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Development and characterization of chitosan-based antimicrobial films incorporated with streptomycin loaded starch nanoparticles

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ABSTRACT

Nowadays, Chitosan has attained more attention due to its potential applications in food, agriculture and pharmaceutics. The cationic nature of chitosan enhances the film forming capacity of this polymer. However, films made only from chitosan lack water resistance and have reduced mechanical properties. The functional properties of chitosan films can be improved when chitosan films are combined with other film forming materials. The objective of this study was to prepare chitosan based antimicrobial films by the incorporation of streptomycin loaded starch Nanocrystals. Different properties of this film such as swelling nature, moisture content, degradation nature and the antimicrobial activity of modified chitosan films were investigated. Drug releasing efficacy of the film was also studied. The addition of streptomycin loaded Starch nanocrystals in chitosan-gelatin film increased crystallinity of the film, lowered the swelling nature of the film to a controlled manner. Moreover the Modified chitosan based antimicrobial film showed almost 90% of *Escherichia coli* inhibition and 80% of *Bacillus subtilis* inhibition and also the film showed a sustained release (60%) of streptomycin for 10 days.

Focal point:

- Benchside
  Synthesis of streptomycin loaded starch nanoparticles (SS-NPs) using nanoprecipitation method and the development of novel chitosan based antimicrobial film by the incorporation of streptomycin loaded starch nanoparticles using solvent casting technique
- Bedside
  Development of potential multifunctional antimicrobial film for medical, pharmaceutical and food based applications due to its excellent film forming ability, biocompatibility, biodegradability and antimicrobial property
- Industry
  The designed unique antimicrobial film, if finely tuned, can be used both in biomedical fields for developing scaffolds in tissue engineering, wound dressing material, capsule material for sustained drug release and immobilization of enzyme and food industry as packaging material
- Government
  Financial investment and support from government would help to develop new novel translational tools which contribute for better health care and also help to reduces disease burden
- Regulatory
  Stringent regulatory principles limit the clinical trials essential for validation of biomaterials which might have turned in to a highly beneficial multifunctional product such as antimicrobial film potentially useful both in biomedical and food industry.

1. Introduction

Organic films and coatings, especially those of natural polymers are very attractive as biomaterial coatings because they offer great versatility in the chemical groups that can be incorporated at surfaces of food items, biomedical devices, filters, and also used as additives for antifouling paints [25]. The relative ease of processing is another attraction for the extensive interest in organic polymer

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films. Recently, increasing attention has been paid to develop functional polymer films with antimicrobial properties to use them for medical packaging and also they are ideal for overlay material that can be used to prevent bacteria growth on any surface, which require antimicrobial protection. Commonly used polymers for the development of antimicrobial film involves polystyrene, polyvinylchloride, poly lactic acid or poly (lactide-co-glycolic acid) and cellulose. Antimicrobial agents are usually stabilized by the addition of cyclodextrin inclusion compounds at high polymer-processing temperature [26].

Among the known biopolymer, chitosan seems to be promising material for the development of bioactive films. Chitosan is natural cationic polysaccharides, formed by the deacetylation of chitin, a major exoskeleton component of crustaceans such as crabs and shrimps [11,35]. Chitosan has many advantages such as biodegradability, biocompatibility, non toxicity, aesthetic appearance, edibility, barrier properties and biofunctionality over other biomaterials [36] Chitosan and its derivatives also possess biological activities such as broad spectrum antimicrobial activity [17,19,20,3] wound healing activity and drug delivery [1,4]. These properties make chitosan highly important in many fields like cosmetics, pharmacy, food, agriculture, biomedical, and material science [14,18,22,32,33]. The cationic property of chitosan allows electrostatic interactions with other compounds, which have been widely used for the development of bioactive films [24]. Cationic antimicrobial polymers, can prevent bacterial infection of implanted devices such as catheters [37]. In the present scenario, improving functional properties of chitosan films by incorporation of antimicrobial agents can be highly advantageous.

Cagri et al., [6] reported the incorporation of different antimicrobial agents such as benzoic acid, streptomycin, lysozyme, nisin, propionic acid, lactic acid, tetracycline, rifampicin etc to polymer films, which helps to enhance antimicrobial activity. They also reveal that the addition of antimicrobial agents in the films helps to target post processing contaminants on the food surfaces.

The present study was aimed to develop chitosan based films incorporated with streptomycin loaded starch nanoparticles. We studied different properties such as swelling nature, degradation property and moisture content of the film and also investigated the antibacterial activity of the developed film.

2. Materials and methods

2.1. Chemicals

Solvents, Streptomycin and Polysorbate 80 (Tween 80) surfactant were purchased from Himedia Laboratories Pvt Ltd., Mumbai, India. Chitosan were provided by Nitta Gelatin India Ltd. Native sago was obtained from a local grocery store.

2.2. Synthesis of streptomycin loaded starch nanoparticles (SS-NPs) embedded Chitosan-gelatin film

Chitosan-gelatin film incorporated with SS-NPs was synthesized by a modified method of solvent casting [5].

2.2.1. Synthesis of Streptomycin loaded starch nanoparticles

The procedure was done as follows: 0.081 *g* of Streptomycin was added to 30 mL of absolute ethanol containing 0.01 M Tween 80. To this suspension, 5 mL of cyclohexane was added and the solutions were stirred continuously for 1 h followed by addition of 1 mL of 1% starch solution. Starch solution was prepared by the dissolution of 10 *g* sago powder in 25 mL of 0.8% NaOH solution and 25 mL of 1% urea solution. These solutions were cooled at −20 °C for 1 h and 50 mL of this solution was stirred for 1 h using magnetic stirrer until all the starch powder was completely dissolved to obtain a homogeneous starch solutions. Streptomycin was loaded *in situ* onto starch nanocrystals as the starch nanocrystals formed during the precipitation process. The solution was centrifuged at 10,000 rpm for 5 min to collect pellet and the supernatant. Subsequently, the collected pellet were washed with absolute ethanol for 3 times and dried at 40 °C for 12 h and stored in sealed containers prior to their use. The absorbance of its supernatant was measured at 195 nm in order to measure the concentration of unloaded Streptomycin.

2.2.2. Preparation of SS-NPs loaded chitosan film

The prepared streptomycin loaded starch nanoparticles (SS-NPs) were then added to 1% chitosan solution. The chitosan solution was prepared by dissolving crab shell chitosan (~400 kDa, 76% deacetylated) in an aqueous solution (1% v/v) of glacial acetic acid (Sisco Research Laboratories pvt. Ltd.). Followed by the addition of gelatin (1%) The resultant chitosan-gelatin solution was filtered through a Whatmann No. 3 filter paper and stored. The solutions were casted on Teflon coated plate by solvent casting technique and placed in oven at 40 °C for 24 h. The films were peeled off and stored in air tight containers.

2.3. 2.3. Swelling study of SS-NPs embedded Chitosan-gelatin film

The swelling study of SS-NPs embedded Chitosan-gelatin was evaluated by immersing each preweighted (8 mg, 1×1 cm^2) test film in 10 mL of 0.1 M 1X phosphate buffer (pH 7.4) at room temperature (30 ± 2 °C) under static conditions. Phosphate buffer (10X) was prepared as stock, which contains 1370 mM NaCl, 27 mM KCl, 100 mM Na_2HPO_4, and 20 mM KH_2PO_4. Then the stock was diluted, to 1X working concentration and used for the study. At every 10 min, the swollen test films were taken out, blotted with a filter paper to remove surface water and the weight of the films were recorded. Swelling capacity (or swelling ratio) (%) was calculated using the Eq. (1)

$$\text{Swelling ratio} = \text{weight of swollen Film} - \text{dry weight/dry weight of film} \quad (1)$$

2.4. Solubility of SS-NPs embedded Chitosan-gelatin film in Water

The solubility of SS-NPs embedded Chitosan-gelatin film was determined according to the method by Nafchi et al. [27] with slight modification. The test films were cut into 1×1 cm^2 size and approximately 0.8 *g* weight was used for the study. Each film was immersed in 25 mL distilled water in a beaker and kept for shaking at a speed of 120 rpm at room temperature (30 ± 2 °C) for 24 h. The soaked pieces were then separated using filtration through cheese cloth followed by oven drying at 50 °C till attaining constant weight. Another experiment was conducted without shaking under similar conditions to compare the effect of shaking on solubility. Final weights of both samples were recorded and solubility calculated using Eq. (2)

$$\text{Solubility} = [(\text{initial weight} - \text{final weight})/\text{initial weight}] \times 100\% \quad (2)$$

2.5. Degradation ability of SS-NPs embedded Chitosan-gelatin film

The initial weight (W_i) of dried samples (0.8 g) were taken. The samples were then immersed in different concentration (20 μg/mL, 40 μg/mL, 60 μg/mL and 100 μg/mL) of enzymes (trypsin and lysozyme), 1X PBS (pH 7.4), 0.1 M HCl and 0.1 M NaOH for 60 min interval. After the incubation period final weight (W_f) of samples was analyzed as described previously and degradation