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Curcumin/cellulose micro crystals/chitosan films: Water absorption behavior and in vitro cytotoxicity

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ABSTRACT

A new technique, called vapor induced phase inversion (VIPI), has been employed to fabricate cellulose micro crystals (CMC)-loaded chitosan (Ch) films. The method involves immediate exposure of CMC-dispersed chitosan solution to NH_3 gas. The films were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) analysis. The swelling ratio (SR) of films showed negative dependence on the cellulose content in the films. The dynamic water uptake data were interpreted by various kinetic models. Finally, the release of curcumin from the films was investigated. The CMC-loaded chitosan film showed slower release as compared to the plain chitosan film, suggesting that cellulose micro crystals acted as diffusion barrier. The films were non-cytotoxic, non-thrombogenic and non-hemolytic.

1. Introduction

Wound healing is the process in which damaged epidermal tissues are replaced/regenerated by living issues [1]. A wound dressing consists of a natural or synthetic polymer matrix, and it absorb exudates with simultaneous release of the entrapped bioactive agent at a pre-determined rate. An ideal wound dressing prevents the wound from microbial contamination and enhances the wound healing process. An ideal wound dressing should be able to permeate the gases and keep the wound environment moist. In addition, it should be biocompatible and preferable biodegradable [2]. In the last decade, there has been sincere concern about saving the aquatic environment from synthetic polymers, and therefore, the focus of research has now switched over to using naturally occurring polysaccharides for biomedical applications [3]. In recent past, bio-polymers such as cellulose, chitin, chitosan, sodium alginate, collagen, gelatin etc. have been used frequently in wound dressings and other biomedical applications [4]. The major advantages of natural polymers include their good cytocompatibility, biocompatibility, and above all, their biodegradable nature which eliminates chances of post-operative surgery for removal of implanted polymeric device. Gelatin, chitosan [5], collagen [6] etc. are among popular dressing materials used for quicker wound healing. Chitosan [-(1–4)-2-amino-2-deoxy-D-glucose], a de-acetylated form of chitin is a naturally occurring linear biodegradable polysaccharide and it is made up of N-acetyl-D-glucosamine and D-glucosamine. It is nontoxic, biocompatible [7], biodegradable polymer [8]. It is used in drug delivery, cell delivery systems, orthopedics, wound healing [9], ophthalmology, and bone healing [10]. It has ability to form complexes with DNA and poly anionic polymers [11], and can open intercellular tight junctions and it is totally biocompatible. It exhibits antimicrobial activity against bacteria [12], fungi, and yeast. A chitosan film with controllable water absorption and drug release properties can be obtained using cross linking agents such as glutaraldehyde [13]. However, a number of reports are available which claim its severe toxicity [14]. Therefore, in place of GTA, other cross linkers such as genipin [15], suberoyl chloride [16] epichlorohydrin [17] etc. have also been used, but they are also reported to be toxic for human [18]. With the aim to fabricate un-cross linked chitosan film, but with controllable physical and chemical properties, we have dispersed cellulose micro crystals uniformly into chitosan film using a novel VIPI approach. In this method, CMC dispersed chitosan solution is exposed to NH_3 gas. This causes a sudden change in pH of the dope and chitosan is precipitated as porous film, with homogeneously distributed cellulose micro crystals. These cellulose crystals act as diffusion barrier to control the swelling, moisture permeation and drug release properties of the resulting films. The CMC/Ch

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films have been loaded with natural bioactive Ingredient curcumin, which is a hydrophobic polyphenolic compound derived from the rhizome of the perennial herb Curcuma *longa* [19]. It is reported to possess a number of biological activities like wound healing, antibacterial, antioxidant, anti-inflammatory, anticancer properties etc. [20].

2. Materials and method

2.1. Materials

Cellulose micro crystals (CMC), chitosan (Ch) (degree of deacetylation 98%, molecular weight 1.23×10^5 g/mol), glacial acetic acid and liquid ammonia were obtained from E. Merck, Mumbai, India. Other chemicals were purchased from SRL, Pune, India and were of analytical grade. The distilled water was used throughout the investigations.

2.2. Fabrication of CMC/Ch film by VIPI approach

An in-lab built apparatus, employed in VIPI approach, is shown in Fig. 1.

A 250 mL conical flask, containing liquid NH_3 was fitted with an airtight wooden cork having a hole of diameter 1 cm. A glass tube, bent through right angle at one place was placed in the cork hole and its other end was jointed below the mouth of the glass jar and made airtight. The conical flask was warmed gently at 40 °C. The NH_3 gas passed into the glass jar and created NH_3 environment. In order to prepare CMC/Ch composite film, 0.5 g of Ch was dissolved in 25 mL of 2% (w/v) solution of acetic acid and to this different amount of cellulose micro crystals (CMC), (i.e. 10, 20 and 30 wt% of the chitosan content) were added. The suspension was agitated on a magnetic stirrer (Remi, India) for 30 min to ensure uniform mixing, poured into a Petri plates and immediately placed in the gas jar. The Petri plates were taken out after 5 min and put in an electric oven at 60 °C for complete evaporation of solvent. In all, four films were fabricated, whose designations were Ch/CMC(0), Ch/CMC(10), Ch/CMC(20), and Ch/CMC(30) respectively. The number in parenthesis denotes the wt% of cellulose micro crystals (CMC) relative to the chitosan in the feed mixture.

2.3. Extraction of curcumin (CC) from turmeric

The extraction of curcumin from turmeric, using polar organic solvents such as alcohols, acetone, ethyl acetate, etc. has been reported by a number of workers [21]. However, according to a report by Revathy et al. [22], out of a number of extraction solvents like acetone, chloroform, hexane, methanol, ethyl acetate, the solvent acetone yielded maximum curcumin recovery of almost 22.8%. Therefore, we also decided to extract curcumin from turmeric using acetone following the method proposed by Saraswathi et al. [23]. In brief, 20 g of fine turmeric powder was suspended in 150 mL of acetone under moderate stirring for 72 h at 37 °C. The mixture was filtered, the filtrate was poured into a Petri plate and the solvent was evaporated under vacuum to obtain semi-dry oily mass. The oily mass was weighed accurately (2.99 g) and dissolved in 50 mL of DMSO to give a reddish-brown curcumin solution. The curcumin content in the above extract was calculated using the following expression

$$\begin{aligned}\text{Curcumin content per mL} &= \frac{(\text{amount of oily mass})}{\text{volume of DMSO}} \\ &= \frac{2.99}{50}\text{g/mL} \\ &= 0.0598\text{g/mL}\end{aligned} \quad (1)$$

In order to prepare a film, 3.0 mL of extract was used, which contained 0.18 g of curcumin.

2.4. Preparation of curcumin (CC) loaded samples

In order to prepare CC loaded films, 3 mL of above extract (containing 0.18 g of CC) was added into CMC/Ch dispersion under constant stirring, followed by transfer into Petri plates. The Petri plates were exposed to NH_3 gas for film formation as described above. The samples were designated as $Ch/CMC(0)_{0.18}$ and $Ch/CMC(20)_{0.18}$ where the samples carry their usual meaning and the sub-script is the amount of CC in g loaded in the film.

2.5. Characterization of films

The Fourier Transform Infrared (FTIR) spectra were recorded with an FTIR spectrophotometer (Shimadzu, 8400, Japan) using KBr. The surface morphology of all the films was determined by Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) at Indian Institute of Technology and Design of Materials, Jabalpur (India).

2.6. Swelling studies of films

The swelling behavior of films was studied in the pseudo extracellular fluid (PEF), with the following composition: 1 L of the solution contained 2.2 g of KCl, 6.8 g of NaCl, 25 g of sodium bicarbonate and 3.5 g of sodium di-hydrogen phosphate. The pH of this solution was 7.36. A pre-weighed film sample was placed in 100 mL of PEF at 37 °C and it was taken out at different time intervals, wiped superficially with tissue paper to remove extra surface water, weighed accurately in an electronic balance (Denber, Germany), and then placed again in water. The swelling ratio (SR) was using the following expression:

$$\text{SR} = \frac{(M_t - M_0)}{M_0}\text{g/g} \quad (2)$$

where M_0 and M_t are the initial mass and mass at different time intervals respectively. To determine equilibrium swelling ratio (ESR), M_t was replaced by M_e which is the weight of the swollen film at equilibrium.

2.7. Dynamics of moisture uptake

The kinetics of moisture uptake was performed using the method used elsewhere [24]. Saturated solution of KNO_3 was put in a plastic jar and a rectangular block of Stainless steel, with its top above the level of the solution, was put in the middle of the jar. Now an aluminum crucible was placed on the top of the steel block. A pre-weighed piece of completely dry film was placed on the crucible, lid of the jar was closed tightly and the jar was placed in an incubator, maintained at 37 °C. The film was taken out at regular time intervals, weighed accurately by an electronic balance (Denber, Germany) and then put back in the jar. The mass measurements were continued till the attainment of equilibrium and expressed as g/g dry films. All the experiments were replicated three times and the average values were inserted in the model developed by Singh and Kulshrestha [25]:

$$\frac{1}{(m - m_0)} = \frac{1}{k(m_e - m_0)} + \frac{1}{(m_e - m_0)} \quad (3)$$

where m_0 and m_e are masses of the dry and fully equilibrated film samples respectively; and m is the mass of the hydrated film at different time intervals.