INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

AQUASOMES AS A CARRIER SYSTEM FOR ORAL DELIVERY OF BROMELAIN

Abhilash Kutlehria *, Peeyush Kaushik, Shaveta Sharma, Arvind Kaur
Chandigarh College of Pharmacy, Landran, Mohali, Punjab, India
*Corresponding Author Email: abhilashkutlehria@gmail.com

Article Received on: 03/08/18 Approved for publication: 30/08/18

DOI: 10.7897/2230-8407.098177

ABSTRACT

The aim of the present study was to investigate the aquasome as potential carriers for efficient oral delivery of Bromelain in order to improve its bioavailability. Aquasomes prepared by inorganic core of calcium phosphate covered with a sugar film and further adsorption of the Bromelain and characterized in terms of the morphological examination, particle size distribution, entrapment efficiency, drug loading and in vitro release. The surface morphology of the freeze-dried aquasomes were smooth, discrete with a regular spherical to near-spherical shape. Size of the aquasomes after freeze-drying was 473 mm and well-distributed. The zeta potential of microspheres was -11.1 mV. Entrapment efficiency and drug loading of different aquasome formulation was found to be 72.0±2.13 to 79.6±0.95 and 2.78±0.05 to 3.98±0.06 respectively. The cumulative release rate of bromelain aquasomes was followed by a sustained release. The release following Korsmeyer-Peppas Model and from the 'n' value we see that the release followed the Non-Fickian release mechanism. That means here the release is occurred by diffusion as well polymeric chain erosion.

Key words: Aquasome, Oral Delivery, Freeze-dried, Zeta potential, Non-Fickian

INTRODUCTION

The effectiveness of several drugs is often limited due to their potential to reach the site of therapeutic action. In most cases (conventional dosage forms), only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biochemical properties. Thus, the fundamental goal of drug therapy is to provide therapeutic amount of drug to targeted site in the body to promptly achieve and maintain optimum plasma drug concentration in order to produce desired effect[1]. In recent years, significant efforts have been devoted for the development of new drug delivery system to improve the therapeutic efficacy and safety of the existing drugs by altering the bio-distribution pattern of the drug, by reducing the amount and frequency of dosing. Drug delivery system using colloidal particulate carriers such as liposomes, niosomes or proniosomes and aquasomes proved to have distinct advantage over conventional dosage with an increasingly important role in drug delivery, on account of their small size, better drug targeting, delivery and release, with their additional potential to combine diagnosis with therapy[2,3,4].

Aquasomes make different from other nanoparticles systems by their conformation and the water absorbent nature which not only makes their aqueous transport permissible but also confers the possibility of establishing non-covalent links with distinct molecules and macromolecules promoting a major stability compared than liposomes; advantages that offer a particularly favorable environment for proteins thereby avoiding their denaturalization. Those properties are possible because aquasomes are inorganic cores, which are coated with polyhydroxyl compounds and these are responsible for their hydrophilic behavior[5,6,7].

In recent years, a variety of naturally occurring dietary compounds have been shown to possess significant anticancer properties[8]. Among them, Bromelain (BL), a cysteine proteinase derived from pineapple (*Ananas comosus*) has iso-electric point of 4.6. It has been used for several therapeutic applications such as inhibition of platelet aggregation, as an anti-inflammatory agent, in modulation of cytokines, in enhancing the absorption of antibiotics and has anti-tumor activity[9,10]. The acceptance of BL as a phyto-therapeutical drug among researchers is now increasing because of its higher efficacy and nontoxic nature. Also, pre-treatment with BL delayed the onset of tumorigenesis and reduced the cumulative number of tumors in a mouse skin cancer model. However, there are only a very few reports which mention BL's anti-cancer activity upon oral delivery. It has been shown that BL's anti-cancer activity is due to its protease components[11]. BL preferentially cleaves off CD44 molecules which are present in metastasized tumor cells by virtue of its proteolytic activity, thus inhibiting the metastatic process[12]. However, upon ingesting, the proteolytic activity of BL gets rapidly deactivated because it forms complexes with anti-proteinases, alpha 2-macroglobulin (AMG) forming the AMG-BL complex which leaves the proteolytic activity of BL intact but reduced[13].Thus, anti-cancer activity of this molecule cannot be demonstrated with the existing conventional drug delivery systems. Therefore, it is necessary to find a new approach for oral delivery of BL, and also to enhance BL concentration at the tumor site.

The present study had the objective of preparing nanoparticles in the form of aquasomes. They were charged with BL, a model drug of low aqueous solubility that presents polymorphism[14]and has lately been used as a study model in micro- and nanoparticulates systems[15,16]. The structural analysis of these nanoparticles was carried out by and electronic microscope.

MATERIALS AND METHODS

Bromelain was obtained as gift from Frensenius Kabi Oncology Ltd, WB, India. Trehalose was obtained from Central Drug House (P) Ltd., India, Cellobiose and sucrose was purchased from alpha chemicals, India, Lactose, Maltose and acetone provided by finar chemicals ltd. Calcium chloride dehydrate was purchased from Qualikems Fine Chemicals Pvt. Ltd, New Delhi, India Potassium dihydrogen phosphate, Disodium hydrogen phosphate and Sodium hydroxide was purchased from Titan Biotech Ltd, India.

Determination of Melting point

Melting point of the drug was determined by using capillary method. Drug was filled into capillary tube by sealing its one end at the height of 3 mm from the closed end. Then, the capillary was introduced into the digital melting point apparatus and the point at which the drug starts melting was noted until the entire sample get melted.

Identification of drug by

FTIR and UV-Visible spectroscopy

Fourier transforms infrared spectral spectroscopy (FTIR) The pure drug was mixed with IR grade potassium bromide in a ratio of (1:100) and pellets were prepared by applying 10 metric ton of pressure in hydrophilic press. The pellets were then scanned over range of 4000-400 cm1 in FTIR spectrometer. FTIR spectrum of BL showed the presence of the peaks which complies with the reference spectra.

Standard plot by UV

100 mg of BL was weighed and transferred to 100 ml volumetric flask. Drug was dissolved in 10 ml methanol and sonicated for 5 min. Final volume was made up to the mark with same solvent and strength of 1000 µg/ml was obtained. Further dilution was made with methanol to get 100 µg/ml solutions and scanned under 200 nm to 400 nm in UV-Visible Spectrophotometer.

Preparation of standard calibration curve of BL

Preparation of stock solutions of BL in methanol 100 mg of BL was weighed and transferred to 100 ml volumetric flask. Drug was dissolved in 100 ml phosphate buffer (pH6.5) and sonicated for 5 min. Final volume was made up to the mark with same solvent and strength of 1000 µg/ml was obtained.

Preparation of serial dilutions

From the standard stock solution, a series of dilutions 10, 20, 30, 40, and 50 µg/ml were prepared by taking 0.1, 0.2, 0.3, 0.4, and 0.5 ml of solution and was transferred into 10 ml volumetric flasks and volume was made up to 10 ml with phosphate buffer and absorbance was taken at 340 nm

Determination of drug-excipients compatibility study

Drug and excipient compatibility studies were conducted to determine the compatibility of the excipients with the drug for the preparation of formulation. The FTIR spectrum was recorded by using FTIR after preparing potassium bromide disk. The finely ground drug powder and excipients powder were mixed with powdered potassium bromide and the mixture was pressed with a specific hydraulic compression. The prepared KBr pellet was then observed under Fourier transform infrared spectrometer (FTIR) and the spectrum of drug and excipients was recorded and compared.

Development of aquasomes

The general procedure consists of an inorganic core formation, which will be coated with Lactose forming the polyhydroxylated core that finally will be loaded by BL, our model drug.

Inorganic cores: core was prepared by precipitation method. In this method 0.75 M solution of disodium hydrogen phosphate in water was slowly added to the 0.25 M solution of calcium chloride and was continuously stirred for 2 hours on magnetic stirrer at low temperature. The precipitated calcium phosphate was separated by filtration and then washed three times with distilled water to remove sodium chloride formed during reaction. The precipitate was resuspended in distilled water and passed through 0.22 µm Millipore filter to collect the particles less than 0.22 µm. The filtered suspension so obtained was freeze dried using Heto drywinner.

Sugar coating: For sugar coating a sample of 1 mg of the inorganic cores was resuspended into 1 ml distilled water and was added to a 100 ml solution of lactose and cellobiose separately, having concentration of 0.03M, 0.06M and 0.09M each. Then the mixture was mechanically agitated for a period of 90 min for effective coating. After the mechanical agitation the resultant suspension was stored at 2-8ºC for further use.

Drug loading: A solution of bromelain was prepared (1mg/ml) by dissolving it in 10% formic acid solution and its pH was adjusted to 6.5 by the drop wise addition of ammonium hydroxide solution. Then 50 ml of drug solution was added to the suspension containing sugar coated ceramics and shook vigorously for 20 min. The suspension was the stored overnight in refrigerator at 2-8ºC for drug loading by partial adsorption. Bromelain loaded aquasomes were added drop wisely into sodium alginate solution having concentration of 10mg/ml under mild agitation for 10 min. The resultant suspension was centrifuged at 3,400 rpm for 5 min, and the supernatant was discarded. Finally, alginate coated aquasomes were re-dispersed into calcium chloride ($CaCl_2$) aqueous solution at concentration of 0.524mmol/L to crosslink the alginate layer presents on the surface of aquasomes. After cross linking, the above solution was freeze dried and placed in refrigerator at 2-8ºC.

Characterization of aquasomes

Entrapment Efficiency and drug loading: Entrapment efficiency is the percentage of actual mass of drug entrapped in the carrier relative to the initial amount of loaded drug. The % entrapment efficiency is calculated by:

$$\%\ \text{Entrapment efficiency} = \frac{\text{Actual drug loaded}}{\text{Theorotical drug loaded}} \times 100$$

$$\%\ \text{Drug loading} = \frac{\text{weight of total added drug} - \text{weight of unentrapped drug}}{\text{weight of aquasome}} \times 100$$

For theoretical drug loading it was assumed that entire drug gets entrapped in sugar coated ceramic core. For practical drug loading, an accurately weighed quantity of aquasomal formulation (equivalent to 10mg of BL) was dispersed in 10 ml of distilled water and centrifuged at 14000rpm for 20 min. The clear supernatant so obtained was filtered using syringe filter and analyzed for the content of free BL at 340 nm by UV spectrophotometer. Actually, loaded drug was given by measuring the difference between amounts of drug added and measured unloaded drug of supernatant after adsorption.

Particle size and zeta potential: The size and zeta potential of the aquasomes formed from the hydration with water was determined using zetasizer (Malvern Instruments, UK) at 25°C using disposable sizing cuvettes. 1 ml sample was taken for particle size analysis and three replicates were taken for each sample. Polystyrene beads were used as a standard to check instrument performance. The polydispersity index (PI) was determined as a measure of homogeneity. Small values of PI (<1) indicate a homogeneous population.

Morphological analysis: The surface morphology of aquasomes was characterized by transmission electron microscopy (TEM) (FEI Netherland, Europe). Samples were diluted with double-distilled water and then negatively stained with 2% (w/v) sodium phosphotungstic acid solution for observation.

***In vitro* drug release study:** *In vitro* release study of BL from aquasomes and pure BL was performed in triplicate using phosphate buffer pH 6.5 as dissolution media (900ml) employing USP type I dissolution test apparatus. Accurately weighed lyophilized aquasomal powder equivalent to 50 mg of BL was filled into hard gelatin capsule. To study the *in vitro* release of BL capsule was placed into dissolution basket and media was stirred at speed of 100 rpm at 37 ±0.5 ºC. Aliquots of 10ml samples were withdrawn at various time intervals, filtered using syringe filter 0.45μ and analyzed for BL content at 340nm using UV spectrophotometer. Sink condition was maintained with 10 ml of fresh dissolution medium.

Table 1: Standard curve data of BL

10	0.156±0.013
20	0.268±0.012
30	0.346±0.011
40	0.454±0.013
50	0.569±0.017

Table 2: BL loaded different Aquasomes formulations

Ingredients	Formulation code						
	F1	F2	F3	F4	F5	F6	F7
Bromelain (mg)	50	50	50	50	50	50	50
Disodium hydrogen phosphate (gm)	4	5	7	3	6	5	7
Calcium chloride (gm)	2	3	5	4	2	3	2
Lactose (mg)	50	100	100	50	50	50	100

Table 3: % Entrapment Efficiency and % drug loading of different Aquasomes formulations

Formulation code	% Drug entrapment efficiency	% Drug loading
F1	72.0±2.13	3.56±0.08
F2	77.8±1.56	3.12±0.04
F3	79.6±0.95	3.98±0.06
F4	76.2±1.45	2.78±0.05
F5	74.4±1.57	2.94±0.03
F6	75.77±1.06	3.47±0.04
F7	78.9±1.84	3.4±0.06

Table 4: In vitro drug release of optimized formulation F3 and Pure drug in phosphate buffer pH 6.5

Time (min.)	% CDR of F3	% CDR of Pure BL
0	0±0	0±0
10	9.5±0.63	3.2±0.08
30	15.9±0.91	8.4±0.12
60	24.78±1.01	14.78±0.14
90	35.69±1.12	21.63±0.18
180	38.46±1.32	24.69±0.17
240	42.69±1.17	27.12±0.16

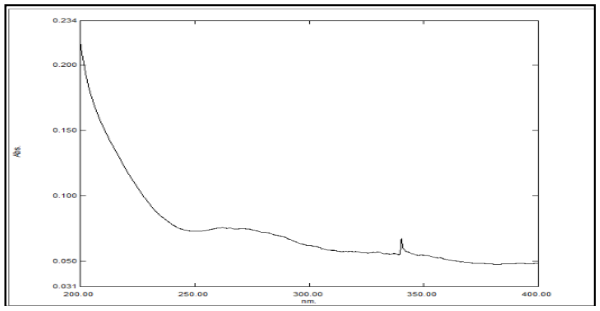

Figure 1: UV Scan of BL

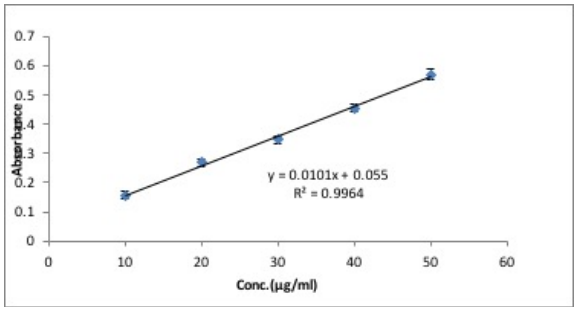

Figure 2: Standard curve of BL in phosphate buffer pH 6.5 at 340 nm

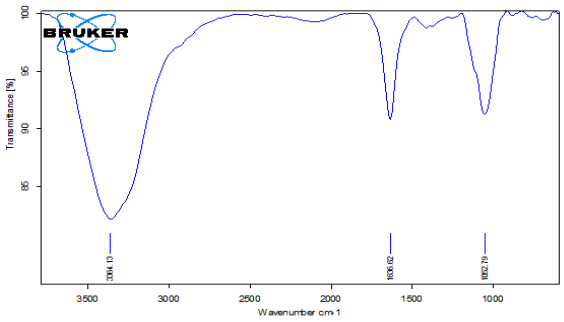

Figure 3: FTIR spectra of pure BL

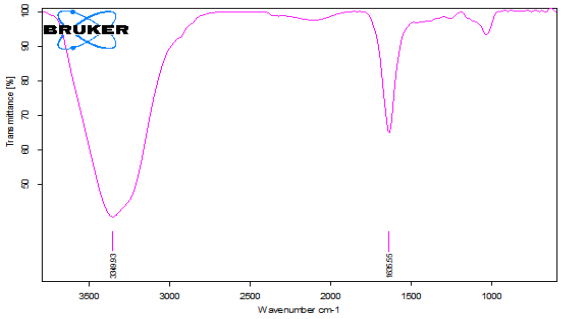

Figure 4: FTIR Spectra of BL with other excipients

Results

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	473	Peak 1:	240.5	100.0	17.16
PdI:	0.930	Peak 2:	0.000	0.0	0.000
Intercept:	1.07	Peak 3:	0.000	0.0	0.000

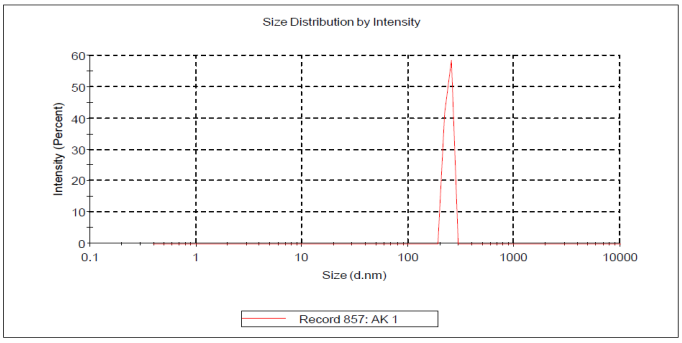

Figure 5: Particle size of optimized formulation (F3)

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-11.1	Peak 1:	-11.1	100.0	4.76
Zeta Deviation (mV):	4.76	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.200	Peak 3:	0.00	0.0	0.00
Result quality :	Good				

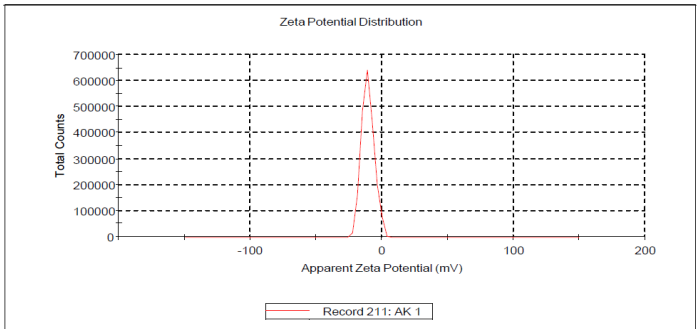

Figure 6: Zeta Potential of optimized formulation (F3)

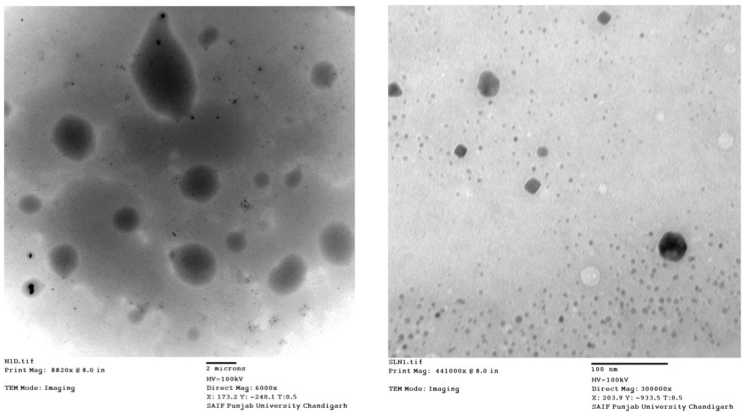

Figure 7: TEM of optimized formulation F3

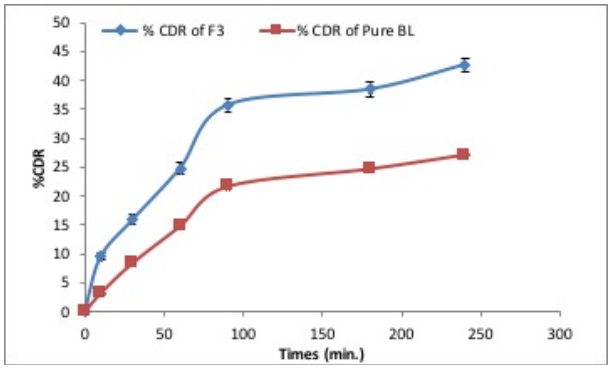

Figure 8: In vitro drug release of optimized formulation F3 and Pure drug in phosphate buffer pH 6.5

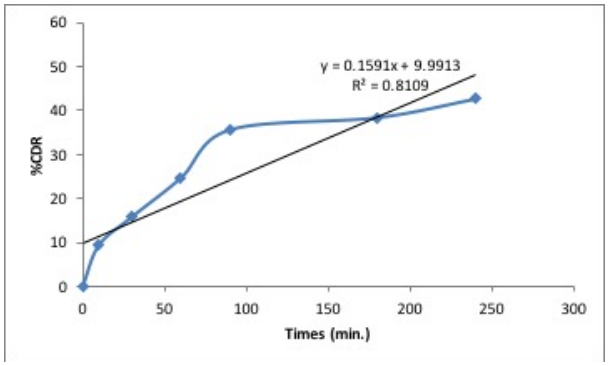

Figure 9: Zero order release kinetics of formulation F3

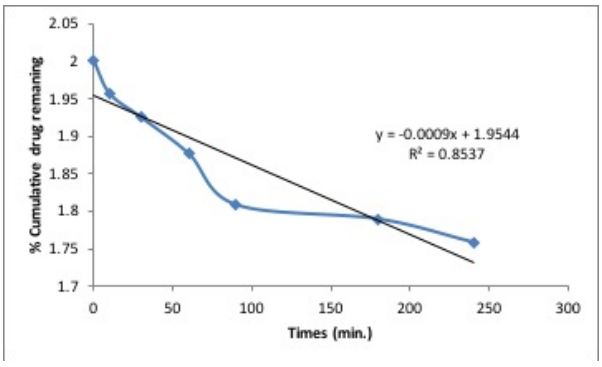

Figure 10: First order release kinetics of formulation F3.

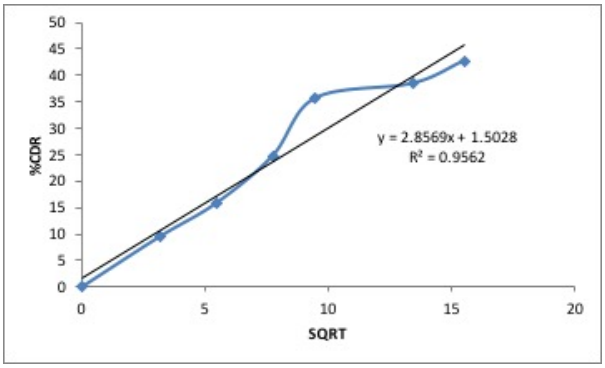

Figure 11: Higuchi release kinetics of formulation F3

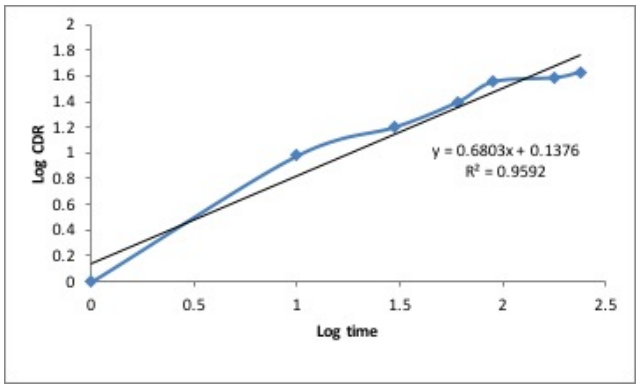

Figure 12: Korsmeyer-Peppas Model of formulation F3

RESULT AND DISCUSSION

Melting point: BL (drug sample) was observed for melting point, the melting range of BL was observed to be 233-236°C which complies with reported melting range i.e. 234-235°C**.**

UV Spectroscopy: The drug was identified with the help of UV exhibited absorption maxima at 340 nm when methanol was used as solvent as mentioned in literature.

Standard plot by UV: Various concentration (10-50 µg/ml) of BL were analysed using UV spectrophotometer and standard curve of BL was plotted.

FTIR Spectroscopy: The FTIR of BL has been shown in Figure No.3-4 intense band at 3277.63 cm^{-1}, 1639.11, cm-1, 1761cm^{-1} and 1036.62 cm^{-1}, corresponding to the functional groups OH, C=C, and C-O.

drug-excipients compatibility study: The peaks observed in FTIR of mixture of bromelain and excipients at 3349.93 cm^{-1}, and 1635.55 cm^{-1}. There was no major shifting in the frequencies of above said functional groups of which indicates that there was no chemical interaction between Bromelain and excipients which were used in the formulation.

Formulation development

Bromelain loaded aquasomes are prepared by a three-step procedure consisting of the formation of an inorganic core, coating with lactose sugar and adsorption of the drug onto this sugar-coated core. Of all the available materials, calcium phosphate was selected as core of choice as it is ceramic (structurally most regular materials) and crystalline in nature surface energy which favors the binding of carbohydrate on surface film. Further drug is adsorbed over sugar loaded core particles through non-covalent and ionic interactions. Composition of different formulation are given in table2.

Characterization of aquasomes

Entrapment Efficiency and Drug Loading: Entrapment efficiency and drug loading of different aquasome formulation was found to be 72.0±2.13 to 79.6±0.95 and 2.78±0.05 to 3.98±0.06 respectively highest entrapment efficiency and % drug loading was found to be F3 formulation which was further evaluated for particle size, zeta potential, morphological study and in-vitro drug release study.

Particle size and zeta potential: The BL loaded aquasomes exhibited 473 nm of particle size. Hence it can be concluded that, the aquasomal formulation had led to reduction of particle size to nanometer range. Zeta potential results showed a negative surface charge (−11.1 mV) for F3

Morphological study: The TEM images revealed that aquasomes (F3) were spherical and within the nano size range, and no drug crystals were visible (Fig. 7).

In-Vitro **drug release:** Improved dissolution was observed with aquasome formulation of BL than that of pure drug, which can be accounted for nanosize and aqueous environment of the aquasomes. The release following Korsmeyer-Peppas Model and from the 'n' value we see that the release followed the Non-Fickian release mechanism. That means here the release is occurred by diffusion as well polymeric chain erosion.

CONCLUSION

The results of our present study clearly represent promising carriers of bromelain loaded aquasomes for orally drug delivery as well as it could be viewed as substitute to conventional dosage form. The microspheres showed the particle size of 473 nm. The aquasomes had not only good sphericity but also uniform distribution of particle size. In vitro release study, only a little drug was released from microspheres in the initial time and then was graduated released from the aquasomes as time lapsed, suggesting that bromelain was well entrapped in aquasomes. The release following Korsmeyer-Peppas Model and from the 'n' value we see that the release followed the Non-Fickian release mechanism. That means here the release is occurred by diffusion as well polymeric chain erosion.

REFERENCES

1. Vora B., Khopade A.J., Jain N.K., Proniosome based transdermal delivery of levonorgestrel for effective contraception. J. Control. Release, 1998, 54, 149–165. https://doi.org/10.1016/S0168-3659(97)00100-4
2. Wong M., Thompson T.E., Aggregation of dipalmitoylphosphatidylcholine vesicles. Biochemistry, 1982, 21, 4133–9 https://doi.org/10.1021/bi00260a032
3. Uchegbu, I.F., Vyas, S.P., Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int. J. Pharm, 1998, 172, 33–70. https://doi.org/10.1016/S0378-5173(98)00169-0
4. Hu C., Rhodes D.G., 1999. Proniosomes: A novel drug carrier preparation. Int. J. Pharm, 1999, 185, 23–35. https://doi.org/ 10.1016/S0378-5173(99)00122-2
5. Kim I.S., Kim S.H., Development of polymeric nanoparticulate drug delivery system. In vitro characterization of nanoparticles based on sugar containing conjugates. Int. J. Pharm, 2002, 245, 67–73. https://doi.org/ 10.1016/S0378-5173(02)00336-8
6. Masatoshi I., Yongning Z., Influence of saccharides on the stabilization of frozen hemeprotein. Chem. Abst, 1998, 45, 539–544.
7. Kossovsky N., et al, Control of molecular polymorphism by a structured carbohydrate/ceramic delivery vehicle-aquasomes. J. Control. Release, 1996, 39, 383–388. https://doi.org/10.1016/0168-3659(95)00169-7
8. Brien S., Lewith G., Walker A., Hicks S. M and Middleton D., Bromelain as a Treatment for Osteoarthritis: a Review of Clinical Studies Alternative Med., 2004, 1, 251–257.
9. Onken J.E, Greer P.K., Calingaert B., Hale L.P., Bromelain treatment decreases secretion of pro-inflammatory cytokines and chemokines by colon biopsies in vitro Clin. Immunol., 2008, 126, 345–352. https://doi.org/10.1016/ j.clim.2007.11.002
10. Hale L.P., Greer P.K., Trinh C.T., Gottfried M.R., Treatment with oral bromelain decreases colonic inflammation in the IL-10-deficient murine model of inflammatory bowel disease, Clin. Immunol., 2005, 116, 135–142. https://doi.org/10.1016/ j.clim.2005.04.011
11. Bhui K., Prasad S., George J., Shukla Y., Bromelain inhibits COX-2 expression by blocking the activation of MAPK regulated NF-kappa B against skin tumor-initiation triggering mitochondrial death pathway Cancer Lett., 2009, 282, 167–176.
12. Fitzhugh D.J., Shan S., Dewhirst M.W., Hale L.P., Bromelain Treatment Decreases Neutrophil Migration to Sites of Inflammation Clin. Immunol., 2008, 128, 66–74.
13. Hale L.P., Greer P.K., Sempowski G.D., Bromelain treatment alters leukocyte expression of cell surface molecules involved in cellular adhesion and activation Clin. Immunol., 2002, 104, 183–190.

14. Del R'ıo, L.A., Estudio de preformulaci ´on para el desarrollo de comprimidos de indometacina como substancia policristalina. Ars Pharmaceutica, 2002, 43 (1–2), 147–171.
15. Chow, G.-M., Gonzalves, K.E., Nanotechnology. Molecularly designed materials. J. Am. Chem. Soc, 1996, 622, 335–348.
16. Ramos-Picos, D., G´ omez-Carril, M., Fern'andez-Mena, D., M'etodos de obtenci ´on de microesferas biodegradables. Rev. Cubana Farm, 2001, 35 (2), 126–135.

Cite this article as:

Abhilash Kutlehria *et al*. Aquasomes as a carrier system for oral delivery of bromelain. Int. Res. J. Pharm. 2018;9(8):123-129 http://dx.doi.org/10.7897/2230-8407.098177

Source of support: Nil, Conflict of interest: None Declared