

Summary

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‘Curcumin Loaded Cellulose Whiskers/ Alginate Composite

Films for Wound Dressing Applications’

Submitted by

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In this project, cellulose whiskers were prepared by acid induced hydrolysis of cellulose powder under controlled conditions to yield nano-sized cellulose whiskers. These cellulose whiskers (CW) were loaded into sodium alginate solution, containing a pre-calculated quantity of Curcumin in aqueous medium under mild stirring to ensure complete mixing of all the ingredients. The above solution was poured into Petri plate and the solvent was evaporated to give the Curcumin loaded CW/SA films. These films were prepared with different compositions.

Characterization of films

The Cur-loaded films were characterized by SEM, XRD, TGA and mechanical analysis. The X-ray diffraction (XRD) method was used to measure the crystalline nature of Alg/CW(20) and Cur-loaded Alg/CW(20)₄₅₀ films. These measurements were carried out on a Rikagu Diffractometer (Cu radiation = 0.1546 nm) running at 40 kV and 40 mA. The diffractogram was recorded in the two theta range of 5–60° at the rate of 2° min⁻¹. The surface morphology of the plain and Cur loaded films

was investigated using a JEOL 6400F microscope operated with an accelerating voltage of 2 kV and a working distance of 4.4 mm. The mechanical properties of the films were determined according to the procedure reported elsewhere . Film samples, with the dimensions of 39 mm × 5.8 mm, were equilibrated under the RH of 50% at 23°C for a period of 24 h and their tensile strength (TS) and percent elongation at break (PE) were measured by using an Instron Universal Testing Instrument (Model 1011). The initial grip separation and crosshead speed were set to 40 and 200 mm per min, respectively.

Determination of moisture sorption isotherm

A gravimetric static method was employed to determine moisture absorption isotherms. Saturated solutions of various salts were prepared in polypropylene chambers to create environments with desired relative humidity (RH) . The chambers were kept in an incubator (Sanyo MIR 152) at 30°C. Now, pre-weighed film samples were put in small crucibles of aluminum foils and placed in air-tight polypropylene chambers. The film samples took almost three days to attain the equilibrium. The equilibrium moisture content (EMC) of the film samples was determined using the vacuum oven method and expressed as g/g dry solids. All the moisture absorption experiments were carried out in triplicate and the average values were used to plot the isotherms.

Blood compatibility

A representative sample Ch/CMC(20)450 was used to determine the total protein adsorption using the procedure given in a report from the International Standard Organization (ISO).

Protein and albumin adsorption study

A piece of the film sample Alg/CW(20)₄₅₀, with surface area of 1 cm², was incubated in 0.9% saline solution at 37°C for a period of 24 h, followed by its transfer into 10 ml of pure frozen plasma (PFP)serum for 3 h under normal stirring. The film was taken out after 3 h and the protein contents was measured using the (Bicinchoninic acid) BCA reagent. This method is based on the fact that protein reduces Cu²⁺ to Cu¹⁺ in the alkaline medium. The BCA combines with Cu¹⁺ to create a purple-colored product with a maximum absorbance at 546 nm.

The albumin content was determined using Bromo cresol green (BCG) test. The Bromocresol green test is based on the fact that at acidic pH (approximately 4.0), albumin act as a cation and binds to the anionic dye Bromo cresol Green (BCG), forming a green colored complex. The absorbance of soluble complex is measured at 630 nm (Shimadzu, Genesis 10-S). The color intensity of the complex was proportional to albumin concentration in the sample.

Antibacterial test by the ‘zone inhibition method’

The antibacterial tests were carried out with curcumin loaded film sample Alg/CW (20)₄₅₀ by ‘zone of inhibition’ method. In brief, the culture medium was prepared by mixing nutrient agar (2.8 g) and agar powder (1 g) in 100 ml of distilled water in a conical flask and autoclaved for 30 min. The medium was transferred into sterilized Petri plates in a laminar air flow. After solidification of media, Escherichia coli culture was streaked on its surface. Now, the film sample Alg/CW (20)₄₅₀ was placed in center of the Petri plate and the Petri plate was incubated for 2 days at 37°C in the incubator.

Antifungal activity

We also carried out the antifungal activity of the film sample Alg/CW (20)₄₅₀ against *Candida albicans*, and *Candida parapsilosis*. As even to fourteen days old culture was obtained from Fungal Dis-ease Diagnostic Center, Jabalpur (India). For disc diffusion test, films were cut into disc shape with a diameter of 5 mm, then sterilized by autoclaving for 30 min at 120°C, and finally placed on different cultured agar plates. The plates were incubated for 1 day at 37°C in an incubation chamber.

Curcumin release studies

The pre-weighed Cur loaded film was placed in 25 ml of physiological fluid (PF) at 37°C. The amounts of curcumin released at different time intervals were determined spectrophotometrically (Shimadzu, Genesis 10-S) at 526 nm. After each measurement, the film was transferred in to 25 ml of fresh physiological fluid. Calibration curve, prepared for the curcumin solutions of known concentrations in the appropriate range, was used to determine the amount of curcumin in the release media.

Results

Characterization of films

It is observed that surface texture of film Alg/CW(20) is quite rough due to the presence of well dispersed cellulose micro crystals throughout the matrix. Overall, a uniform distribution of CMC. It is observed that surface texture of film Alg/CW(20) is quite rough due to the presence of well dispersed cellulose micro crystals throughout the matrix. Overall, a uniform distribution of CMC is visible (although with a few agglomerations). However, the surface texture of Cur loaded film sample Alg/CW (20)₄₅₀ exhibits different texture. It may be noticed that the whole surface have a relatively smoother texture and the curcumin

molecules seems to have superimposed on the cellulose crystals and the grooves visible in the plain film are not so pronounced now. In other words, the pronounced appearance of the cellulose crystals in the film sample Alg/CW(20) is depressed greatly due to presence of cur-cumin which must have formed a layer on the cellulose crystals throughout the film matrix.

Thermal stability of films

It can be seen that cellulose powder suffers a drastic weight loss of nearly 76% in the temperature range of 200–380°C where all cellulose is pyrolyzed. The drastic weight loss is attributable to the liberation of volatile hydrocarbon from rapid thermal decomposition of cellulose chains. However, beyond 400°C there is gradual weight loss probably due to the steady decomposition of the remaining heavy components mainly from lignin. The thermo-gram of chitosan powder showed a two stage degradation behavior. There is weight loss of 46% in the first phase in the temperature range of ambient to 390°C. In the second phase, the weight loss observed was almost 37% in the temperature range of 400–800°C. Such a two stage degradation of chitosan has also been reported previously. The thermogram of Alg/CW(20)₄₅₀ shows an intermediate degradation behavior. The degradation is a little faster than that of chitosan but is slow enough as compared to that of cellulose crystals. More interestingly, the thermogram of Alg/CW (20)₄₅₀ is more similar to that of chitosan in nature, probably due to the relatively much higher content of chitosan in the film as compared to cellulose content which is only 20% to that of chitosan. Finally, it is also noteworthy that presence of curcumin did not affect the thermal stability of the film because of very negligible content of curcumin as compared to the total mass of polymer (see TGA of curcumin in inset as obtained from the literature) [26]. According to the report, curcumin does not show any moisture loss up

to 200°C because of its poor hydrophilic nature and a slower mass loss is observed at 240°C due to thermal degradation and it becomes prominent on reaching at 500°C. The presence of curcumin does not make any influence on the stability of the film because the Cur content is only 450 µg, as compared to the total mass of 1 g of the film.

Tensile strength measurement

It can be seen that the TS of films increases from 5.7 to 13.8 MPa with the increase in cellulose content from 0% to 20%. However, for the sample Ch/CMC (30)450 the TS decrease to 8.1. The observed increase in TS with cellulose content may be attributable to the fact that cellulose crystals, being self-reinforced, possess fair stiffness and as their content in the film increases, the TS also increases. In addition, with the increase in cellulose content, the interfacial interactions between polar groups of cellulose micro crystals and chitosan chains become prominent. These interactions help in transfer of applied stress from the matrix to the cellulose micro crystals, and therefore enhance the tensile strength of resulting films. In a recent work, Zimmermann et al. have reported a threefold increase in the tensile strength of PVA film due to addition of 20% (by weight) of cellulose fibers. In the present work, we have also observed an almost three fold increase in the TS of the chitosan film due to addition of cellulose crystals (20% of chitosan content by weight). Moreover, in a study by Atefa et al., the TS of agar-films increased with addition of nano cellulose up to 2.5%, but later on the TS started to decrease with further increase in the cellulose content. In the present work, the observed decrease in TS with increase in CMC content beyond 20% (by weight) may probably be due to the over exceeded stiffness that could bring internal cracks within the film matrix, making the film brittle and so reducing its mechanical strength. In addition, the heterogeneous size distribution and

agglomeration of CMC (as was also visible in SEM images) could be another reason for the observed decrease in TS of the film Ch/CMC (30)450. Similar results have also been reported by Piyada et al. for the rice starch films, reinforced with rice starch nanocrystals. A close look at the percent elongation (PE) values reveals an almost opposite trend with the exception of the film sample Ch/CMC (30)450. The observed decrease in PE with CMC content may probably be due to the increased nature of nano fillers. These dispersed cellulose crystals restrict the segmental motion of polymeric chains, thus reducing their elongation tendency.

Equilibrium moisture uptake studies

All the curves obtained were sigmoid in shape, exhibiting type-II characteristic isotherms. Such curves are typical for most of the biopolymers like starch, chitosan, etc. The curves may be divided into three zones, i.e. zone-I, zone-II and zone-III with water activity ranges of 0.0–0.2, 0.2–0.7 and 0.7–1.0, respectively. The variation in moisture uptake with water activities for all the films may be explained as follows. In the water activity (a_w) range of 0–0.2 (zone-I), the equilibrium moisture content (EMC) increases with a_w and the film sample Alg/CW (30)₄₅₀ shows the maximum moisture absorption. This is attributable to the fact that in this zone, water vapor molecules are readily absorbed on the active polar groups (i.e. –OH groups) present on the surface of the film. Here, the dispersed cellulose crystals with their surface hydroxyls also contribute towards moisture uptake. In other words, the density of the polar –OH groups on the film surface increases with CMC content in the film. As a_w increases beyond 0.2 and enters into the zone II with a_w range of 0.2–0.7, the EMC increases with relatively slower rate. The reason is that moisture uptake occurs at less active sites. It is reported that, in this region unfolding or relaxation of polymer chains opens up new sites for uptake. Finally, there is sharp increase in the EMC with a_w , in the

range of 0.7–1.0. Here, the moisture molecules diffuse into the voids and capillaries within the film matrix. In addition, a cross over can be clearly observed. The film Alg/CW (0)₄₅₀ shows maximum moisture absorption while the film Alg/CW(30)₄₅₀ absorbs minimum moisture. The relatively faster moisture uptake in this zone is simply because of the capillary action. When water vapor molecules enter into the film matrix, the –OH groups of cellulose crystals in the bulk of the films do not interact with invading water vapor molecules.

Curcumin Release Studies

Cellulose crystals, present within the chitosan films, serve as diffusion barrier and they are expected to retard the release of curcumin from the films. The release profiles of Cur-loaded film samples Alg/CW (0)₄₅₀, Alg/CW(10)₄₅₀, Alg/CW(20)₄₅₀, and Alg/CW(30)₄₅₀ are shown in Fig. 6. It can be seen that the film sample Alg/CW (0)₄₅₀ exhibits maximum release while minimum release of curcumin was obtained for the film sample Alg/CW (30)₄₅₀. This indicates that amount of Cur released decreases with the increase in the cellulose content of the films. The amounts of Cur released from the samples Alg/CW (0)₄₅₀, Alg/CW(10)₄₅₀, Alg/CW(20)₄₅₀, and Alg/CW(30)₄₅₀ in 36 h was 365, 300, 270 and 140 micro g, respectively. Here, it is also noteworthy that the total loading of curcumin in each film was around 450 micro g. Such a lower curcumin release from these films is attributable to the fact that Cur has very poor solubility in water. It has been reported that Cur has a solubility of around 2.67 micro g/ml at pH 7.3. Many preclinical and clinical studies in mice, rats and humans have also revealed a low bioavailability of curcumin.

The curcumin release data was applied on the Higuchi model, which was initially developed for the planar systems, but later extended to

systems with different geometries. It is based on the following assumptions: (1) initial drug concentration in the matrix is much higher than its solubility, (2) thickness of drug particles is much smaller than the thickness of the matrix and (3) drug diffusivity is constant and (4) perfect sink conditions are always maintained. The most simplified form of this model is given as:

$$F = K_H t^{1/2}$$

Where, F may be taken as the fractional release and K_H is the Higuchi constant. The major benefits of this equation include the possibility to: (i) facilitate device optimization, and (ii) to better understand the underlying drug release mechanisms. The equation can also be applied to other types of drug delivery systems than thin ointment films, e.g., controlled release transdermal patches or films for oral controlled drug delivery. The dynamic release data was used to draw plots between F and $t^{1/2}$, which were linear with fair regressions.

Antibacterial activity

The antibacterial action of sample curcumin loaded Alg/CW(20) was investigated qualitatively by using zone inhibition method. The results of antibacterial tests indicated a zone of around 4 cm in the Petri plate supplemented with Cur loaded film.

Conclusion

This study concludes that curcumin loaded Alg/CW films demonstrate controlled release of Cur, extended over a time period of 36 h. The

amount of curcumin released shows a negative dependence on the concentration of cellulose crystals dispersed within the chitosan matrix. The dispersed CMC produce additional physical crosslinks and retard the release rate. The films show fair antimicrobial activities against bacteria and fungi.