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Research article

Formulation and Evaluation of Nanoparticles containing Losartan Potassium

Amar Singh[*] and Amar Deep
[*] *Smt. Tarawati Institute of Bio-Medical & Allied Sciences, Roorkee-Dehradun Road, Saliyar, Roorkee, Distt. Haridwar - 247667,*
[*]***Corresponding author:*** *E-mail: aaadirector@gmail.com*
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ABSTRACT

Losartan potassium is an antihypertensive agent used in the treatment of hypertension which has low peak plasma concentration and half-life. It has been selected as candidate for the formulation of sustained release dosage forms. In the present work losartan potassium loaded chitosan nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions. Nanoparticles of different core: coat ratio were formulated and evaluated for drug content, loading efficiency, particles size, zeta potential, *In vitro* drug release and stability studies. Scanning Electron Microscopy indicated that the nanoparticles were found to be in nanometer range and showed ideal surface morphology. Differ ential scanning calorimetry analysis indicated that there were no chemical interactions between drug and polymer and stability of drug. *In vitro* release behavior from all the drug loaded batches were found to follow zero order and provided sustained release over a period of 24 hours. No appreciable difference was observed in the extent of degradation of product during 60 days in which nanoparticles were stored at various temperatures. The developed formulation overcomes and could possibility be advantageous in terms of sustained release dosage forms of losartan potassium.

KEY WORDS: Nanoparticles, Chitosan, Losartan potassium, Ionic gelation technique.

INTRODUCTION

An essential requirement of modern drug therapy is the controlled delivery of a drug or an active substance to the site of action in the body in an optimal concentration versus time profile. One attempt to achieve this goal was the development of colloidal drug carriers known as nanoparticles, chiefly because of their small particle size. Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. They consist of macromolecular materials in which the active ingredient is dissolved, entrapped, encapsulated and adsorbed or chemically attached [1-2].

Losartan potassium is an angiotensin II receptor antagonist and inverse agonist of angiotensin–II (A-II) receptor. It blocks all over actions of A-II and causes fall in blood pressure in hypertensive patient which last for 24 h [3]. The objective of the work was to formulate chitosan nanoparticles containing losartan potassium by ionic gelation method to evaluate its physicochemical characteristics (particle size, zeta potential, drug loading capacity) and *in vitro* release characteristics for sustained action [4-7].

MATERIAL AND METHODS

Losartan potassium was a gift sample from Cipla Pvt. Ltd Mumbai and chitosan, glacial acetic acid and sodium tripolyphosphate were obtained from Smt Tarawati Institute of Biomedical And Sciences, Saliyar, Roorkee, India. All other chemical used were of analytical grade.

Preparation of nanoparticles

Chitosan nanoparticles were prepared by ionic cross linking of chitosan solution with tripolyphosphate (TPP) anions. Chitosan was dissolved in aqueous solution of acetic acid (0.25v/v) at various concentrations such as 1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml coded as batch number L1, L2, L3, L4 and L5 (Table 1) under magnetic stirring at room temperature, 5ml of 0.85% w/v TPP aqueous solution was added drop wise using syringe needle into 10 ml chitosan solution containing 10mg of losartan potassium. pH was adjusted to 6 by adding 0.1 N NaOH. The stirring was continued for about 30 min. The resultant nanoparticles suspensions were centrifuged at 12000x g for 30 min using C_{24} centrifuge. The formation of the particles was a result of the interaction between the positive groups of the TPP and the negatively charged amino groups of chitosan [8-15].

Characterization of prepared nanoparticles

Particle size, Surface Morphology and Zeta Potential

The surface morphology (roundness, smoothness, and formation of aggregates) and particles size (Figure 1 & 2) were studied by scanning electron microscopy (SEM) {Model: S 4700-1, Make: Hitachi}. Zeta potential of the best formulation (L3) was measured by Zeta-sizer (Model: Zeta-sizer IV, Make: Melvern instruments) [16-17].

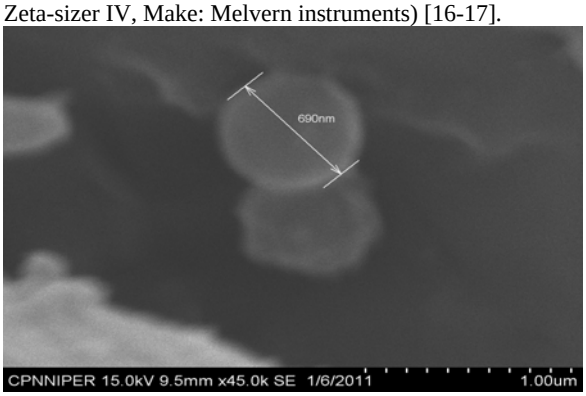

Figure 1: Scanning Electron Microscopy clusters of Losartan Potassium

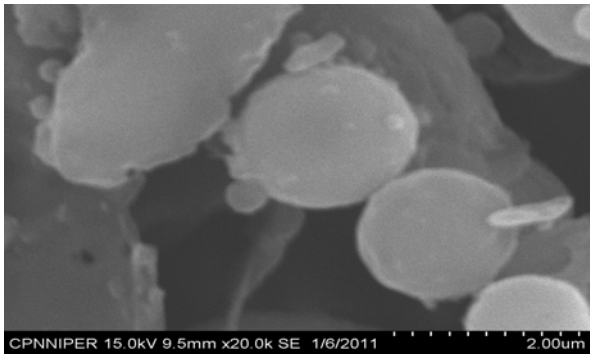

Figure 2: Scanning Electron Microscopy clusters of Losartan Potassium

Drug content

Drug content was determined by centrifugation method. The redispersed nanoparticles suspension was centrifuged at 15,000 rpm for 40 min at 25^{o}C to separate the free drug in the supernatant. Concentration of losartan potassium in the supernatant was determined by using UV- vis spectrophotometer at 232 nm after suitable dilution [18-19].

Differential Scanning Calorimetry Analysis (DSC)

Analysis of losartan potassium was performed by using DSC (Model: DSC 204, Make: HP Phonix). The DSC study reveals that melting point of losartan potassium is similar to that of the as mentioned in the official monograph (Figure 3). There was no incompatibility between drug and polymer has been observed (Figure 4). This explains that there are no any chemical species present in the drug molecule which degrades the original compound and we can go forward with this in our formulation part [20-21].

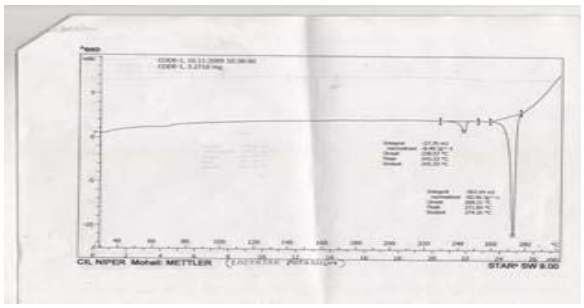

Figure 3: DSC Spectra of Losartan Potassium

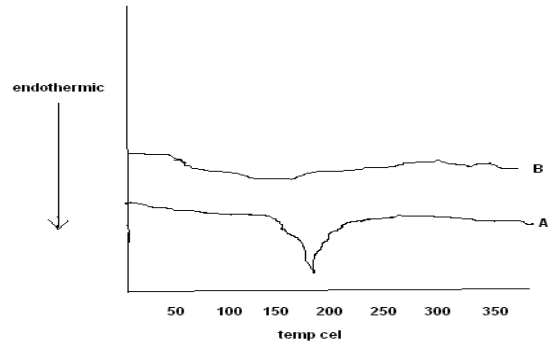

Figure 4: Drug-Polymer Compatibility of Losartan Potassium (DSC thermograms of (A) Losartan potassium and (B) Chitosan).

***In vitro* Release Studies**

In vitro release studies were carried out by using dialysis tubes with an artificial membrane. The prepared losartan potassium nanoparticles and 10 ml of phosphate buffer pH 7.4 was added to the dialysis tube and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 250 ml of phosphate buffer pH 6.8. The medium in the receptor was agitated continuously using a magnetic stirrer and the temperature was maintained at 37±1^{o}C. The sample of receptor compartment (5ml) was taken at various intervals of time over a period of 24h and each time fresh buffer was replaced. The amount of drug released was determined spectrophotometrically at 232 nm [22-24].

Kinetic modeling

In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of nanoparticles were fitted with various kinetic equation like zero order (Graph 1), first order (log % drug remaining Vs time) , Higuchi's model (Cumulative % drug release vs square root of time). The r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots [25-27].

Stability Study

Formulation of batch number L3 was used for stability study. It was divided into 3 set and stored at 4^{o}C in refrigerator. Room temperature (30^{o}C), 45^{o}C ± 2^{o}C and relative humidity at 75 % ± 5 % were maintained in humidity control oven. *In vitro* releases of drug content of all samples were determined after sixty days [28].

RESULT AND DISCUSSION

Nanoparticles were prepared by ionic gelation technique. It was found to be discrete through SEM analysis, their mean size distribution was found to be 690 nm. The drug loading capacity of nanoparticles containing drug-polymer in various ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 were found to be 75.2 ±0.32, 76.0 ±.045, 87.5 ±0.68, 83.5 ±0.62, 78.5 ±0.46%. Thus there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. The formulation L4 registered highest entrapment of drug (87.5 ±0.68%). Cumulative percentage drug released for L1, L2 and L5 after 24 h were more than cumulative release of L3 and L4. The cumulative percentage drug release after 24 h were 74.5 %, 78.2%, 68.0%, 75.5% and 71.8% for L1, L2, L3 L4 and L5 formulation respectively.

Zeta potential for L3 was found to be 30.08 ± 0.4 mV. It was apparent that in vitro release of losartan potassium showed a very rapid initial burst, and then followed by a very slow drug release. An initial, fast release suggests that some drug localized on the surface of the nanoparticles. L3 was showing good sustained release compared to other formulations and it was considered as best formulation. The stability studies showed that maximum drug content and *in vitro* drug release was found in L3 formulation which was stored at 4^{o}C and room temperature. Based on drug content, drug entrapment deficiency, particles size morphology, zeta potential and *in vitro* drug release, batch L3 was selected as an optimum formulation. Thus nanoparticles of losartan potassium with core: coat ration 1:3 was found to be spherical, discrete and free

flowing and able to sustained drug release effectively. Data are shown in Figure 5.

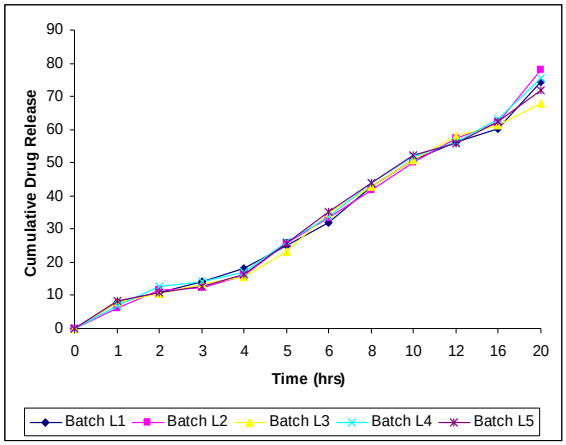

Figure 5: Cumulative percentage release of Losartan Nanoparticles

Analysis of the DSC profiles obtained for pure Losartan potassium suggests no interaction between the drug and excipients used in study. Fig 5 shows the endothermic peak at 22^0C ±0.98^0C with an enthalpy value of 79.9 mj/mg for pure Losartan potassium. An endothermic peak for Chitosan polymer (B) was not observed in the scanning temperature range. A single endothermic peak at 22^0C ±1.12^0C for ethyl cellulose (B) with an enthalpy value of 28.5 mj/mg was observed for Losartan potassium, which suggests no interaction between the drug mixtures [29-30].

CONCLUSION

Chitosan negative ions were cross linked with TPP positive ions which results in formation Chitosan- TPP complex via ionic gelation, results in formation of nanoparticles for sustained release. The resultant nanoparticle shows sustained release for long duration. The formulation (L3) shows better characteristics than other formulations. DSC study explains that there is no any chemical interaction between the drug and polymer. The drug entrapment efficiency of Chitosan- TPP complex shows 87.5 % which is better than other prepared formulation.

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