Abstract—Microparticles carrier systems made from naturally occurring polymers based on chitosan/casein system appears to be a promising carrier for the sustained release of orally and parenteral administered drugs. In the current study we followed a microencapsulation technique based aqueous coacervation method to prepare chitosan/casein microparticles of compositions 1:1, 1:2 and 1:5 incorporated with chloramphenicol. Glutaraldehyde was used as a chemical cross-linking agent. The microparticles were prepared by aerosol method and studied by optical microscopy, infrared spectroscopy, thermo gravimetric analysis, swelling studies and drug release studies at various pH. The percentage swelling of the polymers are found to be in the order pH 4 > pH 10 > pH 7 and the increase in casein composition decrease the swelling percentage. The drug release studies also follow the above order.

Keywords—Chitosan/casein microparticles, Chloramphenicol, Drug release, Microencapsulation.

I. INTRODUCTION

MICROSHERE carrier systems made from the naturally occurring proteins have attracted considerable attention for several years as a matrix for controlled and sustained release delivery of many drugs [1, 2]. There has been some interest in the milk protein, casein, as a drug carrier mainly for the sustained delivery of cytotoxic drugs [3, 4, 5]. Glutaraldehyde cross-linked casein microspheres were found to be resistant in proteolytic tract for more than 24 hours and suggested that it could be used as a matrix for the controlled delivery of oral drugs [6]. Therefore, casein appears to be a promising carrier for the sustained release of many orally as well as parenteral administered drugs. Chitosan is a hydrolyzed derivative of chitin, a biopolymer widely distributed in nature. Chitosan has attracted attention as a matrix for controlled release systems since it possesses reactive functionalities and easily degraded into non-toxic product by enzymes [7]. Chloramphenicol is a bacteriostatic antimicrobial originally derived from the bacterium Streptomyces venezuelae which inhibits bacterial protein synthesis and has a very broad spectrum of activity against Gram-positive bacteria, Gram-negative bacteria and anaerobes [8]. Therefore chloramphenicol was selected as a model drug in this study.

Amine groups of chitosan are mostly protonated in acidic solutions and the resultant soluble polysaccharide is positively charged [9]. Oppositely charged electrolytes will interact rapidly with chitosan in solution to form an insoluble precipitate. This principle has been used for the production of chitosan micro capsule to control drug release [10]. Controlled release microspheres formulated with bovine serum albumin or egg albumin as a naturally occurring polymer have also been extensively studied using different methods of preparation [11]. Microspheres prepared from single polymeric materials for controlled drug delivery are sometime unable to fulfill all the required physical properties, encapsulation efficiency or rate and mechanism of drug release. Combination of chitosan with other polymers such as alginate, pectin, and albumin for the production of sustained drug release oral dosage form was the interest of many investigators [12]. Sustained release of diltiazem hydrochloride from casein-chitosan Microsphere, cross-linked with formaldehyde prepared with colloidal coacervation technique in a completely aqueous environment was studied [13]. This technique avoids the use of organic solvents (e.g. solvent evaporation or organic phase separation method) associated with safety hazards, toxicity and high cost.

The present work was undertaken to prepare chitosan/casein microparticles of varied molar ratio in aqueous medium. Glutaraldehyde was used as a cross-linking agent and chloramphenicol was incorporated in chitosan/casein during the coacervation process. The microparticles were studied by various methods such as FT-IR, optical microscopy, thermogravimetry, swelling studies and drug release studies in varied pH.

II. MATERIALS AND METHODS

A. Preparation of Chitosan/casein Microparticles Containing Chloramphenicol

The chemicals, Casein (Loba Chemie Pvt. Ltd.), chitosan (Acros Organics), Glutaraldehyde (Loba Chemie Pvt. Ltd.), chloramphenicol (Jawa Pharmaceuticals), Sodium hydroxide (E. Merck India Ltd.), Glacial acetic acid (Fischer Inorganics & Aromatics), Citric acid (Fischer Inorganics & Aromatics), Sodium bicarbonate (Ranbaxy Fine Chemicals Ltd.), Potassium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium tetraborate (Merck Specialties Pvt. Ltd.)
and Sodium chloride crystals GR (Merck Ltd.) are of analytical grades, were used without further purifications for the preparation of chitosan/casein microspheres and in conducting the experiments. Buffers of pH = 4 (citric acid/sodium hydroxide/sodium chloride), pH = 7 (potassium dihydrogen phosphate/disodium hydrogen phosphate) and pH = 10 (sodium tetraborate/ sodium hydroxide) were prepared in double distilled water [14].

Chitosan, 1 g, was added to 1 L of 1% acetic acid in water and stirred overnight to form a clear solution. 1%, 2% and 5% casein solutions were prepared separately by adding an appropriate amount of casein powder in 1N NaOH solution and magnetically stirred till a clear solution was obtained. These solutions were preserved in refrigerator for further use.

To a beaker containing 25 ml of 1% casein solution, 25 ml of 1% solution of chitosan solution added with an appropriate amount of the drug (0.05%, 0.01% or 0.015% by weight of polymers) was added by means of a self-designed cross-flow aerosol generator with magnetic stirring. The aerosol generator consisting of two glass tubes with an orifice diameter of 0.1mm were positioned perpendicular to each other on a rectangular glass plate as shown in the Figure 1. The instantaneous product formed was stirred for further 30 minutes. To this reaction mixture, glutaraldehyde (5% by weight of polymers) was added drop wise and stirred for further 30 minutes. The precipitate was filtered through filter paper in a Buckner funnel and washed with distilled water until the wash out becomes neutral pH. The product was dried in a vacuum oven at 800°C and preserved in a desiccator.

Chitosan is insoluble in neutral and alkaline pH and is only soluble in acidic pH. Below pH 6, the amine groups are protonated and positively charged and chitosan is insoluble in water. At high pH, the polymer loses its charge as amine groups become deprotonated and therefore insoluble in water [16]. The dried powder of chitosan – casein microparticles were subjected to Infrared Analysis on a FT-IR instrument (Bruker Tensor 27), coupled to a PC. The samples were placed in the holder directly in the IR laser beam and the transmittance was observed for the Mid-IR range of 400 – 4000 cm\(^{-1}\). The IR spectrum was collected and analyzed using OPUS software.

D. Thermo Gravimetric Analysis

TA Instruments model TGA 2950 was used in the example described here. The samples were weighed in between 3 and 10 mg. Scans were made at a heating rate of 10°C/min under nitrogen gas.

E. Degree of Swelling

The role of pH on the extent of water absorption of polymeric gels is of great importance since a change in pH of swelling media often causes a fluctuation in free volumes accessible to penetrating water molecules, it affects swelling properties of polymers [15]. The pre-weighed samples were packed in dialysis bags and immersed in buffers of pH 4, pH 7 and pH 10. The bags were removed at regular intervals, and the surface water was removed by filter paper and weighed. Degree of swelling could be described as the water absorptivity of sample by the following equation [16].

\[
\text{Degree of swelling (\%)} = \frac{W_t - W_d}{W_t} \times 100
\]

Where, \(W_t\) and \(W_d\) are the weight of the swollen and dry sample, respectively.

F. Drug Release Studies

Samples weighing 0.05 g incorporated with drug were immersed in 5 ml of pH 5, 7 and 10 buffers in separate centrifuge tubes. The samples were centrifuged at regular intervals and the optical absorption of the drug in the supernatant was measured at 227 nm, which is the \(\lambda_{max}\) for chloramphenicol, using Perkin Elmer UV-Vis Spectrometer (Model: Lamda 25). Corresponding drug concentrations in the supernatant were calculated from the calibration plot generated by regression of data taken in triplicate. To determine the amount of drug entrapped, 0.05 g of microparticles were gently heated at 50 °C with 5 ml of pH 5 buffer in a test tube for 15 min and kept for 24 hrs with occasional shaking. The optical absorption of centrifuged solutions was determined at 227 nm and drug concentrations were calculated from the calibration plot.

III. RESULTS AND DISCUSSION

Chitosan is insoluble in neutral and alkaline pH and is only soluble in acidic pH. Below pH 6, the amine groups are protonated and positively charged and chitosan is insoluble in water. At high pH, the polymer loses its charge as amine groups become deprotonated and therefore insoluble in water [16]. Casein is an amphiphilic protein and its isoelectric point is 4.6. At pH above the isoelectric point casein is negatively charged and is soluble in water [3, 4]. The rate of addition of
chitosan was kept as 0.1 ml/min and the air pressure was 1 bar. The mixing of two polymer solution resulted in simultaneous formation of an insoluble phase, which is due to the formation of chitosan/casein coacervation by electrostatic interaction of the negatively charged casein protein with positively charged chitosan. The rapid and instantaneous interaction of chitosan with oppositely charged molecules was already reported by other investigators [17, 18]. The pH of the final aqueous medium in all the cases was below the isoelectric point of casein. Crosslinking is required to harden the surface of the microparticles and thereby to control the drug release. In the previous studies, chitosan/casein microsphere system was cross-linked with formaldehyde [19]. The aldehyde group of crosslinking bifunctional agent used here (i.e. glutaraldehyde) forms covalent imine bonds with amine groups of chitosan and casein via Schiff reaction [20]. The yield of the microparticles ranged between 88 to 93 %. Figure 1 shows the as-prepared chitosan-c-casein (1:1) microparticles and their sizes are ranging from 10 to 45 micron diameter.

Figure 3 (a & b) show the FT-IR spectra of chitosan, casein and chitosan/casein microparticles. Major regions of the FT-IR spectra of chitosan/casein showed absorption peaks at 3365 cm\(^{-1}\) (O-H), 2915 cm\(^{-1}\) (C-H), 1722 cm\(^{-1}\) (C=O), 1585 cm\(^{-1}\) (hydrogen bonded N-H) and 1028 (C-O) [21]. Since the characteristic absorptions of chitosan and casein overlapped with each other the FT-IR spectra of chitosan, casein and chitosan/casein showed similar absorption patterns. A comparison of 1:1, 1:2 and 1:5 chitosan/casein spectra reveals that the absorption peaks dominated by chitosan around 1650, 1585, 1432, 1372 cm\(^{-1}\) gradually decreases, which confirms the incorporation of casein during the formation of chitosan/casein coacervation.

TGA studies of chitosan, casein and chitosan/casein (1:2) are given in Figure 4. The initial weight loss around 100 to 140\(^{\circ}\)C appears to relate to adsorbed water [22]. TGA of chitosan shows a narrow degradation with a maximum degradation temperature at 320\(^{\circ}\)C while casein has a broad degradation pattern with maximum degradation at 360\(^{\circ}\)C. Chitosan/casein (1:2) also shows narrower degradation pattern similar to chitosan but with a maximum degradation temperature at 305\(^{\circ}\)C. It can also be noted that the thermal stability of chitosan/casein has increased, which is in accordance with the trend observed in case of composite films of hydroxyapatite and chitosan [23]. These results further confirm the formation of chitosan/casein coacervation upon addition of a basic solution of casein into an acidic chitosan solution.
As evident from the Figure 5 (a, b & c), the microparticles swelled to great extent at pH 4 followed by pH 10 and pH 7. At pH 4 the amine groups of chitosan are protonated and acquire positive charge [16]. Casein also acquire a net positive charge, since the isoelectric point is 4.6, leading to electrostatic repulsion between the two polymer chains [3, 19]. At pH 7, Chitosan is deprotonated but casein will have a net negative charge whereas, at pH 10, the net negative charge of casein will be more than at pH 7 making the hydrogen bonding interaction of casein with alcohol and amine groups of chitosan stronger, leading to better attraction between the polymers. This can be attributed to the trend observed for the percentage swelling of the polymers, which are in the order pH 4 > pH 10 > pH 7. It could be derived from the Figure 5 (a, b & c), that the increase of casein concentration decreases the swelling of chitosan/casein microparticles.

The reason for the reduced swelling rate could be as a result of increase in matrix density due to increase in casein concentration [24, 25].

As evident from the trends in the Figure 6 (a, b & c), the rate of drug release is more at pH 4, followed by pH 10 and pH 7. These results can be explained as discussed in swelling studies experiments. At pH 4, electrostatic repulsion between the two polymer chains lead to a better water diffusion into the matrix and release of drug. On the other hand at pH 7 and 10, the hydrogen bonding interaction between the polymer networks make the microparticles more compact and therefore the diffusion of water molecule and subsequent release of drug was hindered. The extent of interaction would be expected to be more at pH 7 than at pH10. Therefore the rate of drug release of the chitosan/casein microparticles are observed in the order pH 4 > pH 10 > pH 7.
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