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Potential use of curcumin loaded carboxymethylated guar gum grafted gelatin film for biomedical applications

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ABSTRACT

Present study describes the synthesis of carboxylmethyl guar gum (CMGG) from the native guar gum (GG). Further, the prepared CMGG is grafted with gelatin to form CMGG-g-gelatin and then mixed with curcumin to prepare a biomaterial. The resultant biomaterial is subjected to the analysis of ^{1}H NMR, ATR-FTIR, TGA, SEM and XRD ensure the carboxymethylation and grafting. The results reveal that 45% of the amine groups of gelatin have been reacted with the —COOH group of CMGG and 90–95% of curcumin is released from CMGG-g-gelatin after 96 h of incubation in the phosphate buffer at physiological pH. In vitro cell line studies reveal the biocompatibility of the biomaterial and the antimicrobial studies display the growth inhibition against gram +ve and gram −ve organisms at a considerable level. Overall, the study indicates that the incorporation of curcumin into CMGG-g-gelatin can improve the functional property of guar gum as well as gelatin.

1. Introduction

Gelatin is a hydrolyzed product of a native collagen, an abundant structural protein found in the various parts of the animal body, such as skin, tendon, cartilage and bone [1]. Gelatin has been widely used in the pharmaceutical and medical fields as sealants for vascular prostheses [2–4], drug delivery vehicle material [5–7], wound dressing material [8,9], etc. However, at a temperature above 35 °C, the secondary bonding structure of gelatin is completely broken and thereby destroys the physical network and imparts poor thermal and mechanical properties. Therefore, the use of stabilizers to increase the stability of gelatin by cross-linking is a common practice. The modification of biopolymer (collagen, gelatin, etc.) using an exogenous cross-linker has been studied extensively for a number of biomedical applications. Several physical and chemical methods have been reported for cross-linking of collagenous materials. Physical methods include dehydro-thermal treatment and UV irradiation [10,11]; however, they are generally less efficient. Many chemicals such as carbodiimide [12], formaldehyde [13], glutaraldehyde [14,15] and oxidized polysaccharides such as dextran [16,17], chondroitin sulfate [18,19], starch [20] and genipin have been used to modify the gelatin for biomedical applications [21]. Though the stability of the biopolymer is achieved by cross-linking agents, the low mechanical strength restricts its application. In addition, the biocompatibility of the resultant biopolymer is questionable because of the release of toxic components from some of the cross-linkers during its usage.

Thus, in order to obviate the problems associated with mechanical property and biocompatibility, an attempt is made in the present study by grafting the gelatin molecule with a modified natural polysaccharide, carboxy methylated guargum (CMGG). Guar gum is a polygalactomannan derived from the seeds of a leguminacea plant, *Cyamopsis tetragonolobus*, and its molecular structure shows a backbone of β-D-mannopyranoses linked at 1→4 position to which, on the average, every alternate mannose and α-D-galactose is linked to 1→6 position [22]. GG is a non-toxic and biodegradable polymer and it has found various applications such as emulsifier, suspending and bioadhesive agent [23]. It is well known that curcumin is a natural pigment derived from *Curcuma longa* and has well been explored for its antimicrobial [24], antiviral [25], anticancer [26] and wound healing activities [27]. However, curcumin is sparingly soluble in water; this hydrophobic nature of curcumin inhibits its vascular and oral administration. These

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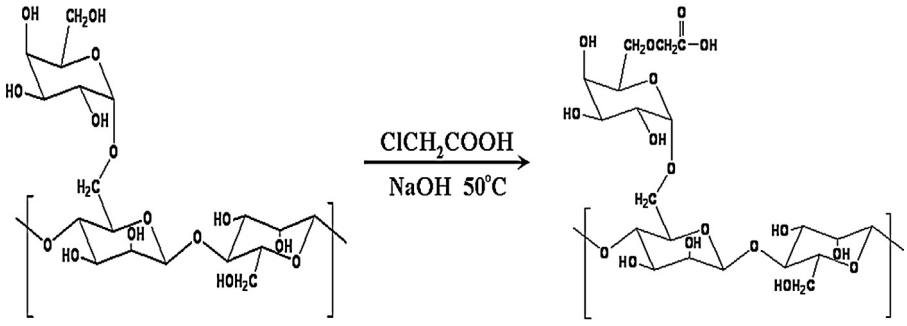

Scheme 1. Carboxymethylation of guar gum.

properties of curcumin can be utilized by incorporating in the film of carboxymethylated guargum grafted gelatin (CMGG-g-gelatin) for a possible application as an antimicrobial patch for wound healing. Wound healing is a complex and multifunctional process that helps in the contraction and closure of the wound and restoration of tissue integrity [28]. Infections at the wound site are cited as one of the reason for delayed wound healing [29]. Currently, the demand for new wound healing materials with inherent antimicrobial properties is on the rise. Guar and xanthan gums were already patented as bioabsorbable materials for wound dressing [30]. Therefore, guar gum has been selected for the present study to protect gelatin from faster degradation by the gelatinase enzyme at the wound environment. Hence, the durability of the CMGG-g-gelatin material will be increased and it can withstand at the wound site for a prolonged time for sustained release of curcumin.

The major objective of the present study is to prepare a polysaccharide (from plant) and protein (from animal) based grafted biomaterial with inherent antimicrobial property as an efficient wound healing material. As far as we know, this is the first kind of report for the preparation of a biomaterial by using a modified plant based material (CMGG) and an animal based material (gelatin), for the delivery of another natural product, curcumin for the application of an effective wound healing material.

2. Experimental details

2.1. Materials

Guargum, chloro acetic acid and gelatin were obtained from Merck (India) and picrylsulfonic acid [2,4,6-trinitrobenzene sulfonic acid (TNBS)], curcumin were obtained from Sigma–Aldrich (USA). 3-[4,5-Dimethylthiazol-2-yl]-2,5-dephenyltetrazolium bromide (MTT), 1-ethyl-3(3-dimethyl aminopropyl) carbodiimide (EDC), N-hydroxy succinamide (NHS) and 2-(N-morpholino) ethanesulfonic acid (MES buffer) were obtained from HiMedia (India). All of the other reagents were of analytical reagent grade and used without further purification.

2.2. GG purification procedure

The commercial guar gum was purified according to the procedure as described in the literature [31] with some modifications. Crude guar gum (10 g) was stirred in 250 ml of cold distilled water for 24 h at room temperature. The clear supernatant was obtained by centrifugation and ethanol was added to the clear supernatant to precipitate out the carbohydrate. The material was washed again with ethanol, followed by distilled water and subsequently freeze-dried.

2.3. Preparation of carboxymethyl guargum (CMGG)

GG was derivatized to sodium carboxymethyl guargum following the method as reported previously [32]. In brief, for carboxymethylation, 6 g of purified guar gum was dispersed in 120 ml of isopropanol and water mixture (8:2, v/v ratio) taken in a 250 ml round bottom flask connected to an oil bath, and fitted out with a magnetic stirrer. Then, about 18 g of NaOH was added and continued the stirring for an hour at 50 °C. An aliquot of 10 ml chloro-acetic acid of specified weight (6 g) was then added to the reaction mixture, over a period of 30 min. The reaction mixture was heated to a specified temperature (50 °C) with continuous stirring for 4 h, to drive the reaction process to completion (Scheme 1). The reaction product was repeatedly extracted with ethanol and separated by centrifugation. After the third extraction, the pH was adjusted to 7 with several drops of glacial acetic acid. Finally, the precipitate was washed with water and 80% ethanol, and subsequently vacuum-dried. The modification of GG to CMGG is shown in Scheme 1.

2.4. Determination of degree of substitution (DS) of CMGG

The degree of substitution of CMGG was estimated using a titrimetric method reported elsewhere [33]. 1.5 g of CMGG was dispersed in 50 ml of 2 M HCl solution using 70% methanol as solvent, and the suspension was stirred continuously for 2 h. During this process, the sodium form of CMGG (Na-CMGG) was converted to its hydrogen form (H-CMGG). The product was then washed with 95% (v/v) ethanol to remove the chlorine and the dispersion was filtered. The filtrate was then dried in a vacuum oven at 60 °C for 2 h. 0.5 g of the dried H-CMGG was dissolved in 50 ml of 0.1 M NaOH solution and stirred for 2 h and the excess of NaOH was back-titrated with 0.1 M HCl solution using phenolphthalein as an indicator. The DS was calculated using the following equation:

$$W_A = \frac{(C_{NaOH}V_{NaOH} - C_{HCl}V_{HCl})}{m}$$

$$DS = \frac{162W_A}{(5900 - 58W_A)}$$

where C_{NaOH} and C_{HCl} are the molar concentration of standard NaOH and HCl solutions, W_A is the mass fraction of $-CH_2COOH$, V_{NaOH} is the volume of NaOH and V_{HCl} is the volume of HCl and m is the weight (g) of polymer taken.