ABSTRACT

Recently efforts have been made to design novel drug dosage formulations so that more and more effectiveness could be expected over conventional dosage forms. In order to achieve this goal, controlled release technology had emerged out as commercial methodology by which method pre-decided and reproducible release of drug up to therapeutic level into a specific environment over a prolonged time period could be maintained. In general, such drug delivery systems function according to the changes in physiological signals with in the body and target the drug for the desired site of action to reduce any side effect. Recent attempts on Nano and micro beads of polymers have been formulated using polymeric material either synthetic or natural origin. Therapeutic drug molecules complexed by beads of polymers capable of forming gel, may also be released by diffusion. Hence, drug delivery system requires essentially a polymeric matrix which would be non toxic, biocompatible and biodegradable.

Deacetylated product of chitin provides a polysaccharide (1→ 4) 2-amino-2 deoxy β – D glucan which is known as the chitosan and is one of the well known biodegradable polymers metabolized by human enzymes. Chitosan can be prepared as hydrogel beads, having a positive charge at metabolic and physiological pH, bioadhesivity and water holding capacity may be enhanced in tissues of human body for extended period of time. In general, three dimensional hydrophilic polymer network of hydrogel beads are capable of retaining large amount of water or bio fluids. Hydrogels are thermodynamically compatible with water and exhibit swelling in aqueous media and they have resemblance with natural living tissues due to their high water retention capacity. Cross linked hydrogel network can be obtained by cross linking chitosan with glutaraldehyde. Their properties depend mainly on the cross linked density. Formation of hydrogel network requires a critical number of cross links per chain and it forms porous structure whose pore size depends upon swelling of beads which in turn depends on external environment.

Our present study is an attempt to develop cross linked beads composed of chitosan and two amino acids as spacer groups cross linked with glutaraldehyde for

sustained release of chlorpheniramine maleate (CPM) as a model drug and to compare it with cross linked beads of chitosan and chitosan- containing one of the amino acid. We have prepared four types of beads cross linked with glutaraldehyde (a) chitosan (b) chitosan-glutamic acid (c) chitosan-glycine (d) chitosan-glycine-glutamic acid having different composition to investigate the comparative swelling behavior and modeling drug release properties.

To prepare chitosan or chitosan-amino acid drug unloaded beads chitosan or chitosan and amino acid taken in acetic acid, stirred at room temperature up to a homogeneous mixture and then extruded in the form of droplets into NaOH and methanol solution using a syringe. So formed beads were then washed and allowed to react with different concentration of glutaraldehyde at 50^{o}C for 10 minutes. Finally cross linked Interpenetrating Network (IPN) beads were washed and air dried. To prepare drug loaded beads of same composition, a known amount of CPM (150 mg, 200 mg) were separately added to the chitosan or chitosan and amino acid homogenous mixture before extruding through a syringe.

The beads were characterized by FTIR spectroscopy to confirm the crosslinking reaction and drug interaction with cross linked polymer in beads. Spectra of chitosan, glutamic acid, glycine, CPM drug unloaded and drug loaded chitosan and chitosan amino acid beads were recorded. The absorption bands in the spectra indicated the formation of imino (-N=CH-) linkage as a crosslink due to a chemical reaction taking place between amino group of chitosan and aldehyde group of glutaraldehyde in beads. Also, the drug CPM has not undergone any chemical change with in the chitosan and chitosan-amino acid beads.

Characterization of beads using Scanning Electron Microscopy (SEM) was carried out to understand their shape, size surface morphology, and internal structure. All the beads were found to be nearly spherical to elongated in shape with varying in size from 83-164 μm. The beads have rough, rubbery, fibrous and folded surfaces. The interior of beads showed micropores and tubular networks. The variable porosity of the beads was noticed depending on their variable composition.

X-ray diffraction investigation on used raw materials, drug unloaded and drug loaded beads were carried out to determine the crystalline nature of drug after loading into chitosan and chitosan-amino acid IPN beads. Results indicated amorphous dispersion of chlorpheniramine maleate (CPM) in the polymeric matrix. No crystals of drug were found in drug loaded beads up to the detection limit exhibiting incorporation of drug as amorphous form in between polymeric networking.

The beads were characterized by thermal analyses to find out their thermal behavior. On comparison between chitosan, chitosan-glycine, chitosan-glutamic acid, chitosan-glutamic acid-glycine beads using thermal gravimetric analysis (TGA) indicated that chitosan-glycine-glutamic acid beads lose 49% weight at 400^{o}C are as thermally stable as that of chitosan beads which loses 46% weight at 400^{o}C and also more stable than chitosan-glutamic acid beads (loses 50% weight) and slightly lesser than chitosan-glycine beads (loses 43% weight). The drug was found to be quite stable with in the beads. DTG analysis concluded that chitosan-glutamic acid-glycine beads were found to be most stable as it loses weight at highest temperature 271^{o}C as compare to chitosan beads at 244^{o}C, chitosan glutamic acid at 249^{o}C and chitosan-glycine beads at 268^{o}C. Similar conclusion has also been made by DTA studies in which exothermic peak for chitosan-glutamic acid-glycine beads move towards higher temperature.

Characterization of chitosan-glycine-glutamic acid beads has also been carried out using thermal analysis by varying their composition. It was indicated that crosslinking of beads with glutaraldehyde increased its thermal stability as the crosslinking density increased with using higher concentration of glutaraldehyde. Variation of chitosan concentration and amino acid composition also gave almost equally thermally stable beads. Drug loaded and unloaded beads gave almost similar thermograms indicating the amorphous dispersion of drug into the beads.

The swelling behavior of bead were observed to be dependent on pH, degree of crosslinking and their composition. At pH 2.0 and 7.4, the swelling of beads was found to be in the following order

At pH 2.0

chitosan-glycine-glutamic acid > chitosan-glycine > chitosan > chitosan-glutamic acid.

At pH 7.4

chitosan-glutamic-acid > chitosan-glycine-glutamic acid > chitosan > chitosan-glycine.

It was observed that percentage of swelling for chitosan, chitosan-glycine and chitosan-glycine-glutamic acid beads is higher in acidic solution (pH 2.0) than in alkaline solution (pH 7.4) but in case of chitosan-glutamic acid beads the rate of swelling was lower in acidic solution than in alkaline solution. It was also observed that swelling rates increases with the decreased concentration of glutaraldehyde because the lower crosslink density results in lower strength of the beads and higher degree of swelling. The percentage of swelling of the beads decreased with increasing concentration of chitosan because increased quantity of chitosan decreased the percentage of amino acid acting as spacer and finally, decreased the pore size of the bead, resulted lesser degree of swelling. Degree of swelling was also affected by changing in amino acid composition. The increase in concentration of glycine decreased the swelling percentage of cross linked beads in basic solution while increased in acidic solution.

On biodegrading in acetic acid solution 0.1 g of drug loaded chitosan amino acid beads actually released 78 μg and 142 μg CPM after 48 hours from the beads incorporated with 150 mg and 200 mg of CPM respectively.

The drug released experiments in acidic (pH 2.0) and (pH 7.4) at 37^{o}C showed a burst release initially for first hour, followed by a moderate release for next four and finally an almost constant release of drug from matrix within the periods of 48 hours. The amount and percentage of drug release followed the order of swelling of beads. So drug release depends on the degree of swelling. More swelling results to faster release of drug. The drug release is also pH dependent. It has been observed that the

amount and percentage of drug released were much higher in acidic medium than in alkaline medium in case of pure chitosan, chitosan-glycine and chitosan-glycine-glutamic acid beads while in case of chitosan-glutamic acid beads the rate of drug release in alkaline medium was higher than in acidic medium.

In case of chitosan-glycine-glutamic acid beads the drug release rate increases with the decrease in crosslink density which increases the pore size of the polymer network. Degree of crosslinking decreased with decreasing concentration of cross linker i.e. glutaraldehyde. Hence, rate of release of CPM drug is highest in case of beads having lowest concentration (3.12 %) of glutaraldehyde and also is lowest in case of beads constituting highest concentration (25%) of glutaraldehyde. It was also observed that the beads having smaller weight of chitosan gave higher release rates due to having more of chitosan, the amount of available amino acid acting as spacer group becomes low and it will formed smaller mesh size volume. The amount of amino acid also affects the drug released rate. It was observed that on increasing the amount of glycine or decreasing the amount of glutamic acid, the rate of drug release increased in acidic solution while decreased in basic solution.

On increasing the amount of incorporated CPM drug (142 µg) in the beads results the similar release pattern of drug as obtained from beads containing lower quantity of CPM drug (78 µg). The amount of drug released from beads containing higher drug quantity were higher but the percentage of CPM release from these beads loaded with higher amount of drug was found to be lower. This concluded that the mechanism of drug release due to the diffusion through swollen beads depends on the percentage of swelling of beads.

Linear plots of percent cumulative amount release versus square root of time for IPN beads containing 78 µg and 142 µg of drug have been obtained which demonstrate that the release from the cross linked polymeric microsphere matrix is diffusion controlled and obeys the Higuchi's Model.

The observations of the present study have shown that chitosan-glycine-glutamic acid beads posses a pH dependent swelling behavior. It can be used

successfully for the formulation of controlled drug delivery devices. They have optimum entrapping capacity for the studied drug and provide a sustained release of drug for extended period which make them appropriate for delivery of drug at a controlled rate.