IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2020 Vol.:18, Issue:2

Paroxetine Hydrochloride Proliposomes: For Enhanced Delivery by Oral Route

IJPPR
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ISSN 2349-7203
HUMAN

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Submission: 23 April 2020
Accepted: 01 May 2020
Published: 30 May 2020

Keywords: Proliposomes, Paroxetine hydrochloride, Bioavailability

ABSTRACT

One of the major contributors to suicide mortality and disability globally is Major Depressive Disorder (MDD). The administration of antidepressants was considered to be the most significant treatment option. Paroxetine hydrochloride, a highly potent antidepressant is a widely used and approved drug for treating MDD. The present systematic study focused to investigate the advantages of proliposomes for improved oral delivery of paroxetine hydrochloride to overcome the disadvantages with existing oral formulations. Paroxetine hydrochloride loaded proliposomes were prepared using stearic acid (SA), cholesterol (CHOL) and hydrogenated soy phosphatidylcholine (HSPC) in different ratios by film deposition method and the optimized formulations were characterized for zeta potential, entrapment efficiency, and micromeritics. Further a dissolution study and in vitro drug release study carried out provide an insight on the stability and enhanced dissolution of paroxetine hydrochloride from proliposomes formulation. The solid-state characterization (DSC, SEM, and PXRD) studies unravel the transformation of paroxetine hydrochloride to molecular state or amorphous from the native crystalline form. Based on the overall results proliposomes are a suitable carrier for improving the solubility of Paroxetine Hydrochloride.

INTRODUCTION

For the past two decades, advancements and progress in drug formulations and innovative routes of administration are made but still the most favored and widely accepted route of administration is oral route due to its advantages such as ease of administration, sustained delivery, suitable for solid dosage forms with long shelf life and intensified immune response. At the same time few challenges such as inadequate solubility and stability in gastrointestinal fluids, insufficient permeation across the gastrointestinal barrier, metabolism in the enterocytes, and liver exist in oral delivery which led the foundation for researchers to work in this arena. Hence several techniques are investigated for such drugs in improving solubility by complexation, drug derivatization, solid-state manipulation, the inclusion of surfactants, increasing the surface area by micronization or nanonization, spray drying and microencapsulation [1,2].

The main challenge remains the same for a few drugs despite enhancing the dissolution, which is poor systemic exposure altering therapeutic efficacy. Thus, the need to develop colloidal carrier systems has raised for such drugs leading to enhanced permeation across the gastrointestinal barrier [3,4]. Colloidal drug delivery systems that fall under the category of novel drug delivery systems solve the problem thus improving solubility and permeation thereby bioavailability. Colloidal drug delivery systems also called vesicular drug delivery systems are classified based on the nature of the composition into liposomes, ethosomes, transferosomes (lipidaceous in nature), polymerosomes (Polymeraceous), niosomes (surfactant) and sphingosomes, cubosomes, virosomes, and pharmacosomes.

Liposomes which are phospho-lipidaceous in nature are considered the most successful when compared with other dosage forms among colloidal drug delivery systems because of site-specific affinity and also act as reservoirs for a drug. Despite various advantages, liposomes also suffer from limitations such as aggregation, sedimentation, fusion, phospholipid oxidation/ hydrolysis as well as difficulty in large scale production [5].

Thus, to overcome above imperfections proliposomes were developed which are stable, free-flowing because of its dry nature making the drug delivery systems more versatile during storage, sterilization, distribution, measuring, and transfer.

Proliposomes are defined as a dry free-flowing powder formulations consisting of the water-soluble carrier along with phospholipids. Liposomes are formed from proliposomes after

reconstitution in body fluids or aqueous medium accompanied by gentle agitation. Thus, formed liposomes are uniform in size and resemble typical liposomes. Also, liposomes are biocompatible and bioadhesive which can adhere to the gastrointestinal tract improving absorption [6].

Major depressive disorders (MDDs) are prevalent and common mental disorders, identified in almost all age groups and regions across the world. World Health Organization (WHO) has ranked MDD as one of the major contributors to disability and suicidal death per year. Globally, the total number of patients with depression increased by 18.4% between 2005 and 2015 and was estimated to exceed 300 million in 2015. Hence, researchers worked to develop a therapy for this ailment and found the administration of antidepressant drugs to be the most effective treatment option [7]. Paroxetine Hydrochloride, a highly potent drug that has a maximum affinity to serotonin reuptake inhibitors is an approved and widely used drug in the treatment of MDDs. The drug is prescribed to treat anxiety disorders, post-traumatic stress disorder, and symptoms of menopause. Considering the past work and studies on proving secular carriers which includes proliposomes [8] in enhancing the dissolution thereby bioavailability and permeation thus the present structured study concentrated to blend the benefits of proliposomes for improved delivery of Paroxetine Hydrochloride by the oral route. The researcher's intent to select paroxetine hydrochloride drug is to improve efficiency by overcoming existing limitations with conventional tablet formulations such as nausea, discontinuation of therapy due to gastrointestinal reactions, and noticeable fluctuations in peak valley due to plasma concentration.

Paroxetine Hydrochloride proliposomes were formulated by the film deposition method and characterized by solid-state characterization to study physical state, morphology, and possible interaction between the formulation's ingredients.

MATERIALS AND METHODS

MATERIALS

Paroxetine Hydrochloride was a kind gift sample from Hetero laboratories, Hyderabad, India. Phospholipon 90H (highly purified hydrogenated soy phosphatidylcholine 90% purity, (HSPC) was generously donated by Lipoid, Ludwigshafen, Germany. Cholesterol (99% purity, CHOL), Spray dried mannitol (pearlitol SD200), Stearic acid (SA) was a generous gift sample from Dr. Reddy's Laboratories, Hyderabad, India. All other chemicals used were of

analytical grade, and solvents were of LR grade. Freshly collected double distilled water was used throughout the experiments.

METHODS

Preparation of Proliposome Powders

Prolioposome formulations were prepared by film deposition by carrier method [9]. The film deposition method was used for the preparation of proliposome formulations and the composition was represented in table no. 1 and 2. In brief, accurately weighed amounts of lipid mixture (250 µM) comprising of HSPC, Cholesterol and stearic acid at different molar ratios such as 1:0, 1:1, 1:2, 2:1 and 3:1 and drug (10 mg) were dissolved in 20 ml of a solvent mixture containing chloroform and methanol (9:1) ratio. The resultant solution was transferred into a 250 ml round bottom flask, and spray dried mannitol (250 mg) was added to form a slurry. The flask was attached to a rotary evaporator, and the organic solvents were evaporated under reduced pressure at a temperature of 45-50ºC. After confirming the complete removal of solvent, the resultant powders were further dried overnight in a hot air oven at room temperature to obtain free-flowing, dry powder products. The obtained proliposome powders were passed through the #60 mesh sieve and stored in a tightly closed container at 4ºC for further evaluation.

Table No. 1: Composition of Paroxetine Hydrochloride Proliposome Powders

Formulation code	API (mg)	Carrier (mg)	Molar Ratio (Phospholipid:Cholesterol)	HSPC (mg)	CHOL (mg)	SA (mg)
PHCL0	10	250	1:0	187.5	-	-
PHCL1	10	250	1:1	94.0	-	35.5
PHCL2	10	250	1:2	62.5	-	47.33
PHCL3	10	250	2:1	125.0	-	23.66
PHCL4	10	250	3:1	140.5	-	17.75
PHCL-HC1	10	250	1:1	94.0	49.0	-
PHCL-HC2	10	250	1:2	62.5	64.5	-

Physicochemical Characterization of Proliposome Powders

Formation of liposomes from proliposome powders and morphological evaluation

The formation and morphology of the liposomes were evaluated by optical microscopy. The proliposome powder was taken on a cavity glass slide and few drops of water were added

along the side of the coverslip. The formation of vesicles on the surface of a solid particle due to hydration was monitored through an optical microscope and photomicrograph was taken for the morphological evaluation [10].

Flow properties of proliposome powders

The content uniformity of the powder formulations is dictated by the flow properties of proliposome powder. The flow properties of powders were assessed through measuring the angle of repose by using conventional fixed funnel method, carr's compressibility index and hausner's ratio was calculated from the bulk and tapped density of the proliposome powders [11].

Number of vesicles per mm^3

The formation of vesicles is one of the important prerequisites to optimize the composition of the proliposome formulations. The liposomes formed after the hydration of the proliposome powders were counted by an optical microscope using a hemocytometer and the number of vesicles per cubic mm was calculated by using the following formula [8].

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

Measurement of vesicle size and zeta potential of liposomes

The proliposome powders were hydrated with the distilled water and agitated manually for 2 min, and the resultant liposome dispersion was used for the determination of particle size, zeta potential, and entrapment efficiency.

The mean size and size distribution of liposomes were determined by photon correlation spectroscopy using Zeta sizer (Nano ZS90, Malvern Instruments, UK). Each sample was diluted to a suitable concentration with distilled water, and analysis was performed at 25ºC with an angle of detection of 90ºC. The size and polydispersity index of liposomes were obtained from the instrument. The zeta potential values were also obtained from Zetasizer and the measurement is based on the Smoluchowski equation [12].

f ¼ UEg=e

Where

'f' is zeta potential,

'UE' is electrophoretic mobility,

'g' is the viscosity of the medium, and

'e' is dielectric constant.

Entrapment efficiency

The entrapment efficiency of the liposomal formulation was determined by measuring the concentration of free drug in the dispersion medium using ultra-filtration [13]. In brief, ultra-filtration was carried out using a centrifuge (centrisart, sartorius AG, Germany) at 3500 rpm for 15 min, which consists of a filter membrane (Molecular weight cut off 20,000 D) at the base of the sample recovery chamber. The amount of the drug in the aqueous phase was quantified by spectrophotometer. The experiment was performed in triplicate, and percentage entrapment of paroxetine hydrochloride in liposomes was calculated from the following equation:

$$\% \text{ Drug entrapment} = \frac{(\textit{Total amount of drug added} - \textit{unentrapped drug})}{\textit{Total amount of drug added}} \text{X } 100$$

***In-vitro* drug release study**

The liposome dispersion formed after hydration of proliposome formulation was subjected to an *in-vitro* drug release study to understand the release behavior of Paroxetine hydrochloride from liposomes using the dialysis membrane. After soaking the dialysis membrane in the release medium for 24 hrs, liposome dispersions 2ml equivalent to 2mg of the drug were placed in the dialysis bag and kept in 20 ml of release medium which was stirred continuously at 200 rpm and maintained at 37°C at preset time intervals (15,30,60,90 and 120 min). 5 ml sample was withdrawn and replenished by an equal volume of fresh medium to maintain the constant volume of the release medium. The sample was analyzed by a UV spectrophotometer, and obtained absorbance was fitted into the mathematical equation.

In-vitro dissolution study

In-vitro dissolution study of the proliposome powders and control formulation was performed by using the dissolution USP type II (paddle) apparatus in simulated gastric fluid (pH-1.2). The volume of the dissolution medium was 900 ml and the temperature was maintained at 37±2°C with paddle speed set at 50 rpm. Throughout the experiment at predetermined time intervals, an aliquot of a 5ml sample was withdrawn and replenished with the fresh dissolution medium to maintain the constant volume and analyzed by UV spectrophotometer.

Solid-state characterization

These studies allow us to understand the properties of formulation and formulation components. The various tests are as follows:

a. Scanning electron microscopy (SEM)

The surface morphology of the pure drug, mannitol, and pro liposome powders was investigated by scanning electron microscope (S-4100, Hitachi, Japan). Samples were fixed on a brass stub using double-sided adhesive tape and were made electrically conductive by coating with a thin layer of gold and SEM images were recorded at 15 keV accelerating voltage.

b. Differential scanning calorimetry (DSC)

The molecular state of the drug in optimized proliposome formulation was evaluated by performing differential Scanning Calorimetry (Mettler DSC 823e, Mettler – Toledo, Germany) analysis of pure drug, mannitol, and proliposome powder. The DSC curves of the samples were obtained by a differential scanning calorimeter. Average sample weight of 5 ± 2 mg was heated in hermetically sealed aluminum pan over a temperature range of 20–300^{0}C under a constant nitrogen gas flow of 30 mL/min at a heating rate of 10^{0}C/min. The instrument was calibrated with indium (calibration standard, purity >99.9%) for melting point and heat of fusion.

c. Fourier transform infrared (FT-IR) spectroscopy

Infrared spectra of a drug, mannitol, and optimized proliposome powder formulation were obtained using the FT-IR spectrophotometer (Paragon 1000, Perkin Elmer, USA) by the

conventional KBr pellet method. The scanning range was 4000–500 $cm^{-1}$and the resolution was 4 cm^{-1}.

d. Powder x-ray diffraction study (PXRD)

The PXRD patterns of a drug, mannitol, and optimized proliposome powder formulation were obtained using an X-ray diffractometer (X' Pert PRO panalytical, Netherlands). The measuring conditions were as follows: Cu Kα radiation, nickel filtered; graphite monochromator; 45 kV voltage; and 40 mA current with X'celerator detector. All samples were run at 1^0(2ø) min^{-1} from $3^0$to 45^0 (2 ø).

Stability studies

The formulations of proliposome stored in a glass vial were covered with the aluminum foil and kept at room temperature and in the refrigerator at (4±2°C) for 90 days at definite time intervals (0,30,60 and 90 days), the sample was withdrawn and hydrated with the distilled water and observed for any sign of drug crystallization under the optical microscope. Further samples were evaluated for vesicle size and % retention of the paroxetine hydrochloride.

Statistical analysis

The data obtained were subjected to a one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Preparation of proliposome powders

Proliposome formulations are found to be efficient carriers for lipophilic and amphiphilic drugs for improving the bioavailability by the oral route of [11,13,14]. In this study the proliposome formulation was formulated and evaluated for their potential in improving the oral delivery. Various methods have been reported for the formulation of proliposome including the film deposition method [15], freeze-drying method, crystal film method [17], powder bed method [18], spray drying method [19] and fluidized –bed method [20]. According to the feasibility and possibility in our laboratory, we have employed the film deposition on the carrier method for the preparation of paroxetine hydrochloride containing proliposomes.

The formulation of liposomes after the reconstitution depends on the ease of dispersibility of the carrier in the aqueous fluids. Among various carriers spray-dried mannitol was preferred because it possesses high porosity and surface area that enabled the formulator for easy adjustment of the amount of carrier required to support the lipid and also to prepare the proliposomes with the high surfactant to carrier mass ratios.

The selection of the phospholipids is important because it dictates the stability of the liposome formation. Since the risk of the oxidation is high in the phosphatidylcholine due to the presence of the unsaturated bonds in a fatty acid tail, hydrogenated soy phosphatidylcholine which is in a powder form was used in the formulations. The high transition phase temperature and solid-state renders more stability in gastric fluid and improve the flow characteristics of proliposomes respectively, which is an important prerequisite for the solid dosage form. Apart from this lipid to carrier load can be increased so that lipophilic drugs with a high dose can be incorporated without any hindrance to the flow properties. The proliposome concept has resolved many stability issues about the aqueous liposome dispersions. The maximum benefits of proliposomes can be achieved when it forms stable vesicles with high entrapment efficiency after hydration in the gastric fluids. In this perspective, the structural lipid, cholesterol were used which is known to increase the stability of the bilayer with high amounts of drug entrapment. However, the formation and stability of the formed liposomes are by and large dependent on the composition of phospholipid-to-cholesterol and phospholipid –to –stearic acid ratio because of any alteration in their composition results in leakage of the drug before the drug diffusion and fusion of vesicles with the gastrointestinal membrane. Therefore, the effect of cholesterol was investigated by varying the HSPC-to-cholesterol and HSPC –to –stearic acid ratio keeping the total lipid constant (250 µM).

Physico-chemical characterization of proliposome powders

Formation of liposomes from proliposome powders and morphological evaluation

The proliposome upon hydration derives the formation of liposomes and was spontaneously suggesting a rapid conversion to liposomes on contact with physiological fluids in the body. It is evident that in the initial stage upon contact with water, the lipids tend to form tubular structures and upon manual agitations they have formed into small multilamellar vesicles acquiring spherical shape. The images of the same are represented in Fig no.1.

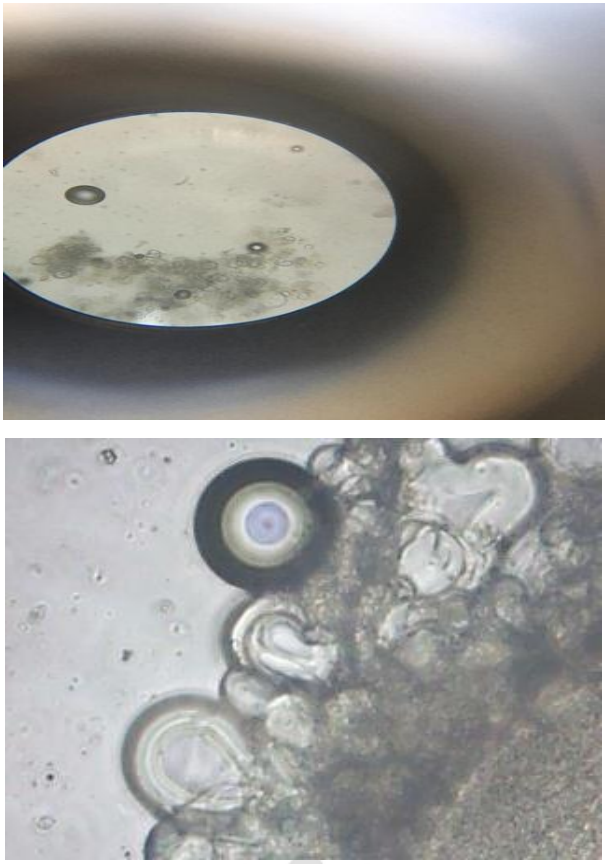


Figure No. 1: Microscopic Images of formation of liposomes from proliposomes under different magnifications

Flow properties of proliposome powders:

The micromeritics of the proliposomes powders is vital in handling and processing operations because the dose uniformity and ease of filling into the container are dictated by the powder flow properties. In general, three types of flow measurements can be used to evaluate the nature of powder flow that is the angle of repose, Carr's index and Hausner's ratio and the results were detailed in Table 2. The smaller the value of angle of repose, the lesser the internal friction or cohesion between the particles and greater the flow characteristics and vice-versa. It is apparent from the results that a small angle of repose assures good flow properties for pro liposome powder formulations. In addition to the angle of repose, Carr's index and Hausner's ratio were also less than 21 and 1.25, respectively, ensuring acceptable flow for proliposomes powder formulations.

Table No. 2: Flow Properties of Paroxetine Hydrochloride Loaded Proliposome Powder Formulations

Formulation code	Angle of repose	Compressibility Index	Hausner's ratio
PHCL0	17.90±0.07	10.40±0.09	1.18±0.01
PHCL1	24.20±0.02	16.40±0.13	1.17±0.13
PHCL2	18.20±0.11	11.30±0.08	1.20±0.13
PHCL3	21.30±0.14	15.50±0.12	1.19±0.18
PHCL4	20.10±0.13	11.30±0.22	1.20±0.13
PHCL-HC1	26.20±0.21	16.80±0.16	1.23±0.16
PHCL-HC2	21.20±0.15	16.90±0.14	1.20±0.11

Average of three determination ± SD

Number of vesicles per mm^3

The distinctive advantages of proliposome formulations can be speculated only when abundant numbers of vesicles are derived from the hydration of proliposome powder in the gastrointestinal tract. Among all the formulations, the proliposome formulation (PHCL-HC1) and (PHCL1) containing the equimolar ratio of HSPC and CHOL (1:1) and HSPC and SA (1:1) demonstrate the good number of vesicles (Table 3).

Measurement of vesicle size and zeta potential of liposomes

One of the important parameters for the vesicular systems is vesicles size and size distribution. The mean size of the vesicles was in the range of nm (Table 3). The size of the vesicles seems to be dependent on the cholesterol and stearic acid concentration. The polydispersity index used as a measure of unimodal size distribution was within the acceptable limits for all the proliposomal formulations (Table No. 3). The zeta potential of the proliposome formulations was also within an acceptable range.

Entrapment efficiency

Among the different methods used for the determination of entrapment efficiency, we have employed the filtration technique because no dilution step involved. The entrapment efficiency of the proliposome formulation was between 70 to 94%. Our result envisages that entrapment efficiency of paroxetine hydrochloride is dependent on the composition of

proliposomes. The entrapment efficiency has increased with an increased concentration of cholesterol and stearic acid (PHCL-0 to PHCL-4).

Table No. 3: Physico-Chemical Characterization of Paroxetine Hydrochloride Proliposome Formulations

Formulation code	Particle size	Polydispersity index	Zeta potential	% drug entrapped	Number of vesicles/mm^3×10^3
PHCL0	252 ± 13	0.187	49.2	77.5±2.4	3.09
PHCL1	171± 14	0.123	50.9	94.6 ± 2.3	3.9
PHCL2	215 ± 16	0.197	52.3	80.1±3.6	3.76
PHCL3	237 ± 11	0.267	48.2	70.2±3.7	4.10
PHCL4	285 ± 09	0.285	52.1	74.1±1.1	3.34
PHCL-HC1	185 ± 12	0.231	48.6	93.8 ± 4.8	3.87
PHCL-HC2	182 ± 19	0.145	53.8	92.5 ± 3.1	3.51

Average of three determination ± SD

***In-vitro* drug release study**

To ascertain the effect of the composition of the proliposomes on the drug release and the stability of liposomes, an *in-vitro* release study was conducted for the reconstituted liposomes across the dialysis membrane. Drug solution shows a very rapid drug diffusion indicating the permeability of a typical biphasic pattern, was observed for liposomes with an initial rapid phase followed by a slow sustained phase for a period of 24 hrs. The initial rapid rise in the release as expected could be due to the burst release of a drug because of the presence of the unentrapped drug in the outer region of liposome membrane and prevalence of sink condition for drugs and is represented in Fig no. 2. The in vitro release data subjected to mathematical modeling reveal that the drug release from proliposome formulations is diffusion-controlled following zero-order kinetics (higher R^2 values).

Table No. 4: *In-vitro* release kinetics of paroxetine hydrochloride from liposome dispersions.

Formulation code	Regression coefficient(R^2)				Release exponent '*n*'
	Zero-order	First-order	Hixson Crowell	Higuchi	
Control	0.8957	0.9276	0.9174	0.9917	0.9092
PHCL0	0.8141	0.8952	0.8728	0.9562	0.9175
PHCL1	0.7106	0.8122	0.6971	0.7838	0.9559
PHCL2	0.7322	0.8479	0.8161	0.9482	0.9959
PHCL3	0.7472	0.8872	0.8445	0.9502	0.9871
PHCL4	0.8057	0.9556	0.9156	0.9795	0.9781
PHCL-HC1	0.4464	0.8349	0.6858	0.7444	0.9346
PHCL-HC2	0.6969	0.9291	0.8673	0.9219	0.9901

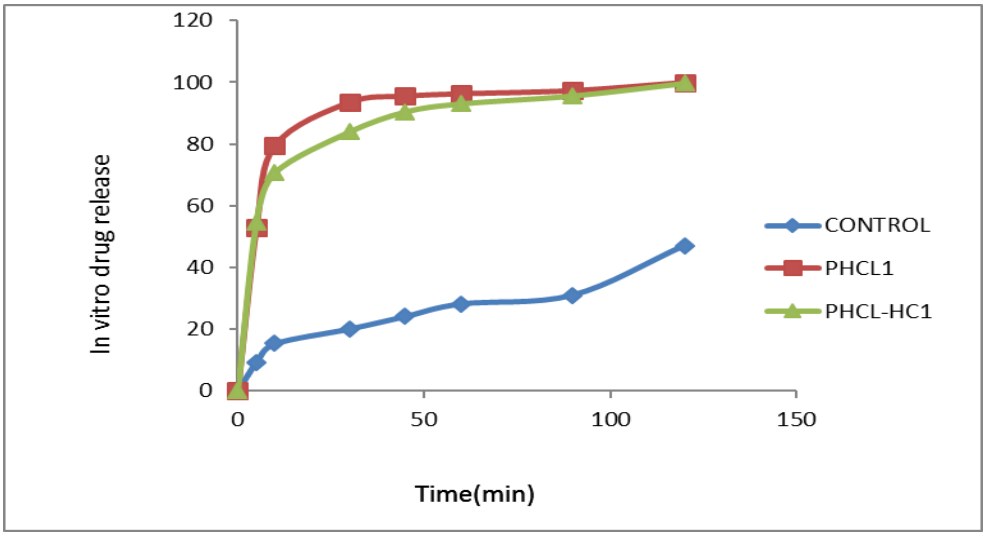


Figure No. 2: *In-vitro* drug release study for control and optimized formulations

***In-vitro* dissolution study**

Dissolution study data revealed that formulations containing equimolar ratios of HSPC to stearic acid and HSPC to cholesterol with formulation codes PHCL1 and PHCL-HC1 have shown maximum drug release within 15mins indicating immediate drug action. Thus, proving improved drug solubility thereby dissolution.

Table No. 5: *In-vitro* dissolution study of paroxetine hydrochloride from proliposomes

Time (min)	CONTROL	PHCL0	PHCL1	PHCL2	PHCL3	PHCL4	PHCL-HC1	PHCL-HC2
0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
15	9.88±0.4	29.32±0.2	71.23±0.7	31.30±0.1	38.07±0.2	37.06±0.7	78.31±0.6	52.76±0.6
30	15.74±0.6	34.21±0.5	80.21±0.3	41.12±0.4	42.07±0.7	45.02±0.3	85.32±0.1	57.46±0.1
45	20.10±0.5	50.17±0.4	83.29±0.6	48.15±0.1	48.06±0.3	56.30±0.8	89.23±0.2	64.52±0.2
60	24.95±0.3	65.32±0.3	90.12±0.3	51.03±0.8	56.02±0.5	61.57±0.7	92.61±0.1	71.03±0.2
90	28.42±0.8	68.12±0.2	91.31±0.4	55.98±0.9	62.06±0.6	71.03±0.3	95.23±0.3	78.04±0.3
120	32.25±0.1	71.22±0.7	93.73±0.3	61.67±0.5	67.77±0.9	78.36±0.1	96.61±0.2	87.09±0.1

Average of three determination ± SD

Solid-state characterization

a. Scanning electron microscopy (SEM)

The surface morphology of the pure drug, pearlite SD200 and proliposome powders was examined by SEM, and the images are represented in Fig no. 3 the absence of typical crystalline structures of paroxetine hydrochloride in a proliposome formulation indicates the transformation of a drug to amorphous and molecular state. Further, a porous structure of spray-dried mannitol as evident in Fig no.3.

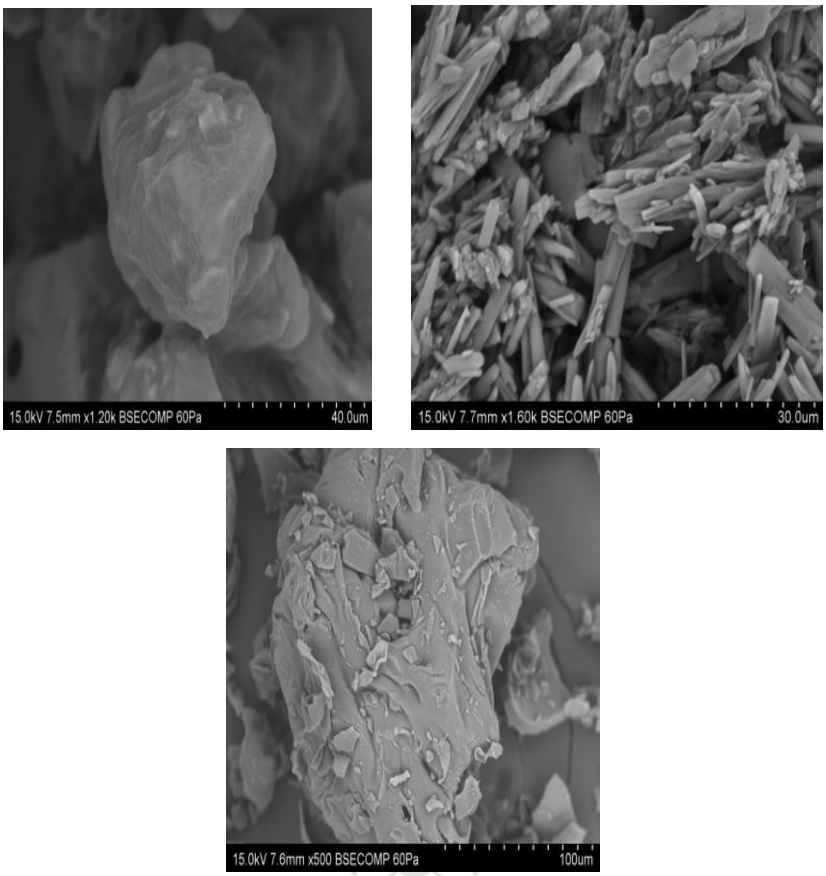
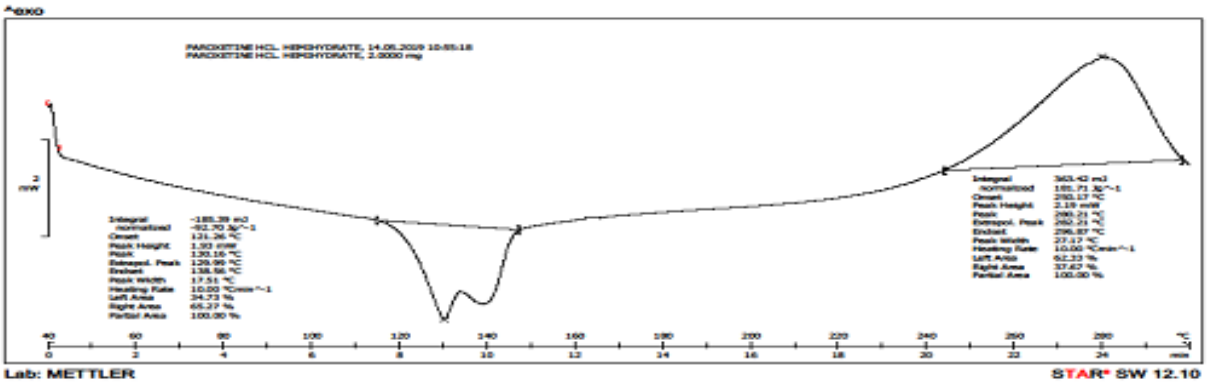


Figure No. 3: SEM Images of (A) Paroxetine hydrochloride (B) Pearlitol SD200 (C) Proliposome powder

b. Differential scanning calorimetry (DSC)

In Fig no. 4 the disappearance of the peak in DSC thermogram of proliposome formulation over the melting range of paroxetine hydrochloride unravels the transformation of the physical state of a drug (crystalline to amorphous) because of the presence of phospholipids.



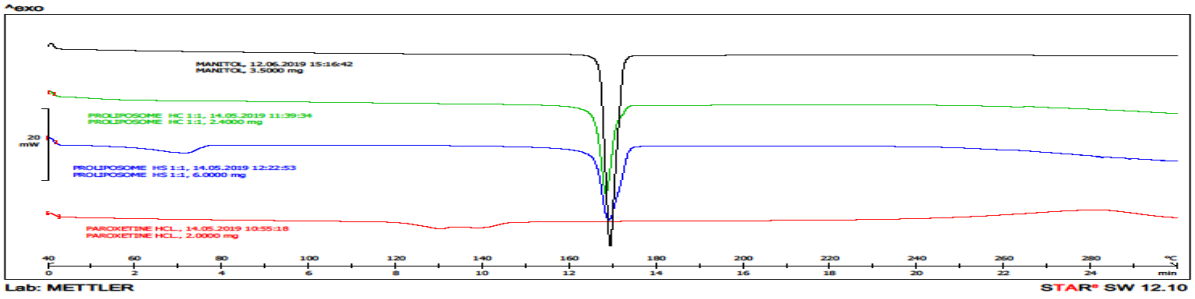
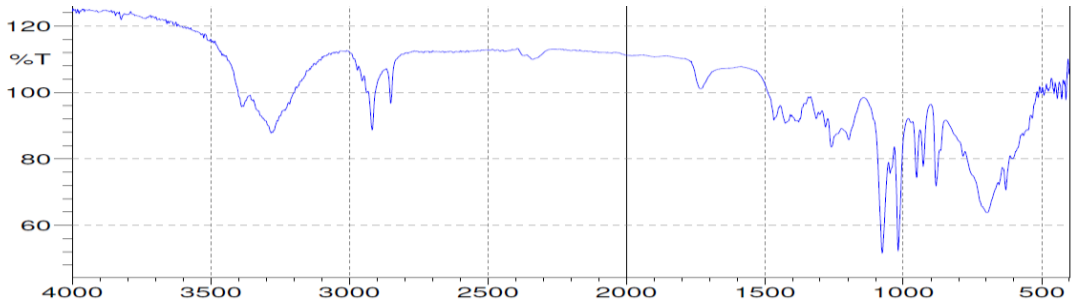


Figure No. 4: DSC Images of Paroxetine hydrochloride and overlay of optimized formulations with mannitol

c. Fourier transform infrared (FT-IR) spectroscopy

No additional peak in the FT-IR spectra of proliposome formulation indicated the absence of chemical interaction between the drug and formulation ingredients as shown in Fig no. 5.



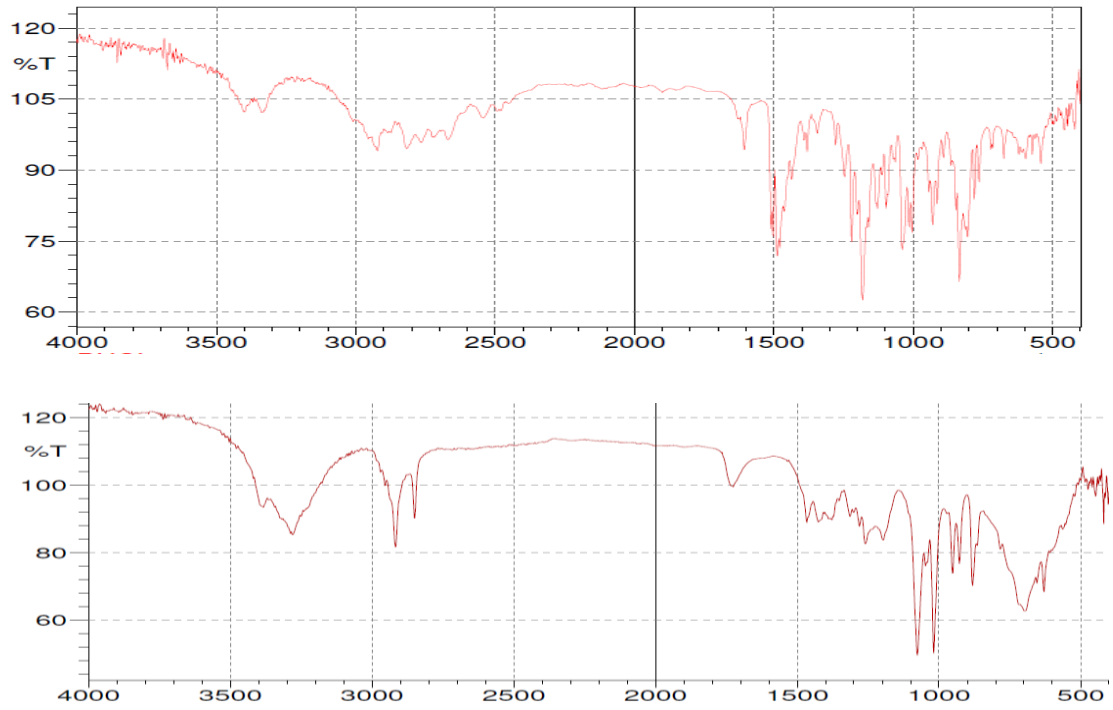


Figure No. 5: IR spectrum of Paroxetine hydrochloride, PHCL1, and PHCL-HC1 formulation respectively

d. Powder x-ray diffraction study (PXRD)

The transformation of crystalline form to amorphous was also confirmed by the PXRD analysis wherein characteristics of paroxetine formulation were reduced in the intensity as shown in Fig no. 6.

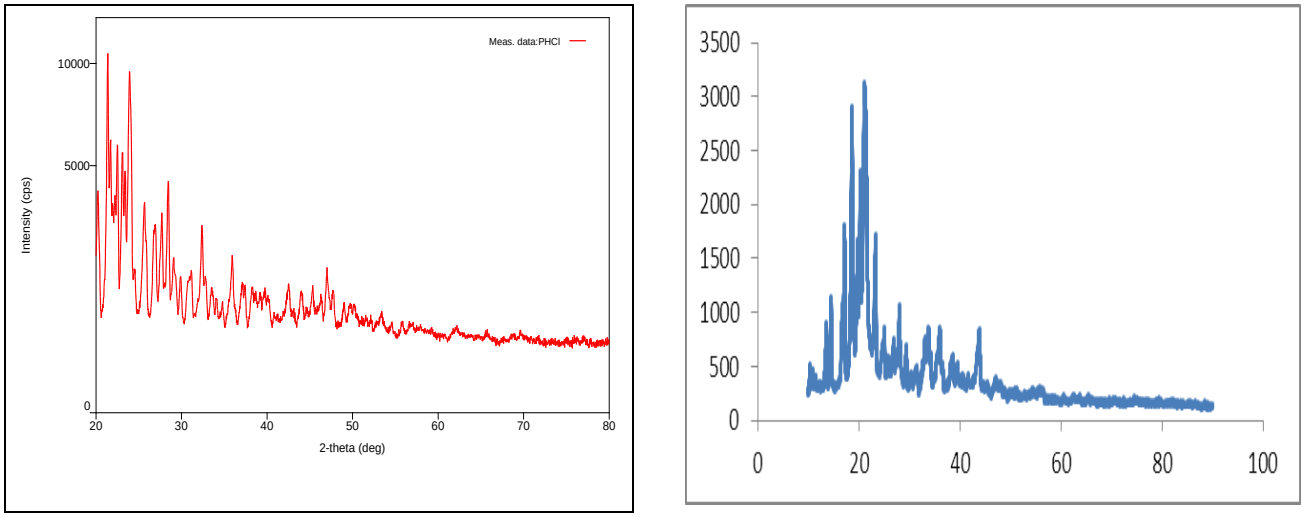


Figure No. 6: PXRD Images of Paroxetine hydrochloride and optimized formulation

Stability studies

The stability of the proliposome formulation was ascertained from reconstituted liposome dispersion by monitoring particle size, physical appearance, and % retention of paroxetine hydrochloride at specific time intervals after the storage for 3 months. The powder was free-flowing without any coagulation, and hydration of the formation of liposomes was rapid without any aggregation problems and also we could not notice any signs of drug crystallization when observed under the optical microscope. Further study, no dramatic change in particle size and size distribution indicated the stability of the proliposome formulation upon storage at refrigerator temperature for 3 months. The results are indicated in Tables 6 and 7. However, a marginal reduction in % retention of the proliposome formulation of paroxetine hydrochloride was observed which is statistically insignificant.

Table No. 6: Stability study data for the particle size of various optimized formulations

Formulation	SIZE (nm)				
	Initial	15 days	30 days	60days	90 days
PHCL1	171±14	192±19	191±12	192±17	189±09
PHCL-HC1	185±12	195±11	194±13	187±15	186±20

Average of three determination ± SD

Table No. 7: Stability study data for % cumulative of drug release of various optimized formulations

Formulation	% cumulative of drug release				
	Initial	15 days	30 days	60days	90 days
PHCL1	99.89±0.3	90.0±3.6	89.0±2.9	90.0±4.2	89.3±3.4
PHCL-HC1	99.73±0.32	88.0±2.6	86.0±2.9	89.0±3.4	88.6±2.5

Average of three determination ± SD

Statistical analysis

The data obtained were subjected to a one-way analysis of variance (ANOVA), and the significance of the difference between formulations was calculated by Student-Newman-

Keuls (compare all pairs) with Instat Graphpad Prism software (version 4.00; Graphpad software). The level of the statistical significance was chosen as $p < 0.0001$.

CONCLUSION

In this study, the advantages of the proliposome formulation have been explored for the enhanced bioavailability by oral delivery of paroxetine hydrochloride. The paroxetine hydrochloride proliposomes were prepared by the film deposition method using the spray-dried mannitol as a carrier varying the ratios of hydrogenated soya phophotidylcholine, cholesterol, and stearic acid. The formulation was optimized by In vitro drug release study, dissolution study, and physicochemical characterization studies. In conclusion, the improved bioavailability of paroxetine hydrochloride by oral delivery proves the potential of proliposomes as a suitable carrier for poorly water-soluble drugs.

ACKNOWLEDGMENT

The authors express sincere gratitude to the management and principal of Marathwada Mitramandal's college of pharmacy for providing necessary facilities to carry out the research work.

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