AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Formulation and Characterization of Polysorbate 80 Coated Chitosan Nanoparticles of Serratiopeptidase

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ABSTRACT

Nanoparticles act as a promising system for targeted delivery of drugs and as an effective route of drug administration. In this study, polysorbate 80 coated nanoparticles of serratiopeptidase were formulated and aimed for the treatment of blood clots in brain. Serratiopeptidase exerts effective activity against blood clotting and has ability to dissolve non-living tissues, blood clots, cysts, atherosclerotic clots. Different nanoparticle formulations of serratiopeptidase were prepared with different concentrations of chitosan and tripolyphosphate using ionic gelation method. The nanoparticles were coated using polysorbate 80 and were characterized and evaluated for different parameters such as particle size, entrapment efficiency, zeta potential and transmission electron microscopy. The *in vitro* drug release of prepared nanoparticles was studied in phosphate buffer (pH 7.4). The results indicated that the developed nanoparticle formulation could be established as a promising carrier for active targeting into brain to dissolve blood clots.

Keywords: Serratiopeptidase, chitosan, tripolyphosphate, nanoparticles, polysorbate 80, ionic gelation method.

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Received 14 March 2016, Accepted 15 May 2016

Please cite this article as: Sharma K *et al*., Formulation and Characterization of Polysorbate 80 Coated Chitosan Nanoparticles of Serratiopeptidase. American Journal of PharmTech Research 2016.

INTRODUCTION

Development of novel and effective formulations for drug delivery to brain has become a necessity, due to poor delivery of drugs by conventional formulations. One of the most promising technologies for brain delivery of drug is colloidal carriers which aid to enhance the permeability and bioavailability of drugs[1]. Nanoparticles can be used for drug delivery into brain to protect drug from degradation and prolonging blood circulation. Nanopartricles can cross blood brain barrier (BBB). The modern approaches such as ligand binding and nanotechnology are being used for brain targeting of drug and can be used as potential carriers for brain[2].

Serratiopeptidase is an enzyme derived from *enterobacteria Serratia* E 15. It is having fibrinolytic, anti-inflammatory and anti-oedemic activity. It is a bacterial enzyme used for various pharmacological activities such as reduction of pain and inflammation e.g. in arthritis[3] . Because of its anti-inflammatory and fibrinolytic activity it may be possibly used for removal of blood clots from brain in case of brain injury.

In this study ionic gelation method is used to prepare nanoparticles[4]. For achieving maximum drug entrapment in the nanoparticles, the chitosan : tripolyphosphate (TPP) ratios selected between range of 3:1 to 6:1[5]. This method has many advantages such as the use of polymers as carriers of drug because they are biocompatible and biodegradable. Chitosan contains certain cations on its chemical structure which form meshwork structure by combining with TPP counter ions and cross linking will induce gelation. This polymer also acts as rate retardant of drug release in controlled release formulations[6]. Chitosan has ability to open epithelial tight junctions for increasing the paracellular transport of macromolecular drug, TPP is a polyanion cross linker in polysaccharide based drug delivery[4]. Nanoparticles coated with polysorbate 80 increase the permeation across BBB and acts as efficient brain delivery carriers[7]. It is reported to cause BBB disruption and provide successful delivery of drug into the brain[8].

MATERIALS AND METHOD

Materials

Serratiopeptidase was kindly gifted by Spanker Biotech Ltd. Solan. All other chemicals used in present study such as chitosan, Tri poly phosphate, polysorbate 80, acetic acid, disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, HCl, sodium hydroxide were purchased from LOBA chemicals Pvt. Ltd. Mumbai, India.

Method

Preparation of Polysorbate 80 coated serratiopeptidase nanoparticles

Nanoparticles were prepared by using ionic gelation technique[4]. In this method two phases were prepared one was acidic phase having chitosan (0.1%) in aqueous acetic acid and other was basic phase having TPP (0.1%). Nanoparticles were formed by drop by drop addition of basic phase (TPP) into acidic phase (chitosan) at room temperature with continuous stirring at 1000 rpm for 60 minutes resulting in intramolecular and intermolecular linkages created between phosphate groups of TPP and amino groups of chitosan. The 1:1 ratio of polymer and drug was used. After addition of drug stirring was done for 1 hr. at 4000 rpm at room temperature. Then centrifugation was done for 30 minutes at 12000 rpm to remove the unbounded drug from the drug entrapped in prepared nanoparticles[9,10]. Different ratios of chitosan : TPP polymer were prepared ranging from 3:1 to 6:1 and 10 mg drug was incorporated into it.

Characterization of the prepared chitosan nanoparticles

Particle size and size distribution

Particle size and size distribution was determined by using zetasizer (Beckman Coulter). The samples were placed in clear disposable zeta cells and results were recorded. The Poly dispersity index (PDI) was observed to determine particle size distribution Among all the different formulations, the formulation which is having the minimum particle size was selected for further studies[11].

Entrapment efficiency

The nanoparticle suspension was centrifuged at 12000 rpm for 30 minutes at room temperature to separate the free drug in the supernatant. The absorbance was checked by UV spectrophotometer (Shimadzu Co Ltd. Japan) at 252 nm[12].The amount of unentrapped drug (supernatant) was calculated. The amount of entrapped drug was determined by subtracting free unentrapped drug from total drug amount taken[13]. Then, Percent drug entrapment efficiency was calculated from formula given

% Entrapment efficiency = Total drug amount – Free entrapped drug/ Total drug amount× 100

Zeta potential

Zeta potential represents the characteristics of the electronic properties of the formulation and is used as a method to assess the stability of prepared formulations. The zeta potential of the drug loaded nanoparticles was measured using zetasizer by placing the sample in clear disposable zeta cells[4] .

Transmission electron microscopy

The structure of the optimized formulation was observed with the help of transmission electron microscopy (TEM). One drop of sample was put on a carbon coated grid (200 mesh) for few minutes and removed the excess solution by filter paper. One drop of phosphotungstic acid (1.0%) was stratified for staining. The extra phosphotungstic acid on the sample was removed by filter paper and observed after drying for 30 min at room temperature. The grid was observed by TEM and by using imaging viewer software. The images were analyzed and captured[3].

In vitro drug release

In vitro drug release was determined by dialysis bag diffusion technique using a dialysis membrane[14]. An accurately weighed amount of nanoparticles equivalent to 10 mg of drug was transferred to dialysis bag and sealed. The sealed bag was then suspended in a beaker containing 100 ml of phosphate buffer pH 7.4 and stirred at a constant speed at 37^0C. Aliquots were withdrawn at preselected time intervals up to 12 hours from receiver compartment and replaced with an equal volume of fresh medium to maintain a sink condition. The samples were analyzed by UV spectrophotometer, against phosphate buffer (pH 7.4) blank, at 252 nm[15,16,17]. The result obtained from *in vitro* permeability studies were treated by different mathematical models (zero-order, 1st-order, higuchi, korsmeyer-peppas and hixson-crowell) to check the drug release kinetics from the prepared nanoparticles. Selection of a suitable model was done on the basis of R^2 (correlation coefficient) values calculated from the mathematical data [18,19].

RESULTS AND DISCUSSION

Serratiopeptidase nanoparticles were prepared using different concentrations of chitosan and TPP (Table 1). The prepared formulations were characterized to determine the effect of different ratios used on particle size, zeta potential and entrapment efficiency. All formulations were found to be in nanorange. Particle size was found to be minimum in 4:1 polymer ratio. On further increasing the concentration of chitosan with respect to TPP the particle size was found to increase significantly (Table 2). TPP acts as cross linking and condensing agent, which forms hydrogen bonds with free amine groups on both SER and CS molecules and hence leads to the formation of compact nanoparticles. The formulation F3 prepared by taking 4:1 polymer ratio of chitosan and TPP was found to have minimum particle size and

maximum entrapment efficiency. The zeta potential of the selected formulation was found to be within acceptable limits (+61±0.03). So, it was selected for further studies.

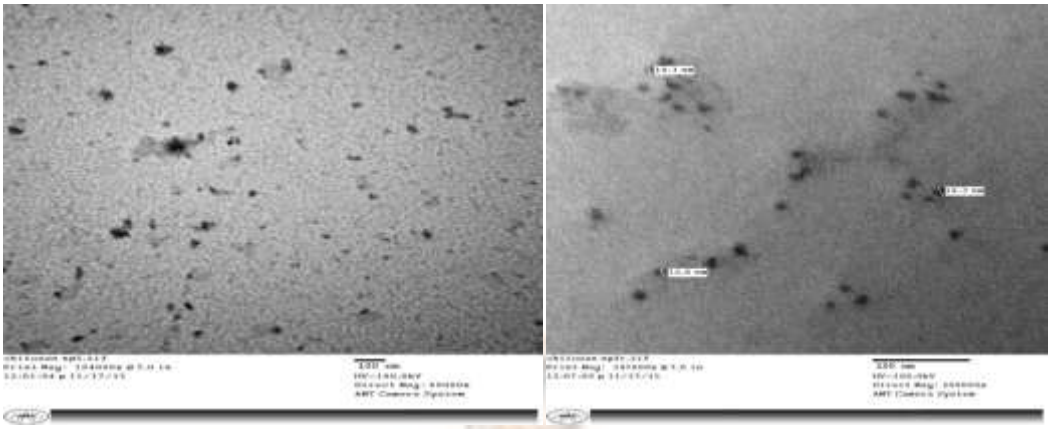

Figure 1(a) and (b): TEM images of polysorbate 80 coated chitosan nanoparticles loaded with drug at 60,000X and 20,000X.

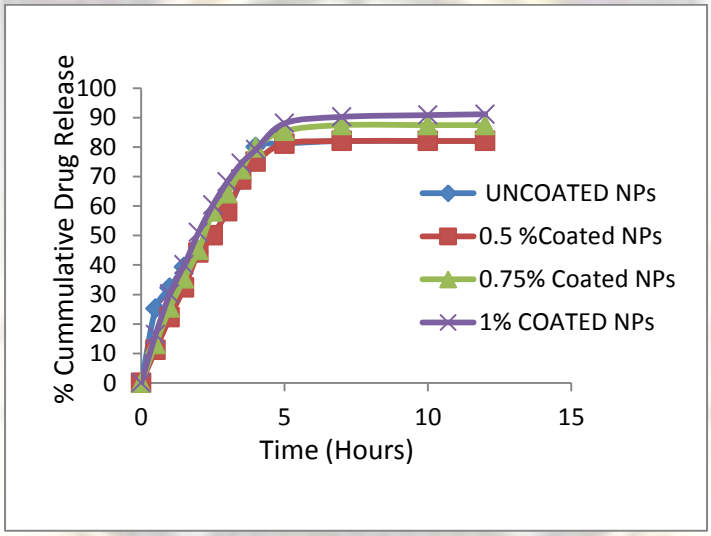

Figure 2: *In vitro* drug release studyof uncoated NPs & coated NPs (0.5%, 0.75% and 1.0%)

Table 1: Composition of prepared formulations.

S.no	Formulation	Drug	Chitosan (0.1%)	TPP (0.1%)	Polysorbate 80
1	F1	10 mg	30 ml	10 ml	-
2	F2	10 mg	35 ml	10 ml	-
3	F3	10 mg	40 ml	10 ml	-
4	F4	10 mg	45 ml	10 ml	-
5	F5	10 mg	50 ml	10 ml	-
6	F6	10 mg	55 ml	10 ml	-
7	F7	10 mg	60 ml	10ml	-
8	F8	10 mg	40 ml	10ml	0.50%
9	F9		40 ml	10 ml	0.75%
10	F10		40 ml	10 ml	1.0%

Table 2: Particle size and entrapment efficiency of nanoparticles prepared using different ratios of polymer.

S.No	Formulation Code	Particle Size (nm)	% Entrapment efficiency
1	F1 (3.0:1)	180.0 ± 0.05	61.0% ± 0.05%
2	F2 (3.5:1)	211.6 ± 0.02	35.4% ± 0.04%
3	**F3 (4.0:1)**	**173.8 ± 0.01**	**73.7% ± 0.01%**
4	F4 (4.5:1)	196.7 ± 0.03	50.7% ± 0.03%
5	F5(5.0:1)	178.5 ± 0.02	73.1% ± 0.02%
6	F6 (5.5:1)	183.2 ± 0.04	59.2% ± 0.04%
7	F7 (6.0:1)	190.5 ± 0.05	57.5% ± 0.05%

Table 3: Particle size of formulations having different concentrations of polysorbate 80.

S.No	Formulation	Polysorbate 80	Particle Size (nm)	% Drug release
1	F8 (4.0:1)	0.5%	245.2 ± 0.03	82.3%
2	F9 (4.0:1)	0.75%	258.5 ± 0.02	87.5%
3	**F10 (4.0:1)**	**1.0%**	**239.4 ± 0.01**	**91.55%**

Table 4 : Kinetic parameters of drug release from prepared formulation (F10).

S.no.	Mathematical model	R2 value
1	Zero order	0.844
2	First order	0.931
3	Higuchi	0.986
4	Korsmeyer-Peppas	0.956
5	Hixson – Crowell	0.979

Preparation of polysorbate 80 coated nanoparticles

The selected formulation (F3) was coated using different concentrations of polysorbate 80 and the effect of coating on the particle size was determined (Table 3).

Characterization of polysorbate 80 coated nanoparticles

Particle size

F10 formulation coated with 1.0% polysorbate 80 was found to have minimum particle size. So, it was selected for further studies.

Transmission electron microscopy

The surface morphology of NP formulation (F10) was characterized using TEM (Figure 1(a) and 1(b).The nanoparticles appeared non-aggregated, spherical in shape and found to be within nanorange.

***In vitro* drug release**

The formulation (F10) was subjected to *in vitro* drug release across cellophane membrane (Figure 2). At pH 7.4, the nanoparticles are swollen to a great extent, showing fast release of drug. The nanoparticle formulation has release of 80% within the initial 5 hours followed by sustained drug release up to 12 hours. The burst release could be due to the

release of drug absorbed or present on the surface of the nanoparticles which released immediately on contact with dissolution media [20]. After initial 5 hours the drug release extended upto 12 hrs. Coated nanoparticles has higher percentage cumulative release which was maintained upto 80% for 12 hours. Also the effect of different concentrations of polysorbate 80 on drug release was studied. It was found that as the concentration of polysorbate 80 is increased the drug release also increases (Figure 2)

To study the release kinetics various kinetic models including zero-order, 1st-order, higuchi, korsmeyer-peppas and hixson-crowell were applied (Table 4). The R^2 value of the higuchi model was highest indicating that drug release from nanoparticles is following diffusion mechanism[19,21].

CONCLUSION

The serratiopeptidase loaded chitosan nanoparticles were prepared using different ratios of polymers and optimized on the basis of entrapment efficiency, particle size and zeta potential. Formulation (F3) having maximum entrapment efficiency, minimum particle size and optimum zeta potential value was selected, that were found to be 173.8 ± 0.01%, 73.7% ± 0.01 nm and +61.22 mv ± 0.03 respectively. The selected nanoparticle formulation was coated with polysorbate 80 and the effect of surfactant coating on particle size and drug release was evaluated. Formulation (F10) prepared using 1.0% surfactant concentration for coating showing optimum particle size and drug release was selected and analyzed by TEM. The *in vitro* studies indicated that the polysorbate coated nanoparticles are able to release about 87% of drug within initial 5 hours following a slow release during the 12 hours study. The release kinetics was found to follow higuchi model indicating diffusion dependent mechanism of drug release. The results show the potential of prepared nanoparticles as carrier for effective transport and drug release and could be further studied for their brain targeting property using a suitable animal model.

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