International Journal of Biological Macromolecules 77 (2015) 322–329

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Controlled drug release from cross-linked κ-carrageenan/hyaluronic acid membranes

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ARTICLE INFO

Article history:
Received 15 February 2015
Received in revised form 9 March 2015
Accepted 14 March 2015
Available online 1 April 2015

Keywords:
κ-Carrageenan
Hyaluronic acid
Hydrogels
L-Carnosine
drug release

ABSTRACT

In this work, hydrogel membrane composed of; kappa carrageenan (κC) and hyaluronic acid (HA) crosslinked with epichlorohydrine is produced. The optimum condition has been established based on their water absorption properties. Tensile strength (TS) and elongation (*E*%) for the formed films are evaluated. The obtained films were characterized by FTIR, scanning electron microscopy (SEM) and thermal analysis. All membranes were loaded with L-carnosine as a drug model. The swelling properties and kinetics of the release of the model drug from the crosslinked hydrogel membrane were monitored in buffer medium at 37 °C. The equilibrium swelling of films showed fair dependency on the high presence of HA in the hydrogel. Moreover, the cumulative release profile increased significantly and ranged from 28% to 93%, as HA increases. SEM explored that, the porosity increased by increasing HA content; consequently, drug release into the pores and channels of the membranes is facilitated. In addition, water uptake % increased as well. A slight change in TS occurred by increasing the HA% to κC, while the highest value of strain for κC membrane was 498.38% by using 3% HA. The thermal stability of the κC/HA was higher than that of HA.

1. Introduction

Hydrogels are mostly crosslinked polymers with a three dimensional hydrophilic network that have the ability to swell but not to dissolve in water when it is brought into contact with it [1]. They can absorb large amounts of liquid without dissolving and retain the liquid even under pressure. Hydrogels have been proven effective in facilitating repair of pressure, fungal infections [2], vascular, burn and other wounds [3]. This hydrophilicity is due to the existence of chemical residues such as hydroxylic, carboxylic, amidic, and some other chemicals that might be found within the polymer backbone [4]. Producing hydrophobic hydrogels may be possible using blended or copolymerized hydrophilic and hydrophobic polymers; interpenetrating or semi-interpenetrating polymer networks [4]. Hydrogels can be prepared using natural or synthetic polymers. Many approaches have been reported in order to synthesize biomedical hydrogels [5,6].

Carrageenan is the generic name for a family of high molecular weight sulphated polysaccharides obtained by extraction of definite species of red seaweeds [7]. It is composed of alternating copolymer of α-(1–3)-D-galactose and β-(1–4)-3,6-anhydro-D or L-galactose and classified into three sub groups (κ, λ, and I) based on the number and distribution of sulfated ester pattern on 3,6-anhydro-D or L-galactose residues. Hydrogels formed from carrageenan are suitable for drug delivery systems due to their nontoxicity and most importantly, to the easy gelling, thermo reversibility of the gel network and appropriate viscoelastic properties [8] that enable them to undergo harsh conditions. Kappa-carrageenan (κC) is a linear sulfated polysaccharide with a repeated D-galactose and 3,6-anhydro-D-galactose units [9]. It is believed that κC can form micro particles using *in situ* ionic gelation, and have successfully encapsulated some model drugs such as ibuprofen and verapamil [10]. Therefore, it can be said that they are capable of being used to encapsulate macromolecular drugs in oral drug delivery. Although κC are capable of encapsulating drugs to prevent premature release and/or degradation in

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http://dx.doi.org/10.1016/j.ijbiomac.2015.03.055
0141-8130/

the stomach environment, an efficient drug carrier is necessary, as this will ensure the drug is released into the intestinal region. κC synthesis and characterization based superabsorbent hydrogels such as persulphate-induced graft copolymerization of acrylamide, acrylic acid and methacrylic acid onto κC have been reported [11]. κC had been shown better film forming property with high water barrier and mechanical properties [12,13]. In food industry, κC is widely utilized due to its excellent physical functional properties, such as gelling, thickening, emulsifying and stabilizing abilities [14]. In recent years, it is increasingly used in pharmaceutical formulations [7]. However compared with commonly used pharmaceutical excipients such as hydroxypropyl methylcellulose (HPMC), hydroxyethyl cellulose [15], chitosan (CS) [16–20], carboxymethyl starch [21,22], carbomer and alginate [23–26], the utilization of κC in the discipline of pharmaceutics is not frequently reported. In addition, only limited reviews are available about the evaluations of κC in pharmaceutical industry [27,28].

Hyaluronic acid (HA) is a linear polysaccharide formed by repeating disaccharide units of D-glucuronic acid and *N*-acetyl glucosamine linked by β(1,4) and β(1,3) glycoside bonds [29,30]. In physiological conditions, HA appears in the form of sodium salt (HAs), negatively charged and highly hydrophilic [31]. HA is known to be a major constituent of vertebrate tissue and body fluid, and also play an important role in various biological processes, such as cell adhesion, cell migration, wound repair, cell proliferation, and innate immunity[32]. The architecture of this natural biopolymer exhibits excellent physicochemical properties such as biocompatibility, non-toxicity, nonimmunogenic, non-inflammatory and totally biodegradable features [33]. Also, HA is intensively studied for its unique features, such as high water-binding capacity and interesting viscoelastic behavior [34,35]. HA derivatives are currently used in a various number of biomedical applications: arthritis treatment [36], ophthalmic surgery [37], tissue engineering [38], wound healing [39] and drug delivery [40,41].

In the present work, the study of the swelling properties and kinetics of the release of a model drug from crosslinked κC/HA, kappa carrageenan (κC) and hyaluronic acid (HA) are crosslinked with epichlorohydrine to produce hydrogel membranes has been reported. The optimum conditions for the formation of films have been established based on their water absorption properties. The crosslinking reaction will be confirmed by FTIR analysis. The membranes will be also characterized by scanning electron microscopy (SEM) and thermal analysis. The equilibrium swelling of membranes showed fair dependency on the high presence of HA in the hydrogel. In addition to, the release of the drug increased simultaneously by increasing the HA% in the membrane composition which is considered one of the novel results in this research work relevant to the prior research work in the literature. On the contrary, the κC membranes blended with HA, the cumulative release profile increased significantly and range from 28% to 93%, as HA content increases, due to the hydrogel membranes surface hydrophilicity improved after addition HA in different portions, which are accompanied with swelling and sustained zero-order-release behavior improvement. Finally, a representative membrane sample was loaded with drug L-carnosine and release was monitored kinetically in the buffer medium at 37 °C.

2. Materials and methods

2.1. Materials

Kappa-carrageenan (κC), Epichlorohydrine, L-carnosine (M_W: 226.23) were purchased from Sigma-Aldrich. Hyaluronic acid (HA) was provided from china. Distilled water was used as the solvent to prepare hydrogel samples. All other chemicals were analytical grade and used without any further purification.

2.2. Preparation of κC/HA/epichlorohydrine hydrogel

The solution casting method was used for the preparation of κC/HA based hydrogel membranes. Preliminary experiments were conducted to determine the appropriate concentration of plasticizer (glycerol) for preparing membranes. Results showed that membrane solutions containing 25% (w/w) glycerol (based on carrageenan weight) were easily removed from the plate. Membranes solutions were prepared by dissolving hot κC solution was blended with different concentration of hyaluronic acid (HA) (1, 3, 5, 7 and 10%) to final weight 1% in distilled water under magnetic stirring for 15 min at 75 °C. Following the addition of glycerol at constant concentration (25%, w/w based on final weight). Finally, addition of epichlorohydrine (0.1, 0.2, 0.3 and 0.4%) and stirring was continued for a further 15 min at 75 °C. The final solutions were cast on the center of a rimmed circular area (177 cm^2) of clean and leveled plastic plates, then dried at 30 °C for 24 h. The formulated membranes were dried in an oven at 37 °C for about 24 h.

2.3. Swelling study

Water uptake of the κC–HA membranes are usually defined as the weight percent with respect to the weight of the dried membrane. For measuring the swelling ratio of κC–HA composite membranes, membrane samples were cut into 3.5 cm × 3.5 cm pieces and vacuum-dried for 12 h, at room temperature, the dried sample weight is determined (W_{dry}). The dried samples were soaked in distilled water at room temperature, then weighted (W_{wet}) at specific interval times. The water uptake of κC–HA membranes was given as the following equation [42]:

$$\text{water uptake}\,(\%) = \left[\frac{(W_{wet} - W_{dry})}{W_{dry}}\right] \times 100 \quad (1)$$

2.4. Release profile study

For *in vitro* release studies, κC–HA membranes loaded L-carnosine as a drug model, were cut into (50 mm × 50 mm) into small pieces. These specimens were soaked in 100 ml phosphate-buffered saline (PBS) solution (pH 7.4), and incubated at 37 °C during the entire release time probe. At practical time interval, aliquots of samples (1.5 ml) were taken from the release medium and that quantity substituted with a like fresh release medium. The L-carnosine was detected spectroscopy at 285 nm with the support of its calibration curve of L-carnosine. The maximum released L-carnosine can be estimated according the following equation:

$$\text{release}\,(\%) = \left[\frac{\text{released carnosine}}{\text{total loaded carnosine}}\right] \times 100 \quad (2)$$

3. Characterization of the formed membranes

3.1. Tensile strength and % elongation at break

Both tensile strength and elongation at break were determined using Shidmadzu Universal Testing (model AG-I, Shidmadzu, Japan) according to ASTM ID: D882-12. All tested membrane strips were cute in diameter 1 cm × 6 cm. Membrane strips were fixed between the grips and the cross-head speed was set at 10 mm/min. Membrane thickness was calculated using a manual digital micrometer (Mituto, Tokyo, Japan) to the nearest 0.001 mm. Reported values were average of at least 5 random locations for each membrane sheet. Tensile strength was evaluated by dividing the peak load by the cross-sectional area of the initial membrane specimen. Elongation was determined by percent change in the length of the specimen from the original distance between the grips. The data