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Research Article

DEVELOPMENT AND EVALUATION OF BAZEDOXIFENE ACETATE LOADED PROLIPOSOMES FOR EFFECTIVE ORAL DELIVERY

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ABSTRACT

The main objective of the present study was to develop proliposomal formulations to enhance the oral bioavailability of bazedoxifene acetate by improving solubility, dissolution and/or intestinal permeability. Proliposomal powder formulations were prepared with bazedoxifene acetate drug varying the Phospholipon 90H and cholesterol ratio in the range of 1:0 to 1:1 using pearlitol SD200 as carrier by film deposition method. The prepared proliposomal powder was filled into capsules. The bioavailability enhancement of proliposomes loaded with drug was studied focusing on phospholipid composition and drug:lipid ratio. Prepared proliposomes were characterized for their particle size distribution, zeta potential, entrapment efficiency, in vitro dissolution study and thermal characteristics to understand the phase transition behavior. Further, the formulated proliposomes were subjected to stability behaviour, ex vivo permeation studies using rat intestine followed by in vivo studies. Physico-chemical studies help in optimization of formulations. Enhancement in dissolution is due to incorporation of bazedoxifene acetate into the phospholipids and change in the physical state from crystalline to amorphous, thus improving oral bioavailability. Ex vivo studies show significant permeation enhancement across gastrointestinal membrane compared to control. In conclusion, proliposomes provide a powerful and functional way of distribution of inadequately soluble bazedoxifene acetate drug which is proved from in vivo studies based on the enhanced oral delivery.

KEYWORDS: Proliposomes, liposomes, bazedoxifene acetate, permeability, pharmacokinetics, bioavailability.

INTRODUCTION

The improvement of oral extent of absorption for poorly aqueous soluble active pharmaceutical ingredient is still the most crucial feature in development of dosage forms. The conventional techniques of improving the bioavailability act by enhancing the dissolution behaviour[1] such as reducing the particle size by co-grinding technique[2], micronization[3] but can't preclude or alter the gastrointestinal (GI) tract barrier function and presystemic metabolism. In order to overcome these concerns and improve the oral bioavailability provesicular system was introduced without altering or affecting intrinsic properties of the drugs entrapped. Provesicular systems[4] namely proliposomes consists of phospho-lipids loaded with drug; along with readily aqueous soluble carrier which offers to form liposomal vesicles upon contact with water or biological fluids. Proliposomes which are free flowing dry powder in nature are dispensed in the form of beads, tablets and capsules. Bazedoxifene acetate, a novel third generation Selective Estrogen Receptor Modulator (SERM) is used in the prevention and treatment of postmenopausal osteoporosis[5] by adhering to estrogen receptors in tissues of bone as agonist promoting bone mineral and density preservation. In the similar manner, bazedoxifene acetate acts as antagonist for tissue in uterine and breast thus preventing stimulation and proliferative action[6].

MATERIALS AND METHODS

Bazedoxifene acetate was a kind gift sample from MSN labs, Hyderabad, India. Phospholipon 90H (Hydrogenated Soy Phosphatidylcholine - HSPC, 90% purity) was generously donated by lipoid, Ludwigshafen, Germany. Cholesterol (>99%) procured from Sigma. Spray dried mannitol (Pearlitol SD200) was a generous gift sample from Dr. Reddy's laboratories, Hyderabad. The study was conducted at Albino research center (Registration No. 1722/RO/Ere/S/13/CPCSEA) with prior approval of institutional animal ethical committee. Euthanasia and disposal of carcass were in accordance with the guidelines.

Formulation of Proliposomes

The proliposome formulations were prepared by using film deposition method[7] and the composition was represented in Table 1. In a 250mL round bottomed flask weighed amounts of lipid mixture (250µM) containing HSPC and cholesterol at various molar ratios and drug (10mg) were dissolved in 20mL solvent mixture of chloroform and methanol (1:1). To this spray dried mannitol (250mg) was added and subjected to evaporation at 45±2^{0}C using rotary vacuum evaporator (Rotavap PBU-6, India). The resultant powder was further dried overnight in a vacuum oven at room temperature so as to obtain dry, free flowing product and were passed through US 60 mesh sieve (250 µm) and stored in tightly closed glass container at 4^{o}C. For comparison, control formulation devoid of phospholipids and cholesterol is processed.

Evaluation/Characterization of Proliposomes

Hydration of Proliposomal Systems

Hydration of Proliposomal systems is judged by studying the shape of the liposomes formed from the proliposomes by optical microscopy (Olympus-CH20i).

Measurement of Micromeritic Properties of Proliposomal Systems

Flow properties are reviewed from angle of repose studied by fixed funnel technique[8]. And also, Carr's index and Hausner's ratio are obtained from bulk and tapped density calculations.

Determination of Size of liposomes, Zeta potential, Entrapment Efficiency and Number of liposomes

For quantifying these parameters proliposome powders are to be converted into liposome dispersions, by hydration followed by 3min bath sonication (Soltec 2200MH, India). For the thus formed liposomes average size and its distribution is checked by Nano particle Analyzer (Horiba SZ-100, Japan). And entrapment efficiency was carried out using Centrisart (Sartorius AG Gottingen, Germany) equipment. % Entrapment efficiency is obtained by subtracting unentrapped drug from total amount of drug. The liposomes were counted by optical microscope (Olympus CH20i, India) using a hemocytometer, and the number per cubic mm was calculated by using the following formula[9].

$$\text{Total number of liposomes per mm}^3 = \frac{\text{Total number of liposomes counted} \times \text{dilution factor} \times 4000}{\text{Total number squares counted}}$$

In vitro dissolution study

In vitro dissolution studies (Lab India DS 8000, India) for proliposome powders compared against control; filled in capsule dosage form are performed in USP type-I (basket) apparatus set at 50RPM containing 900mL of pH 1.2 simulated gastric fluid as medium at 37±0.5ºC. Samples was removed maintaining the sink conditions at definite time points, filtered through 0.45µm millipore membrane and drug release was analyzed by U.V Visible Spectrophotometer (Lab India UV 3000+, India) determining the absorbance at 299nm[10].

Scanning Electron Microscopy (SEM)

By SEM (Hitachi S-3700N, Japan) technique morphology of surface for both the pure drugs and developed optimized proliposomes was identified[11].

Transmission Electron Microscopy (TEM)

TEM (JEOL-100CX-II, Tokyo, Japan) technique also helps in knowing the form, shape and structure of samples as in SEM but in more detail[12].

Differential Scanning Calorimetry (DSC)

DSC (Shimadzu 60H, Tokyo) analysis of optimized formulation and pure drug are studied for the molecular state of the compound. DSC curves help in deducing heat of fusion and melting point.

Powder X-ray Diffractometry (PXRD)

PXRD (Shimadzu 7000, Tokyo) for drug and most effective powder formulation are studied.

Fourier Transform Infrared Spectroscopy (FT-IR)

IR spectra of drug, best powder formulation and phospholipon 90H are acquired from FT-IR spectrophotometer (Shimadzu 8400S, Japan) at a scanning range of 4000–400cm^{-1} with a resolution of 4cm^{-1} to study the drug-excipient interactions.

Stability Studies

Stability for the best formulations is studied for a period of 180 days for various parameters. At predetermined time points that is 0,30,60,90,120 and 180 days samples kept at 24±2ºC and 4±2ºC in aluminum foil covered glass vials are taken out studied.

Ex vivo Absorption Study using Rat Intestine

Albino wistar rats (male) approximately weighing 200gm are taken and study was performed as reported. Control (drug dispersion equivalent to 2mg) and proliposome systems are compared[13].

In vivo Bioavailability Studies

High performance liquid chromatography (HPLC - Waters separation module - Model No:2690; Detector: PDA – Model No: 2996, USA) method is developed and validated for estimation of respective sample drug in serum[14]. Albino wistar rats (male) approximately weighing 200gm are selected for these investigations which are fasted for overnight. These wistar rats are split up into 2 groups one for control and one for optimized proliposome formulation with 6 in every group and delivered with treatment at a random basis. Accurately weighed dose of 10mg per kg body weight the drug or optimized formulation are administered. 250µL of blood sample is taken or withdrawn into micro centrifuge tubes retro orbital plexus at definite time points. Serum was obtained from the collected blood which was left to clot by centrifugation process for 10minutes at 10,000 rpm using centrifuge (Remi R-24, India). Thus, obtained drug containing serum is stored at a temperature of -20^{0}C until further analysis. Cmax and Tmax are picked up from the graph plotted between concentrations of drug in serum at various time points. Trapezoidal rule technique is followed to determine AUC_{0-t}. In the same way $AUC_{t-\infty}$ was calculated by dividing concentration of drug at last time point in the serum with Ke. The relative bioavailability was estimated by dividing the $AUC_{0-\infty}$ of proliposome formulation with control oral suspension. The data obtained were subjected to student's 't' test and one-way analysis of variance (ANOVA), and the significance of difference between formulations was calculated by student-Newman–Keuls (compare all pairs) with Instat Graphpad prism software (version 4.00; GraphPad Software, San Diego California). The level of statistical significance was chosen as $p < 0.05$.

RESULTS

Preparation of Proliposome Powders

In the present study, proliposomes are formulated, developed and assessed for their scope in raising the quality of the delivery of bazedoxifene acetate drug by oral route.

Evaluation/Characterization of Proliposomes

Evaluation of proliposomes after preparation aids in characterizing the formulation and identifying the optimized formulation.

Hydration of Proliposome Systems

Upon hydration of proliposomes, liposomes were derived and were formed immediately upon contact with water and the photomicrographic images at different magnifications are represented in fig. 1.

Measurement of micromeritic properties

All the formulations were having excellent flow property considering micromeritic properties and the values are represented in Table 2.

Determination of Size, Zeta Potential, Entrapment Efficiency and Number of liposomes

The results of all above mentioned physico – chemical parameters are represented in Table 3.

In vitro dissolution study

The percent drug release was more for all proliposomal formulations when measured against control indicating transformation of the crystalline state of the drug to amorphous state.

Scanning Electron Microscopy

SEM images as in fig. 3 shows conversion to amorphous state from crystalline form.

Transmission Electron Microscopy

TEM analysis confirms the spherical shape after hydration as shown in fig.4 resembling the drug-enriched core model.

Differential Scanning Calorimetry

Endotherm peak fades away around melting point which is visible in pure drug indicating the metamorphosis of crystalline state to amorphous state in formulation as shown in fig. 5a and 5b.

Powder X-RAY Diffractometry

Reduction in the intensity of characteristic drug peaks in the optimised formulation compared to pure drug confirms the amorphization of the drug as shown in fig.6a and 6b.

FT-IR Analysis

FTIR spectrum without any extra peaks when compared to pure drug spectrum extrapolates the lack of chemical interaction as indicated in fig.7a, 7b, 7c.

Stability Studies

The stability studies, dictating shelf life are represented in Table 5 suggest that the proliposomal powder was comparatively more stable when stored at 4^0C that at 24^0C.

Ex vivo Absorption Study using Rat Intestine

The amount of drug permeated from proliposomal systems for bazedoxifene acetate has enhanced when compared with respective control within a period of 2 h as shown in fig. 8.

In vivo bioavailability study

Pharmacokinetic study

Various pharmacokinetic parameters are calculated and represented in Table 6. Results obtained show a higher Cmax and Tmax for proliposomes when compared against control. Slower excretion of bazedoxifene acetate drug from proliposomes is evidently the reason for higher mean residence time (MRT) when compared against control. Excellent Area under the Curve (AUC) values indicating higher systemic exposure thus overcoming the bioavailability problem; which is raised as a result of increased hepatic metabolism. Overall improvement in the relative bioavailability (RA) with a significant difference of $p<0.001$deduces the potential of proliposomes as a suitable carrier for improved oral delivery of bazedoxifene acetate.

TABLE 1: COMPOSITION FOR BAZEDOXIFENE ACETATE LOADED PROLIPOSOME POWDER USING SPRAY DRIED MANNITOL

Formulation Code	API (mg)	Carrier (mg)	Molar Ratio (Phospholipid: Cholesterol)	Phospholipid (mg)	Cholesterol (mg)
BPL_0	10	250	1:0	187.5	-
BPL_1	10	250	0.8:0.2	150.0	19.3
BPL_2	10	250	0.6:0.4	112.5	38.6
BPL_3	10	250	0.2:0.8	37.5	77.3
BPL_4	10	250	0.4:0.6	75.0	57.9
BPL_5	10	250	1:1	187.5	96.5

API – Active Pharmaceutical Ingredient (Bazedoxifene acetate drug)

TABLE 2: FLOW PROPERTIES OF VARIOUS PROLIPOSOMAL FORMULATIONS

Formulation Code	Angle of Repose*	Compressibility Index*	Hausner's Ratio*
BPL_0	23.01±0.11	12.66±0.11	1.09±0.28
BPL_1	21.48±0.10	11.63±0.12	1.16±0.21
BPL_2	19.29±0.18	14.40±0.15	1.25±0.09
BPL_3	18.60±0.15	12.10±0.20	1.18±0.24
BPL_4	18.25±0.19	15.70±0.03	1.25±0.08
BPL_5	19.06±0.22	13.90±0.06	1.19±0.25

*Average of three determinations ± Standard Deviation

TABLE 3: PHYSICO-CHEMICAL CHARACTERIZATION OF VARIOUS PROLIPOSOMAL FORMULATIONS

Formulation Code	Particle Size*	Polydispersity Index	Zeta Potential*	% Drug entrapped*	Number of Vesicles/ mm^3 x 10^3
BPL_0	233±08	0.211	54.6±3.6	79.9±3.9	4.11
BPL_1	208±16	0.265	53.5±4.5	81.6±3.2	3.87
BPL_2	214±25	0.293	52.2±2.7	84.7±3.4	3.74
BPL_3	198±22	0.281	49.5±2.6	83.6±1.5	4.15
BPL_4	193±23	0.318	52.5±1.9	84.9±4.2	4.32
BPL_5	184±09	0.338	54.8±2.4	89.6±2.7	4.49

* Average of three determinations ± Standard Deviation

TABLE 4: DISSOLUTION DATA

Time (min)	Cumulative Percent Drug Released*±SD						
	Control	BPL_0	BPL_1	BPL_2	BPL_3	BPL_4	BPL_5
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5	4.25±0.44	42.75±0.31	44.75±0.24	48.75±0.58	48.25±0.25	55.25±0.21	69.25±0.71
10	6.27±0.31	51.49±0.29	54.25±0.51	52.77±0.21	55.02±0.94	70.81±0.39	84.63±0.78
20	8.06±0.29	79.52±0.18	80.80±0.76	82.06±0.28	86.57±0.16	86.70±0.57	90.85±0.74
30	12.10±0.57	86.21±0.74	85.99±0.45	86.02±0.92	91.30±0.18	93.43±0.81	95.10±0.64
45	16.17±0.64	93.43±0.55	93.97±0.98	92.99±0.76	96.55±0.31	96.94±0.77	98.37±0.49
60	20.01±0.18	99.45±0.63	99.73±0.32	98.75±0.88	98.83±0.38	99.97±0.92	101.41±0.33

* Average of three determinations ± Standard Deviation

TABLE 5: STABILITY STUDY DATA FOR OPTIMIZED FORMULATION

Stability evaluation parameters*	Initial	30d	60d	90d	120d	180d
Particle Size	184±09	192±19	191±12	192±17	189±09	190±12
%Retention of drug	89.6±2.7	90.0±3.6	89.0±2.9	90.0±4.2	89.3±3.4	89.1±4.1
Cumulative amount drug permeated (µg/cm2)	188.8±29	200.5±27	197.2±29	199.8±25	193.7±21	203.6±19

*Average of three determinations ± Standard Deviation

TABLE 6: PHARMACOKINETIC PARAMETERS FOLLOWING ORAL ADMINISTRATION (MEAN ± STANDARD DEVIATION, N=6)

Pharmacokinetic parameters	Control	BPL_5
C_{max} (µg/ml)	0.639 ± 0.02	0.949±0.02
T_{max} (h)	2.0 ± 0.00	2.0 ± 0.00
$T_{1/2}$ (h)	31.735 ± 0.663	27.44 ± 0.55
Ke (h^{-1})	0.022 ± 0.605	0.025±0.001
AUC_{0-t} (µg.h.ml^{-1})	17.011 ± 0.264	22.676 ± 0.3
$AUC_{0-\infty}$(µg.h.ml^{-1})	19.346 ± 0.242	25.351±0.4
$MRT_{0-\infty}$(h)	17.475 ± 0.194	19.948 ± 0.23
RA		1.3±0.02

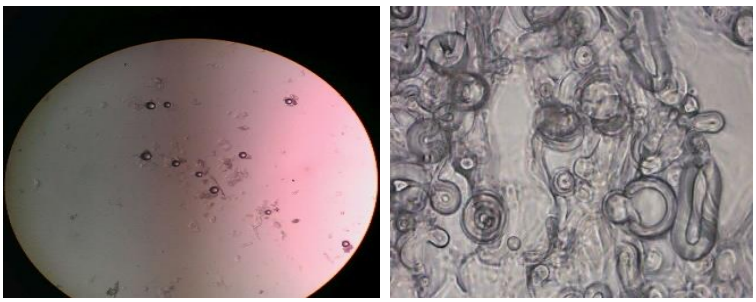


Fig. 1: Photomicrographic Images under magnification 10X and 450X.

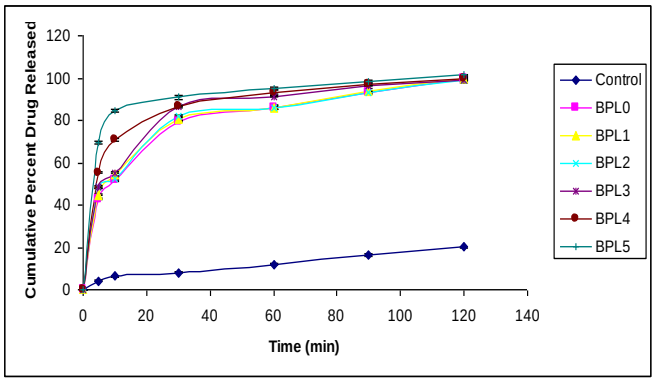
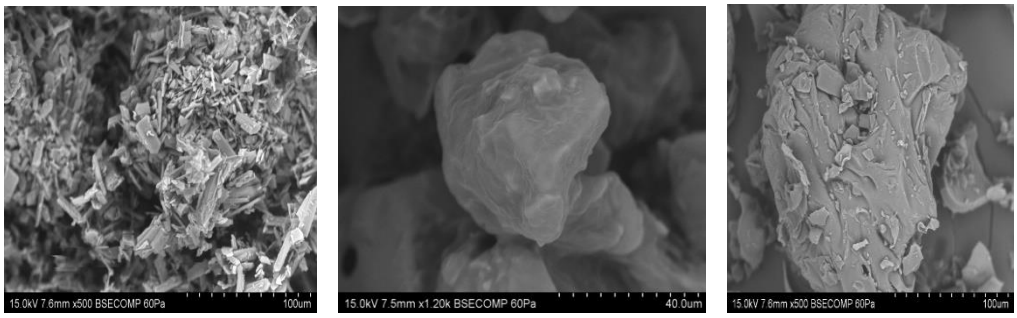


Fig. 2: Cumulative percent drug release versus time plot



(a) (b) (c)

Fig. 3: SEM Images of (a) Bazedoxifene acetate (b) Proliposome powder (c) Pearlitol.

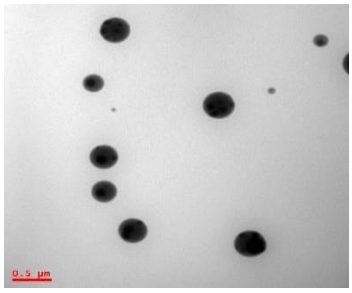


Fig. 4: TEM images of Bazedoxifene acetate Proliposome powder

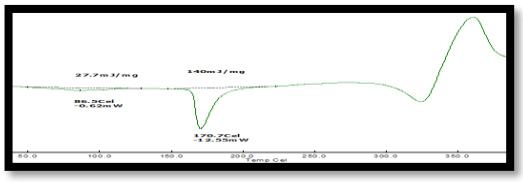


Fig. 5a: DSC of bazedoxifene acetate

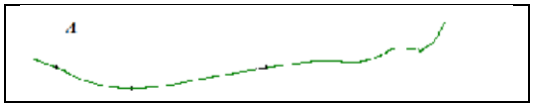


Fig. 5b: DSC of Optimised formulation of BPL_5.

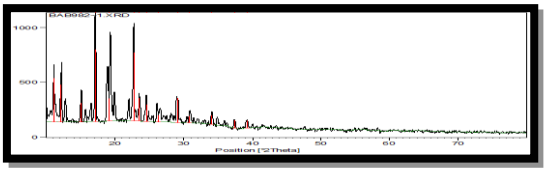


Fig. 6a: PXRD of Bazedoxifene acetate

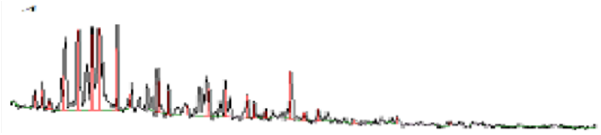
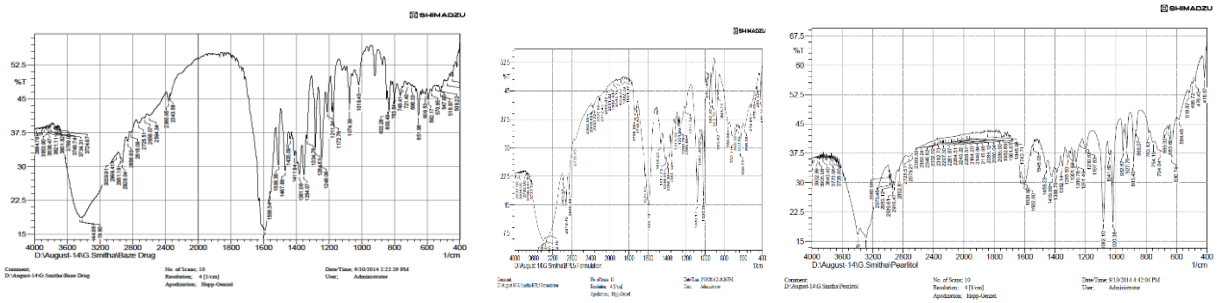


Fig. 6b: PXRD of Optimised formulation of BPL_5



(a) (b) (c)

Fig. 7: IR spectrum of (a) bazedoxifene acetate, (b) BPL_5 formulation, (c) Pearlitol.

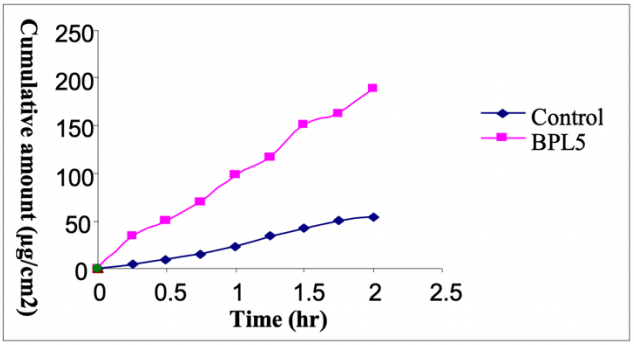


Fig.8: Ex-vivo permeation of Bazedoxifene acetate across rat intestine from proliposome systems (Mean ± Standard deviation, n = 6).

DISCUSSION

The bazedoxifene acetate proliposomes were prepared by film deposition method using spray dried mannitol as carrier at varying ratios of HSPC and cholesterol. The formulation containing equimolar ratio of HSPC and cholesterol was optimized based on the physicochemical characterization and dissolution studies. The in-vivo pharmacokinetic studies reveal potential of proliposomes as suitable carriers for poorly soluble drugs. The results of this study clearly indicate the improvement in intestinal absorption and oral bioavailability of bazedoxifene acetate. The improved delivery of liposome entrapped drug is mediated by vesicle adsorption onto the cell surface followed by endocytosis. The permeability and potential uptake of slightly soluble drugs is increased, thus enhancing the bioavailability.

CONCLUSION

From the extensive study the following conclusion is drawn that proliposomes a type of novel drug delivery has improved the oral bioavailability of inadequately soluble bazedoxifene acetate.

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