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Research Article

Development and Evaluation of novel maltodextrin proniosomes for oral delivery of abacavir Sulphate

Helen Sonia A[1], Sambath Kumar R*[2], Ruckmani K[3], Gover Antoniraj M[3], Venkateswra Murthy N[2], Bhama S[2]

[1]S.A. Raja Pharmacy College, Vadakkangulam, Tirunelveli District, 627116, India
[2]J.K.K.Nattraja College of Pharmacy, Komarapalayam, Namakkal District, 643183, India
[3]Dept of Pharmaceutical technology, Anna University, Tiruchirappalli, 620024, India

ABSTRACT

The aim of the present work was to investigate feasibility of proniosomes in the encapsulation of hydrophilic anti-retroviral drug abacavir sulphate. This was performed and optimized by slurry method using different surfactants, cholesterol and dicetyl phosphate. Blank formulations comprising all the excipients were prepared and subsequently drug loaded formulations also prepared. The formulations were evaluated for vesicle formation, vesicle size, size distribution, zeta potential, encapsulation efficiency, drug content, *in vitro* drug release, release kinetics and micromeritic properties. The optimized formulation was evaluated for scanning electron microscopy, fourier transform infrared (FTIR) spectroscopy, osmotic shock studies and stability study. The tween series formulations non significantly (P=0.12>0.05) enhanced the encapsulation of abacavir sulphate compared with the formulations with span series surfactants. Among all the proniosome formulations, CDPF7 containing tween 60 was selected as an optimized formulation due to high encapsulation efficiency of 85.02 ±1.560%, prolonged drug release of 94.46 (±1.396)% after 24 hours and better stability.

Keywords: Encapsulation; Retroviral; Hydrophilic; Vesicle; Micromeritic; Spectroscopy.

INTRODUCTION

The progress in nanotechnology helps in developing newer formulations. One of the succession in nanotechnology is the development of colloidal drug carrier. Colloidal drug transporter systems such as vesicles, micellar solutions, nanoparticles and liquid crystal dispersions seem to be promising drug delivery systems and have led to increasing interest over the past 20 years (Rita et al., 2007). Vesicles are concentric bilayer made-up of amphiphilic molecules surrounds an aqueous compartment. The components of vesicles affect their physicochemical properties such as their charge, size, elasticity, lamellarity, and thermodynamic phase (Gannu et al., 2011). Encapsulation of API in vesicular system can be predicted to extend the existence of the drug in systemic circulation and thus augment penetration into target tissue and reduce toxicity (Prajapati et al., 2012). The vesicular structure can also be modified to provide sustained or controlled drug delivery for prolonged periods (Gannu et al., 2011).

Vesicular systems like liposomes or niosomes have definite advantages while avoiding shortcomings associated with conventional dosage forms and can carry hydrophilic drugs by encapsulation or hydrophobic drugs by partitioning into hydrophobic domains (Thejaswi et al., 2011). Although niosomes and liposomes are excellent approaches of drug delivery, both are dispersed aqueous systems and have a trouble of degradation by oxidation or hydrolysis, need of special storage and handling, shows sedimentation, aggregation or fusion on storage, and difficulties in sterilization (Ashwani et al., 2011). Thus the advancements in the niosome lead to the development of proniosomal delivery systems to overcome the demerits of niosomes and liposomes. The proniosomes are hopeful drug carriers, because they have greater chemical stability and lack of many disadvantages associated with liposomes (Thejaswi et al., 2011). Also these are dry products, which could be hydrated immediately before use, would avoid many of the problems associated with aqueous niosome dispersions and problems of physical stability (Viviane et al., 2012). In the work accounted here, we describe the formulation of dry niosomes called as “proniosomes” for antiretroviral drug abacavir sulphate.

AIDS is the most widespread problem around the world because of quick increase in the number of victims (Sembulingam et al., 2005). HIV mainly infects helper T cells, macrophages and dendritic cells, which are essential to the human immune system. Macrophages are located at tactical points like spleen, liver, lungs and connective tissues where microbial attacks

* Corresponding Author
Email: sambathju2002@yahoo.co.in
Contact: +99-9842779911
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are likely to take place (Julie et al., 2008) and can turn into a reservoir for HIV through viral replication taking place in the macrophages after infection (Arun et al., 2000). Particles or vesicles from 150 nm to 2 µm show maximal phagocytosis by macrophages (Fang et al., 2006).

Abacavir sulphate, the anti-HIV compound approved in 1998 (Thomas et al., 2013) is still generally used alone or in combination with other anti-retroviral agents for treatment of AIDS and AIDS-related complex. It is the only approved antiretroviral that is active as a guanosine analog (Laurence et al., 2011). Rapid diminution in plasma HIV -RNA count and quick rise in CD4 cell count has been well-known when abacavir was given to HIV infected patient (Tripathy et al., 2013). Abacavir has been reported to produce severe hypersensitivity reactions and adverse effects. The half-life of hydrophilic abacavir sulphate is 1 to 1.5 hours (Tripathy et al., 2013). Marketed preparations of abacavir sulphate currently available are there in conventional dosage form. The objective of the current study was to develop a prolonged release proniosome formulation of abacavir sulphate for targeted delivery and to minimize the undesirable effects.

MATERIALS AND METHODS

Abacavir Sulphate was obtained as a gift sample from Cipla Limited Mumbai. Span 60 was supplied by Loba Chemie pvt. Ltd, Mumbai. Span 20, Tween 40 and Tween 80 were provided by Tokyo Chemical Industry Co., Ltd, Japan. Cholesterol, Span 40, Span 80 and Chloroform were bought from SDFCL S D Fine - Chem. Limited, Mumbai. Tween 20, Tween 60 and Methanol were obtained from Ultra International, Bangalore. All other chemicals and solvents were of analytical grade.

Preformulation studies

Preformulation studies were carried out to develop the stable dosage form with different nonionic surfactants (spans and tweens) at 100, 150, 200 and 250 µM concentration with an equal ratio of cholesterol. Although vesicles formed in all concentrations, the encapsulation efficiency was found to be very less except at 250 µM ratio. Thus 250:250µM ratio of surfactant:cholesterol was preferred for present formulation. The process-related variables like speed of rotation of flask, hydration medium and hydration time were optimized by trial and error method. Drug - excipient compatibility studies also carried out.

Preparation of proniosomes

Proniosome formulations were prepared by the slurry method. The slurry method is comparatively simple and is mostly useful for the carriers which are not dissolving in organic solvents (Agaiah et al., 2012). In brief, accurately weighed amounts of lipid mixture (500 µM) comprising of surfactant and cholesterol, with 5 µM DCP were dissolved in 4ml chloroform. The drug was dissolved in 6 ml methanol and the resultant solutions were transferred to a 250 ml round bottom flask having maltodextrin carrier. Additional chloroform: methanol solution was added to form slurry in the case of inferior surfactant loading. The flask was attached to a rotary flash evaporator to evaporate solvent at 100-150 rpm, a temperature of 60°C, and a reduced pressure of 600mmHg until the mass in the flask had become a dry, free flowing product. After ensuring the complete removal of solvent, the resultant materials were further dried overnight in a desiccator under vacuum at room temperature. This dry preparation is referred to as ”proniosomes” and was used for preparations and for further study on powder properties. These proniosome granules were stored in a tightly closed container at refrigerator temperature until further evaluated. Blank proniosomes were made in the same way without incorporating drug (Almira et al., 2001; Viviane et al., 2012). The composition of different batches of abacavir sulphate proniosomal formulations are represented in Table 1.

Preparation of niosomes from proniosome

The proniosomal powder was transformed to niosome vesicles by hydrating with phosphate buffer (pH 7.4) at 80°C by means of vortexing using vortex mixer for 2 min. The niosomal dispersion was placed over a glass slide and the vesicle formation was observed under optical microscope. There resultant niosomal dispersion was subsequently subjected to evaluation of vesicle formation, zeta potential, vesicle size, size distribution and encapsulation efficacy (Ranjan et al., 2014; Preethy et al., 2015).

Optical microscopy

The proniosome derived niosomal formulations were confirmed for vesicle formation by optical microscopy at suitable magnification. The niosome dispersion obtained was mounted over a microscopic slide and fixed over by drying at room temperature. The dried thin film of niosome suspension was observed for the formation of vesicles. Photo microscopic images of the formulations have been taken by a digital camera and shown in figure 1 (Tank Chintankumar et al., 2009; Viviane et al., 2012).

Zetapotential, vesicle size, size distribution

Vesicle size, size distribution and zeta potential of niosomes derived from proniosome samples were determined by photon correlation spectroscopy using the Malvern Zetasizer. A zeta cell was washed several times with deionized water before being loaded with niosome suspensions gained from proniosome to measure the zeta potential. Each sample was diluted to appropriate concentration with demineralized water and the vesicle size was analyzed with an angle of detection of 90° at 25°C. Size of the vesicles, polydispersity index and their mean zeta potential values were obtained from the instrument (Table 2) (Mohammad Najlah et al., 2014).

Encapsulation Efficacy

From the pharmaceuticals and cosmetics point of view the percentage encapsulation efficiency is one of the most important consideration in vesicle formulations (Balakrishnan et al., 2009). Free abacavir sulphate was separated from niosome attained from proniosome entrapped abacavir sulphate by centrifugation at 15,000 rpm and 4°C for 1 hour using a cooling centrifuge. The supernatant was taken and diluted with phosphate buffer pH 7.4, for spectrophotometric estimation of free drug at 285 nm. The concentration of encapsulated abacavir sulphate was calculated by subtracting the concentration of free drug in the supernatant from the total drug incorporated as follows and the obtained results were adjusted with the data attained from blank formulations (Mohamed Nasr et al., 2010).

$$\%Encapsulation\ efficiency = \left[\frac{Total\ drug - Free\ drug}{Total\ drug}\right] \times 100$$

Drug content

Abacavir sulphate content in proniosomes was obtained by an UV spectrophotometric method. Niosomes obtained from proniosomal formulation containing 10 mg Abacavir was taken into a standard volumetric flask. The vesicles were destructed with 50ml propane-1-ol by shaking and 1ml of the mixture subsequently diluted with phosphate buffer pH 7.4. The absorbance was measured spectrophotometrically against blank at 285 nm. The average abacavir content of three determinations was reported in Table 3 (Preethy et al., 2015).

***In vitro* drug release**

Dissolution is the procedure of extracting the active pharmaceutical ingredient out of the solid pharmaceutical dosage form matrix into solution within the GIT. Dissolution study is an *in vitro* method that describes how an active pharmaceutical ingredient is taking out of a solid dosage form. It is an acceptable tool that predicts and offers rough assessment of the *in vivo* routine of the formulation (Fotaki et al., 2010).

The dissolution studies were carried out according to the US Pharmacopeia (USP) type I apparatus (basket method). The abacavir sulphate proniosome formulations corresponding to 10 mg abacavir were filled into hard gelatin capsule. The dissolution medium was 900ml 0.1N HCl/ phosphate buffer solution (pH 6.8) in six dissolution jars to maintain sink conditions. The capsules were placed in basket and immersed in dissolution medium. The stirring speed was 50 rpm, and the temperature was maintained at 37°C±0.5°C. The samples (3 ml) were withdrawn at fixed time intervals using a syringe and passed through 0.2 µm membrane filter. Withdrawn samples from dissolution jars were replaced by fresh medium. The abacavir content was evaluated by UV spectrophotometer at 285 nm in case of pH 6.8 phosphate buffer as dissolution medium and 295nm in case of 0.1N HCl as medium. The cumulative percentage of abacavir release from formulation was plotted as a function of time (Abd-Elbary et al., 2008; Mohamed Nasr et al., 2010).

Release kinetics

Release kinetics is an essential part for the dosage form development. Mathematical approach is important scientific method to evaluate and optimize the error in terms of deviation in the drug release profiles of formulated dosage form during the formulation development phase. In formulation and development mathematical model approach used to diminish the number of trials in ultimate optimization (Benika Sharma et al., 2014). In order to realize the kinetic of drug release, the release data got from different formulations were subjected with various kinetic equation models like zero order, first order, Higuchi's model and Hixson model. Abacavir release from proniosome formulations were integrated into Korsmeyer & Peppa's equation and the exponent n was computed from slope of the straight line (Table 4) (Tank Chintankumar et al., 2009; Preethy et al., 2015)

Osmotic shock studies

The consequence of osmotic shock on optimized proniosome formulations was evaluated by incubating of niosomal suspensions obtained from proniosomes in media of diverse tonicities. The formulation was divided into three parts incubated with hypotonic (0.5%NaCl), isotonic (0.9%NaCl), and hypertonic solutions (1mol/L sodium iodide solution) for 3 hours. Then the changes in the vesicle size in the formulations were observed and specified in table 5 (Ranjan et al., 2014).

Stability study

Physical stability study was carried out to investigate the degradation of drug from proniosome during storage. The optimized proniosome formulation with the composition of Tween 60 and cholesterol in 250:250 µM ratio with 5 µM DCP was divided into 3 sets of samples. The samples were sealed in glass vials and stored at (2-8°C) in refrigerator, room temperature 25±2°C and 45±2°C for a period of 3 months. Samples were withdrawn at definite periods of time and analyzed for vesicle size, % drug remaining and percent drug entrapment (Table 6). The results obtained were compared to the freshly prepared niosomes (Bhushan Arun et al., 2015).

Solid state characterization

Micromeritic properties

The flow properties of powder are essential in handling and processing procedures. The flow properties of abacavir sulphate proniosome granules were studied through assessing the angle of repose, Carr's compressibility index and Hausner's ratio. (Tank Chintankumar et al., 2009; Mona Hassan et al., 2012).

Angle of repose: The angle of repose was determined by usual fixed funnel method. Briefly, proniosome powder and the pure drug were poured into a funnel which was placed at a height of 2.5 cm from black horizontal surface. The powders were flowed down from the funnel to form a cone shape on the horizontal surface. The angle of repose was determined by measuring the elevation of the cone (h) and the diameter of its base (d). Each trial was done in triplicate and outcomes were only considered suitable, when a symmetrical cone of dry powder was formed.

The angle of repose (ɵ) was calculated from the subsequent equation:

$$Tan\theta = \frac{2h}{d}$$

Carr's compressibility index and Hausner's ratio

The Carr's compressibility index and Hausner's ratio were calculated from the bulk and tapped density of the proniosome powders.

Scanning electron microscopy

The exterior characteristics of the proniosome powder and maltodextrin was examined by scanning electron microscope (JSM 6390LA, Jeol, Tokyo, Japan). Each sample was smeared on a small piece of adhesive carbon tape which was fixed on a brass stub and subjected to gold coating using sputtering unit for 10 sec at 10mA of current. The gold coated samples were placed in chamber of SEM and images were recorded (Alekhya Gurrapu et al., 2012).

Fourier transform infrared (FT-IR) spectroscopy

FTIR spectra of pure abacavir sulphate, surfactants, cholesterol, maltodextrin, blank proniosome formulation (CBPF7) and optimized proniosome formulation (CDPF7) were obtained using FT-IR spectrophotometer (Jasco) by the usual KBr pellet method to scrutinize the interactions between drug and excipients in formulation. The results of pure drug and optimized formulation were illstrated in Figure 4. (Ranjan et al., 2014).

Statistical analysis

Data are presented as mean ± SD. Statistical analysis was performed by Students' *t* test using Graph Pad software. Significance was defined at *p* values <0.05.

RESULT AND DISCUSSION

The present study was undertaken to formulate proniosome carrier system for antiviral drug abacavir sulphate by slurry method using commonly available surfactants like spans and tweens. 250:250µM ratio of surfactant:cholesterol was selected for present formulations from preformulation studies. Drug excipient compatibility was confirmed by compatibility study using FT IR analysis.

The morphology of prepared abacavir sulphate proniosome derived noisome formulations was studied using optical microscopy and the images are illustrated in Figure 1. The niosome vesicles were multi-lamellar, isolated and spherical without much aggregation.

The size of niosome vesicles gained from abacavir sulphate proniosomes was found to be in acceptable limit and the mean size is presented in Table 2. The niosomes formed from blank formulations were smaller (not statistically significant P=0.7241>0.05) in size than niosomes formed from drug loaded formulations. The size of blank formulations and the drug loaded formulations were in the range of 128.6 ±10.79 to 175.8 ±9.94 and 131.8 ±8.45 to 178.8 ±6.87 respectively. The liaison observed between blank and drug loaded formulations has been attributed the role of drug encapsulation in vesicle size. The niosome vesicles produced from span proniosomes were extremely statistically significant (P=0.0003<0.05) smaller in size than the vesicles produced from tween proniosomes. In the 16 formulations smallest mean size 128.6 nm ±10.79 was observed in the case of span 80 based blank formulation whereas niosomes were of bigger size 178.8 nm ± 6.87 in the case of tween 40 based proniosomes. The smaller vesicles produced from span formulations might be due to low HLB value, higher hydrophobicity, and low surface energy of span series surfactants. Furthermore increasing alkyl chain length and hydrophilicity of surfactants may be increase the vesicle size of proniosome derived niosomes.

The data of zeta potential of the formulations were found in range of -33.0 ±1.17 to -44.3 ±1.74. The vesicles formulated with span formulations were found to have the more zeta values, ranging from -40.5±1.08 to -44.3 ±1.74 compared to the proniosome vesicles formulated with tween, showing extremely statistically significant (P=0.0001<0.05) low zeta values ranging from -33.0 ±1.17 to -38.1±1.04. The charges of the proniosome derived vesicles were found to be more negative (> -30 mV) due to the presence of anionic charge inducer DCP. It indicates the stability of vesicles with sufficient electric repulsion.

Polydispersity index was determined to analyze the uniformity of vesicle. As shown in Table 2, PDI of the formulations ranged from 0.342 ±0.02 to 0.398 ±0.07 which implied that the vesicles were comparatively homogenous.

Encapsulation efficiency was analyzed for all the formulations to identify the most excellent in terms of encapsulation efficiency after subtracting blank interference and the data are presented in table 3. The larger vesicle size formed from tween proniosomes may made a payment to the higher entrapment efficiency of abacavir sulphate. From these results encapsulation efficiency was found to be more with the formulation CDPF7 (85.02% ±1.560) which may have suitable surfactant Tween 60 to encapsulate hydrophilic

Table 1: Composition of abacavir sulphate proniosomal formulation

S.No	Formulation code	Surfactant used	250µM surfactant	250µM Cholesterol	DCP	Maltodextrin
1	CBPF1	Span 20	84.302 µl	96.66 mg	2.73 mg	500 mg
2	CBPF2	Span 40	100.64 mg	96.66 mg	2.73 mg	500 mg
3	CBPF3	Span 60	107.66 mg	96.66 mg	2.73 mg	500 mg
4	CBPF4	Span 80	108 µl	96.66 mg	2.73 mg	500 mg
5	CBPF5	Tween 20	278.9 µl	96.66 mg	2.73 mg	500 mg
6	CBPF6	Tween 40	396.32 µl	96.66 mg	2.73 mg	500 mg
7	CBPF7	Tween 60	312.5 µl	96.66 mg	2.73 mg	500 mg
8	CBPF8	Tween 80	303.24 µl	96.66 mg	2.73 mg	500 mg
9	CDPF1	Span 20	84.302 µl	96.66 mg	2.73 mg	500 mg
10	CDPF2	Span 40	100.64 mg	96.66 mg	2.73 mg	500 mg
11	CDPF3	Span 60	107.66 mg	96.66 mg	2.73 mg	500 mg
12	CDPF4	Span 80	108 µl	96.66 mg	2.73 mg	500 mg
13	CDPF5	Tween 20	278.9 µl	96.66 mg	2.73 mg	500 mg
14	CDPF6	Tween 40	396.32 µl	96.66 mg	2.73 mg	500 mg
15	CDPF7	Tween 60	312.5 µl	96.66 mg	2.73 mg	500 mg
16	CDPF8	Tween 80	303.24 µl	96.66 mg	2.73 mg	500 mg

Drug content used 25 mg per batch, CBP-Blank formulations, CDP- Drug loaded formulations.

Table 2: Vesicle size, size distribution and zeta potential of proniosome derived niosomes

S.No	Formulation code	Zeta potential mV*	Vesicle Size (nm)*	PDI*
1	CBPF1	-40.5(±1.082)	146.9(±7.475)	0.395(±0.064)
2	CBPF2	-40.9(±1.343)	141.3(±8.773)	0.372(±0.016)
3	CBPF3	-42.1(±0.985)	136.0(±6.354)	0.350(±0.028)
4	CBPF4	-41.8(±0.896)	128.6(±10.792)	0.359(±0.029)
5	CBPF5	-35.7(±1.124)	168.1(±7.790)	0.368(±0.013)
6	CBPF6	-37.5(±1.026)	175.8(±9.943)	0.351(±0.027)
7	CBPF7	-37.6(±0.802)	171.4(±6.047)	0.362(±0.055)
8	CBPF8	-38.1(±1.039)	164.5(±8.054)	0.369(±0.012)
9	CDPF1	-42.1(±0.519)	149.2(±8.864)	0.359(±0.036)
10	CDPF2	-43.2(±1.365)	144.5(±7.436)	0.342(±0.015)
11	CDPF3	-44.3(±1.735)	138.5(±6.548)	0.390(±0.035)
12	CDPF4	-43.4(±0.954)	131.8(±8.450)	0.371(±0.056)
13	CDPF5	-33.0(±1.172)	172.2(±6.396)	0.397(±0.029)
14	CDPF6	-35.5(±1.137)	178.8(±6.872)	0.398(±0.066)
15	CDPF7	-34.4 (±1.159)	175.0 (±5.122)	0.372 (±0.046)
16	CDPF8	-34.5(±1.114)	168.8(±8.435)	0.375(±0.051)

#*n=3

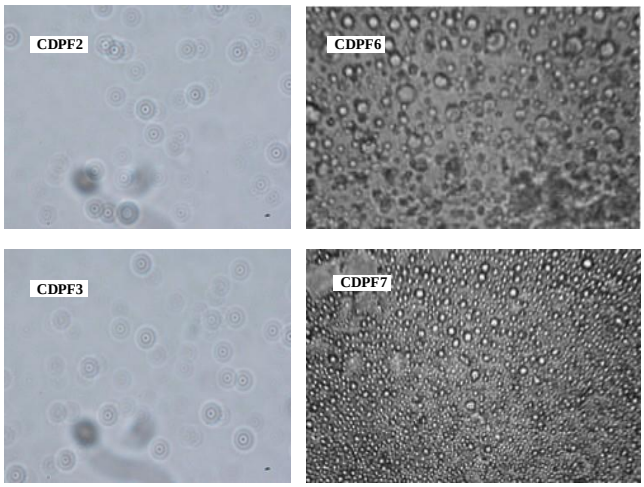

Figure 1: Optical photomicrograph of various batches of proniosome derived niosomes

Table 3: Characterization of Abacavir Sulphate proniosomes

Formulation code	%Drug Encapsulated*	Drug content*	Angle of repose*	CI*	HR*
CDPF1	45.38 ±1.924	99.03 ±0.245	32.31±1.649	13.29 ±1.448	1.15 ±0.019
CDPF2	63.62 ±1.541	99.94 ±0.652	28.36±0.849	8.47 ±1.434	1.09 ±0.017
CDPF3	70.72 ±2.479	99.19 ±0.854	28.51±1.054	7.92 ±1.199	1.09 ±0.014
CDPF4	35.13 ±1.404	100.13 ±1.234	31.84 ±0.787	12.61 ±1.461	1.14 ±0.019
CDPF5	68.38 ±2.395	99.04 ±0.573	32.09 ±1.426	12.87 ±1.083	1.15 ±0.014
CDPF6	77.32 ±2.113	99.01 ±0.949	33.33 ±1.296	13.07 ±1.261	1.15 ±0.017
CDPF7	85.02 ±1.560	99.67 ±0.963	32.43 ±0.866	13.44 ±0.465	1.16 ±0.006
CDPF8	57.59 ±2.025	99.03 ±0.245	31.66 ±1.363	11.84 ±1.050	1.13 ±0.014

#Pure drug: Angle of repose - 56.88 ±0.265, CI - 35.23 ±0.914, HR - 1.54 ±0.022 *n=3 #Maltodextrin: Angle of repose - 38.23 (±1.589), CI - 16.95 (±0.619), HR - 1.20 (±0.009) **CI-** Compressibility index, **HR –** Hausner's ratio.

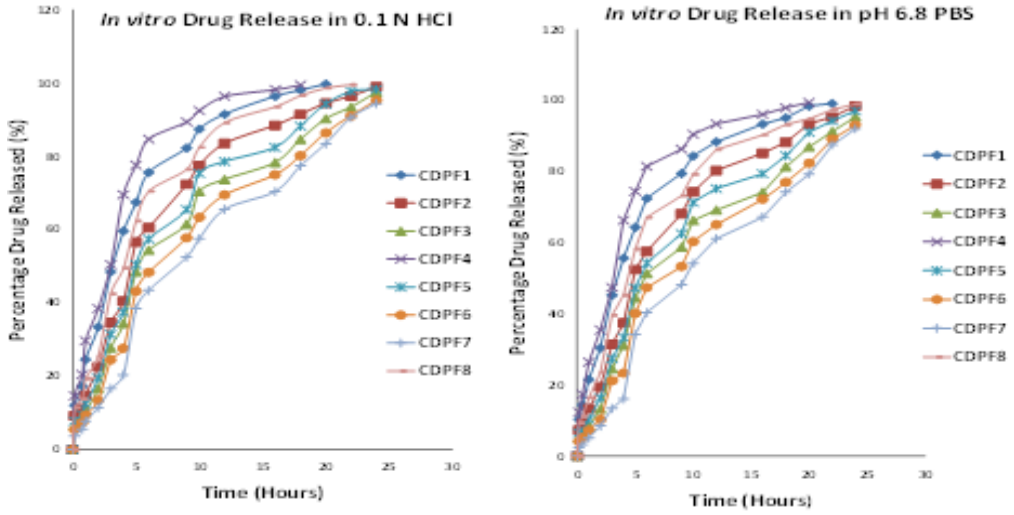

Figure 2: Invitro release of abacavir sulphate proniosome formulations

Table 4: Release kinetics data of selected formulations

Formulation code	Zero order		First order		Higuchi' model		Hixson Crowell		Korsmeyer & Peppas equation	
	0.1N HCl	pH 6.8 PBS	0.1N HCl	pH 6.8 PBS	0.1N HCl	pH 6.8 PBS	0.1N HCl	pH 6.8 PBS	0.1N HCl	pH 6.8 PBS
CDPF5	0.896	0.907	0.949	0.963	0.979	0.979	0.984	0.959	0.966 n= 0.576	0.959 n=0.621
CDPF6	0.933	0.938	0.962	0.967	0.981	0.976	0.986	0.984	0.963 n=0.636	0.956 n=0.701
CDPF7	0.953	0.960	0.952	0.958	0.973	0.965	0.984	0.984	0.959 n=0.733	0.962 n=0.812

PBS – Phosphate Buffer Solution

Table 5: Osmotic shock studies data

Formulation	Average Vesicle size			
	PBS pH 7.4	Hypertonic (1 mol/L NaI)	Isotonic (0.9% NaCl)	Hypotonic (0.5%NaCl)
CBPF7	171.4 ±6.047	Shrunk	176.2 ±10.627	197.4 ±15.615
CDPF7	175.0 ±5.122	Shrunk	182.7 ±8.059	203.0 ±10.104

PBS – Phosphate Buffer Solution # *n=3

drug abacavir sulphate. This higher encapsulation efficiency may be due to the presence of cholesterol, dicetyl phosphate in the formulation and also due to the large hydrophilic head region with long alkyl chain of the surfactant tween 60. The presence of cholesterol and dicetyl phosphate in formulation increase the stability of vesicular membrane and the long alkyl chain reduce the leakage of encapsulated drug from vesicles.

Equivalence of abacavir sulphate content in proniosome formulations were confirmed to assure uniformity in dosages and given in table 3. The drug content was found to be higher in all formulations in the range of 99.01 (±0.949)% to 100.13 (±1.234)%.

In vitro drug release studies are habitually performed to predict how a drug delivery system might work in an ideal situation as well as provide some signs of its *in*

Table 6: Stability study data of optimized formulation

Temperature	Refrigerator Temperature (2-8°C)*			Room Temperature (25±2°C)*			Elevated Temperature (45°±2°C)*		
Sampling period	1 month	2 months	3 months	1 month	2 months	3 months	1 month	2 months	3 months
Percentage drug retained	99.23 ±0.89	98.71 ±0.80	98.55 ±1.17	98.56 ±0.81	98.12 ±0.72	97.69 ±0.98	98.66 ±0.92	95.13 ± 1.35	90.98 ±1.45
Percentage drug encapsulated	84.69 ±1.14	84.07 ±1.45	83.16 ±1.24	83.39 ±0.81	81.89 ±1.18	80.08 ±1.40	80.52 ±1.57	73.42 ±1.63	68.02 ±2.03
Vesicle size	178.63 ±7.16	182.5 ±8.18	188.97 ±8.91	182.97 ±8.91	189.67 ±7.83	196.83 ±11.95	189.67 ±12.24	202.63 ±17.38	224.4 ±18.04

*n=3

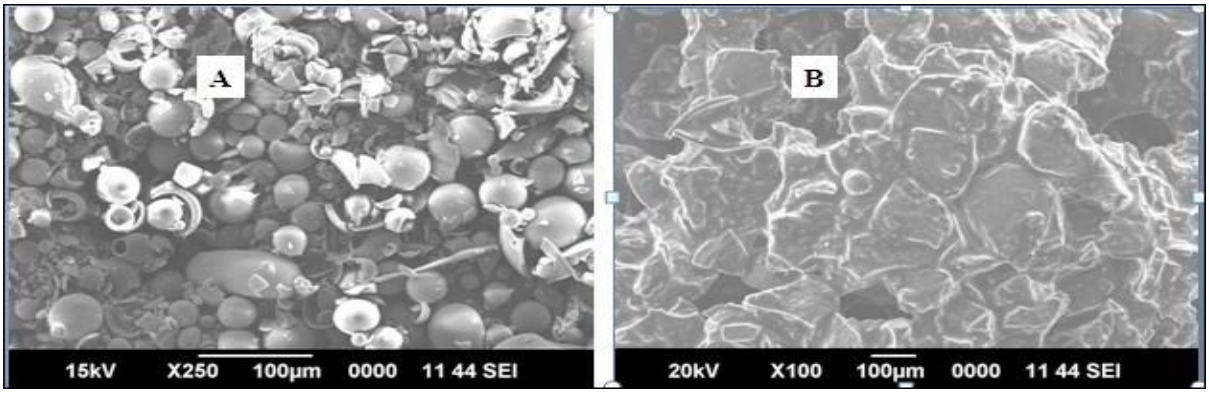

Figure 3: SEM image; A- Maltodextrin, B- Optimized formulation (CDPF7)

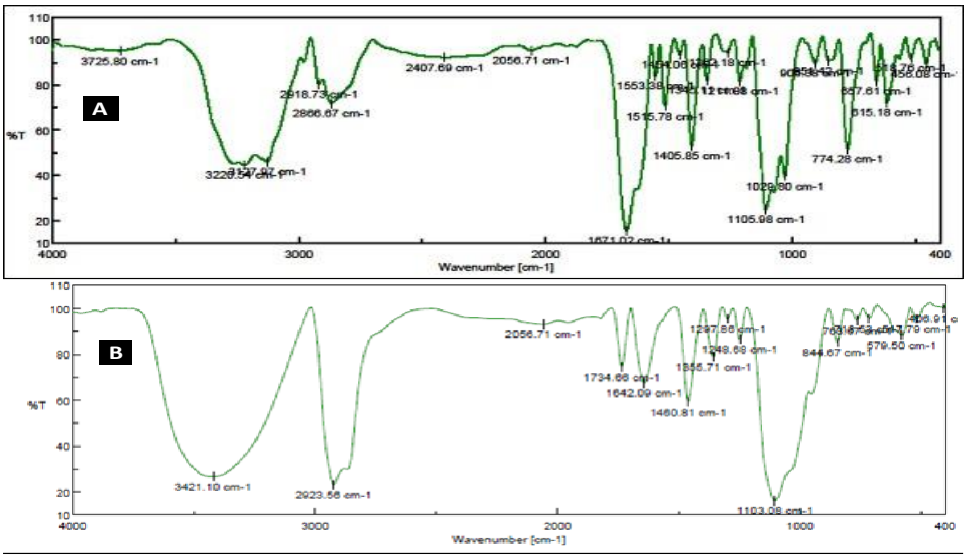

Figure 4: FT-IR spectrum. A-Pure drug, B-Optimized formulation (CDPF7)

vivo performance since drug release indicates the amount of drug existing for absorption (Gupta et al., 2007). The release rate of abacavir sulphate proniosome preparations in simulated gastric fluid (0.1N HCl) is compared with abacavir released from proniosomes in simulated intestinal fluid. The release rate of abacavir sulphate from proniosome preparations in intestinal fluid is considerably lower than that released in gastric fluid. This may be due to the higher solubility of abacavir sulphate in acidic pH. *In vitro* release was found to be inverse to encapsulation efficiency. The optimized formulation had shown the prolonged *in vitro* release of 94.46 ±1.396% in 0.1N HCl and 92.12 ±1.429% in pH 6.8 phosphate buffer solution. A suitable explanation of these results is connected to the ability of cholesterol to close down the gel to liquid phase transition of proniosomal system and thus improved the encapsulation of hydrophilic drug abacavir sulphate. Furthermore it reduces the niosomal vesicle membrane fluidity and improves the rigidity by compacting the packing of surfactants into the bilayer membranes (Viviane et al., 2012).

The zero order plots confirmed the zero order abacavir release characteristics of the optimized formulation

(CDPF7), which was proved by the R^2 value which found to be closer to 1. By incorporating release data in Higuchi and Hixon models, the R^2 values of all the formulations were found to be closer to 1 in both models. Thus the plot indicated that the drug release follow combined desorption and diffusion mechanism. The *in vitro* kinetic was subjected to Peppa's model, all the n values ranged from 0.576 to 0.812 ($0.5 < n < 0.89$) revealed the truth that the abacavir release mechanism follows non-fickian.

The results of osmotic shock on blank and optimized proniosome formulations of abacavir sulphate were presented in Table 5. It was observed that shrinkage occurred while the formulation was incubated in hypertonic solution whereas an increase in vesicle size occurred in hypotonic solution. When incubated in normal saline (0.9% NaCl), the formulation showed a small increase in vesicle size.

Physical stability of abacavir sulphate optimized proniosome formulation is as shown in the table 6. Percentage drug retained, percentage drug encapsulated and vesicle size at the end of the 3 months storage period at refrigerated (2-8°C) and ambient conditions (25±2°C), not shown any obvious changes and it was retained loosely with uniform appearance. Obtained results proved that abacavir sulphate proniosome granule was quite stable at refrigeration and ordinary room temperature.

Angle of repose of maltodextrin and pure abacavir sulphate were compared with proniosome formulation of abacavir sulphate by fixed funnel method and the results of measurements are summarized in Table 3. The results indicated that the angle of repose of dry proniosome powder is smaller than that of maltodextrin powder and the free flowing property of pure abacavir sulphate was very poor. It shown the flow property of proniosome granules is appreciable than that of maltodextrin powder and pure drug. The compressibility index and hausner ratio results moreover supported to the angle of repose data. Thus further processing of dry proniosome granules as a tablets, capsules or beads is possible to provide suitable unit dosing.

Shape and surface feature of proniosome were observed by scanning electronic microscopy analysis. Scanning electron microscopy of uncoated maltodextrin (Figure 3A) and dry best proniosome powder (Figure 3B) reveal that the difference in the exterior of the surfaces. The changes in surface morphology proved the coating of surfactant on carrier.

FTIR spectra of pure abacavir sulphate (Figure 4A) and the CDPF7 formulation (Figure 4B) were very comparable indicating that no significant interaction occurred between abacavir sulphate and the other excipients.

CONCLUSION

The original intention of our proniosome development for hydrophilic antiretroviral drug was to afford an alternative novel drug delivery vehicle to niosomes and liposomes in targeted drug delivery. To summarize the above mentioned outcomes, water soluble drug abacavir sulphate was fruitfully incorporated into proniosome powders with agreeable flow properties. The optimized proniosomal powder CDPF7, composed of CH: T60: DCP in micromolar ratio of 250:250:5 loaded on maltodextrin, showed abacavir EE% of nearly 85.02 % and vesicle size of 175.0 nm after reconstitution. The hydration of dry proniosome powder was found to be much easier than the long shaking process necessary to hydrate thin film in the conventional niosome preparation. This formulation provided prolonged dissolution profile due to the inclusion of drug into the vesicles. Combined with the immense advantages the vesicular drug delivery systems invoked, the optimized formulation represents a predominantly attractive carrier for abacavir sulphate due to its confirmed stability along with the established ability to prolong drug release and as it is dry free flowing granule form, facilitates the possibility of comfortable unit dosing through further processing to make tablets and beads or capsules.

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