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Synthesis, characterization and properties of carboxymethyl kappa carrageenan

Lihong Fan[a,*], Libo Wang[a], Song Gao[a], Penghui Wu[a], Mingjia Li[a], Weiguo Xie[b], Shuhua Liu[b], Weiping Wang[c]

[a] College of Chemical Engineering, Wuhan University of Technology, Wuhan 430070, China
[b] The Third Hospital of Wuhan, Wuhan 430000, China
[c] College of Bioengineering, Hubei University of Technology, Wuhan 430068, China

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ABSTRACT

In order to develop a wound healing material possessing anticoagulant activity, antimicrobial activity and moisture absorbability and moisture-retention capacity, carboxymethyl κ-carrageenan (CMKC) was synthesized by the reaction of κ-carrageenan with monochloroacetic acid. The products were characterized by FT-IR, ^{13}C NMR, degree of substitution (DS) and molecular weight. Anticoagulant activity of CMKC was investigated by APTT, TT and PT assays. The results showed that CMKC with a low DS promoted anticoagulant activity in comparison with κ-carrageenan, but as the DS further increased from 0.42 to 1.09, the activity decreased. Antibacterial activity was evaluated and we found that the introduction of carboxymethyl groups conferred antibacterial activity onto κ-carrageenan. Results indicated that CMKC exhibited good antimicrobial properties against *Escherichia coli* and *Staphylococcus aureus*, and the antibacterial activity of CMKC enhanced as the DS increased. CMKC displayed better moisture-absorption and water-retention ability than κ-carrageenan and as the DS increased, these properties of CMKC increased.

1. Introduction

Carrageenans are a family of linear, sulfated galactans, extracted from a certain species of marine red algae (Knutsen, Myladobodski, Larsen, & Usov, 1994). Due to their excellent physical functional properties and biological activities, carrageenans are extensively utilized in the food, cosmetics, textile and pharmaceutical industries (Campo, Kawano, da Silva, & Carvalho, 2009). To extend the use of κ-carrageenan, the industrial and scientific interests in the carboxymethylation of κ-carrageenan have increased significantly in recent years. Products such as metal adsorbent, magnetic nanospheres and encapsulant for oral delivery have been developed from carboxymethyl carrageenan (Aranilla, 2008; Leong et al., 2011; Yagi et al., 2010). However, the properties of carboxymethyl κ-carrageenan, especially the anticoagulant activity, antibacterial activity and moisture absorbability and moisture-retention capacity, to our knowledge, have not been studied.

Some sulfated polysaccharides, such as the sulfates of chitin, chitosan and alginate (Huang, Mendis, & Kim, 2005; Nishimura et al., 1998), which exhibit potent biological activities including anticoagulant, antithrombotic, antiinflammatory and antitumor activities, present advantages as an alternative source for heparin (Alban, Schauerte, & Franz, 2002; Fareed, Hoppensteadt, & Bick, 2000). However, the traditional methods of preparing sulfated polysaccharides have provided problems, e.g., high cost, strict preparation conditions, and toxic waste, owing to the use of strongly hydrolytic sulfating agents, such as chlorosulfonic acid, together with organic solvents such as pyridine (Bajdik et al., 2009; Cirelli & Covian, 1989; Guiseley, 1978; Wolfrom & Juliano, 1960). Kappa carrageenan, as a prominent natural marine sulfated polysaccharide, exhibits potent anticoagulant, antithrombotic and anti-inflammatory activities (Mourão, 2004; Silva et al., 2010; Yamada et al., 1997). Compared to other synthesized substitutes of heparin, synthesis of carboxymethyl κ-carrageenan can avoid the problems of the synthesis of sulfated polysaccharides which we have mentioned above, and also provide great advantages such as technical simplicity, low cost and environmental protection. Moreover, after carboxymethylation, the carboxymethyl κ-carrageenan would contain sulfate and carboxyl groups, as the nearest structural analogues of heparin. However, there are no reports on the anticoagulant activity of carboxymethyl κ-carrageenan.

The usual wound healing materials with antimicrobial effects against infection have been developed by incorporating antimicrobial agents, including iodine, silver ions and antibiotics (Murphy, Lee, & Herndon, 2004). Despite frequent use, there is growing evidence that silver is highly toxic to keratinocytes and fibroblasts,

* Corresponding author. Tel.: +86 27 62773634; fax: +86 27 87859019.
E-mail address: lihongfan2000@hotmail.com (L. Fan).

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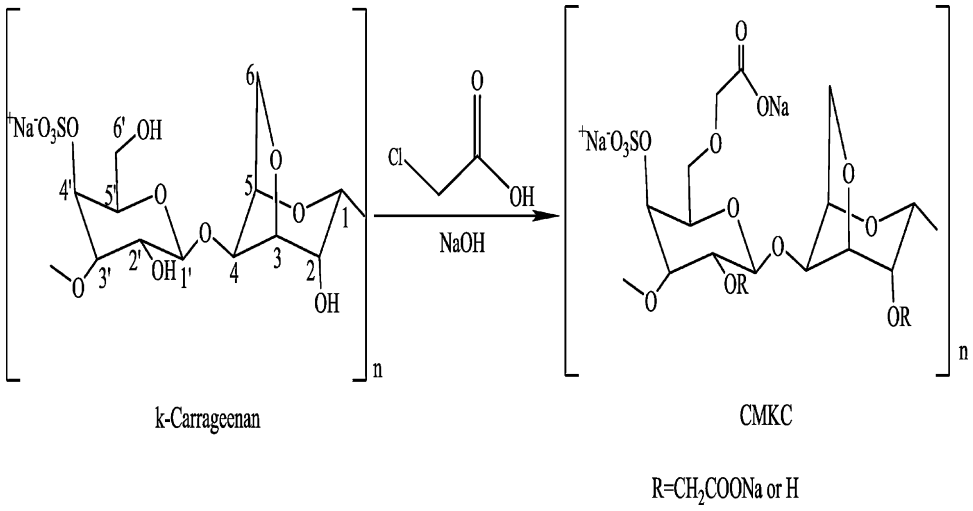

Scheme 1. The synthesis of carboxymethyl κ-carrageenan.

and the use of antibiotics may lead to bacterial resistance (Burd et al., 2007; Poon & Burd, 2004). For these reasons there was strong motivation to find new wound healing materials with inherent antibacterial activity. Heparin, the most sulfated and acidic glycosaminoglycan, has been widely utilized in treating wounds for more than 40 years (Saliba, 1967). However, there are some potential adverse effects with heparin use including thrombocytopenia, allergy and inducing diseases such as bovine spongiform encephalopathy (Michael, 2001). As we have mentioned above, carboxymethyl κ-carrageenan is the nearest structural analogue to heparin. Thus, the carboxymethylation of κ-carrageenan offers possibilities to produce excellent wound healing materials with antibacterial activity.

In addition, some reports reveal that when wounds are retained in a moist but not wet condition, the migration of epithelial cells from the edge of the wound to the wounded area is faster than when wounds are kept in a dry state (Chen, Wang, Chen, Ho, & Sheu, 2006). The aim of modern "moist healing" wound dressings is to create the moist conditions that can facilitate optimum healing (Qin, 2008). Carboxymethyl groups are strongly hydrophilic, and the introduction of carboxymethyl groups into κ-carrageenan is a convenient and effective method to make this polysaccharide have moisture-absorption and moisture-retention abilities.

In this study, carboxymethyl κ-carrageenan was synthesized (Scheme 1), and the products were characterized by FT-IR, ^{13}C NMR, degree of substitution (DS) and molecular weight (M_w). The properties including anticoagulant activity, antibacterial activity and moisture absorbability and moisture-retention capacity of carboxymethyl κ-carrageenan were evaluated, and the influences of carboxymethylation on these properties of κ-carrageenan were investigated.

2. Materials and methods

2.1. Materials

Kappa-carrageenan was purchased from Dehui Ocean Biotechnological Co. Ltd. (Qingdao, China). Monochloroacetic acid, sodium hydroxide and other reagents were kindly supplied by Sinopharm Group Chemical Reagent Corp. And all the reagents were of analytical grade and were used without further purification. Activated partial thromboplastin, prothrombin and thrombin were applied by Shanghai Sun Bio. Corp. Human plasma was bought from Blood Center of Wuhan.

2.2. Carboxymethylation of κ-carrageenan

Kappa-carrageenan (5 g) was suspended in 100 ml 80% EtOH–H_2O solution, and then 10 ml 20% NaOH solution was added dropwise over a period of 15 min. The reaction mixture was kept at 35 °C for 1 h with vigorous stirring. $ClCH_2CO_2H$ was added in NaOH solution, after stirring at room temperature for 30 min, the mixture was added to the reaction mixture which was then heated to 55 °C for 4 h. Next, CH_3COOH was added to the mixture to adjust the pH to 7.0. The carboxymethyl κ-carrageenan salt was obtained through vacuum filtration and washed three times with 80% ethanol solution. The product was dried in oven at 50 °C. By changing the molar ratios of monochloroacetic acid (MCA) to κ-carrageenan, a series of CM-κ-carrageenan with various DSs were prepared.

2.3. FT-IR measurements

IR spectra of samples were performed with a Nicolet 170SX Fourier transform infrared spectrometer. The test specimens were prepared by the KBr-disk method.

2.4. ^{13}C NMR spectra of the carboxymethyl κ-carrageenan

^{13}C NMR spectra were recorded on a Bruker AMX-500 NMR spectrometer at an ambient temperature. The samples were dissolved in D_2O. Tetramethylsilane (TMS) was used as internal standard.

2.5. Preparation of CMKC with different molecular weights

Carboxymethyl κ-carrageenan (CMKC) was degraded by an oxidative method involving hydrogen peroxide (H_2O_2) (Murinov, Romanko, Kuramshina, Kabalnova, & Murinov, 2007). CMKC (10 g) was added to 400 ml distilled water, then heated to 80 °C, and stirred until a homogeneous solution was formed. H_2O_2 solution (30%, w/w) of a desired volume was dropped into the CMKC solution within 30 min. After degradation for 4 h, 3 volumes of ethanol were added to the CMKC solution to cause a precipitate. The precipitate was centrifuged at 3000 rpm for 10 min, collected, and then refined three times by the dissolution-precipitation process. Finally, the precipitate was collected and dried in vacuum at room temperature to obtain CMKC with different molecular weights.