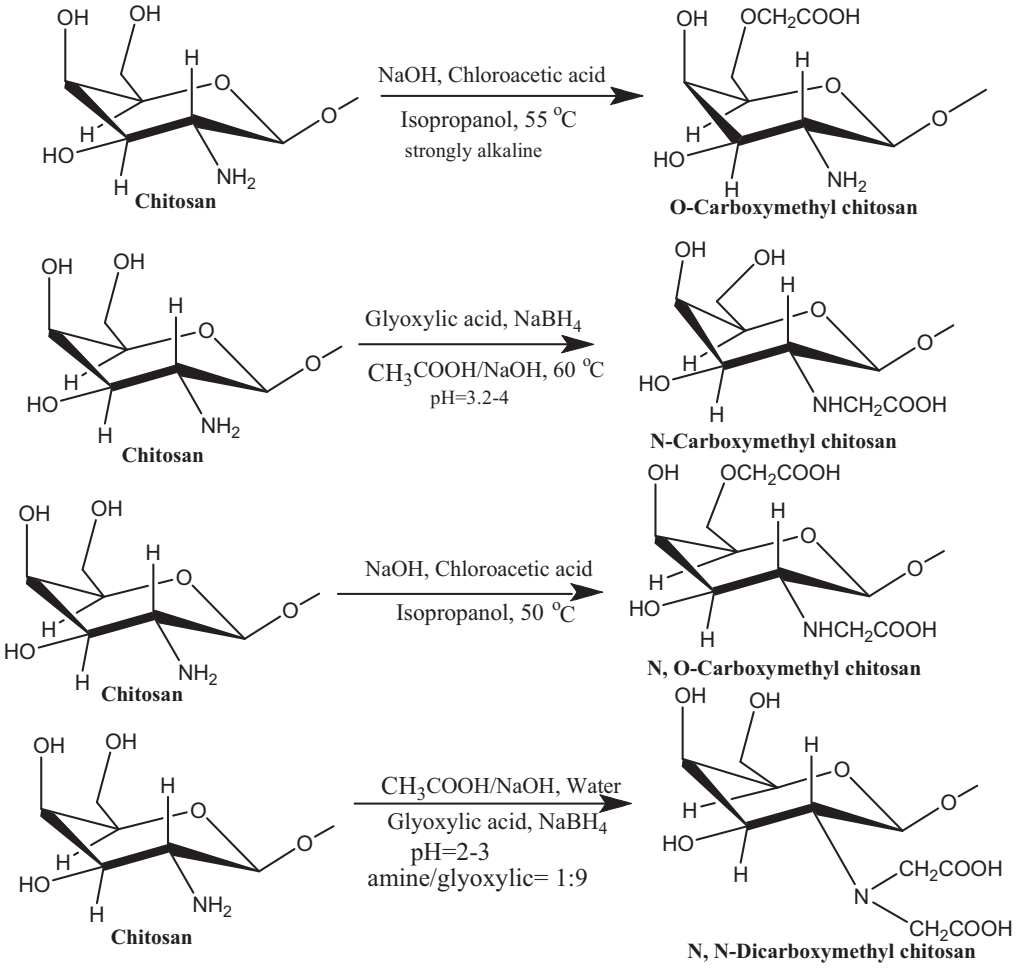

Fig. 1. Preparative methods of carboxymethyl derivatives of chitosan.

acid determine the ratio of acetyl, carboxymethyl and free amino groups; an advantage of this reaction is that it does not require heating. The application of this polymer derivative in the field of removal of trace metal ions from industrial water has been reported earlier (Muzzarelli, Weckx, Filippini, & Sigon, 1989).

2.1.3. Preparation of N,O-carboxymethyl chitosan

NOCM-chitosan is a chitosan derivative bearing carboxymethyl substituent at some of the amino and primary hydroxyl sites of the glucosamine units of the chitosan structure. Moisture retention, gel formation and good biocompatibility are the major attractive physico-chemical properties of NOCM-chitosan which make this hydrophilic and amphoteric polyelectrolyte with prevalent cationic character suitable for biomedical applications. The application of NOCM-chitosan in adsorption of Congo red (Wang & Wang, 2008) and superabsorbent polymer (Yu, Yun-fei, Hui-min, & Jian-xin, 2009) has also been reported. NOCM-chitosan can be prepared by using chitosan, sodium hydroxide, isopropanol and chloroacetic acid (Chen et al., 2003).

2.1.4. Preparation of N,N-dicarboxymethyl chitosan

While preparing NNDCM-chitosan, the concentration of chitosan, water, glacial acetic acid, glyoxylic acid, and sodium borohydride are very crucial parameters. To a fixed concentration of chitosan (30 g) suspended in demineralized water (3 l), 27 g of glacial acetic acid is added and stirred for 20 min. Then glyoxylic acid is added (178 ml 50% (v/v) corresponding to 119 g of glyoxylic acid) and the molar ratio of amine/glyoxylic acid is set to 1:9 at pH 2–3. Finally, with the help of peristaltic pump (1.2 ml min^{-1}), sodium borohydride (90 g) in water (2.5 l) is delivered as a 3.6% solution to the reaction vessel. The NNDCM-chitosan thus prepared has a good film forming ability, good capacity to chelate metal ions and possesses excellent osteoinductive properties with calcium phosphate (Muzzarelli et al., 1998).

2.1.5. Characterization of various carboxymethyl chitosans

Recently, a novel method for simultaneously determining DD, DS, and the distribution factor of —COONa or —COOH in CM-chitosan has been developed by potentiometric titration (Kong, 2012). Table 1 shows the characterization of NOCM-chitosan and OCM-chitosan by various spectroscopy techniques.

2.2. Physicochemical properties of carboxymethyl chitosan

2.2.1. Solubility and moisture retention properties

Carboxymethylation is the preferred modification aimed at increasing the solubility of chitosan in water while imparting novel functionalities. Apart from establishing relationship between water solubility of OCM-chitosan, pH, temperature and fraction of carboxymethylation, the relationship between the moisture absorption and moisture retention by CM-chitosan with its DD and DS has been experimentally demonstrated (Chen et al., 2003). In this context, the possible solubility and aggregation mechanism of OCM-chitosan in water is worth mentioning. The researchers explained that while H-bonding between water and polymer and presence of COO^- group on OCM-chitosan chains are the driving

Table 1
Characterization of carboxymethyl chitosan.

Characterization technique	Comment	Ref.
FTIR spectroscopy	**NOCM-chitosan** (i) Intrinsic peaks of —COOH group at 1741– 1737 cm^{-1} (ii) The bands at 1597–1650 cm^{-1} (carboxy group which overlaps with N—H bend) and 1414–1401 cm^{-1} (—CH_2COOH group) are intense indicating carboxymethylation on both the amino and hydroxyl groups of chitosan	De Britto, Frederico, and Garrido Assis (2011)
	OCM-chitosan (i) The peaks at the 1154–1029 cm^{-1} (C—O stretch) also increase (ii) 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 (iii) In the H form of OCM-chitosan, the characteristic peaks are 1741 cm^{-1} (—COOH), 1154–1029 cm^{-1} (—C—O—), and 1624 and 1506 cm^{-1} (NH_3^+)	Chen and Park (2003)
Differential scanning calorimetry (DSC)	(i) Lower decomposition temperature for NOCM-chitosan (283.3 °C) than O-CM-chitosan (302 °C) (ii) An extra thermal event (second thermal degradation peak) for N-CM-chitosan at 600 °C, with an additional weight loss (iii) Higher stability of NCM-chitosan	Kittur, Harish Prashanth, Udaya Sankar, and Tharanathan (2002) Miranda et al. (2006)
Gel permeation chromatography	Miranda et al. utilized 0.1 mol/L sodium nitrite containing 200 ppm of sodium azide as an eluent at a flux of 0.6 mL/min. The degree of polydispersion was 3.4, indicating a high degree of dispersion of the sample, and the average molecular mass (Mw) obtained was 1.45×106 g/mol for NCM-chitosan of 18.5% DS	Miranda et al. (2006)
Capillary zone electrophoresis	Experimental study for optimization demonstrated that when 50 mM sodium phosphate at pH 2.0 and untreated fused silica capillary are used, high-molecular weight chitosan and CM-chitosan were baseline separated with ultraviolet detector with satisfactory repeatability and excellent linear responses	Fu, Huang, Zhai, and Li (2007)
Titrimetry	The DS of CM-chitosan was determined by using potentiometric titration	Ge and Luo (2005)
^{1}H NMR spectroscopy	(i)The substitution of carboxymethyl groups on O-6, O-3 and N-2 can be determined from the ^{1}H NMR spectrum using the method of Hjerde et al. (ii) The resonance signal of NCM-chitosan on ^{1}H NMR spectrum has been also described by Muzzarelli et al.	Hjerde, Varum, Grasdalen, Tokura, and Smidsrod (1997) Muzzarelli, Ilari, and Petrarulo (1994)
^{13}C NMR spectroscopy	(i) The ^{13}C NMR spectrums at 500 MHz are reported for CM-chitosan in D_2O (ii) The signals for CH_2COOH substituted on-OH and —NH is 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	Chen and Park (2003) Rinaudo, Dung, Gey, and Milas (1992)

forces found for the solubility of OCM-chitosan in water, the intermolecular hydrogen bonding of OCM-chitosan and the electrostatic repulsion between them are responsible forces for aggregation of OCM-chitosan in water (Zhu et al., 2005). A similar study of the effects of acid, pH and ionic concentrations on aggregation behaviors of deoxycholate chitosan and CM-chitosan in aqueous systems has been carried out (Pang, Chen, Park, Cha, & Kennedy, 2007). The researchers found that both the modification of hydrophobic groups and hydrophilic groups affected the rheology properties of the polymers while ionic strength was found to have no influence on CM-chitosan as well as the parent chitosan in respect of the self-aggregation. In addition to this, polyampholytic (zwitter ionic) character of CM-chitosan in DS range of 87–90%, it forms clear gels or solutions depending on the polymer concentration at neutral and alkaline pH values was reported (Thanou, Verhoef, & Junginger, 2001). Among many derivatives of chitosan, CM-chitosan appears to be more suitable for preparing hyaluronan-like substances. Hyaluronan is known for its excellent moisture retention properties and is an important functional ingredient in cosmetics; the moisture retention properties of CM-chitosan have led to its industrial production as a moisturizing agent for skin care is considerable attention.

2.2.2. Chelating and sorption properties

The presence of large number of hydroxyl groups and primary amino groups in the chitosan and ability of chitosan to adopt the suitable configuration for complexation with metal ions due to flexible structure of the polymer chain is the main factors responsible for excellent adsorption properties of chitosan (Wang, Du, & Liu, 2004). Two hypotheses have been proposed for the interpretation of uptake mechanisms of various metal ions. The metal ions are bound with several amine groups from the same chain or from different chains, via inter or intramolecular complexation in the bridge model while it is bound to an amine group in a pendant fashion in the pendant model (Nieto, Peniche-Covas, & Del Bosque, 1992). The earliest work which showed chelation of NCM-chitosan with transition metal ions where addition of NCM-chitosan to solutions of NCM-chitosan (0.2–0.5 mM) transition metal ions produced immediate insolubilization of NCM-chitosan-metal ion chelates (Muzzarelli, Tanfani, Emanuelli, & Mariotti, 1982) The adsorption properties of CM-chitosan and cross-linked CM-chitosan resin with Cu(II) as template revealed that the template CM-chitosan resin can selectively adsorb the Cu(II) ions from the mixed solution containing three kinds of metal ions. It was also found that the template CM-chitosan resin has good reusability and adsorption capacities

Table 2
Adsorption of metal ions/compounds adsorbed by carboxymethyl chitosan based adsorption matrix.

Adsorption matrix	Metal ion/compound adsorbed	Ref.
NOCM-chitosan	Cu(II) ions	Sun and Wang (2006b)
Chitosan hydrogel	Congo red dye	Chatterjee, Lee, and Woo (2010)
Poly(L-lactic acid) surface modified by chitosan and its derivatives	Protein	Peng et al. (2009)
NCM-chitosan	Divalent metal ions	Delben and Muzzarelli (1989)
CM-chitosan hydrogels	Fe(III) ion	Wang et al. (2008)
Crosslinked CM-chitosan resin	Zn(II) ion	Sun and Wang (2006a)
CM-chitosan and Cross linked CM-chitosan resin	Cu(II) ions	Sun and Wang (2006c)

for Cu(II) ions after reuse for 10 times (Sun, Du, Fan, Chen, & Yang, 2006). Similarly, the adsorption of Fe(III) ions onto CM-chitosan hydrogels was found to be very fast (ca. 20 min) due to the chelation of Fe(III) ions with amino, hydroxyl and carboxyl groups of CM-chitosan (Wang et al., 2008). Recently, the ternary copper(II) complexes involving NOCM-chitosan and various biologically relevant ligands containing different functional groups, as amino acids and DNA constituents have been investigated (Shoukry & Hosny, 2012). Table 2 gives a brief account of different metal ions and other compounds by CM-chitosan based adsorption matrices.

2.3. Biological properties of carboxymethyl chitosan

2.3.1. Antimicrobial activity

The antibacterial activity of chitosan and different types of CM-chitosan against *Escherichia coli*, was found to increase in the order NOCM-chitosan < chitosan < OCM-chitosan owing to the reduced number of protonated amino groups in NOCM-chitosan. In OCM-chitosan, instead, substitution occurs only at —OH groups so that the number of amino groups does not change: therefore OCM-chitosan has enhanced antibacterial activity (Liu et al., 2001).

Apart from $-NH_2$ content, MW, DD, concentration in solution, and pH of the medium are the main factors that govern the antimicrobial activity of CM-chitosan. A study has also been carried out to establish relationship between the structure and the antimicrobial activity of quaternized CM-chitosan (Sun et al., 2006). It was observed that the DS of CM-chitosan has little effect on its antimicrobial activity while the increase of their DS of quaternization or the decrease of their MW increases the antimicrobial activity of quaternized CM-chitosan. Recently, antibacterial property of chitosan, OCM-chitosan and NOCM-chitosan nanoparticles against *Staphylococcus aureus* has been experimentally demonstrated (Anitha et al., 2009). The results showed that the antibacterial activity of chitosan nanoparticles is less than NOCM-chitosan and OCM-chitosan nanoparticles. The antifungal effect of CM-chitosan was evaluated experimentally against *Candida albicans*, *Candida krusei*, and *Candida glabrata* which were found to be low (Seyfarth, Schliemann, Elsner, & Hipler, 2008). Recently, a study on antibacterial activity of OCM-chitosan sodium salt and spin ability of OCM-chitosan sodium salt/cellulose blends in N-methylmorpholine-N oxide systems has shown promising results (Liu et al., 2012).

2.3.2. Antioxidant activity

CM-chitosan is reported to exhibit enhanced antioxidant activity (Zhao et al., 2011) where antioxidant activity is mainly related to the content of active hydroxyl and amino groups in the polymer chains (Feng et al., 2008). With the decrease of MW, the antioxidant activity of CM-chitosan increases due to the partial loosening of intermolecular and intramolecular hydrogen bonds. Low molecular weight CM-chitosans were prepared via oxidative degradation and their superoxide anion scavenging activity was evaluated. The results showed that CM-chitosan with lower molecular weight had better superoxide anion scavenging activity (Sun et al., 2007). On the other hand, the Schiff bases of CM-chitosan did not show any improvement in the antioxidant activity due to destruction of part of the hydrogen bonds, formation of new hydrogen bonds, and the change of $-NH_2$ group to C=N (Guo et al., 2005). Contrary to this, as a result of the positive charge, quaternized CM-chitosan showed better hydroxyl radicals scavenging activity than that of CM-chitosan where degree of quaternization ranged from 34.3% to 59.5% (Guo, Xing, Liu, Zhong, & Li, 2008).

2.3.3. Apoptosis inhibitory activity

The potential value of chitosan in cancer therapy has been demonstrated by studying its growth inhibitory effect on human bladder tumor cells 5637 examined by WST-1 colorimetric assay and cell counting (Hasegawa, Yagi, Iwakawa, & Hirai, 2001). The DNA fragmentation and elevated caspase-3-like activity observed in chitosan-treated cancer cells also supported the apoptosis inducing ability of chitosan. However, inhibition of IL-1β-induced chondrocyte apoptosis in a dose dependent manner by CM-chitosan, possibly due to the protection of mitochondrial function, the reduction in the levels of nitric oxide and ROS have been reported (Chen, Liu, Du, Peng, & Sun, 2006). Moreover, reduction in the severity of cartilage degradation and reduction in the expression of matrix metalloproteinase-1 by intra articularly administered CM-chitin demonstrated that CM-chitin may be a potential drug for the treatment of osteoarthritis (Hongbin, Jingyuan, Linyun, & Yuming, 2004). Similarly, the effect of intra-articular injection of CM-chitosan on m-RNA expression of matrix metalloproteinases 1 and 3 and tissue inhibitor of metalloproteinase-1 (TIMP-1) in cartilage of osteoarthritis patients has been evaluated (Liu, Qiu, Chen, Peng, & Du, 2005). Such actions of CM-chitosan portend its use in osteoarthritis. The in vitro degradation behaviors of CM-chitin and CM-chitosan have also been experimentally studied to find out the extent of control on its degradation and their potential biomedical applications (Guangyuan et al., 2009). The effect of surface modification of poly(D,L-lactic acid) film by OCM-chitosan on rat osteoblast and that of poly(lactide-co-glycolide) film by OCM-chitosan on chondrocyte have been evaluated (Kayiongcai, Li, Yang, & Li, 2001). Radiographic evidence of bone regeneration by exploiting NNDCM-chitosan for the delivery of bone morphogenetic protein to bones in human patients undergoing apicectomies and avulsions was observed (Mattioli-Belmonte et al., 1999). The efficacy of NOCM-chitosan to reduce post operative adhesion formation has also been demonstrated in rabbit by a cardiac injury model and an abdominal surgery model (Zhou, Liwski, Elson, & Lee, 2008).

2.4. Derivatives and modifications of carboxymethyl chitosan

Recently a number of derivatives whose physicochemical and biological properties are known to be similar to that of CM-chitosan have attracted the attention of researchers due to their potential applications in clinical medicine and other biomedical applications. While carboxyethyl chitosan and carboxybutyl chitosan are homologues of CM-chitosan, carboxymethyl chitin is analogous derivative of CM-chitosan. A brief description of these derivatives is given below.

2.4.1. Carboxyethyl chitosan

The NCE-chitosan derivative can be prepared by using the 3-halopropionic acids in mild alkaline media (pH 8–9) with $NaHCO_3$. The first synthesis of 1-carboxyethyl chitosan from chitin and chitosan by reaction with 2-chloropropionic acid and its activation effect on canine polymorphonuclear cells was reported by Shigemasa et al. (1995). Although the reaction of chitin with 2-chloropropionic acid was known to introduce 1-carboxyethyl group at 3-O and 6-O positions of chitin, not only 3-O and 6-O carboxyethylation but also N-carboxyethylation proceeded due to simultaneous N-deacetylation. Similarly, N-(2-carboxyethyl) chitosans were synthesized by reaction of low MW chitosan with a low DA and 3-halopropionic acids under mild alkaline media (pH 8–9, $NaHCO_3$) at 60 °C (Skorik, Gomes, Vasconcelos, & Yatluk, 2003). A fundamentally new procedure of synthesis of CE-chitosan in a physical gel, ensuring higher DS at lower temperatures and lower consumption of time and chemicals, compared to the existing procedures has been claimed recently (Pestov, Zhuravlev, & Yatluk, 2007).

Out of a number of additional useful characteristics of CE-chitosan, solubility in water in a wide pH range (Skorik et al., 2003), better biodegradability (Sashiwa, Yamamori, Ichinose, Sunamoto, & Aiba, 2003) and excellent antioxidant characteristics, significant antimutagenic activity (Kogan et al., 2004) than chitosan which are most important for biomedical applications. Recently, semi-IPN hydrogels were prepared by UV irradiation of water soluble NCE-chitosan and 2-hydroxyethyl methacrylate aqueous solutions in the presence of D-2959 as photo initiator (Yingshan et al., 2009). The results observed which demonstrated that the thermal stability and equilibrium degree of swelling improved obviously with increase of CE-chitosan content and the cytotoxicity tests showed that the hydrogels had good biocompatibility. The CM-chitin also turned out to be a suitable mediator for the transport of hydrophilic drugs through the skin (Zhou et al., 2009).

2.4.2. Carboxybutyl chitosan

NCB-chitosan is soluble under acidic, neutral and basic conditions. The reaction of chitosan with levulinic acid in the presence of a reducing agent gives three main compounds: N-monocarboxybutyl chitosan, N,N-dicarboxybutyl chitosan or 5-methyl pyrrolidinone chitosan when the conditions permit the penta-atomic ring formation (Rinaudo, Desbrieres, Le Dung, Thuy Binh, & Dong, 2001). The MP-chitosan has been a protagonist in the applications of chitosan in the medical field (Muzzarelli, 1992; Muzzarelli, Ilari, & Tomasetti, 1993). NCB-chitosan obtained from levulinic acid and five crustacean chitosans with degree of N-carboxyalkylation 0.27 were prepared and characterized. NCB-chitosan showed bacteriostatic activity, along with some other favorable properties such as viscosifying action, enhanced film forming ability, the moisturizing effect and the stabilization of emulsions which demonstrated suitability of NCB-chitosan as functional cosmetic ingredients. (Muzzarelli, Weckx, Filippini, & Lough, 1989). The NCB-chitosan derivative has been proposed for application in many fields such as wound dressing (Skorik et al., 2003), tissue expanders and regeneration of cutaneous tissues (Zhao et al., 2008). It is found to be a good film-forming polymer and a good moisturizing agent and to have good bacteriostatic capacity (Gibbs, Tobin, & Guibal, 2003). The inhibitory, bactericidal and candidacidal activity of NCB-chitosan and its suitability as a wound healing biomaterial has been experimentally demonstrated (Muzzarelli et al., 1990).

2.4.3. Carboxymethyl chitin

An experimental study on the influence of processing parameters which included temperature and concentration of alkali, on the production of CM-chitin was made by (Sini, Santhosh, & Mathew, 2005). The results demonstrated that the reaction temperature has a more profound influence on the properties of the product than alkali concentration. Also optimum conditions for the production of CM-chitin (with MW = 4.11×10^6 Da, viscosity = 1926 cP and DA = 45%) were found to be 60% NaOH concentration at 35–40 °C reaction temperature. The effect of DS of insoluble CM-chitin on the calcium uptake has been investigated and it was concluded that CM-chitin may be useful as a platform for in vivo calcification (Wan, Khor, Wong, & Hastings, 1996) .The suitability of CM-chitin as material for biomedicine particularly in wound healing applications (Wongpanit et al., 2005) and as bioactive bone repairing material has also been shown (Kokubo et al., 2004).

CM-chitosan has been modified by introducing alkyl or acyl groups or by modification with the carboxyl group or grafting with different monomers in order to achieve a number of objectives like increased solubility in water and organic solvents, enhanced entrapment efficiency of various drugs and better control of size of modified CM-chitosan nanoparticles. CM-chitosan has been chemically modified by graft copolymerization of N-acryloylglycine, using 2,2 dimethoxy-2-phenyl acetophenone as photoinitiator. The effect of various reaction parameters on the grafting yield was investigated. The prepared graft copolymers showed that they may be tailored and exploited to expand the utilization of these systems in metal ions uptake and treatment of waste water (El-Sherbiny, 2010). Also CM-chitosan has been modified with acylation where linoleic-acid was covalently conjugated to CM-chitosan via 1-ethyl-3-(3-dimethylaminopropyyl)-carbodiimide-mediated reaction. Stable self aggregated chitosan nanoparticles were formed by sonication which showed feasibility as a potential carrier of adriamycin (Liu et al., 2007). Table 3 gives a brief description of different mechanisms of modification of CM-chitosan and the properties generated by the modification.

3. Biomedical and pharmaceutical applications of carboxymethyl chitosan

3.1. Carboxymethyl chitosan in wound healing applications

Healing of wounds aims at restoration of integrity of the injured tissue and prevention of deregulation of homeostasis. The ideal wound dressing material should maintain moisture at the wound interface, allow gaseous exchange, act as barrier against pathogens and remove excess exudates. It should also be non-toxic, non allergenic, and non-adherent and must be removed without causing trauma. The biochemical significance of exogenous chitins and chitosan as wound dressing material has been earlier reported (Muzzarelli, 1993). The improved biocompatibility, increased biodegradability, lack of toxicity, enhanced antimicrobial activity and moisturizing properties of CM-chitosan make it an excellent biomaterial for wound healing.

The capacity of NOCM-chitosan to stimulate extracellular lysozyme activity of fibroblasts and to promote the proliferation of skin fibroblasts has also been reported (Chen et al., 2002). An in vivo study carried out in rabbit model revealed that NOCM-chitosan gel and solution can reduce adhesion formation and re-formation. It was concluded that NOCM-chitosan may act as major biophysical barrier as the fibroblasts were unable to adhere to NOCM-chitosan solution coated surfaces (Zhou, Elson, & Lee, 2004).

The enhanced wound healing with the NOCM-chitosan incorporated collagen matrices containing chondroitin sulfate has been experimentally evaluated (Chen, Wang, Chen, Ho, & Sheu, 2006). The potential application of microwave treated CM-chitin and CM-chitosan based films for wound care have been tested (Wongpanit et al., 2005). The in vitro study of 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride cross linked

Table 3
Modification of carboxymethyl chitosan by different mechanisms.

Mechanism of modification	Type of CM-chitosan modified	Modifying moiety	Comment	Ref.
Modification with acylation	OCM-chitosan	Succinic anhydride	The ceric ammonium citrate of N-succinyl-O,CM-chitosan in water (0.2–0.3 mg/ml) was determined to be much higher than O,CM-chitosan (0.05 mg/ml)	Zhu, Yuan, and Lu (2007)
	OCM-chitosan	Cholesterol	The size of novel self aggregated cholesterol succinyl-O,CM-chitosan could be controlled by DS of cholesterol moiety.	Yinsong, Lingrong, Jian, and Zhang (2007)
	NOCM-chitosan	Hexanoyl anhydride	The encapsulation efficiency of ibuprofen was significantly enhanced with hexanoyl-NOCM-chitosan.	Liu, Chen, Lin, and Liu (2006)
	CM-chitosan	Linoleic acid	The drug loading capacity and efficiency increased with increasing concentration of adriamycin	Liu et al. (2007)
Modification with alkylation	CM-chitosan	3-Chloro-2-hydroxypropyl trimethylammonium chloride (CTA)	Quaternized CM-chitosan has better antimicrobial activity than CM-chitosan and increase in DS of quaternization and decrease in MW increases the antimicrobial activity of quaternized CM-chitosan	Sun et al. (2006)
	CM-chitosan	Glycidyl octadecyl dimethyl ammonium chloride	Octadecyl quaternized CM-chitosan showed a good solubility both in water and organic solvents, which extends its range of applications	Liang, Wang, Tian, et al. (2008)
Modification with grafting		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	Sun, Xu, Liu, Xue, and Xie (2003)
		2-Hydroxyethylmethacrylate using ceric ammonium nitrate as an initiator	The optimum grafting conditions was: OC-chitosan = 2 g; caricammonium nitrate = 0.2 M; and HEMA = 0.384 mol/l; reaction temperature = 40 °C; and reaction time = 4.5 h	Joshi and Sinha (2006)
		Polyacrylamide using carricammonium nitrate as an initiator	The optimum grafting conditions were: NCMC = 2 g, carricammonium nitrate = 0.2 M, and acrylamide = 0.563 mol/L, reaction temperature = 40 °C, and reaction time = 4.5 h	Joshi and Sinha (2007)
		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	Chen, Liu, Tan, and Jiang (2009)
		N-acryloylglycine using 2,2 dimethoxy-2-phenyl acetophenone (PI) as photoinitiator	Optimum grafting conditions were: CM-chitosan = 0.1 g, N-acryloylglycine = 0.4 g, PI = 0.02 g and reaction time = 1 h	El-Sherbiny (2010)

CM-chitosan prepared showed improved biodegradability while the in vivo studies carried out using a 10 mm rat sciatic nerve defect model demonstrated that CM-chitosan nerve guide had better nerve regeneration performance than chitosan tubes (Wang, Lu, Ao, Gong, & Zhang, 2010). In this context an experimental study on the enhancement in the spread of Neuro-2a cells by 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride cross-linked CM-chitosan films which provided good proliferation substratum for Neuro-2a cells in comparison to chitosan films is worth mentioning (Lu et al., 2007). A series of hydrogels prepared from oxidized dextran and NCE-chitosan without any extraneous cross-linking agent when applied to mice full-thickness transcutaneous wound models suggested that it was capable of enhancing wound healing (Weng, Romanov, Rooney, & Chen, 2008).

3.2. Carboxymethyl chitosan in tissue engineering applications

Tissue engineering has emerged as a major area of regenerative medicine replacing conventional replacement therapies which suffer from some of the demerits of using synthetic materials. The common tissue engineering research concepts are based on construction of hybrid materials that are obtained from the incorporation of cells into 3D porous scaffolds. Cell anchorage, proliferation and tissue formation in three dimensions being essential function of scaffold, porosity plays very crucial role and porosity characteristic is application specific. In this regard, derivative of chitosan like CM-chitosan has emerged as an attractive candidate for scaffolding material as they are capable of degradation as new tissue formation takes place while minimizing inflammatory reactions and toxic degradation products. The usage of NNDCM-chitosan as delivery agent for bone morphogenetic protein in the repair of articular cartilage has been reported earlier (Mattioli-Belmonte et al., 1999). On the other hand, successful achievements of the dual functions of bacterial adhesion reduction and cell function promotion by the CM-chitosan-BMP-2 modified titanium substrates has illustrated good potential of CM-chitosan for the enhancement of tissue integration and implant longevity (Shi, Neoh, Kang, Poh, & Wang, 2009). Similarly, the application potential of injectable gels such as CM-chitosan/gelatin/nano-hydroxyapatite in treating irregular small bone defects with minimal clinical invasion as well as for bone cell delivery has been experimentally demonstrated: application of injectable gels in mice revealed that stability of the in situ formed gels depends on the degree of cross linking and CM-chitosan concentration (Mishra et al., 2011). In addition to this, in vivo evidence to suggest the efficacy of NOCM-chitosan gel and solution to satisfy the requirements

of ideal physical barrier by reducing adhesion formation and reformation in the rabbit model system has also been reported earlier (Zhou et al., 2004). Also, the adsorption of nickel, zinc and copper ions onto biological membranes (CM-chitosan-*graft*-D-glucuronic acid) showed their potential use for tissue engineering, environmental and water purification applications (Jayakumar et al., 2009). The good potential of hydoxyapatite-coated CM-chitosan scaffold as osteogenic scaffolds to stimulate bone healing has been experimentally demonstrated (Budiraharjo, Neoh, & Kang, 2012). Recently, radiation synthesis of gelatin/CM-chitosan/β tricalcium phosphate composite scaffold for bone tissue engineering (Zhou, Xu, et al., 2012) and an improved complex gel of modified gellan gum and CM-chitosan as a candidate material for cartilage tissue engineering has been reported (Tang, Sun, Fan, & Zhang, 2012).

3.3. Carboxymethyl chitosan in drug delivery applications

In the recent times, drug delivery has been a very active area, especially for CM-chitosan as a carrier for various active biological compounds (Khan, Vishakante, Bathool, & Kumar, 2012; Liang, Wang, Tian, Luo, & Chang, 2008; Luo, Teng, & Wang, 2012; Wang, Chen, et al., 2010). In fact, preparation of OCM-chitosan microparticles by emulsification cross linking method has been also recently reported (Shang, Du, & Zhang, 2012). In this regards, hydrogel system, drug conjugate, biodegradable release system, and polyelectrolyte complexes are the main carrier systems for delivery of drugs by CM-chitosan for many components that have been employed till date.

Moreover CM-chitosans are used for the delivery of anticancer drugs, antibacterial drugs, antifungal drugs, anti-inflammatory drugs, proteins/peptides, as well as for DNA/gene delivery.

3.3.1. Carboxymethyl chitosan based systems for the delivery of anti-cancer drugs

Nanoparticulate drug delivery systems are suitable for the treatment of various types of cancers. Doxorubicin·HCl, a water-soluble anticancer drug was loaded in calcium carbonate/CM-chitosan ($CaCO_3$/CM-chitosan) hybrid microspheres and nanospheres. The hybrid microspheres and nanospheres with high encapsulation efficiency, and effective sustain in vitro drug release (Wang, Chen, et al., 2010). Methoxy poly(ethylene glycol)-grafted CM-chitosan was synthesized to make nanoparticles with incorporated doxorubicin and tested with doxorubicin-resistant C6 glioma cells. The result showed that the doxorubicin-incorporated nanoparticles entered into tumor cells more promptly than doxorubicin alone, and it was concluded that doxorubicin-incorporated nanoparticles of CM-chitosan-poly(ethylene glycol) are superior for antitumor drug delivery (Jeong et al., 2010). Vincristine was encapsulated with high drug encapsulation efficiency (>90%) in liposomes made of octadecyl quaternized CM-chitosan/cholesterol that can be applied in cancer diagnosis and treatment (Liang, Wang, Luo, et al., 2008).

A new concept of a localized drug delivery has been proposed by researchers who described the aggregates of OCM-chitosan for the delivery of well known anticancer drug camptothecin (Zhu, Jianhong, & Wenhui, 2006). The results demonstrated that OCM-chitosan can help to enhance the solubility of camptothecin in water, and that the release of camptothecin is significantly sustained. Recently, biological safety of CM-chitosan prepared and characterized by FTIR and NMR spectroscopy, in tumor application was investigated both in vitro and in vivo. The in vitro cytotoxicity studies by MTT assay indicated that CM-chitosan was safe both on normal cell L02 and three tumor cell lines: Bel-7402, SGC-7901 and HeLa. The in vivo study suggested that CM-chitosan was safe and slightly inhibited growth of sarcoma 180 and enhanced body immunity (Zheng, Han, Yang, & Liu, 2011). The OCM-chitosan nanoparticles loaded with curcumin were found to be toxic to the cancer cells and non-toxic to the healthy cells experimentally. Moreover, the nanoformulation of OCM-chitosan enhances the solubility of curcumin in water while curcumin as such is insoluble (Anitha et al., 2011).

The formation of linoleic acid modified CM-chitosan and evaluation of effect of linoleic acid DS on loading capacity, adriamycin loading efficiency and in vitro release profile of linoleic acid modified CM-chitosan nanoparticles has been carried out. The anticancer activity of adriamycin loaded linoleic acid modified CM-chitosan nanoparticles determined experimentally against HeLa cells was found to be comparable to the activity of free adriamycin (Tan & Liu, 2009). Folate modified CM-chitosan coordinated to manganese doped zinc sulfide quantum dot nanoparticles and encapsulated 5-fluorouracil has been developed. The absence of toxicity of the nanoparticles were studied using L929 cells and imaging, specific targeting, and cytotoxicity of the drug loaded nanoparticles were evaluated by breast cancer cell line MCF-7 (Mathew et al., 2010). Also, a novel folate conjugated CM-chitosan ferroferric oxide doped cadmium telluride quantum dot nanoparticles possess a high drug loading efficiency, low cytotoxicity and favorable cell compatibility, and are promising candidates for targeted drug delivery (Shen, Tang, Zhang, Chen, & Zhang, 2012).

Folate modified CM-chitosan hydrogel nanoparticles were prepared the sonication, and doxorubicin was encapsulated to evaluate the loading capacity, loading efficiency and in vitro release profile which was found to be sustained. Also the experimental data suggested that due to the folate-receptor-mediated endocytosis, cellular uptake of folate-modified CM-chitosan nanoparticles was found to be higher than that of nanoparticles based on linoleic acid modified CM-chitosan thereby providing higher cytotoxicity against HeLa cells (Tan & Liu, 2011). The potential of Fe_3O_4/chitosan and Fe_3O_4/OCM-chitosan nanoparticles as good thermo seeds for localized hyperthermia treatment of cancer has been currently demonstrated (Mai et al., 2012).

3.3.2. Carboxymethyl chitosan based systems for the delivery of anti-inflammatory drugs

The encapsulation and release study of ketoprofen, a model anti-inflammatory hydrophobic drug from modified CM-chitosan containing phosphatidylethanolamine (PEA) was investigated. The results suggested that the amount of the drug release was much higher in acidic solution than in alkaline solution due to the swelling properties of the matrix at acidic pH and hence the ampiphillic matrix had much potential for controlled drug delivery of hydrophobic drugs (Prabaharan, Reis, & Mano, 2007). A comparative study of release of Ibuprofen in simulated gastric and intestinal media was carried out between OCM-chitosan/β-cyclodextrin nanocomposites and chitosan/β-cyclodextrin nanocomposites. The results indicated that the release rate of ibuprofen from OCM-chitosan/β-cyclodextrin nanocomposites was slower than that of chitosan/β-cyclodextrin nanocarrier in simulated gastric medium, but the converse was true with simulated intestinal medium (Ji et al., 2011). Recently a new bioadhesive and gastroprotective hybrid particle composed of NCM-chitosan and poly acrylic acid has been developed: it was experimentally demonstrated that the hybrid particle prepared had potential application for delivery to stomach especially in treatment of ulcers and in the prevention of ethanol induced ulcers (Agostini-Junior, Petkowicz, Couto, de Andrade, & Freitas, 2011). The release of sulfasalazine, an anti-inflammatory drug used in the treatment of inflammatory bowel disease, was carried out from chitosan coated alginate–NOCM-chitosan beads which revealed a release of approximately 40% of encapsulated drug in simulated gastric and small intestine tract fluid (Tavakol, Farahani, Farahani, & Najafabadi, 2009). The study established the potential use of chitosan coated alginate–NOCM-chitosan hydrogel as polymeric

carrier for colon specific delivery of sulfasalazine. The non-steroidal anti-inflammatory drug indomethacin has been prepared in magnetic N-benzyl-OCM-chitosan nanoparticles (Debrassi et al., 2011).

3.3.3. Carboxymethyl chitosan based systems for anti-bacterial and anti-fungal activity

A novel delivery system of gatifloxacin, a fourth-generation fluoroquinolone, from OCM-chitosan was prepared and characterized. The in vitro release study of gatifloxacin from OCM-chitosan formulation was found to be slower than that from gatifloxacin in phosphate-buffered saline solution while the bacteria antiproliferative activity assay revealed that MIC of OCM-chitosan formulation against Gram-negative bacteria is fourfold lower than the system without OCM-chitosan (Zhu, Jin, et al., 2007). In this context the effect of chitosan and of NCM-chitosan on intraocular penetration of ofloxacin experimentally investigated (DiColo, Zambito, Burgalassi, Nardini, & Saettone, 2004) is worth mentioning. Table 4 shows various CM-chitosan based compositions with antibacterial and antifungal applications.

3.3.4. Carboxymethyl chitosan based systems for the delivery of proteins/peptides and Vaccines

A novel pH-sensitive hydrogel system composed of NOCM-chitosan and alginate cross-linked with genipin was developed: the release of the model protein bovine serum albumin was investigated in simulated gastric and intestinal media (Chen, Tian, & Du, 2004; Chen, Wu, et al., 2004). At pH 1.2 the amount of BSA released was small, while at pH 7.4 the amount of BSA released was large. Similarly, the release studies of BSA from pH-responsive hydrogel microspheres for oral delivery of proteins have been investigated (El-Sherbiny, 2010). The functionalization of nanodiamond particles with NOCM-chitosan for protein drug delivery applications has been recently demonstrated (Wang, Yang, et al., 2010). A study on the preparation of physically cross linked alginate–NOCM-chitosan microencapsulated beads was carried out (Lin, Liang, Chung, Chen, & Sung, 2005). In the last few years, development of nanocarrier-based vaccines have received much attention in order to provide effective immunization through better targeting and by triggering antibody response at the cellular level. Nanoparticle based vaccine delivery systems are known to improve the effectiveness of vaccines in order to provide optimal immunization. Due to poor immune response in the case of mucosal application, nanoparticulate systems have been developed in order to promote the immune response. The mono-NCM-chitosan and trimethyl chitosan based nanoparticles were synthesized and characterized by ionic gelation: they were used to investigate the higher loading efficiency of tetanus toxoid. Also the in vivo studies were carried out in Balb/c mice. The mouse Balb/c monocyte macrophages that were employed for cellular uptake studies indicated that Fluorescein isothiocyanate-BSA loaded nanoparticles were effectively taken up by them (Sayın et al., 2008). Also the higher cellular viability, higher loading efficacy and nasal immunization of mice with tetanus toxoid loaded complex nanoparticles developed showed that the formulation has potential for mucosal administration of vaccines. The results demonstrated that enhanced immune responses were obtained with intranasal application of nanoparticle formulations and hence the nanoparticulate system developed showed promising potential for mucosal immunization (Sayın et al., 2009). Recently, stimuli-responsive CM-chitosan/poly (γ-glutamic acid) cross-linked with genipin was prepared and BSA was loaded onto it to demonstrate its potential in protein delivery (Yu et al., 2012).

3.4. Carboxymethyl chitosan in targeted drug delivery

Targeted drug delivery is a technique of delivering pharmaceutical compound to a patient in a manner that increases the concentration of the pharmaceutical compound in some parts of the body relative to others in order to prolong, localize, target and have a protected drug interaction with the diseased tissue. In the recent years, CM-chitosan has emerged as an attractive polymer derivative for site specific drug delivery with number of advantages like pH sensitivity, bioadhesive ability, solubility and absorbability, controllable biodegradability, nontoxicity of the degradation end products, sustained release potential and ease of administration (Chen, Tian, et al., 2004; Chen, Wu, et al., 2004).

The CM-chitosan hydrogels which showed excellent pH-sensitivity indicated higher pH-dependent swelling and drug release at pH 6.8 and 7.4. The results suggested that these hydrogels can be used as promising carriers for the administration of colon specific drug delivery of ornidazole (Vaghani, Patel, & Satish, 2012). The spraying co-precipitation method is also disclosed for preparation of Fe_3O_4 nanoparticles functionalized with CM-chitosan (Zhang et al., 2008) while well-dispersed suspension of superparamagnetic Fe_3O_4 nanoparticles stabilized by chitosan and CM-chitosan have also been prepared by which had the potential in the targeted drug delivery application (Zhu, Yuan, & Liao, 2008). Recently the liver targeting and controlled release nanoparticles based on novel thiolated lactosaminated CM-chitosan nanoparticles were prepared and glycyrrhizic acid, a model drug, was encapsulated in these nanoparticles prepared by ionic gelification method. In order to evaluate its intracellular delivery potential, its in vitro drug release study was carried out in the presence of glutathione while the in vivo studies revealed that the cross-linked nanoparticles modify the tissue distribution profile of the glycyrrhizic acid solution, the kidney excretion rate is reduced and the drug accumulation in the liver is increased. The data of study suggested that the cross-linked nanoparticles have potential of drug delivery system with hepatic targeting and controlled release properties (Zheng, Zhang, et al., 2011). Recently, octreotide-modified N-octyl-NOCM-chitosan micelles have been synthesized and emerged as promising intracellular targeting carrier for efficient delivery of antitumor drugs (Zou et al., 2012).

3.5. Carboxymethyl chitosan in gene therapy

The reduction in the thermotropic enthalpy of dipalmitoyl-sn-glycero-3-phosphocholine bi-layer which is a major cell membrane constituent and a standard experimental membrane model, during the gel to liquid crystalline transition and induction of fusion of small dipalmitoyl-sn-glycero-3-phosphocholine vesicles to form large lamellar structures has already been reported earlier (Chan, Mao, & Leong, 2001; Fang, Chan, Hai, & Kam, 2001). Many nucleic acid delivery vehicles have been investigated due to the low transfection efficiency of naked nucleic acid injection in vitro and in vivo. The interaction between OCM-chitosan and dipalmitoyl-sn-glycero-3-phosphocholine bi-layer has been investigated where CM-chitosan emerged as strong polymeric biomembrane perturbant in comparison with chitosan, not only in neutral but also in acidic and basic conditions. The results indicated that electrostatic interactions are the main driving forces in acidic and basic conditions while hydrophobic interactions are the dominating forces in case of neutral conditions and hence OCM-chitosan have good potential for increasing the effectiveness of OCM-chitosan for gene therapy (Zhu, Fang, Chan-Park, & Chan, 2009). With the aim of improving the transfection efficiency of, OCM-chitosan–organosilica hybrid nanoparticles were synthesized through a rapid one-step aqueous synthetic approach for gene delivery (Zhang et al., 2009). The hybrid nanoparticles prepared

Table 4
Carboxymethyl chitosan based compositions for antibacterial and antifungal applications.

CMCS based composition	Target bacteria/fungus	Ref.
Hydroxyl benzene sulfonailides derivatives of chitosan, chitosan sulfate and CM-chitosan	*E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Sarcina*	Zhong, Li, Xing, and Liu (2009)
ZnO/CM-chitosan binano composite	*Staphylococcus aureus*, *E. coli*	Shafei and El Okeil (2011)
Quaternized CM-chitosan	*E. coli*, *Staphylococcus aureus*	Sun et al. (2006)
Poly(N-vinyl imidazole) grafted CM-chitosan	*Staphylococcus aureus*, *E. coli*	Sabaa, Mohamed, Mohamed, Khalil, and Abd El Latif (2010)
Silver–NCM-chitosan nanocomposite	*E. coli ATCC 25922*, *Pseudomonas aeruginosa VM201*, *Staphylococcus aureus ATCC 1128*	An et al. (2010)
Chitosan, OCM-chitosan and NOCM-chitosan nanoparticles	*Staphylococcus aureus ATCC 25923*	Anitha et al. (2009)
O,N,CM-chitosan complex	*Staphylococcus aureus*, *E. coli*	Patale and Patravale (2011)
High and low MW chitosan-HCl, CM-chitosan, chitosan oligosaccharide and *N*-acetyl-D-glucosamine	*Candida albicans, Candida krusei*, *Candida glabrata*	Seyfarth et al. (2008)
Chitosan-EDTA	*Enterococcus faecalis ATCC29212*, *Staphylococcus aureus29213. E. coli 25922*, *Candida albicans ATCC 10231*, *Pseudomonas aureginosa ATCC 27853*	El-Sharif and Hussain, 2011 (2011)
Five chemically modified chitosans (i) 5-methylpyrrolidinone chitosan (ii) N-carboxymethyl chitosan (iii) N-dicarboxymethyl chitosan (iv) N-dimethylaminopropyl chitosan (v) N-phosphonomethyl chitosan	5-Methylpyrrolidinone chitosan, N-carboxymethyl chitosan and N-phosphonomethyl chitosan exerted effective fungistatic action against *Saprolegina parasitica*	Muzzarelli, Muzzarelli, Tarsi, Miliani, and Cartolari (2001)
Schiff bases of CM-chitosan	*Fusarium oxysporium f.* sp. *vasinfectum*, *Alternaria solani* and *Valsa mali*	Guo et al. (2006)

also indicated positive results by showing low toxicity in MTT assay and the nanoparticles were capable of protecting DNA from DNAase I and serum degradation. Recently, multifunctional vectors of OCM-chitosan-cationic liposome-coated DNA/protamine/DNA complexes have been constructed. The in vitro and in vivo transfection results demonstrated that the vector had pH sensitivity and the outermost layer of CM-chitosan fell off in the tumor tissue. This could not only protect the vector from serum interaction but also enhance gene transfection efficiency (Li, Liu, et al., 2012).

3.6. Carboxymethyl chitosan based hydrogels

Crosslinks in hydrogels can be created either chemically or physically. Physically crosslinked calcium–alginate–NOCM-chitosan beads have been prepared by Lin and co-workers for oral delivery of protein drugs to different regions of the intestinal tract. The results revealed that the effective cross-linking density of test beads increased significantly and a higher quantity (up to 77%) of drug was entrapped in the polymer chains as the total concentration of alginate–NOCM-chitosan increased (Lin et al., 2005). On the other hand, novel polyampholyte hydrogels based on CM-chitosan of various DD and DS were prepared by cross-linking with glutaraldehyde. The study revealed that the pH sensitivity can be tailored by varying the molecular structure parameters such as DD and DS (Chen, Tian, et al., 2004; Chen, Wu, et al., 2004). Similarly, chemically cross-linked thermoresponsive and biocompatible core–shell microgels based on N-isopropylacrylamide and CM-chitosan (Chen et al., 2010), and alginate–CM-chitosan (Abreu, Bianchini, Kist, Madalena, & Forte, 2009) have also been developed. A cross-linked polymer network when allowed to swell in an aqueous solution of monomers, these monomers polymerize to form a physically entangled polymer mesh which is referred to as Interpenetrating Network (IPN). Chen and co-workers fabricated novel type of IPN hydrogel membrane based on poly (N-isopropylacrylamide)/CM chitosan and evaluated the effects of the feed ratio of components, swelling medium and irradiation dose on the swelling and de-swelling behavior of the hydrogel. It was also found that the parameters of pH and temperature can be coupled to control the responsive behavior of the hydrogels (Chen et al., 2007). Similarly, the preparation and characterization of super porous hydrogels containing poly(acrylic acid-co acrylamide)/OCM-chitosan where OCM-chitosan is cross-linked with glutaraldehyde which showed improved mechanical properties (Yin et al., 2007). Semi-IPN involves one cross-linked polymer network with another polymer in the linear states. Guo and co-workers fabricated thermo- and pH-responsive semi-IPN polyampholyte hydrogels by using CM-chitosan and poly (N-isopropylacrylamide) with N,N′-methylenebisacrylamide as the cross-linking agent. The results showed that these semi-IPN hydrogels seems to be of great promise in pH-temperature oral drug delivery systems (Guo & Gao, 2007). Similarly, Yang and co-workers fabricated gelatin/CM chitosan hybrid hydrogels which due to high water absorption capacity, a similar compressive modulus with soft tissue, controllable biodegradation, and excellent biocompatibility have the potential for skin scaffolds and wound healing materials (Yang et al., 2010). Recently, in vitro and in vivo cytotoxicity and biocompatibility evaluation of covalently crosslinked NOCM-chitosan and oxidized alginate hydogels have revealed their suitability as a promising drug delivery carrier (Li, Kong, et al., 2012). Although the usage of chemical reagents is effective in yielding a high degree of cross-linking, their full exploitation is restricted due to the cytotoxicity of chemical residues. Therefore radiation cross-linking is a green method to develop hydrogels as the entire sample preparation procedure is prepared in water system. Hence it has been proved to be a safe, clean and effective method for hydrogel synthesis. In this regard, the CM-chitosan based hydrogels synthesized by γ-ray induced cross-linking at paste like state by Wang and co-workers (Wang et al., 2008) is worth mentioning. Similarly, blend hydrogels were prepared by γ-irradiation of highly concentrated carboxymethyl cellulose/CM-chitosan aqueous solution by Hiroki and co-workers (Hiroki, Tran, Nagasawa, Yagi, & Tamada, 2009). Recently, radiation synthesis of nanosilver/gelatin/CM-chitosan hydrogels and their potential as antibacterial wound dressing material has been demonstrated (Zhou, Xu, et al., 2012).

3.7. Biosensors based on carboxymethyl chitosan

A biosensor is a device that measures the presence or concentration of biological molecules, by translating a biochemical interaction at the sensor surface into a quantifiable physical response. CM-chitosan has attracted a great deal of attention over

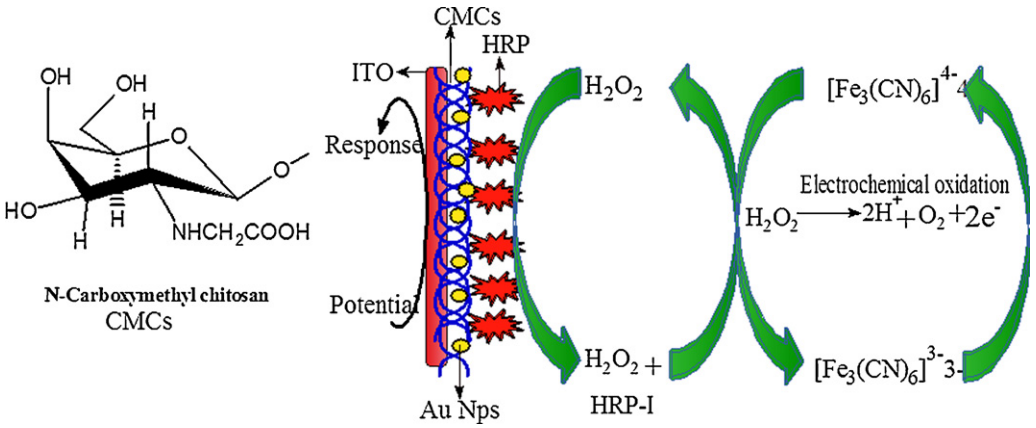

Fig. 2. The schemes of the structure of carboxymethyl chitosan and the electrostatic interaction of CMCs with gold nanoparticles nanocomposite and horse radish peroxidase (HRP) and the electron transfer between the electrode and HRP (Xu, Mao, Liu, Zhu, & Sheng, 2006).

the past two decades in the field of biosensors due to their inherent desirable properties which are suitable for biosensors construction. Due to good film forming capability and excellent biocompatibility of OCM-chitosan as compared to chitosan, it was combined with silica sol–gel method for the immobilization of horse radish peroxidase (HRP) enzyme to construct a H_2O_2 biosensor (Xu, Mao, Liu, Zhu, & Shen, 2006). Similarly, CM-chitosan/gold nanocomposite were prepared and HRP enzyme was immobilized on silica sol–gel matrix containing the nanocomposite, for construction of a novel H_2O_2 biosensor (Xu, Mao, Liu, Zhu, & Sheng, 2006) which is shown in (Fig. 2). Another research on the synthesis of room temperature ionic liquids from 1-ethyl-3-methylimidazolium hydroxide and different MW CM-chitosans by acid–base neutralization reaction, which have potential application in biosensors, was carried out by Huang and co-workers. It was found that ionic liquids composed of CM-chitosan with low MW have good ionic conductivities and thermal stability (Huang et al., 2008). Apart from this, recently, highly sensitive, reversible and reproducible biosensor based on amphoteric 6-CM-chitosan was integrated into the glass conical nanopore channel by a novel covalent modification method with the purpose of controlling ionic transport through nanometer scale responsive to a broad range of pH stimuli (Zhang, Cao, Zheng, & Li, 2010).

3.8. Carboxymethyl chitosan in bioimaging applications

Bioimaging is the technique of visualizing biological processes often on 3D structure in real time non-invasively. Apart from other sources, it utilizes light, electrons, fluorescence, ultrasound, X-rays, magnetic resonance and positrons as a source of imaging. The construction of CdSe/ZnS-labeled OCM-chitosan bioprobes which were highly biocompatible, photostable, suitable for live cell imaging, and useful in the study of polysaccharide function by a simple and convenient method was reported by Xie and co-workers (Xie et al., 2005). An efficient approach of cell labeling using CM-chitosan modified superparamagnetic iron oxide nanoparticles (CM-chitosan-SPIONS) as contrast agent in Magnetic Resonance Imaging (MRI) has also been reported (Shi, Neoh, Kang, &, Shuter, 2009). The free amine groups of OCM-chitosan allowed covalent attachment of fluorescent dye such as rhodamine isothiocyanate to develop a magneto fluorescent nanoprobe for optical imaging. Folic acid (FA) is a high affinity ligand to folate receptors, that was conjugated onto these magneto-fluorescent nanoparticles using different pendant groups (—NH_2, —COOH, —CHO). The experimental data clearly demonstrated the potential of these nano-conjugates as T2-weighted negative contrast MRI agent were evaluated in folate-over expressed HeLa and normal L929 fibroblast cells (Bhattacharya et al. (2011). Recently, CM-chitosan and N, lauryl-CM-chitosan was labeled with ^{99m}Tc with the aim of using them for targeted delivery to some organs in vivo for nuclear imaging and to follow their bio distribution within the body (Dalia, Mohamed, Hamed, Osiris, & Maher, 2011).

4. Carboxymethyl chitosan in green chemistry

Green chemistry which encourages the design of products and processes that minimize the use and generation of hazardous substances. Although using chemical cross-linking agents yield a high degree of cross-linking, the chemical residuals are highly cytotoxic, which restrict their potential applications in the biomedical field. In this regard, a radiation cross-linking technique is a green approach to fabricate biomedical products such as hydrogels because the entire sample preparation procedure is performed in a pure water system and the product obtained is additive-free and sterilized. Plenty of CM-chitosan based hydrogels are prepared by gamma irradiation (Hiroki et al., 2009; Yang et al., 2010) and electron beam irradiation (Zhao et al., 2003; Zhao, Xu, Mitomo, & Yoshii, 2006). Ionizing radiations have been used for cross-linking polysaccharides and their derivatives which included CM-chitosan also, at high concentration (more than 10%, paste like state) for fabricating hydrogels. The results suggested that the hydrogels showed good swelling in water, good biodegradability and satisfying antibacterial activity (Yoshii et al., 2003). Recently, antibacterial wound dressing material have been prepared by using nanosilver/gelatin/CM-chitosan hydrogels via radiation induced-reduction and cross-linking at ambient temperature which demonstrates excellent example of application of green chemistry (Zhou, Zhao, et al., 2012). Similarly, this approach has been exploited in the fabrication of biodegradable gelatin/CM-chitosan/β-tricalcium phosphate composite scaffolds used as bone tissue engineering material by radiation-induced cross-linking (Zhou, Xu, et al., 2012). In this context the exploitation of this green chemistry approach in the preparation of noble metal nanoparticles like silver, gold and platinum by various researchers using CM-chitosan as reducing agent is worth mentioning (Huang et al., 2007; Laudenslager, Schiffman, & Schauer (2008), Wang, Zhuang, Deng, & Cheng, 2010).

5. Conclusion

This review summarized the preparation, characterization, physicochemical and biological properties of CM-chitosan along with the recent developments on biomedical and pharmaceutical applications of this chitosan derivative. The various preparation

techniques presented herein for synthesis of different types of CM-chitosans can be helpful in deciding the context of using CM-chitosan in selectively capturing therapeutic agents and control release in a target site as well as for tissue engineering purposes. Carboxymethylation of chitosan increases its solubility in water and other relevant solvents which make it a promising candidate for delivery of numerous drugs that are otherwise poorly soluble, thereby showing their improved dissolution rate and hence improved bioavailability. The enhanced antibacterial property makes this chitosan derivative particularly suitable for use in wound healing management. The capacity of CM-chitosan to generate biomaterials with predictable pore size and biodegradation rate along with its ability to bind with anionic molecules like growth factors and glucosamine glycans makes it favorable for tissue engineering application. Because of the increased moisture retention ability, improved antimicrobial property, antioxidant activity, non-toxicity, biocompatibility and biodegradability, the derivative has been exploited in CM-chitosan based systems like hydrogels, biosensors, bioimaging applications as well as for gene therapy and targeted and controlled release of different therapeutic agents. Recently, the chief contribution of CM-chitosan in green chemistry is its application in the fabrication of hydrogels by radiation crosslinking technique. But all these applications of CM-chitosan are still at laboratory level and additional studies are necessary before we can expect clinical applications and commercialization of CM-chitosan based pharmaceutical and biomedical products. We hope that this review will help promote new innovative types of CM-chitosan based systems for pharmaceutical and biomedical applications in the near future.

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