Research Article

Design and Characterization of Trehalose Coated Aquasomes Loaden with Ciprofloxacin

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ABSTRACT

Aquasome is a submicronic structure (diameter below 300 nm) made up with carbohydrates. They received much attention to develop a drug delivery system as an alternative to liposome technology in order to overcome the problems related to the stability of these vesicles in biological fluids. The carbohydrate coating functions as a dehydroprotectant and stabilizes subsequently non-covalently bound drug molecule. Ciprofloxacin is an antibiotic agent in the fluoroquinolone class used to treat bacterial infections such as lung infection, typhoid, urinary tract infections and pneumonia. In the present study aquasomes was charged with ciprofloxacin to enhance its stability and solubility were obtained through the formation of an inorganic core of calcium phosphate covered with trehalose film and further adsorption of the ciprofloxacin. The Aquasomes were prepared by inorganic co-precipitation method. The Aquasomal suspension is subjected to lyophilization. The prepared aquasomes were evaluated for the different parameters such as SEM, FTIR, XRD, Zeta potential, Particle size, Drug entrapment efficiency and In vitro drug release. The aquasomes were showed definite characteristic structure and size range of around 100 nm. The formulation Showed smaller particle size of 161.9 nm. Coating of trehalose on the surface of core was further confirmed by zeta potential measurement. It was noted that, the zeta potential of coated particle -18.7 mv. The release of drug from aquasome is about 88.254 % over a period of 5 hours. The aquasome shows a loading efficacy up to 93.5%. Thus, aquasomes of ciprofloxacin were successfully developed.

Keywords: Ciprofloxacin, Trehalose, Aquasomes, Lyophilization, Ceramic core.

INTRODUCTION

One of the most recently created delivery methods for poorly water-soluble drugs, bioactive molecules such as peptides and proteins, drugs classified as BCS II and IV, hormones, antigens, genes, and vaccines to specific areas is the use of aquasomes. Because of their water-like qualities that shield and maintain delicate organic components, aquasomes are also known as "water bodies".[1] Aquasomes have a spherical form and range in size from 60 to 300 nm. Aquasomes are nanoparticulate carrier systems; however, they are three-layered self-assemble structures rather than simple nanoparticles. They are made of a solid phase nanocrystalline core coated in an oligomeric film, to which biochemically active molecules are adsorbed, either fully or partially. These structures self-assemble by ionic and non-covalent bonding. The structural stability is provided by the solid core, and the carbohydrate coating protects against dehydration and stabilize the biochemically active molecules.[2]

Aquasomes are mainly developed as drug delivery system as an alternative to liposome in order to over-come the problems related to the stability of these vesicles in biological fluids and during storage. Nanoparticles were first designed using albumin and non-biodegradable synthetic polymer such as polyacrylamide and poly methyl methacrylate.[2] Within the last decade diverse technological strategies have been proposed in order to obtain nanoparticles, of a distinct nature, charged with drugs which in turn have revolutionized the systems of drug administration, particularly those of controlled liberation and the one oriented at the vectoring of the active principle for liberation of target tissue or organs.[3] In case of liver disease therapy, there has been growing interest in the area of liver-cell specific drug delivery systems in recent years. This may be due to a failure of other pharmacological approaches to the liver disease site as a result of the nonspecific delivery mechanism towards the other organs and several side effects. A great deal of effort has been made to achieve an appropriate liver targeting of chemotherapeutic agents with liposomes.[4] Kossovsky proposed a system to prepare nanoparticles transporting the so-called aquasomes[5], whose particle size (lower than 1000 nm), is appropriate to parenterally administration because it prevents the obstruction into the bloodstream capillaries.[6]

Self-assembly implies that the constituent parts of some final product assume spontaneously prescribed structural orientations in two or three-dimensional space. The self-assembly of macromolecules in the aqueous environment, either for the purpose of creating smart nanostructure materials or in the course of naturally occurring biochemistry, is governed basically by three physicochemical processes: the interactions of charged groups, dehydration effects and structural stability.[7]

Aquasomes are nanoparticulate carrier system but instead of being simple nanoparticle these are three layered self-assembled structures, comprised of a solid phase nanocrystalline core coated with oligomeric film on which biochemically active molecules are adsorbed with or without modification.[8-9]

Ciprofloxacin is an antibiotic agent BCS class IV in the fluoroquinolone class used to treat bacterial infections such as urinary tract infections and pneumonia. Ciprofloxacin has FDA approval to treat urinary tract infections, sexually transmitted infections (gonorrhea and chancroid), skin, bone, joint infections, prostatitis, typhoid fever, gastrointestinal infections, lower respiratory tract infections, anthrax, plague and salmonellosis. In addition, ciprofloxacin is an appropriate treatment option in patients with mixed infections or patients with predisposing factors for Gram-negative infections. This activity covers ciprofloxacin, a broad-spectrum quinolone antibiotic that members of the interprofessional team need to review its indications, coverage, contraindications, and adverse event profile to optimally manage patients' infectious diseases.[10]

Fate of aquasomes: The drug delivery vehicle aquasome is colloidal range biodegradable nanoparticles, so that they will be more concentrated in liver and muscles. Since the drug is adsorbed on to the surface of the system without further surface modification, they may not find any difficulty in receptor recognition on the active site so that the pharmacological or biological activity can be achieved immediately. In normal system, the calcium phosphate is a biodegradable ceramic. Biodegradation of ceramic in vivo is achieved essentially by monocytes and multicellular cells called osteoclasts because they intervene first at the biomaterial implantation site during inflammatory reaction. Two types of phagocytosis were reported when cells come in contact with biomaterial; either calcium phosphate crystals were taken up alone and then dissolved in the cytoplasm after disappearance of the phagosome membrane or dissolution after formation of heterophagosomes. Phagocytosis of calcium phosphate coincided with autophagy and the accumulation of residual bodies in the cell.[9]

Application of aquasomes:

➢ In the delivery of poorly water-soluble drug, BCS class II and IV drug

➢ Insulin and Insulin mimetics delivery

➢ Delivery of enzymes, Delivery of antigens

➢ Delivery of gene, Delivery of Non-Protein Molecules. [10]

MATERIALS AND METHODS

Materials: Ciprofloxacin were procured from Yarrow Chem Product, Mumbai, Tikamgarh and Trehalose were procured from Research Lab Fine Chem Industries, Mumbai. Other required chemicals were provided by college lab store which were of laboratory and analytical grade.

Methods:

Formation of inorganic core: by co-precipitation technique by sonication A solution of disodium hydrogen phosphate (Na2→HPO4, 0.75 M) was steadily added to a solution of calcium chloride (CaCl2, 0.25M) in sonication / magnetic stirring at 2-4°C for 2hrs.The precipitate so obtained was isolated by centrifugation (15,000 rpm for 2 hours) and then washed multiple times with bi-distilled water to remove the sodium chloride formed during the reaction. The precipitate was redispersed in bi-distilled water and filtered using a 0.22 or 0.45 µm Millipore filter to obtain core particles of sizes less than 0.22 or 0.45 µm. A freeze drying / lyophilization technique was applied over the filter value.[2,8]

Coating of inorganic core: For sugar coating a sample of 100 mg of the inorganic cores was resuspended into 100 ml distilled water and was added to a 100 ml solution having concentration of (lactose 0.03M, cellobiose 0.06M, Trehalose 0.06M). Then the mixture was mechanically agitated for a period of 90 min for effective coating. After a period of 90 min of mechanical agitation the dispersions were filtered through a membrane filter (pore size 0.22 or 0.45µm) and then freeze dried.[2,11]

Drug loading: The final stage involves the loading of drug to the coated particles by adsorption. For that, a solution of known concentration of drug were prepared in suitable solvent then coated particles are dispersed into it. After that mechanical agitation, which was maintained for 90 min. Then dispersion was kept overnight at low temperature 2-4 ˚C for drug loading or lyophilized after mechanical agitation some time so as to obtain the drug-loaded formulation (i.e., aquasomes).[8,12]

Table 1: Formulation Table of Ciprofloxacin Loaded Aquasomes

Sr.No.	Ingredients	Batch A	Batch B	Batch C	Batch D	Batch E
1	Ciprofloxacin (mg)	40	40	40	40	40
2	Calcium chloride (gm)	1	1	1	1	1
3	Disodium hydrogen phosphate (gm)	1	1	1	1	1
4	Trehalose (gm)	1	1.5	2	2.5	3

Characterization of Aquasomes:

Scanning electron microscopy (SEM): Scanning electron microscopy (SEM JEOL, Japan) was used to examine the aquasomes morphology. In a first stage, Aquasomes formulation were applied to a 10 mm glass slide and dried overnight at room temperature in a vacuum desiccator till SEM examination was done. Aquasomes were mounted on appropriate support and coated with gold using a gold sputter module in a higher vacuum evaporator for analysis. At a voltage of 15 v, observations were made at various magnifications. [2,13]

X-ray diffraction (XRD): X-ray diffraction analysis was also performed to confirm the formation of the drug-phospholipid conjugate. In X-ray diffraction pattern the crystalline drugs demonstrate characteristic intense peaks while phospholipid which is amorphous, shows wide peaks. Due to the prevalence of the both free drug as well as phospholipids, the physical mixtures get both sharp as well as wide peaks. The production of a drug-phospholipid conjugate is indicated by the absence or depletion in the intensity of sharp peaks. [2,13]

Fourier transform infrared spectroscopy (FTIR): FTIR study was carried out to check compatibility of drug with excipients. Infrared spectrum of ceramic core calcium phosphate was determined on Fourier transform infrared spectrophotometer (Bruker) using KBr dispersion method. The spectrum of dried calcium phosphate was run followed by drug with excipients in the wavelength region between 4000 and 500 cm-1. [2,13]

Zeta potential: The zeta potential was determined using the Zeta sizer (HORIBA Zeta Sizer) and laser Doppler electrophoretic mobility measurements.[2,13]

Drug entrapment efficiency: Drug entrapment was determined using a method reported by Loukas and Gregoriadis . The various plain aquasome formulations (without drug coating) were incubated with the known concentration of drug for 24 hr at 4°C. The supernatant was separated after centrifugation at 15,000 rpm for 1 hr below 4°C in a refrigerated centrifuge (Remi Equipment Pvt. Ltd). The drug remaining in the supernatant liquid after loading was estimated by measuring absorbance at 276 nm using a spectrophotometer (Shimadzu, Japan UV-1800). Aquasomes was separated and the supernatant was scanned with a UV-visible spectrophotometer in this parameter 276 nm.[2,13] The drug entrapment efficiency was determined by using the relation in this equation,

$$\text{Drug entrapment efficiency (\%)} = \frac{Actual\ loaded\ drug}{\text{Theoratical drug loaded}} \times 100$$

$$\text{Drug Loading (\%)} = \frac{\text{Total added drug} - \text{wt. of unentrapped drug}}{\text{Weight of aquasomes}} x\ 100$$

Particle size: The particle sizes of ciprofloxacin aquasome were assessed using a laser diffraction technique (HORIBA Zeta Sizer), which can measure samples in the range of 0.05-20,000 nm. [2,13]

***In-vitro* drug release studies:** *In-vitro* release was evaluated using a dialysis bag technique. 40 mg prepared aquasomes were subjected to in vitro analysis in phosphate buffer 10 ml (pH7.4) then add in dialysis bag. Phosphate buffer pH 7.4 solution 300 ml add in dissolution apparatus maintain at 37±0.5°c and stirred continually at 100 rpm using sigma dialysis sack. At different time interval (1hr, 2hr,3hr,4hr,5hr) 5ml of sample were withdrawn and drug contain was determine spectrophotometricaly at 276 nm (Shimadzu, Japan UV-1800) using the simultaneous estimation method. [2,13]

RESULTS AND DISCUSSIONS

Scanning Electron Microscopy:

Scanning electron microscopy (SEM JEOL) was carried out to check the morphological changes of ceramic core. The ceramic core powder with assymetric morphological structure and size range around 60 nm to 300 nm. The ceramic core was showed definite characteristic structure and size range of around 100 nm. [15]

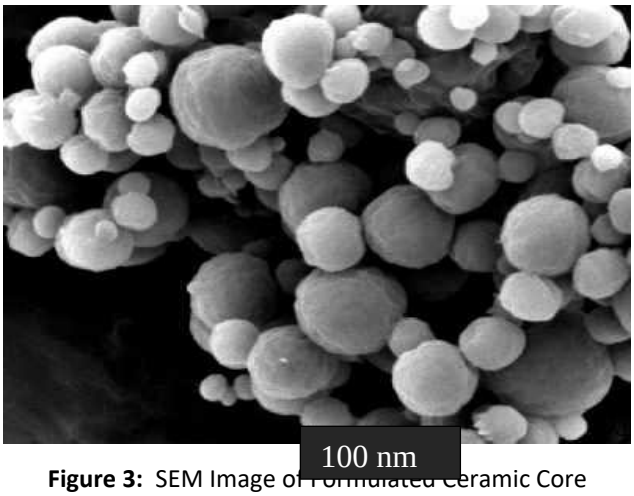

Figure 3: SEM Image of Formulated Ceramic Core

X-Ray Diffraction Study:

X-ray diffractogram of ceramic core exhibits several sharp peaks of different intensities between 10° and 60°.

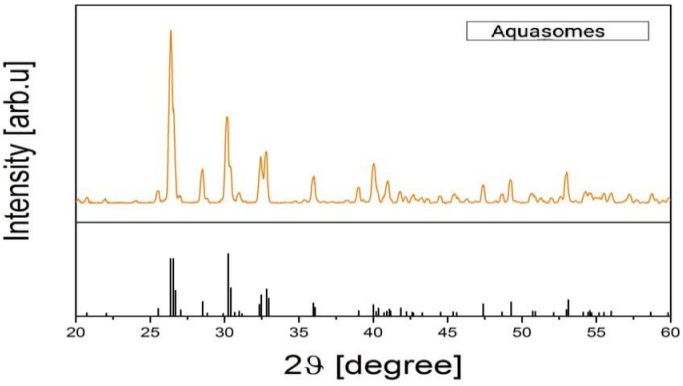

Figure 1: XRD of Formulated Ceramic Core

Fourier transform infrared spectroscopy (FTIR):

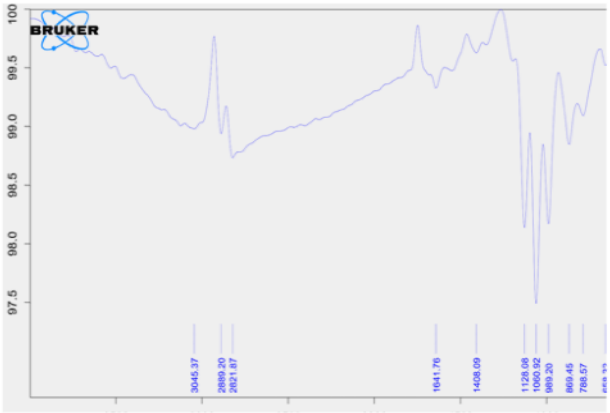

Figure 2: FTIR Spectrum of Formulated Ceramic Core

Zeta Potential of Coated Core:

The Zeta Potential analysis of the coated core was determined by HORIBA Zeta sizer. The average size of the coated core was shown to be – 18.7 mV indicating good physical stability.[16]

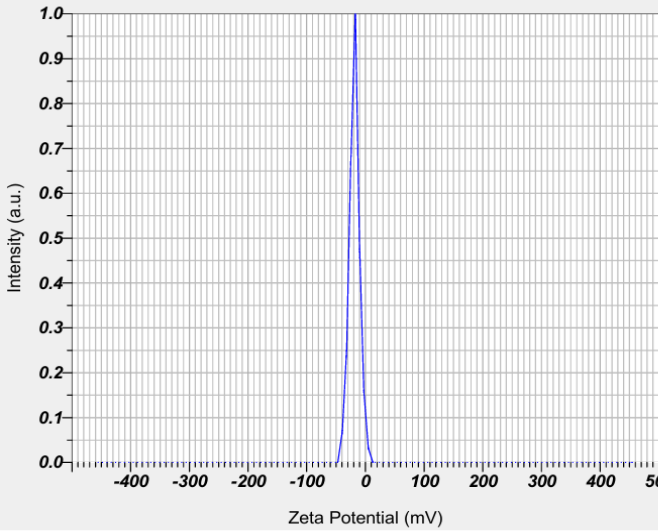

Figure 4: Zeta Potential of Formulated Coated Core

Drug Entrapment Efficiency:

The entrapment efficiency was found to be 58.21 % to 93.5 %. The highest entrapment efficiency was observed with 93.5 % for Batch D.[17]

Particle Size of Batch D Aquasomes:

The Particle Size analysis of the optimized formulation Batch D was determined by (HORIBA Zeta sizer). The optimized formulation Showed smaller particle size of 161.9 nm with Polydispersity index of 0.358 .[16,18]

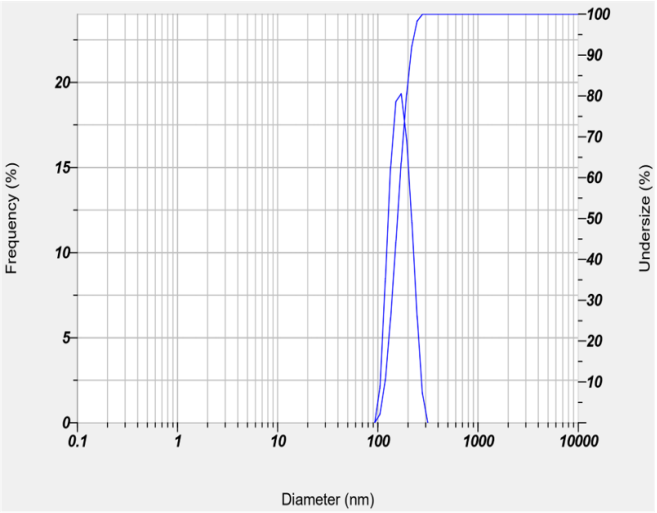

Figure 5: Particle Size of Formulated Aquasomes

***In-Vitro* Drug Release Studies:**

The optimized Batch D showed highest drug release 88.254 %. All the formulations of aquasomes showed drug dissolution within the range of 77.672 % to 88.254 % at the end of 5 hrs.[19]

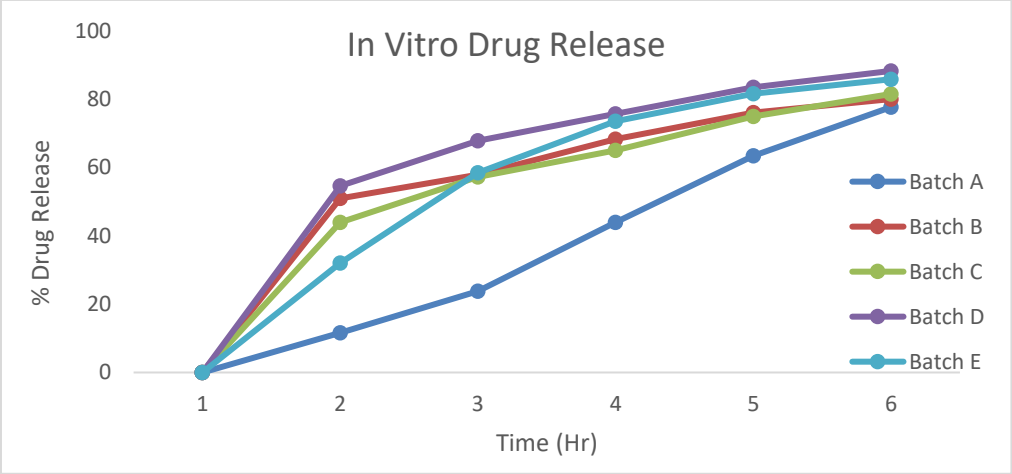

Figure 6: *In Vitro* % Cumulative Drug Release of Formulation Batch A to E

Table 2: % Cumulative Drug Release in Phosphate Buffer (Ph 7.4) For Formulation Batch A to E

Sr. No.	Time (hr)	% Cumulative Drug Release				
		Batch A	Batch B	Batch C	Batch D	Batch E
0	0 hr	0	0	0	0	0
1	1 hr	11.63	50.994	43.97	54.622	32.074
2	2 hr	23.784	57.922	57.2415	67.82	58.49
3	3 hr	43.922	68.31	64.962	75.66	73.49
4	4 hr	63.368	76.102	74.889	83.415	81.603
5	5 hr	77.672	80.01	81.507	88.254	85.8295

CONCLUSION

In the present research work from results and discussion it can be concluded that ciprofloxacin loaded aquasomes prepared by sonication method showed good results. Aquasomes charged with ciprofloxacin a low solubility drug was obtained through the formation of an inorganic core of calcium phosphate covered with trehalose further adsorbed with the ciprofloxacin. On the basis of obtained results it was concluded that the bioavailability of ciprofloxacin was improved by deposition of ceramic inorganic core of calcium phosphate.[2,20] It was confirmed with Scanning electron microscopy and Zeta potential or Particle size that the used condition in this work obtained spherical aquasomes, which were further study for drug release.

Ciprofloxacin loaded aquasomes were successfully developed. Total of 5 batches was prepared out of which the Batch D was considered as optimized batch which showed a particle size of 161.9 nm and zeta potential of –18.7 mV. *In vitro* drug release showed 88.254 % up to 5 hrs. It was noted that trehalose coated aquasomes are best coating material. The present work focused on the development and evaluation of ciprofloxacin loaded aquasomes as the drug delivery system for the management of lung infection, pneumonia, bone and joint infections, intra-abdominal infections, certain types of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections and Prostatitis. The findings of these investigations suggest that ciprofloxacin in the form of aquasomes can be effectively delivered and finds a possible role in the treatment of bacterial infection. The studies also suggest that delivering ciprofloxacin in the form of aquasomes enhances the bioavailability and solubility of the drug.

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