ORIGINAL ARTICLE

Carbohydrate Stabilized Ceramic Nanoparticles for the Delivery of a Poorly Soluble Drug, Lornoxicam

Pavani Vengala, Rudreswar Vanamala, C. V. S. Subrahmanyam

Department of Pharmaceutics, Gokaraju Rangaraju College of Pharmacy, Hyderabad, Andhra Pradesh, India

Abstract

Introduction: To enhance the delivery of poorly-soluble drugs, we have explored aquasomes (three-layered, ceramic core based, and oligosaccharide-coated nanoparticles) as potential carriers for the delivery of model hydrophobic drug lornoxicam (log P = 3.15). **Materials and Methods:** Ceramic nanoparticles were prepared using coprecipitation by sonication method. Cellobiose was used for coating onto ceramic core followed by loading of the lornoxicam by partial adsorption mechanism. The prepared system was characterized for size, shape, drug loading efficiency, and *in vitro* release profile (both 0.1 N hydrochloric acid solution and phosphate buffer solution, pH 6.8). Colorimetric analysis of sugar coating was done using phenol sulfuric acid method. **Results and Discussion:** The formed particles were spherical with an average particle size in the range of 60-300 nm, with a media of 87 nm. The *in vitro* dissolution performance was compared with that of pure drug and better results were observed. The cumulative lornoxicam release for the aquasome formulation (49%) was found to be higher than that of pure drug (34%) and was found to be gradual and linear in acidic media. Whereas, in phosphate buffer solution, pH 6.8, an incomplete release was observed with the pure drug (51% in 2 h) and 95% release was observed within 90 min from the formulation. **Conclusion:** Ceramic nanoparticles can be used for the enhancement of dissolution profile of poorly soluble drugs.

Key words: Cellobiose, ceramic nanoparticles, coprecipitation, lornoxicam, sonication

INTRODUCTION

Particulate drug carriers have a variety of advantages for use in drug delivery. They have large surface area to volume ratios that allow for a high drug payload and a prolonged drug release profile and can deliver drugs through minimally invasive routes identical to their polymeric counterparts. With the advent of nanotechnology, ceramic materials are now showing much promise for numerous drug delivery applications, especially as particulate drug carriers. Indeed, researchers are realizing that the extraordinary characteristics of nanophase ceramics (including size, structural advantages, highly active surfaces, unique physical and chemical properties, and ease of modification) imply that they can be excellent platforms for the drug transportation and controlled prolonged release compared with polymeric platforms.[1]

Calcium phosphate (CAP) owing to its natural presence in the bones and teeth, has been considered as ideal biomaterial with excellent biocompatibility[2] and has been extensively used in many biomedical applications such as dental composites,[3] bone tissue engineering or bone graft substitution (scaffolds),[4-7] orthopedic implants,[8,9] coatings,[10] and antibacterial agents.[11] Recently, more efforts have been made to explore the potential of using CAP nanoparticles as vehicles for drug and gene delivery for their great affinity to DNA and various drugs and good release property.[12-16] Ceramics were also investigated for the adsorption of proteins,[17] delivery of hemoglobin,[18] insulin,[19] enzymes,[20,21] antigens,[22,23] and vaccines.[24,25]

Different strategies were tested to fortify the characteristics of ceramics.[26,27] A myriad of methods has been reported

Address for correspondence:
Dr. Pavani Vengala, Department of Pharmaceutics,
Gokaraju Rangaraju College of Pharmacy,
Hyderabad - 500 090, Telangana, India.
Phone: +91-9959538383. E-mail: pavani181@gmail.com

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to prepare nanostructured CAP, and various morphologies including nanoparticles, plate-like nanocrystals, nanoneedles, nanotubes, and nanoblades.[28]

Solubility is one of the important parameter to achieve the desired concentration of drug in systemic circulation for pharmacological response to be shown. Increasing the bioavailability of poorly soluble drugs is one of the biggest challenges faced by formulation scientists. Nowadays, many of the new drugs exhibit such a low solubility that micronization does not lead to a sufficiently high bioavailability, and so the next step was going down to the Nanolevel, may be necessary.[29,30]

A polymer-free system, proposed by Kossovsky *et al*. containing carbohydrate stabilized nanoparticles of ceramics known as aquasomes,[31-33] has been explored as a potential application for the delivery of poorly soluble drugs. Aquasomes consists of a ceramic nanocrystalline core whose surface is non-covalently modified with the polyhydroxy oligomer to obtain a sugar ball, which is then adsorbed with therapeutically active molecules with or without modification.[34] The polyhydroxyl compounds coated to the core impart a hydrophilic nature to aquasomes. These three-layered structures are fabricated by self-assembly principle, the bottom-up approach[35,36] for the preparation of nanoparticles.

The objective of the present study was formulation and evaluation of nanoparticles in the form of aquasomes loaded with lornoxicam (log P=1.8), a model drug of low aqueous solubility belonging to BCS Class II. The prepared nanoparticles were then characterized with respect to shape, size, pay load, and *in vitro* drug release profile.

MATERIALS AND METHODS

Materials

Lornoxicam was obtained as a gift sample from Aristo Pharmaceuticals Pvt. Ltd. (Hyderabad, India). Calcium chloride was obtained from SD Fine-Chem Ltd. (Mumbai, India). Disodium hydrogen phosphate was obtained from Qualigens Fine Chemicals (Mumbai, India). Lactose monohydrate was obtained from SD Fine-Chem Ltd. (Mumbai, India). Cellobiose was obtained from Sisco Research Laboratories Pvt. Ltd. (Hyderabad, India).

Methods

Preparation of aquasomes

These three-layered structures are prepared by a three-step procedure, consisting of an inorganic core formation, which will be coated with sugar forming the polyhydroxylated core that will be finally loaded with lornoxicam, a poorly soluble drug.

Preparation of ceramic core

The cores were fabricated by a procedure as proposed by Kossovsky *et al*. by the reaction of disodium hydrogen phosphate with calcium chloride to yield the colloidal precipitate[19,37,38] with little modification. Based on the reaction stoichiometry, equivalent moles were reacted in a reaction volume of 120 ml specifically, disodium hydrogen phosphate (1 mole = 8.90 g) and calcium chloride (1 mole = 7.35 g) were taken in 60 ml of water each separately and mixed. A bath sonicator was used for sonication of the mixture for 2 h at 4°C. Following sonication, it was centrifuged using a C-24 Remi centrifuge (4°C, 15000 rpm) for 1 h. After centrifugation, supernatant was decanted; the precipitate was washed 3 times with double-distilled water. The precipitate was resuspended in distilled water (50 ml) and then filtered through a membrane filter (pore size 0.22 μ) of nitrocellulose. The core was dried (100°C, 2 days) to get ceramic nanoparticles. After drying, the percentage yield was calculated. The chemical reaction involved is as follows,

$$3Na_2HPO_4 + 3CaCl_2 \xrightarrow[2\ h,\ 4^\circ C]{\text{Sonication}} Ca_3(PO_4)_2 + 6\ NaCl + H_3PO_4$$

Sugar coating on the ceramic core particles

The core particles (prepared as above) were coated with polyhydroxy oligomer by adsorption method using sonication, a modified method adopted from Patil *et al*.[39] About 300 mg of sugar (cellobiose) was weighed and dissolved in 100 ml of double-distilled water. In a separate beaker, 150 mg ceramic core was taken and 100 ml of sugar solution was added (1:2, core:sugar coat) and sonicated for variable time periods; 10, 20, 30, and 40 min using Bandelin Sonoplus HD 2070 probe sonicator (at 30% pulse and 18 W). This suspension was shaken in an orbital shaker incubator for 3 h (at 100 rpm and 25°C). Here, acetone (non-solvent, 1 ml) was added to the suspension and kept aside for some time. Then, the solution was centrifuged (2000 rpm, 25°C and 15 min). The supernatant was decanted off, and the sugar-coated core was washed twice with water and dried at 70°C in a hot air oven. Cellobiose-coated core was quantified by a colorimetric reaction, phenol sulfuric acid method.

Quantification of sugar coating on core using phenol sulfuric acid method

A volume of 50 mg of sugar-coated core was accurately weighed and dissolved in 5 ml distilled water. From this stock, 2 ml of solution was taken and 5 ml ice cold sulfuric acid and 1 ml of phenol were added and boiled (10 min, 1000°C). The solution was cooled rapidly and the absorbance was measured at λ_{max} 490 nm.[40,41]

Adsorption of drug on the cellobiose-coated ceramic core

The procedure was general and similar as reported earlier.[39] Drug solution of 0.5% w/v (phosphate buffer solution, pH 6.8, and few drops of 1 N NaOH) was added to volumetric flasks containing an accurately weighed amount of sugar-coated core. The flasks were stoppered and shaken vigorously in an orbital shaker incubator (130 rpm for 24 h at 25°C and 30°C). The suspension was centrifuged (15,000 rpm). Ceramic nanoparticles were filtered through 0.2 µ filter using vacuum pump and dried at 70°C for 24 h.

Evaluation of aquasomes

Fourier transform infrared (FTIR) analysis

For final aquasomes, FTIR spectroscopy was performed for the confirmation of the presence of all three components, that is, ceramic core, sugar coating on the ceramic core, and drug on the sugar-coated ceramic core.

Particle size analysis and morphology using scanning electron microscopy (SEM)

The average size and size distribution of lornoxicam-loaded ceramic nanoparticles was determined using zetasizer. Morphology and size were confirmed by SEM (Hitachi S-3000N) in which the samples were mounted rigidly on the surface of a bronze-specimen holder called a specimen stub using a double-sided adhesive tape and coated with an ultrathin coating of electrically-conducting material, gold, deposited on the sample either by low vacuum sputter coating or by high vacuum evaporation with gold and observed under suitable magnification.[42]

Determination of drug content on sugar-coated core

The payload (loading efficiency) of drug in the aquasome formulation was determined by transferring 10 mg of the formulated aquasomes to 10 ml cleaned and dried volumetric flask and the drug was allowed to dissolve in phosphate buffer solution, pH 6.8 containing few drops of 1 M NaOH and volume was made up to the mark. Then, the solution was transferred to 100 ml volumetric flask containing the media (0.05 N NaOH) and the particles were solubilized to get a clear solution. The absorbance of the solution was determined spectrophotometrically at respective λ_{max} of 376 nm.[43]

In vitro drug release

In vitro drug release of the formulations was carried out using USP-type I dissolution apparatus (basket type) in two dissolution media (0.1 N hydrochloric acid solution and phosphate buffer solution, pH 6.8). The temperature of the medium was maintained at 37 ± 0.5°C. The apparatus was allowed to run for 50 rpm. Aliquots of 5 ml samples were withdrawn at various intervals. The samples were filtered through Whatman filter. The fresh dissolution medium (0.1 N hydrochloric acid solutions and phosphate buffer solution pH 6.8) was replaced every time with the same quantity of the sample. Collected samples were analyzed spectrophotometrically at λ_{max} of the drug. The percentage cumulative drug release (%) was calculated.

Drug release kinetics

To study the release kinetics, data obtained from *in vitro* drug release studies were fitted in various kinetics models to understand the linear relationship, that is, kinetic principles. The data were processed for regression analysis using MS Excel statistical functions. To study the release mechanisms, the data of *in vitro* drug release was verified using Higuchi's model and Hixson-Crowell Cube root law models.

RESULTS AND DISCUSSION

Effect of sonication time

The ceramic core to sugar ratio was kept constant (1:2) and the sonication time was varied (10, 20, 30, and 40 min), and the extent of sugar loading was determined using phenol sulfuric acid method and given in Table 1. In a hot acidic medium, glucose is dehydrated to hydroxymethylfurfural. This forms a yellow-brown colored product with phenol and has an absorption maximum at 490 nm. A study of Table 1 specified that 30-min sonication time showed fairly high sugar adsorption. Sonication resulted in size reduction, which further leads to increased surface area available for the sugars to get adsorbed. With further increase in sonication time to 40 min, though there was an increase in sugar loading, slight generation of heat was observed and there is a chance for aggregation of particles if they are sonicated for longer time.

Determination of lornoxicam content on cellobiose-coated ceramic core

The lornoxicam content on the cellobiose-coated ceramic core was determined by accurately weighing 10 mg of aquasomes and dissolving them in 10 ml of phosphate buffer solution,

Table 1: Effect of sonication time on sugar loading onto ceramic core

Sonication time (min)	Cellobiose loading (µg/100 mg core) AM±SD*
10	584.78±2.80
20	615.78±4.80
30	654.01±3.94
40	665.91±4.11

*Each value represents the mean of three determinations.
SD: Standard deviation

pH 6.8, and few drops of 1 M NaOH and finally making up the volume up to 100 ml with medium (0.05 N NaOH). This solution was analyzed spectrophotometrically and the absorbance was observed at the λ_{max} (376 nm). Percentage drug loading was calculated and found to be 8.53%.

FTIR spectroscopic analysis

KBr pellets of lornoxicam cellobiose aquasomes (LCA) were prepared and the characteristic spectra were compared with literature values.[44,45] The FTIR spectra are shown in Figure 1 and the characteristic bands were reported in Table 2.

FTIR spectroscopy confirmed the presence of three layered aquasomal structure, that is, ceramic core, sugar coating on the ceramic core, and drug on sugar-coated ceramic core.

Particle size analysis and morphology

The SEM images of lornoxicam-loaded aquasomes showed spherical nanoparticles. The particle size was uniform and particles were mostly single; however, a few aggregates were also visible [Figure 2]. The average particle size for LCA and the pure drug was determined using zetasizer [Figures 3 and 4; Table 3].

A perusal to Table 3 indicated that the lornoxicam-loaded aquasomes exhibited smaller particle size when compared to that of pure drug, which proves that the aquasomal fabrication had led to reduction of particle size to nanometer range.

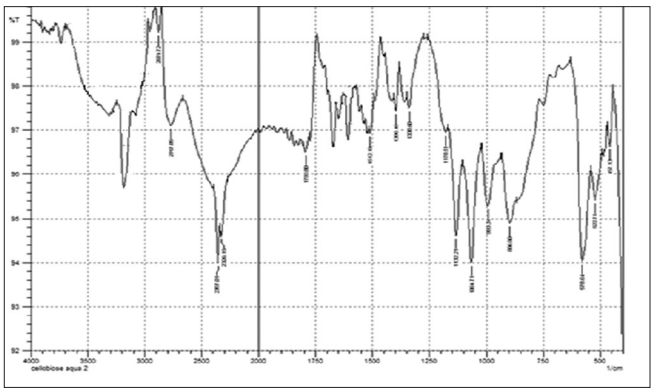

Figure 1: Fourier transform infrared spectra of lornoxicam cellobiose aquasomes

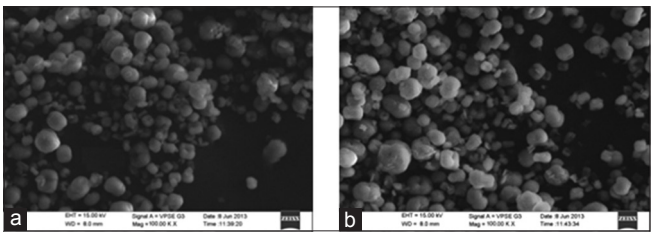

Figure 2: (a and b) Scanning electron microscopy images of lornoxicam cellobiose aquasomes

In vitro drug release study

The *in vitro* drug release for lornoxicam from the formulation and pure drug was studied in 0.1 N hydrochloric acid solution.

Table 2: Comparison of characteristic FTIR bands of LCA

Characteristic bands	LCA, Observed values cm^{-1}	Literature values, cm^{-1}
Phosphate (P-O)	896.97	845-725
Phosphate (P=O)	1178.79	1300-1240
OH stretching	3309.24	3200-3600
CH_2 stretching, asymmetrical	2921.56	2926
CH_2 stretching, symmetrical	2879.12	2853
C=O bonding	1683.79	1630-1690
NH bending	1650.85	1550-1640
S=O bending	1301.54	1175-1350
C-Cl bending	750.96	600-800

LCA: Lornoxicam cellobiose aquasomes, FTIR: Fourier transform infrared

Table 3: Average particle size of lornoxicam aquasomes and pure drug

Formulation	Particle size (nm)*
LCA	87.2
Lornoxicam	1010.0

*Average particle size was determined for 100 particles.
LCA: Lornoxicam cellobiose aquasomes

Table 4: Cumulative percentage release for lornoxicam from pure lornoxicam and LCA in 0.1 N hydrochloric acid solution

Time (min)	% Cumulative lornoxicam release	
	Pure lornoxicam AM±SD*	Lornoxicam cellobiose formulation AM±SD*
0	0	0
5	11.42±6.42	8.10±3.13
10	20.94±4.20	12.32±3.14
20	31.44±6.66	21.84±1.06
30	32.48±3.68	31.90±1.11
40	34.86±4.63	38.09±1.29
50	32.72±2.59	41.86±0.78
60	33.02±0.00	44.79±1.13
70	32.58±1.22	46.87±0.96
80	35.58±3.83	48.85±1.31
90	34.54±2.46	49.11±4.45
100	34.60±1.95	49.37±2.73

*Each value represents a mean of three determinations.
LCA: Lornoxicam cellobiose aquasomes, SD: Standard deviation

The data were shown in Table 4, and the comparative release profiles were recorded in Figure 5. A perusal to Table 4 and Figure 5 indicates that the drug release was slow and found to be incomplete for formulation as well as for the pure drug. The cumulative lornoxicam release for the aquasome formulation (49%) was found to be higher than that of pure drug (34%) and was found to be gradual and linear.

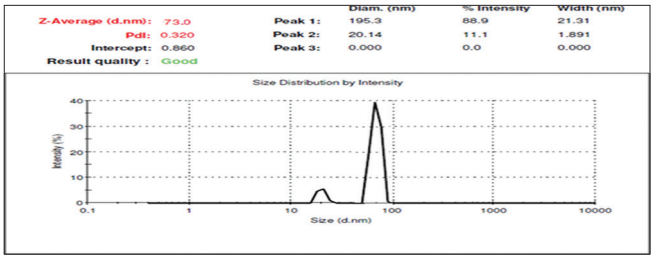

Figure 3: Particle size distribution analysis of lornoxicam cellobiose aquasomes

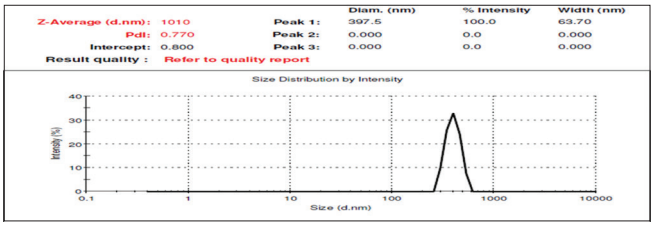

Figure 4: Particle size distribution analysis of pure drug lornoxicam

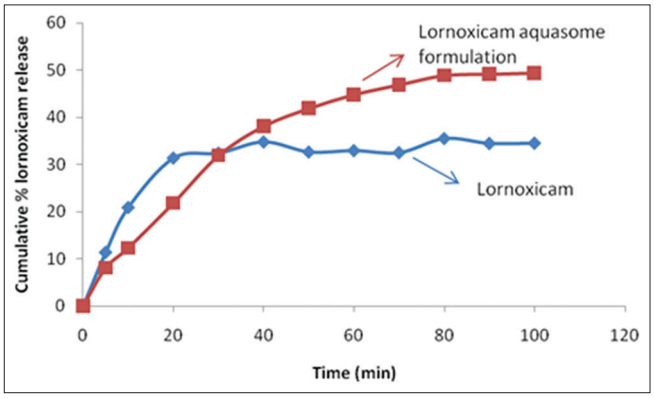

Figure 5: *In vitro* lornoxicam release profile from the pure drug and aquasome formulation in 0.1 N hydrochloric acid solution

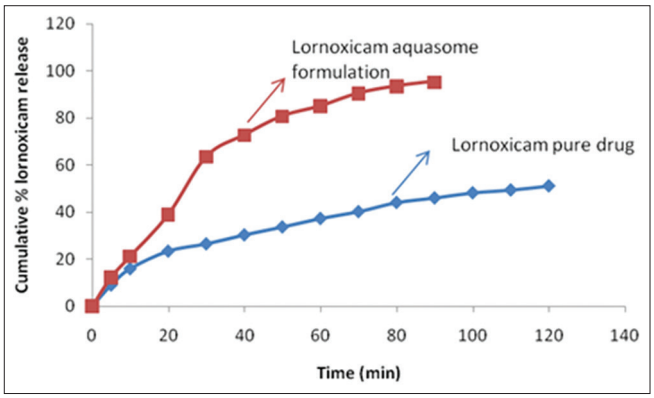

Figure 6: *In vitro* lornoxicam release profiles from pure drug and aquasome formulations in phosphate buffer solution, pH 6.8

Whereas, in phosphate buffer solution, pH 6.8, an incomplete release was observed with pure drug (51% in 2 h) and 95% release was observed within 90 min from the formulation. The data were shown in Table 5, and the comparative release profiles were recorded in Figure 6. A perusal to Table 5 and Figure 6 indicates that drug release from the formulation was rapid and complete than the pure drug.

The release kinetics followed first-order uniformly for all the samples, that is, concentration-dependent kinetics, in both the media. The release mechanism was observed to be Higuchi diffusion controlled.

CONCLUSION

Ceramic nanoparticles are a technological innovation for the delivery of therapeutic agents specifically, poorly soluble drugs. Lornoxicam in the form of ceramic nanoparticles (aquasomes) showed better release profile than the pure drug. These aquasomes with their nanometric dimensions, low drug dose, and hydrophilic properties are a novel drug delivery

Table 5: Cumulative percentage release for lornoxicam from pure lornoxicam and LCA in phosphate buffer solution, pH 6.8

Time (min)	% Cumulative lornoxicam release	
	Pure lornoxicam* AM±SD	Lornoxicam cellobiose formulation* AM±SD
0	0	0
5	9.08±0.67	12.28±1.04
10	16.01±1.28	21.19±1.39
20	23.46±1.07	38.90±2.10
30	26.54±0.89	63.58±2.31
40	30.37±0.68	72.77±3.71
50	33.73±1.13	80.78±0.95
60	37.35±1.09	85.03±1.13
70	40.25±0.86	90.49±2.40
80	44.12±0.83	93.53±2.96
90	46.06±0.52	95.49±2.67
100	48.24±0.99	
110	49.44±1.08	
120	51.16±0.86	

*Each value represents a mean of three determinations.
LCA: Lornoxicam cellobiose aquasomes, SD: Standard deviation

system that has the potential to enhance the dissolution of the less soluble drug.

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