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Research Article

Formulation and characterization of nonionic surfactant/ cholesterol niosomes for abacavir sulphate encapsulation

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ABSTRACT

The purpose of the current study was to develop a niosome formulation of guanosine analog antiretroviral drug abacavir Sulphate for controlled drug delivery. Niosome formulations were prepared by thin film hydration method using surfactants like span 20, 40, 60 & 80 and tween 20, 40, 60&80 with cholesterol as membrane stabilizer and dicetyl phosphate as a negative charge inducer. The formulations were evaluated for vesicle formation, vesicle size, size distribution, zeta potential, encapsulation efficiency, drug content and *in-viro* drug release. Vesicle size and size distribution were evaluated by zetasizer revealed that particle size of 135.0±8.837 to 185.0±13.402 nm and uniform size distribution of niosome. Encapsulation efficacy study report indicated that tween 60 with DCP niosomes exhibited highest encapsulation of 83.02 (±1.085)% and release study demonstrated that 89.56 (±2.090) % of abacavir released over a period of 24 hours. The optimized niosomes showed spherical morphology with smooth exterior under transmission electron microscope (TEM). FT-IR studies, confirmed that absence of chemical interaction between abacavir sulphate and other formulation components of niosome. The stability studies suggested that the more stability of niosome formulation at refrigerated conditions than room temperature. It is evident from this study that niosomal formulation could be a gifted delivery system for abacavir sulphate with prolonged drug release profiles.

Keywords: Abacavir Sulphate; Antiretroviral; Encapsulation; Guanosine; Niosome; Zeta Potential.

INTRODUCTION

A number of novel drug delivery systems have developed encompassing different routes of administration, to attain controlled and targeted drug delivery (Akhilesh et al., 2012). Drug targeting is the release of drug in a specific site for its maximum therapeutic action with reduced toxicity (Arul jothy et al., 2015). Vesicular drug delivery is one of the tools which encapsulate the active pharmaceutical ingredient and releases the encapsulated drug from the vesicle to the target site. eg. Liposomes, niosomes and pharmacosomes (Kumar Sumit et al., 2012; Arul jothy et al., 2015). Drug delivery systems by means of vesicular carriers such as niosomes and liposomes have a variety of advantages over conventional pharmaceutical dosage forms (Ashish Kute et al., 2012). Liposomes and niosomes can hold hydrophilic drugs by encapsulation and hydrophobic drugs by partitioning of these drugs into hydrophobic domains (Chengjiu et al., 1999). Liposomes are basically unilamellar/multilamellar spheroid structures that consist of lipid molecules, commonly phospholipids, bring together into bilayers (Ashwani Singh et al., 2011). Although the purpose of liposomes for improved drug delivery is hopeful, liposomes exhibit few difficulties, including the unsteadiness of aqueous suspensions on storage and the leakage of the encapsulated drugs. Moreover, the high price of synthetic phospholipids and uneven purity of ordinary phospholipids have raised distress over the adoption of liposomal drug delivery systems (Gamal et al., 2010). These basic demerits have given rise to the development of the innovative carrier system named 'Niosomes' (Ashwani et al., 2011). Niosomal vesicles are equivalent to liposomes and serve as drug carriers since they can encapsulate both water soluble and lipid soluble drugs (Mohamed Nasr et al., 2010). Preliminary studies point out that niosomes may augment the absorption of certain drugs from the gastrointestinal tract subsequent oral ingestion and extend the survival of the drug in systemic circulation (Bairwa et al., 2011).

Acquired immune deficiency syndrome (AIDS) is the most common problem throughout the world because of rapid increase in the number of victims (Sembulingam et al., 2005). Over the past 30 years, a number of virus specific targets have been identified and dugs for these developed (Tripathy, 2013). abacavir sulphate

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was approved in 1998 as a nucleoside reverse transcriptase inhibitor (Thomas et al., 2013). This guanosine analogue is a clinically potent ARV drug and the plasma half-life is 1 - 1.5 hours. Rapid reduction in plasma HIV –RNA count and rapid rise in CD4 cell count has been noted when abacavir was given to AIDS patient (Tripathy, 2013).

Drugs frequently used for the treatment of the retroviral infection are mostly available as conventional dosage forms. The main disadvantage of these dosage forms are non-specific or non-targeting delivery of the drug in the site of action. Niosomes extending the circulation of encapsulated drug with changing its organ distribution, metabolic stability, enhance the efficacy and reduce the toxicity of encapsulated antiviral agents. The aim of the present study was to develop a low dose niosomal drug delivery system for anti-viral drug abacavir sulphate. The use of noisome vesicles for targeted drug delivery of abacavir sulphate to HIV infected cells and to achieve prolonged drug release kinetics may permit for the improved effectiveness, reduced drug resistance, a diminution in dosage, a reduction in systemic toxicity and side effects, and upgrading in patient compliance.

MATERIALS AND METHODS

Materials

Abacavir Sulphate was a gift sample kindly supplied by 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 and Span 80 were bought from SDFCL sd fine – chem. Limited, Mumbai. Tween 20, Tween 60 and Dialysis membrane were procured from HiMedia Laboratories Pvt. Ltd, Mumbai. Laboratory grade chemicals without further purification were used as supplied in all cases. Doubly distilled and deionized water was used for the preparation of the solutions. All additional chemicals and solvents were of analytical rank.

Preformulation study

Preformulation studies such as particle size analysis, solubility studies and partition co-efficient were carried out to evaluate the physico chemical properties of pure drug.

The drug – excipients Compatibility Studies were carried out in order to prove absence of any interaction amid drug and excipients by FT-IR analysis and by confirming the absence of caking, liquefaction, discoloration and odor formation of physical mixture.

Niosome preparation

Niosome containing abacavir sulphate formulations were prepared by thin film hydration method. The surfactants, cholesterol and dicetyl phosphate in 250: 250: 5µM ratios were accurately weighed and transferred into a long necked 100 ml round-bottom flask and dissolved in 10 ml chloroform. The flask was attached to a rotary evaporator and the organic solvent was slowly evaporated at 60°C under reduced pressure at 100-150 rpm such that a thin dry film of the constituents was formed on the inner wall of the flask. Any excess chloroform was removed by leaving the flask in a desiccator under vacuum overnight. The dried thin film was then hydrated with 10 ml pH 7.4 phosphate buffered saline containing 25 mg abacavir sulphate, (or 10 ml pH 7.4 phosphate buffered saline) by rotating the flask in the same rotary evaporator in ordinary pressure at 60°C in order to make sure complete hydration of the film. The prepared niosomal preparations (Drug loaded/ Blank) were stored in a refrigerator for the further evaluations (Yong-Mei Hao et al., 2011). The formulation code particulars illustrated in the table.1.

Optical microscopy

The niosomal formulations were confirmed for vesicle formation by optical microscopy at suitable magnification. The niosome dispersion was mounted over a microscopic slide and fixed over by drying at ambient temperature. The dried thin film of noisome suspension was observed for the formation of vesicles. Photo microscopic images of the formulations have been taken by using a digital camera (Akhilesh et al., 2012; Tank et al., 2009).

Vesicle size, size distribution, zeta potential determination

Size and charge of noisome vesicles have a significant outcome on their stability and drug encapsulation (Gannu et al., 2011). Zeta potential was evaluated to determine the stability of niosome by studying its colloidal property (Akhilesh et al., 2012). The polydispersity index was determined as a measure of homogeneicity (Mohd Akhtar et al., 2014). Miniature values of PI (<0.1) indicate a homogeneous population, while PI values >0.3 indicate high heterogeneity (Hanan et al., 2011).

Vesicle size, size distribution and zeta potential of niosome samples were determined by photon correlation spectroscopy using the Malvern ZetaSizer. A zetacell was washed several times with deionized water before being loaded with niosome suspensions to measure the zeta potential. Each sample was diluted to a appropriate concentration with demineralized water and the vesicle size was estimated with an angle of detection of 90° at 25°C. Size of the vesicles, polydispersity index of niosomes, and their mean zeta potential values were obtained from the instrument. Three replicates were taken for each formulation (Ranjan et al., 2014).

Entrapment Efficiency

Free abacavir sulphate was separated from noisome 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:

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

Lintu et al., 2010; Ruckmani et al., 2010).

Transmission electron microscopy (TEM)

The morphology of abacavir sulphate optimized niosomal formulation was investigated by transmission electron microscopy. A drop of niosomal dispersion was diluted 10 folds with deionized water and a drop was spread to a carbon-coated 300 mesh copper grid and left for a minute to stick on the carbon substrate. The excess diluted formulation has been then drawn off by using piece of filter paper and observed under the transmission electron microscope and by using imaging viewer software the images were examined and captured (Hitendra et al., 2012; Anchal et al., 2012).

Drug Content

Abacavir sulphate content in niosomes was obtained by an UV spectrophotometric method. Niosomal 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 consequently diluted with phosphate buffer pH 7.4. The absorbance was measured spectrophotometrically against blank at 285 nm and drug content was calculated from the calibration curve of abacavir sulphate in phosphatebuffer pH 7.4. The average abacavir content of three determinations was reported in Table.4 (Sami· et al., 2014; Ranjan et al., 2014; Preethy et al., 2015)

***In vitro* drug release**

In-vitro release model of niosomal dispersion was carried out by dialysis bag method. 3ml of abacavir sulphate noisome dispersion was taken in dialysis bag (Hi media). Dialysis bag was mounted in a beaker containing 100ml of 0.1N HCl and pH 6.8-phosphate buffer. Magnetic stirrer was used and the temperature was maintained at 37±1ºC. Samples were collected periodically up to 24 hours. The sink condition was continued throughout the experiment. The withdrawn samples were suitably diluted and analyzed for drug content using U.V. spectrophotometer at 285nm keeping phosphate buffer pH 7.4 as blank. (Manivannan et al., 2008; Parthibarajan et al., 2011).

Release kinetics

Release kinetics is an essential part for the dosage form development. Mathematical approach is important scientific methods 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 order to realize the kinetic of drug release, the observation of *in-vitro* drug release study of niosomes were subjected with various kinetic equation models like zero order (cumulative percentage release vs. time), first order (log percentage drug remaining vs time), Higuchi's model (cumulative percentage drug release vs. square root of time) and Erosion (cubic root of the unreleased fraction of the drug vs time). To confirm the mechanism of abacavir release from formulation, the drug release data was integrated into Korsmeyer and Peppas equation (log cumulative percentage of drug released *vs.* log time) (Tank et al.,2009; Ashish Kute et al., 2012; Benika et al., 2014; Preethy et al.,2015).

Lyophilization

Two milliliter of the noisome formulation was prefrozen at -20°C for 1hour and then frozen at -70°C for 2 hours. Vaccum manifolds were closed in the freeze dryer, and the temperature was brought down to -40°C. Vacuum was applied to 0.01M Pascal and the frozen samples were now attached to vaccum manifold and the process was continued for 8 hours. At the end of operation, vaccum was reduced and the freeze dried samples were taken out. The sample was found to be sticky even after overnight storage in desiccators (Lintu et al., 2010).

Fourier transform infrared (FTIR) spectroscopy

FT-IR spectrum of optimized niosome formulation CDNF7 and pure drug were obtained using FT-IR spectrophotometer by the KBr pellet method to examine interactions between drug and excipients in the formulation. The lyophilized noisome formulation was grounded properly with anhydrous KBr and compressed to make pellet. The scanning limit was 400 and 4000 cm^{-1}. The results were accounted in Figure. 5 & 6 and compared with the IR spectrum of pure abacavir sulphate (Ranjan et al., 2014; Preethy et al., 2015).

Osmotic shock studies

The consequence of osmotic shock on optimized noisome formulations was evaluated by incubating of niosomal suspensions in media of diverse tonicities. The niosomal formulations were 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 (Ranjan et al., 2014; Santosh et al., 2015).

Stability study

Physical stability study was carried out to investigate the degradation of drug from niosome during storage. The optimized noisome formulation with the composition of Tween 60 and cholesterol in 250:250 µM ratio with 5 µM DCP was divided into two sets of samples. The samples were sealed in glass vials and stored at 2-

8°C in refrigerator and room temperature 25±2°C for a period of 3 months. Samples were withdrawn at definite periods of time and analyzed for vesicle size, zeta potential, % drug remaining and percent drug entrapment. The results obtained were compared to the freshly prepared niosomes (Tank et al., 2009; Hanan et al., 2011; Anchal et al., 2012).

Statistical analysis

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

RESULTS AND DISCUSSION

The present study was undertaken to formulate noisome carrier system for antiviral drug abacavir sulphate by thin film hydration techniques using commonly available surfactants like span 20, span 40, span 60, span 80, tween 20, tween 40, tween 60 and tween 80.

The particle size of the API was found to be less than 125 microns. The drug was found to be soluble in aqueous solvents such as 0.1 N Hydrochloric acid and phosphate buffer (pH 6.8 & 7.4), slightly soluble in methanol and very slightly soluble in ethanol, chloroform & n-butanol. The partition co-efficient results revealed that the pure abacavir sulphate exhibits highy hydrophilic nature.

From the result of drug excipient compatibility study, the chosen excipients did not show any characteristic changes. Thus it was proved that the excipients selected for noisome formulations were compatible with abacavir sulphate.

The morphology of prepared abacavir Sulphate noisome formulations was studied using optical microscopy and the images are illustrated in Fig.1. Most of the noisome vesicles are discrete and spherical with sharp boundaries.

Mean zeta potential, vesicle size, and PDI data of different abacavir sulphate niosomal formulations prepared using different surfactants were determined by zetasizer and the data are given in Table 2.

The vesicle size of niosomes formulated using tween as surfactant was larger while compared with that of span, this is due to higher hydrophobicity of spans than tweens. It showed that increasing in hydrophobicity reduces surface energy of surfactants ensuing in smaller vesicle size. The size range was found to be 135.0 ± 8.837 to 183.2 ±13.390 (without DCP) and from 137.4 ±7.267 to 185.0 ±13.402 (with DCP) formulations. Vesicle size increases in the following manner Span 80 < Span 60 < Span 40 < Span 20 <Tween 80<Tween 20<Tween 60<Tween 40. This might be explained on the basis of HLB value and alkyl chain length of surfactants incorporated in formulation. In span based niosomes HLB value dominate the alkyl chain length of surfactant and the vesicle size was increased with HLB value because hydrophilicity of niosomes increased with HLB value. While increased the hydrophilicity, the water intake of noisome bilayer will increased and resulting in larger vesicles (Carafa et al., 1998). In tween based niosomes, due to the least difference between the HLB values of surfactance, the length of the alkyl chain also contributed their role in the size of the vesicle. Thus tween 60 (HLB 14.9) formulations exhibited larger vesicles than that of tween 20 (HLB 16.7) formulations. The presence of DCP in formulation was found to be considerably efficient on increasing the noisome vesicle size (P=0.81). Incorporation of a anionic surfactant such as DCP into the niosome membrane leads water efflux into the bilayer and increases separation between bilayers (Zerrin. et al., 2012).

The charges of the noisome vesicles were found to be more negative (> -30 mV) in the presence of DCP. Statistically significant changes were observed between DCP incorporated formulations and the formulations without DCP (P=0.0001<0.05). The values of zeta potential showed that the noisomes prepared with charge inducer have sufficient charge to inhibit aggregation of vesicles due to electric repulsion and these outcome proved the effectual stability of noisome vesicles.

The PDI of the formulations ranged from 0.182 ±0.016 to 0.469 ±0.045 which implied that the vesicles were relatively homogenous.

A comparison between encapsulation efficacy of two categories of formulations such as formulations with charge inducer and the formulations without charge inducer in equimolar (1:1) concentration of surfactant/cholesterol noisome system is scheduled in Table 3. The data shows that the encapsulation efficiency of abacavir sulphate was higher in the charge inducer incorporated formulations. The EE of formulation (CDNF7) was significantly differing (p=0.04) from the formulation (DNF7). This may be due to the reality that cholesterol in the existence of DCP more effectively able to stabilize the organization of the niosomal vesicular membrane in 1:1 molar ratio of non-ionic surfactant and cholesterol, (coinside the result of gentamycin) (Ghada et al., 2008) and also two cetyl chains present in the dicetyl phosphate (Bhavana et al., 1998). Surfactant is an important element in the construction of noisome vesicles and the variation in the surfactants may affect the encapsulation efficiency.

The Table 3 also shows the effect of various sorbitan fatty acid esters and polyoxy ethylene sorbitan fatty acid esters on the encapsulation of abacavir sulphate in niosomal vesicular system. Encapsulation efficiency of noisome formulations formed from Tween series were exhibited high value than that of from span series because of larger vesicle size and highly hydrophilic drugs like abacavir sulphate are encapsulated mostly within the polar head facing hydrophilic region. The surfactant tween is the framework of span molecule with 20 mol-

ecules of ethylene oxide. Accordingly the tween molecules showing more hydrophilicity and larger hydrophilic head region than span molecule (Alaa et al., 2010). The formulation containing Tween-60 (CDNF7) had efficient encapsulation efficiency than those formulations containing other surfactants. This is may be due to the presence of larger hydrophilic head region as well as longer hydrophobic alkyl chain. This longer alkyl chain prevents the leakage of encapsulated drug from the noisome vesicle. In the same time the noisome vesicles prepared with span 80 showed least encapsulation efficiency, even the presence of same head group in all span series surfactants. This may be due to the lowest HLB value and also the presence of unsaturated, bended alkyl chain. Lowest HLB value leads to small vesicle size and bended, double bonded alkyl chain prevent the tight binding of adjacent molecules during the membrane of noisome vesicle formation.

The morphology of noisome vesicles investigated using Transmission electron microscopy. Photograph of TEM depicted in figure 3 reveals that the niosomal vesicles appear as spherical nano vesicles.

The drug content was found to be in the range of 98.62 (±0.863) - 99.97 (±0.740) % and the results are reported in Table no.4. The differences in drug content among DNF7 and CDNF7 were found to be non-significant (P=0.3996>0.05).

The release study was carried out for all the abacavir sulphate loaded noisome formulations in 0.1N HCl and phosphate buffer solution pH 6.8 as shown graphically in the Fig.3. The *in vitro* drug release was affected by the pH of the drug release medium. The drug release in 0.1N HCl was slightly higher than that in the phosphate buffer solution pH 6.8. The increasing drug release as the pH decrease is may be due to pH dependent solubility of abacavir sulphate. The release of abacavir sulphate from all noisome formulations was actually biphasic process where an primary rapid drug release phase was observed in the first 2 hours where about 19.67 (±1.754) – 82.47 (±2.205)% of the abacavir sulphate was released from various niosome preparations followed by a slow drug release phase. The early rapid phase might be owing to the release of unencapsulated drug and desorption of abacavir sulphate from the surface of noisome vesicles while the drug release in the subsequent time consuming phase was limited by diffusion through the niosomal bilayers (Shuangshuang et al., 2013; Sara et al., 2016). By comparing the drug release data of abacavir sulphate niosomes containing dicetyl phosphate (DCP) with that of drug loaded DCP free-niosomes, it is cleared that the drug release is slow down in the presence of DCP. This authenticates that DCP stabilizes the structure of Abacavir sulphate loaded niosomal membrane and turns into less permeable. The formulation with DCP (CDNF7) showed a significant slow drug release (P=0.01<0.05) with formulation without DCP (DNF7). The impact in the abacavir sulphate release varies according to the change in composition of the non-ionic surfactant. The amount of abacavir sulphate released from different niosomal formulations were found in the array of DNF4 > DNF1 > DNF8> DNF2 > DNF5 > DNF3> DNF6> DNF7. This may be due to the inverse relationship between encapsulation efficiency and drug release, ie, higher encapsulation efficiency slower the drug release. *Invitro* drug release from Tween 60 formulation with DCP was found to be prolonged. The tween 60 formulation (CDNF7) showed prolonged drug release of 85.59% (±1.311) after 24 hours.

Out of 16 drug loaded formulations 6 desired formulations (DNF5, DNF6, DNF7, CDNF5, CDNF6 and CDNF7) were selected and taken forward for drug release kinetics study. For all the selected formulations, zero order, first order, Higuchi plot, Hixson Crowell and Korsmeyer and Peppas equation were plotted separately. In each models, R^2 value was determined from the graph and reported in Table 5. While the R^2 values of regression plots for zero order and first order were considered, R^2 values of zero order plots were found to be higher than first order plots in case of the formulations without charge inducer and it was higher for first order plots in case of charge inducer incorporated formulations. Hence it is clear that the drug release from these abacavir sulphate niosomal formulations without and with charge inducer followed zero order kinetics and first order kinetics respectively. By incorporating drug release data in Higuchi as well as Erosion models, the R^2 values of all the noisome formulations were found to be more for higuchi model. The linearity of graph indicated that the release model was diffusion controlled. To additional confirmation of the exact drug release mechanism, the data was incorporated in to kores meyer- peppas model and the drug release mechanism was indicated based on the value of exponent 'n'. For all the niosomal formulations the drug release exponent 'n' value found near to 0.5. This shows the drug released from all the niosomal formulations followed fickian diffusion.

CDNF7 formulation with tween 60 as surfactant and dicetyl phosphate as charge inducer was selected as best formulation based on the high percentage encapsulation efficiency and prolonged drug release. The best formulation and its blank formulation were lyophilized for promoting the stability during storage. But after lyophilization the freeze dried niosome was found to be not flowing and sticky.

FT-IR Spectra of pure abacavir sulphate and optimized formulation (CDNF7) were recorded. The FTIR **s**pectra of pure abacavir sulphate and optimized formulation are shown in Fig. 5 and 6. The presence of peaks at 3220.54 cm-1 (O-H stretching), 2918.73 cm-1 (C-H stretching), 2866.67 cm-1(C-H stretching), 1671.02 cm-1(C=C stretching), 1553.38 cm-1(N-H bending), 1515.78 cm-1 (Aromatic C-C stretching), 1405.85 cm-1 (Aromatic C-C stretching), 1105.98 (secondary amine C-N stretching), 851.42 cm-1& 774.28 cm-1 (Aromatic C-H

Table 1: Formulation code particulars

Formulations without DCP				Formulations with DCP			
BNF1	Span 20 Blank	**DNF1**	Span 20 drug loaded	**CBNF1**	Span 20 Blank	**CDNF1**	Span 20 Drug loaded
BNF2	Span 40 Blank	**DNF2**	Span 40 Drug loaded.	**CBNF2**	Span 40 Blank	**CDNF2**	Span 40 Drug loaded.
BNF3	Span 60 Blank	**DNF3**	Span 60 Drug loaded.	**CBNF3**	Span 60 Blank	**CDNF3**	Span 60 Drug loaded.
BNF4	Span 80 Blank	**DNF4**	Span 80 Drug loaded.	**CBNF4**	Span 80 Blank	**CDNF4**	Span 80 Drug loaded.
BNF5	Tween 20 Blank	**DNF5**	Tween 20 Drug loaded.	**CBNF5**	Tween 20 Blank	**CDNF5**	Tween20 Drug loaded.
BNF6	Tween 40 Blank	**DNF6**	Tween 40 Drug loaded.	**CBNF6**	Tween 40 Blank	**CDNF6**	Tween40 Drug loaded.
BNF7	Tween 60 Blank	**DNF7**	Tween 60 Drug loaded.	**CBNF7**	Tween 60 Blank	**CDNF7**	Tween60 Drug loaded.
BNF8	Tween 80 Blank	**DNF8**	Tween 80 Drug loaded.	**CBNF8**	Tween 80 Blank	**CDNF8**	Tween80 Drug loaded.

Table 2: Vesicle size, size distribution and zeta potential of various formulations

S.No	Formulation code	Zeta potential mV	Vesicle Size (nm)	PDI
1	BNF1	-30.7 (±1.375)	150.9 (±11.260)	0.326 (±0.027)
2	BNF2	-28.9(±1.569)	145.9 (±14.363)	0.432 (±0.029)
3	BNF3	-33.6 (±0.611)	138.8 (±12.095)	0.349 (±0.033)
4	BNF4	-30.6(±1.217)	135.0 (±8.837)	0.182 (±0.016)
5	BNF5	-2.04 (±0.477)	172.8 (±8.361)	0. 253(±0.013)
6	BNF6	-3.69 (±0.539)	181.1 (±10.617)	0.332 (±0.026)
7	BNF7	-3.53 (±0.601)	174.4 (±13.712)	0.414 (±0.031)
8	BNF8	-3.57 (±0.465)	169.3 (±12.106)	0.406 (±0.027)
9	DNF1	-26.5 (±1.401)	152.8 (±7.159)	0.332 (±0.023)
10	DNF2	-28.3 (±0.850)	149.2 (±9.611)	0.384 (±0.032)
11	DNF3	-32.9 (±0.954)	141.1 (±11.849)	0.439 (±0.057)
12	DNF4	-29.5(±1.464)	137.0 (±7.753)	0.314 (±0.033)
13	DNF5	-2.11 (±0.252)	176.2 (±10.018)	0.368 (±0.049)
14	DNF6	-2.89 (±0.506)	183.2 (±13.390)	0.347 (±0.042)
15	DNF7	-2.72 (±0.733)	179.4 (±7.619)	0.277 (±0.039)
16	DNF8	-2.65 (±0.671)	173.2 (±14.853)	0.435 (±0.043)
17	CBNF1	-41.7 (±1.305)	153.2 (±13.453)	0.402 (±0.066)
18	CBNF2	-41.8 (±1.206)	148.3(±10.553)	0.361 (±0.036)
19	CBNF3	-42.4 (±0.833)	141.5(±13.403)	0.373 (±0.023)
20	CBNF4	-41.8 (±1.192)	137.4(±7.267)	0.382 (±0.030)
21	CBNF5	-36.6 (±1.137)	175.2(±12.217)	0.369 (±0.055)
22	CBNF6	-38.5 (±0.751)	183.3 (±9.340)	0.385 (±0.063)
23	CBNF7	-38.2(±1.106)	179.1 (±7.427)	0.377 (±0.047)
24	CBNF8	-38.3 (±0.802)	173.2 (±10.352)	0.384 (±0.075)
25	CDNF1	-43.6 (±1.550)	155.8 (±11.920)	0.377 (±0.048)
26	CDNF2	-44.1 (±0.656)	151.9 (±10.007)	0.347 (±0.045)
27	CDNF3	-44.6 (±1.464)	144.2 (±9.304)	0.344 (±0.042)
28	CDNF4	-44.8 (±1.206)	139.9 (±7.251)	0.362 (±0.024)
29	CDNF5	-34.2 (±0.656)	179.3 (±13.892)	0.411 (±0.034)
30	CDNF6	-36.6 (±0.954)	185.0 (±13.402)	0.371 (±0.028)
31	CDNF7	-37.2 (±0.757)	182.1 (±16.690)	0.469 (±0.045)
32	CDNF8	-36.6 (±1.200)	176.8 (±15.222)	0.415 (±0.054)

All the values are expressed as mean±SD, n=3.

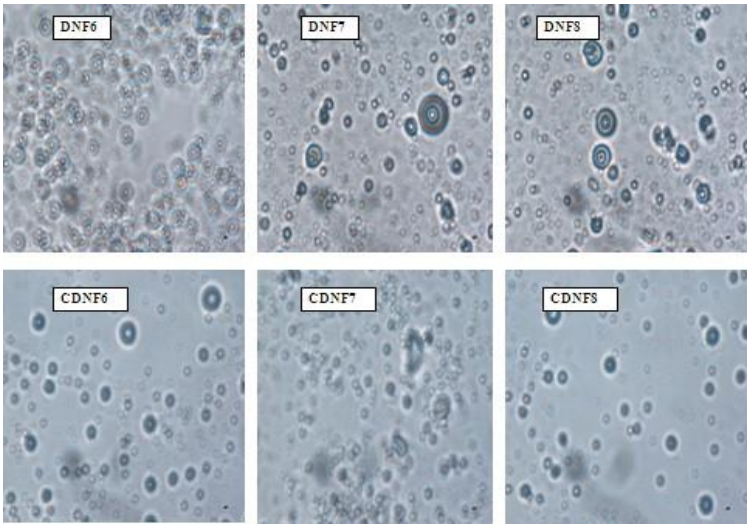

Figure 1: Optical photomicrograph of various batches of niosomes

Table 3: Encapsulation efficiency of various formulations

Formulation code	% Drug Encapsu-lated	Formulation code	%Drug Encapsu-lated
DNF1	34.10 (±2.352)	CDNF1	38.73 (±1.518)
DNF2	58.23 (±0.777)	CDNF2	61.93 (±1.793)
DNF3	66.85 (±1.232)	CDNF3	68.67 (±0.666)
DNF4	29.43 (±1.692)	CDNF4	32.12 (±2.535)
DNF5	61.87 (±2.715)	CDNF5	65.78 (±1.650)
DNF6	70.77 (±1.644)	CDNF6	76.57 (±0.839)
DNF7	77.81 (±2.837)	CDNF7	83.02 (±1.085)
DNF8	47.9 (±2.007)	CDNF8	54.98 (±2.628)

All the values are expressed as mean±SD, n=3.

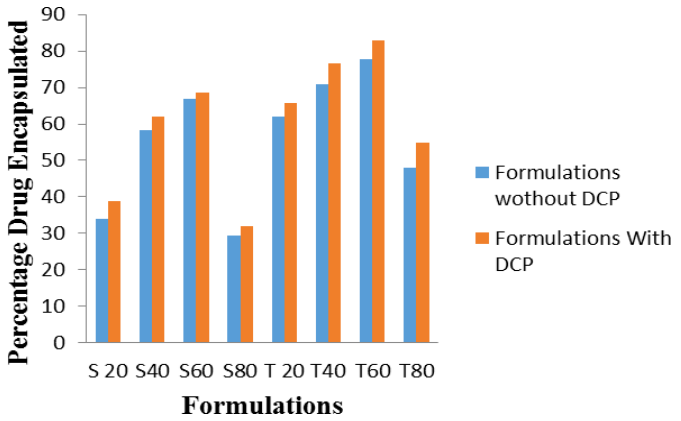

Figure 2: Encapsulation efficiency of niosomal formulations

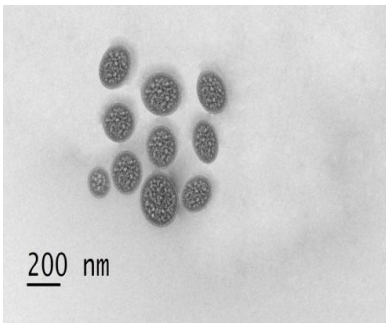

Figure 3: TEM image of CDNF7 formulation

Table 4: Drug content of niosomal formulations

Formulation code	Drug content	Formulation code	Drug content
DNF1	99.3(±0.794)	CDNF1	99.64 (±0.922)
DNF2	98.75 (±1.073)	CDNF2	98.86 (±1.332)
DNF3	98.85(±1.283)	CDNF3	99.54 (±1.082
DNF4	99.16 (±0.661)	CDNF4	99.97 (±0.740)
DNF5	99.72 (±0.940)	CDNF5	98.62 (±0.863)
DNF6	98.99 (±1.610)	CDNF6	98.72 (±0.927)
DNF7	99.91 (±0.986)	CDNF7	99.25 (±0.708)
DNF8	99.02 (±1.226)	CDNF8	99.62 (±1.063)

All the values are expressed as mean±SD, n=3.

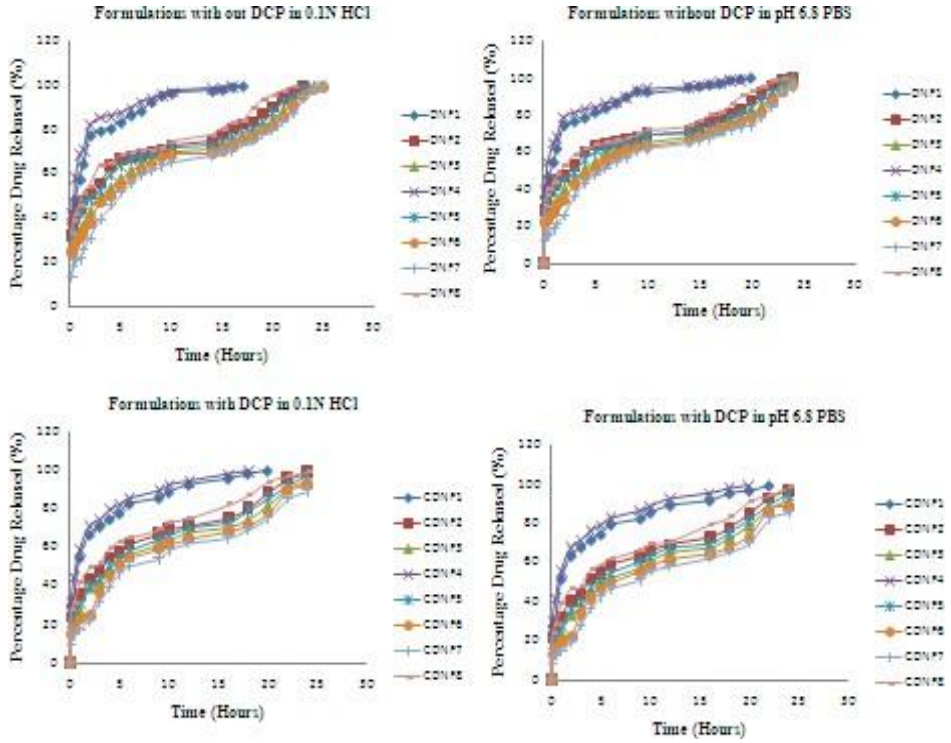

Figure 4: Invitro drug release profile of abacavir Sulphate from Niosome formulations

Table 5: Kinetics data of selected formulations

Formulation code	Zero order		First order R^2		Higuchi' Model R^2		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
DNF5	0.818	0.840	0.734	0.713	0.921	0.931	0.866	0.856	0.974 n=0.226	0.971 n=0.238
DNF6	0.874	0.876	0.781	0.816	0.960	0.967	0.892	0.903	0.971 n=0.290	0.971 n=0.306
DNF7	0.889	0.900	0.819	0.830	0.972	0.973	0.908	0.908	0.981 n=0.379	0.976 n=0.411
CDNF5	0.868	0.879	0.831	0.906	0.973	0.976	0.925	0.940	0.983 n=0.314	0.980 n=0.327
CDNF6	0.882	0.893	0.910	0.923	0.973	0.877	0.935	0.939	0.965 n=0.383	0.964 n=0.406
CDNF7	0.900	0.909	0.939	0.944	0.982	0.981	0.951	0.951	0.981 n=0.446	0.975 n=0.467

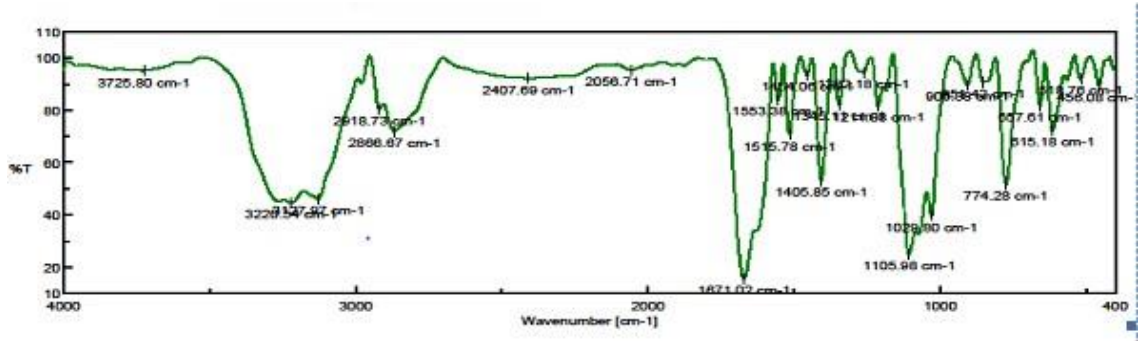

Figure 5: FT-IR Spectra of Pure abacavir sulphate

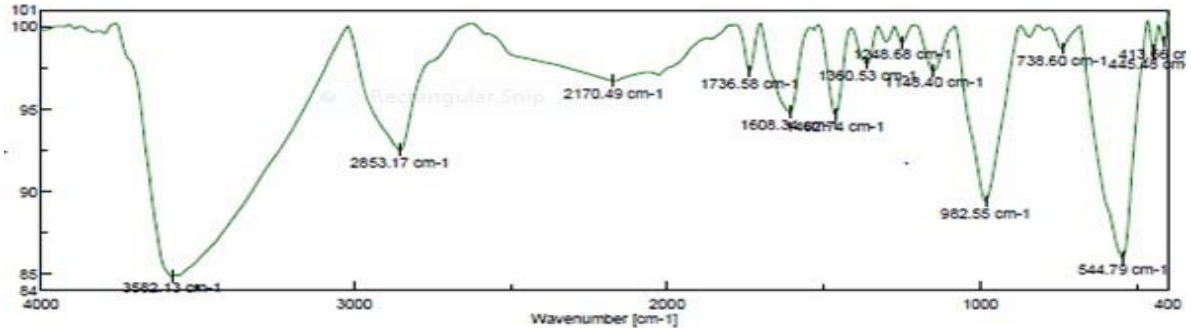

Figure 6: FT-IR Spectra of optimized formulation (CDNF7)

Table 6: Effect of osmotic shock on abacavir sulphate formulations

Formulation	Average Vesicle size			
	PBS pH 7.4	Hypertonic (1 mol/L NaI)	Isotonic (0.9% NaCl)	Hypotonic (0.5%NaCl)
BNF7	174.4 (±13.712)	Shrunk	181.7(±10.347)	207.8 (±16.579)
DNF7	179.4 (±7.619)	Shrunk	188.8 (±8.302)	214.7 (±23.232)
CBNF7	179.1 (±7.427)	Shrunk	185.7 (±9.493)	208.3(±19.354)
CDNF7	182.1 (±16.690)	Shrunk	189.4(±12.646)	212.5(±15.989)

All the values are expressed as mean±SD, n=3.

Table 7: Stability data of optimized formulation

Temperature		Refrigeration temperature			Room Temperature		
Sampling period	Initial	1st month	2nd month	3rd month	1st month	2nd month	3rd month
Percentage Drug retained	99.25 (±0.708)	98.23 (±1.854)	97.70 (±1.268)	96.91 (±1.591)	97.90 (±1.078)	95.67 (±1.297)	93.75 (±0.972)
Percentage drug Encapsulated	83.02 (±1.085)	80.97 (±1.325)	79.54 (±0.891)	78.65 (±1.532)	74.75 (±1.311)	65.52 (±1.925)	53.42 (±1.565)
Vesicle Size	182.1 (±16.609)	187.03 (±11.834)	192.11 (±14.512)	200.29 (±17.045)	198.31 (±17.788)	215.71 (±16.691)	229.22 (±21.746)

All the values are expressed as mean±SD, n=3.

bending), were characteristic to the pure abacavir Sulphate. The characteristic peaks due to pure abacavir sulphate have appeared in formulation and indicating no chemical interaction between abacavir sulphate and excipients. It also confirmed that the stability of drug in formulation.

It was found that shrinkage of vesicles occurred for abacavir sulphate formulations incubated in hypertonic solution whereas an increase in vesicle size occurred in hypotonic solution. When incubated in normal saline (0.9% NaCl), formulations showed a small increase in vesicle size.

The preliminary stability study of the abacavir sulphate loaded niosomes (CDNF7) was performed by analyzing the drug content, Encapsulation efficiency, vesicle size at 0 day, and after being stored for 1 month, 2months & 3 months at refrigeration temperature and room temperature. Statistically significant changes were observed between initial sample and after 3 months at room temperature (DC: $p = 0.0014 < 0.05$; EE: p =

0.0001 < 0.05; VS: p=0.04<0.05). No significant changes were observed at refrigeration temperature (DC: p = 0.08 > 0.05; VS: p=0.26>0.05) indicating excellent stability of niosomes at refrigeration temperature.

CONCLUSION

The present study illustrated that the niosomal vesicle is a suitable carrier for the targeted delivery of hydrophilic antiretroviral drug abacavir sulphate. The outcomes of the study showed that type of surfactant and presence of charge inducer alter the vesicle size, encapsulation efficiency and drug release of niosomes. abacavir sulphate was successfully encapsulated within the polar head facing hydrophilic region of the niosomal vesicles with high efficiency due to the influence of equimolar concentration of surfactant and cholesterol with or without DCP by thin film hydration method. Niosomes formulated with Tween 60 encapsulated large amounts of abacavir sulphate, and the addition of DCP promoted the encapsulation efficiency as well as prolonged the drug release for a longer time. Thus niosomes could be used as a drug carrier vesicle for abacavir sulphate, for producing targeted delivery and prolonged activity.

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