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Research Article

FORMULATION OF NANOPARTICLES OF EPROSARTAN MESYLATE FOR THE BETTER DRUG DELIVERY BY IMPROVING SOLUBILITY

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ABSTRACT

Objective: In the present study, we have made an attempt to the developed formulation of nanoparticles (NPs) of eprosartan mesylate (EM) incorporated in carboxymethyl chitosan using reverse micelle technique for the better drug delivery by improving solubility.

Methods: The NPs size and morphology were investigated by high-resolution transmission electron microscopy and field emission scanning electron microscopy, respectively. The physical and chemical aggregation state of eprosartan was analyzed using ultraviolet spectroscopy, and Fourier transforms infrared spectroscopy.

Results: To increase the solubility of eprosartan by reverse micelle technique of the drug through polymeric NPs is an alternative efficient, option for increasing the solubility. Eprosartan nanosuspension was successfully formulated for dissolution and bioavailability enhancement of the drug. The percentage drug release pattern of both formulations was compared against that of pure drug. It shows that in 10 min 39% and 17% of drug was released from the NPs made by RM method and microemulsion method, respectively, as compared to that of 1.3% of the pure drug. In 50 min almost more than half 51% of the drug was released from NPs by microemulsion method whereas only 2.5% of the drug was released from NPs containing the pure drug. In 120 min 67% of the drug was released from NPs by microemulsion method whereas only 5.8% of drug release was shown by NPs with the pure drug. We are paying attention on evaluating the influence of particle size and crystalline state on the *in vitro* performance of eprosartan.

Conclusion: In summary, we have developed a new approach toward the delivery of poorly water-soluble drug eprosartan by reverse micellar method. The particle size of NPs obtained by the reverse micellar method was significantly reduced as compared to the other method.

Keywords: Eprosartan, Nanoparticle, Carboxymethyl chitosan, Reverse micellar method.

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INTRODUCTION

Eprosartan mesylate (EM) is an "angiotensin II receptor antagonist" which is used for treating high blood pressure. As compared to other angiotensin II receptor antagonists, eprosartan is reported to be better tolerated than enalapril [1]. EM antagonizes the effect of angiotensin II (vasoconstriction and aldosterone secretion) by blocking the angiotensin II receptor in vascular smooth muscle and the adrenal gland producing decreased blood pressure [2]. It is a BCS Class II, insoluble in water, antihypertensive drug having 13% oral bioavailability [3,4]. It has been observed and proved that the bioavailability of poorly soluble drug improves by nanonization, following the production of nanoparticles (NPs) by Sucker *et al.* in 1980, the nanosizing has gained a lot of attention [5-14]. According to Noyes Whitney equation, it has been observed experimentally that the reduction in particle size of a sparingly soluble material results in an increased rate of solution. Noyes Whitney is regularly used to describe the process of dissolution of solid drugs:

$$Dm/dt=DA (C_s-C)/h$$

Where the rate of change of mass dissolved (m) with time (t) is related to the diffusion coefficient (D) through a static layer of liquid of thickness h, and C_s is the equilibrium solubility and the amount dissolved at time t (C) in that an increase in the surface area of a drug will result in a more rapid dissolution process, particularly under sink conditions (where $C << C_s$) [15].

The concept of reducing particle size to nanorange and comparing two different methods for the preparation of NPs has been explored in this experiment. Ionic gelation method and reverse micellar method were compared for the preparation of NPs, and their effect on particle size and hence solubility has been determined. Chitosan NP prepared by ionotropic gelation technique was first reported by Calvo and has been widely examined and developed by Janes. The electrostatic interaction between amine group of chitosan and negatively charge group of polyanion such as tripolyphosphate is the mechanism on which formation of chitosan NP is based. This technique offers a simple and mild preparation method in the aqueous environment. First, chitosan can be dissolved in acetic acid in the absence or presence of stabilizing agents, such as poloxamer, which can be added in the chitosan solution before or after the addition of polyanion. Polyanion or anionic polymers were then added, and NPs were spontaneously formed under mechanical stirring at room temperature. The size and surface charge of particles can be modified by varying the ratio of chitosan and stabilizer. Chitosan NP prepared by microemulsion technique was first developed by Maitra. This technique is based on the formation of chitosan NP in the aqueous core of reverse micellar droplets and subsequently cross-linked through glutaraldehyde. This technique offers a narrow size distribution of <100 nm, and the particle size can be controlled by varying the amount of glutaraldehyde that alters the degree of cross-linking. Nevertheless, some disadvantages exist such as the use of organic solvent, time-consuming preparation process, and complexity in the washing step [16].

METHODS

Drugs and chemical

Chitosan (CS) (Mw = 100,000-300,000, deacetylation degree ≥ 90% as determined by free amine groups) and monochloroacetic acid were

acquired from Sigma-Aldrich. Calcium chloride, isopropanol, methanol, and ethanol were obtained from Merck India; water was Millipore water. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and EM were purchased from Sigma-Aldrich, USA. Dimethyl sulfoxide and N, N˙-dimethylformamide were obtained from Merck India and purified by vacuum distillation. Fetal bovine serum and minimum essential medium were procured from Hyclone, USA, and HiMedia, India, respectively. Chitosan supplied by HiMedia laboratories; Isooctane supplied by Sigma Laboratories, Hexanol supplied by SD-fine chemicals, and cetyltrimethylammonium bromide (CTAB) supplied by SD-fine chemicals. All Chemicals are used without further purification.

Preparation of carboxymethyl chitosan (CMC)

CMC was synthesized by our formerly reported protocol with a slight modification. Briefly, 1.0 g of chitosan is swelled in 40 ml of 50% sodium hydroxide solution at 0°C overnight. Then, the chitosan is washed with isopropanol and dissolved in 30 ml of isopropanol and the monochloracetic acid (3.0 g) was dissolved in isopropanol (5 ml) and added into the reaction mixture dropwise for 20 min and reacted for 12 h at the 40°C. Then, the reaction was stopped by adding 70% ethyl alcohol (25 ml). The solid was filtered and rinsed in 70–90% ethyl alcohol, and vacuum dried at room temperature. The resulted product was the sodium salt of CMC [17].

Preparation of CMC NPs by ionic gelation method

CMC NPs were synthesized by ion tropic gelation of CMC solution with calcium chloride solution. About 0.5% CMC solution was prepared in distilled water. To 5 ml of this solution, 1 ml of 1.5% $CaCl_2$ solution was added under constant stirring. The resulting NPs were purified by centrifugation for 10 min at 15,000 rpm and lyophilized [18]. A diagrammatical representation is given in Fig. 1 for better insight into the development of NCs.

Drug loading into CMC NPs

EM (1 mg/mL) is poorly water-soluble drug was dissolved in 0.05% 10 ml glacial acetic acid solution. 10 mg CMC NPs were (per ml CMC NPs) introduced into the previously prepared drug solutions. The solution was shaken in an orbital shaker for 24 h. The suspension was then centrifuged at 15,000 rpm min to separate the NPs from the solutions. The procedure was repeated 3 times to eliminate any unloaded eprosartan from the suspension. The amount of loaded eprosartan was measured spectrophotometrically at 234 nm with a UV-1700 spectrophotometer (Shimadzu). The drug loading content and entrapment efficiency were calculated according to the following equations.

$$\text{Drug loading contents } (\%) = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of nanoparticles taken}} \times 100$$

$$\text{Drug entrapment efficiency } (\%) = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of nanoparticles taken}} \times 100$$

Preparation of CS NPs using microemulsion/reverse micelle technique

Eprosartan loaded chitosan NPs were prepared using cetyltrimethylammonium bromide as a surfactant, isooctane as oil, 1-hexanol as cosolvent, eprosartan (concentration 5 mg/ml) as the model drug, and chitosan-eprosartan solution as the aqueous phase. To prepare the reversed micelles, CTAB (used in a concentration of 5 mg/ml), isooctane oil, and cosolvent were poured into a test tube. The chitosan (in concentration of 5 mg/ml) in acetic acid solution, glutaraldehyde and eprosartan were then added to the mixture of CTAB and solvent under continuous stirring at room temperature.

NPs were formed in the presence of a surfactant. The system was stirred overnight to complete the cross-linking process, which the free amine group of chitosan conjugates with glutaraldehyde. The organic solvent is then removed by evaporation under low pressure. The formation of reversed micelles was inferred the mixed emulsion became transparent or semi-transparent. The completion of the reaction was recognized when the reverse micelle solvent becomes turbid. The yields obtained were the cross-linked chitosan NP and excess surfactant. The excess surfactant was then removed by precipitate with $CaCl_2$ and then the precipitant was removed by centrifugation at 6000 rpm for 15 min. Finally, the obtained NPs were dried for 24 h in the air at the laboratory ambient temperature.

The eprosartan loaded chitosan NPs obtained from both the process were collected and compared for particle size, particle size distribution, zeta potential, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and dissolution studies.

Characterization

Particle size and zeta potential measurements

Mean particle size and its distribution were determined using dynamic light scattering (DLS) technique. Zeta potential was evaluated by measuring electrophoretic mobility of the particle using a laser-based multiple angle electrophoresis analyzer. Eprosartan NPs were sufficiently diluted with deionized water to reduce the count rate up to 100–250 kcps and analyzed by employing Malvern Zetasizer (Nano ZS, Malvern Instruments, UK). Observations were recorded in triplicate for each sample at 25°C±1°C.

Particle size measurement by SEM and TEM

Particle's morphological characterization of eprosartan-NPs was executed by SEM and TEM. Samples for SEM studies were prepared by clinging the NPs on a double adhesive tape stuck to an aluminum stub and were coated with gold and palladium under an argon atmosphere utilizing a high-vacuum evaporator (Polaron SEM coating system). Samples were scanned through an electron microscope (EVO-50, ZEISS; UK) and photomicrographs were captured.

For TEM, a drop of optimized NPs suspension in aqueous solution was placed on a carbon film coated on a copper grid and freeze-dried. Then, NPs were observed at 80 kV using TEM TECNAI G^2 20 S-TWIN (FEI, the Netherlands) instrument, Indian Institute of Toxicology Research, Lucknow.

UV and Fourier transform infrared (FTIR) studies of eprosartan CS NPs

The physical and chemical aggregation state of eprosartan was evaluated by UV and FTIR spectroscopy. The FTIR spectrums of eprosartan and drug-loaded chitosan NPs (freeze-dried) were obtained using spectrometer (Perkin Elmer). A total of 2 mg of sample was mixed with 100 mg of dry potassium bromide (KBr), and the mixture was ground into fine powder using a mortar before compressing into (KBr) disk under a hydraulic press at 10,000 psi. Each KBr disk was then scanned at 4 mm/s at a resolution of 2/cm over a wave number region of 400–4000/cm using IR solution (software ver. 1.10). The characteristics peaks of functional groups in the drug samples were determined and compared with pharmacopeial standards, and the

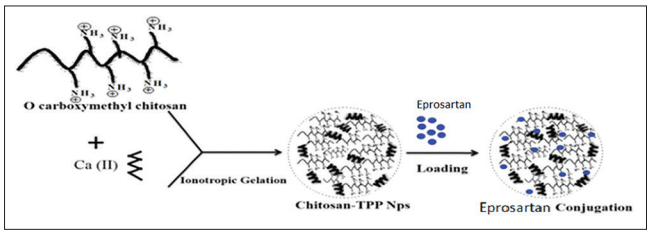

Fig. 1: Schematic representation of synthetic procedure of carboxymethyl chitosan-eprosartan

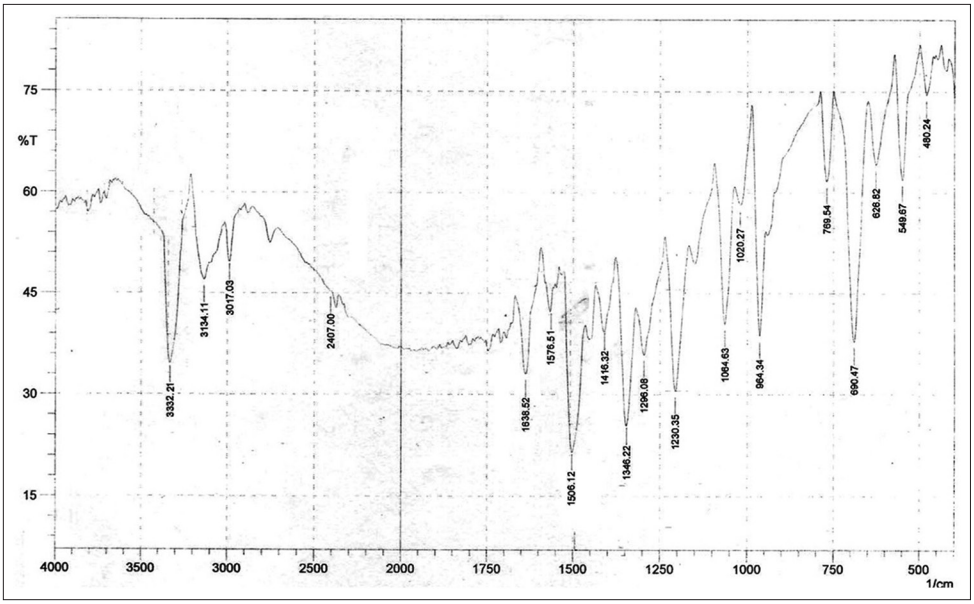

Fig. 2: Fourier transforms infrared spectrum of eprosartan mesylate

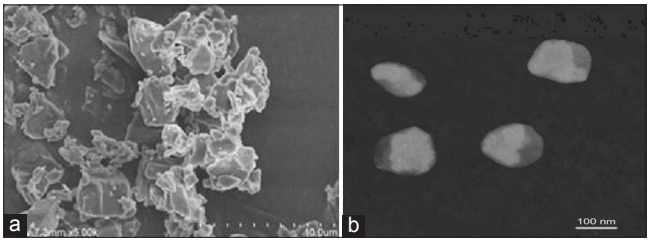

Fig. 3: (a) Scanning electron microscopy of eprosartan nanoparticles; (b) transmission electron microscopy of eprosartan nanoparticles

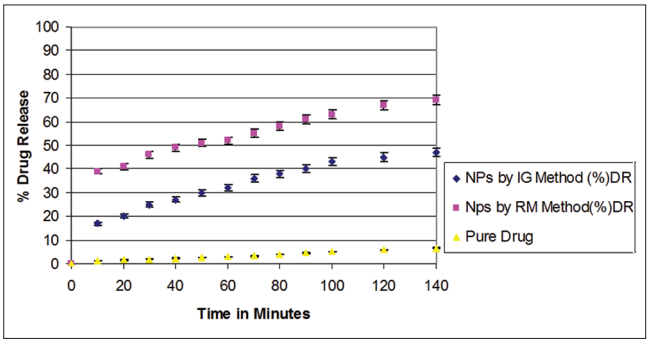

Fig. 4: *In vitro* release studies of eprosartan nanoparticles

possible interactions between drug and excipients were analyzed by obtained data. The electronic configuration of eprosartan NPs was elucidated by a UV spectrophotometer (UV 1700, Shimadzu, Japan). Lyophilized DTX-NCs were dispersed in TDW (3.0 mL) and scanned in the range from 200 to 400 nm. The UV spectrum of pure eprosartan was taken after solubilizing it in methanol.

Evaluation studies

Dissolution studies

In vitro drug release study for optimized formulations of eprosartan loaded polymeric NPs was conducted in triplicate by equilibrium dialysis membrane method. The amount of drug release from the formulation was measured by a spectroscopic method. The experiment was performed by taking the specified volume of formulation in hermetically sealed dialysis membrane. The dialysis bag is suspended in pH 7.4 phosphate buffer saline (PBS) at 37°C being stirred at 240 rpm speed. Samples were collected at predetermined time points, and subsequently, media were replenished with pH 7.4 PBS after each sampling to maintain sink conditions.

RESULTS AND DISCUSSION

FTIR analysis

FTIR study was conducted to characterize any possible interaction between drug and CMCS NPs. The characteristic peaks at 3332.21 and 3134.11/cm (O–H stretching and N–H stretching vibrations), 2407.00/cm (C=N stretching vibration), and 1638.52/cm (corresponding to the C=C) confirm the formation of CMCS, as shown in Fig. 2.

DLS

The hydrodynamic diameter of eprosartan chitosan NPs prepared by reverse micellar technique is measured by DLS which is 80±10 nm, whereas the eprosartan chitosan NPs prepared by ionic gelation method were 450±10 nm. Results represent that there is a significant difference between the two types of NPs, which demonstrate that the NPs prepared by reverse micellar technique were significantly smaller in particle size. Zeta potential α is an electric potential of the shear plane attached to the moving particles in the medium, and it is generally utilized for the prediction of stability of nanosuspensions. The decrease in zeta potential value with a decrease in concentration of stabilizer may be attributed to the inability of stabilizer to prevent particle agglomeration at lower concentrations. The results of the optimized batches of eprosartan-loaded nanosuspension prepared from both the processes depicted the zeta potential values of 35 mV and 50 mV of eprosartan chitosan NPs by ionic gelation process and microemulsion process, respectively, which suggested physically stable formulations were developed.

Particle size measurement by SEM and TEM (Fig. 3)

The SEM study reveals that the surface of the NPs was smooth and no aggregate were found from the sample used for SEM analysis. The particle size analysis done by TEM shows that the average particle size of the NPs was found to be 450 and 100 nm by ionic gelation and reverse micellar technique respectively. The study results are shown in Fig. 3.

Dissolution studies

The *in vitro* drug release pattern of NPs prepared by both the method against the pure drug is shown in Fig. 4. The solubility of the pure drug is very low because of which <10% drug is released in 140 min.

According to the drug release pattern of NPs by IG method, there is a significant increase in percentage drug release as compared to the pure drug while the percentage drug release of NPs by RM method is increased significantly as compared to that of IG method. This increase in percentage drug release could be attributed to the increase in solubility of the drug when formulated as NPs. The percentage drug release pattern of both the NPs shows that the NPs prepared by RM method have increased solubility as compared to the IG method. The reason behind the enhancement in the solubility could be the reduction in particle size. According to Noyes Whitney equation, it has been observed experimentally that the reduction in particle size of a sparingly soluble material results in an increased rate of solution.

Drug release pattern

The percentage drug release pattern of both formulations was compared against that of pure drug. It shows that in 10 min 39% and 17% of the drug was released from the NPs made by RM method and microemulsion method, respectively, as compared to that of 1.3% of the pure drug. In 50 min almost more than half 51% of the drug was released from NPs by microemulsion method whereas only 2.5% of the drug was released from NPs containing pure drug. In 120 min 67% of the drug was released from NPs by microemulsion method whereas only 5.8% of drug release was shown by NPs with the pure drug.

CONCLUSION

The Chitosan NPs prepared by reverse micellar method were found to be more promising then ionic gelation method. The particle size of NPs obtained by the reverse micellar method was significantly reduced as compared to the other method. The reverse micellar method was found to be the best method for the preparation of eprosartan loaded chitosan NPs.

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AUTHOR'S CONTRIBUTIONS

UY, NH, and QR planned the study. UY and QR designed the research protocol. UY compiled the data. UY and QR formulate and interpreted the relevant data. UY drafted the manuscript. UY, NH, and QR critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. UY, NH, and QR are guarantors of the paper.

CONFLICTS OF INTEREST

None of the author has any conflicts of interest in the context of this work.

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