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FORMULATION DEVELOPMENT, OPTIMIZATION, EVALUATION, AND STABILITY STUDIES OF ARIPIPRAZOLE NIOSOMES.

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Abstract

Niosomes were prepared using thin-film hydration and sonication techniques. For the film hydration method, surfactant, cholesterol, stearylamine (65.0:30.0:5.0 mM), and aripiprazole (2 mg) were dissolved in a mixture of chloroform:ethanol (3:1).The solvent was evaporated in vacuo on a field evaporator to form a thin film. Then hydrate the film with 10 ml of phosphate buffer (pH 7.4) using a stirrer. Surfactant, cholesterol, and stearylamine for sonication method (65.0:30.0:5.0 mM) and aripiprazole (2 mg) were placed in a beaker and 10 mL of phosphate buffer (pH 7.4) was added. I let it sit for 5 hours. A free dispersion (niosome) is synthesized 7 minutes before sonication is required.In the ether injection process, the solution containing the mixture of ether and surfactant slowly enters the aqueous solution (preheated to 60 °C) to evaporate the ether and synthesize the milky pest. Various parameters of the product such as percent encapsulation efficiency, vesicle size, polydispersity index and zeta potential were analyzed and selected as the best method for liposome production according to specification #1.

Introduction

The transdermal drug delivery method has many advantages for close drug delivery and clinical applications. However, due to the impermeability of the stratum corneum, the skin is recognized for its strong protective properties compared to other biofilms. The low permeability of the skin makes it an entry point for drugs. Various drug delivery methods can be used to increase the penetration of molecules such as active and passive products, including iontophoresis, sonophoresis, enhanced penetration, and other delivery methods. Vesicular drug delivery would be beneficial because vesicles tend to coalesce and act like mobile devices, and this is thought to increase the thermodynamic activity of the drug at the interface of the stratum corneum and pore on the skin and

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generally provides better penetration.

Optimization of formulation ingredients

Nanobodies have been created in many groups with the help of the availability and type of non-ionic surfactants. Therefore, after choosing the optimized formulation, various indicators such as the product's polydispersity index, zeta power, vesicle length and capture efficiency were evaluated..

MATERIALS AND METHODS

The different materials and equipment used in the present study are given in table 1 and 2.

Table1: List of materials

S.No.	Materials	Name
1.	Drug	Aripiprazole was received as a gift sample from Torrent Research Centre (Ahmadabad, India)
2.	Solvents	Ethanol, ether, acetonitrile, n-octanol (E. Merck, Mumbai, India)
3.	Lipids	Cholesterol, Soya-Lecithin (SD Fine Chemicals Mumbai, India)
4.	Surfactants	Span40, span60, span80, tween20, tween60, tween80,disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride (SD Fine Chemicals Mumbai, India)

Table2: List of equipment

S.No	Equipment	Names
1.	Electronic balance	Sartorius, Mettler Toledo, Germany
2.	UV spectrophotometer	UV-VIS spectrophotometer (Shimadzu UV-1800)
3.	Particle size analyzer	Malvern zeta sizer nano ZS (Malvern Instruments,UK
4.	Magnetic stirrer	Remi Motors Ltd., Mumbai.
5.	Melting point apparatus	Melting point apparatus[Veego,VMP-D]
7.	SEM	JSM6100/Jeol/Japan

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8.	DSC	Netezch DSC 204 F1 Phoenix Differential Scanning calorimeter chamber
9.	FTIR	Alpha-T(Brucker) FTIR
10.	Turbidity meter	Systronics(s)μC turbidity meter135
11.	HPLC	HPLC system(LC-2010CHT Shimadzu, Japan) Equipped with UV-detector
12.	Centrifuge	Pico21 centrifuge,Thermo Scientific Herae US

EXPERIMENTAL

Formulation and Development of Niosomes

Selection of preparation method of niosomes

Liposomes were prepared by thin-film hydration and sonication. In the film hydration method, surfactant, cholesterol, stearylamine (65.0:30.0:5.0 mM) and aripiprazole (2 mg) are dissolved in a mixture of chloroform and ethanol (3:1).The organic solvent was evaporated under vacuum in the field evaporator to form a thin film. The membrane was further hydrated with 10 ml of phosphate buffer (pH 7.4) using mechanical shaking. Surfactant, cholesterol, stearylamine in ultrasonic treatment (65.0:30.Dispense 0:5.0 mM) and aripiprazole (2 mg) into the beaker, add 10 ml of phosphate buffer (pH 7.4) and sonicate the probe for 5-7 minutes at room temperature, if possible, until a milky white dispersion (niosomes). In the ether injection method, the ether solution containing the mixture of surfactants is slowly entered into the preheated aqueous solution (60 °C) through a fine needle, allowing the ether to evaporate, and then milk-free liposomes are produced. Since the best method was chosen for nanobody preparation, various parameters of the formulation were evaluated, such as % encapsulation efficiency, vesicle size, polydispersity index and zeta potential.

Optimization of formulation ingredients

Different liposome compositions were prepared with different properties and types of non-ionic surfactants. The formulation is then evaluated for various parameters such as vesicle size, polydispersity index, zeta potential and encapsulation efficiency, and an optimized formulation is selected.

Evaluation of noisome

a. Entrapment efficiency (%EE)

The percent encapsulation of aripiprazole in the vesicles was determined by

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centrifugation. Eppendorf-containing vesicles were centrifuged at 14,000 rpm for 15 minutes at 4 °C (Pico 21 centrifuge, Thermo Scientific Herae US), the supernatant containing unembedded drug was removed and phosphate detected using a UV spectrophotometer. B.7. 30% v/v ethanol) was used as a blank. The pellet was also analyzed after blood vessels were lysed with Triton X-100. The encapsulation rate is calculated according to the standard: -

$$\% \text{ Entrapment Efficiency} = \frac{\text{Total amount of drug added- free drug}}{\text{Total amount of drug added}} \times 100$$

b. Vesicle size, polydispersity index and zeta potential

Vesicle size analysis was performed using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). The vesicle preparation was diluted with distilled water and the sizes of the vesicles were determined. Determine the polydispersity index (PDI) as a measure of homogeneity. The zeta potential of the liposome formulation was also measured to confirm the stability of the formulation.

c. *In-vitro* release study

In vitro release studies of liposomal formulations were performed using a locally grown vertical Franz diffusion cell. The prepared vesicle (400 μg aripiprazole equivalent) was stored in the donor area separated from the recipient by the egg membrane. The receiving chamber consisted of pH 7.4 and 30% v/v ethanol (to control the water in the sink) continuously at 100 rpm on a magnetic stirrer, and the temperature was maintained at 37 ± 1 °C. The 5 ml sample is removed at various times and replaced with fresh samples.Samples were analyzed for aripiprazole content by UV spectrophotometer at 280 nm using a neutral. Percentage of drug release planned for time.

d. Stability of niosomes in the presence of bile salts

The stability of the liposomes in the presence of bile salt solution was determined. Solutions of sodium deoxycholate in double distilled water were prepared and diluted to obtain concentrations of 2.5 mM, 5 mM, 7.5 mM, 10 mM, 15 mM, and 20 mM. Add 5 ml of niosome preparation to 50 ml of diluted bile salt solution and incubate for 1 hour at 37 °C.

The turbidity of the samples was measured using a Systronics (s) uC Nephelometer 135 and expressed in Nephelometric Turbidity Units (NTU).Well-designed models are selected and further evaluated based on initial screens.

e. Morphology

Morphological features of the prepared liposomes were analyzed by transmission electron microscopy. A small amount of liposome formulation is mixed with 1%

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phosphotungstic acid, a drop of this mixture is placed on a carbon-coated grid and more samples are aspirated using filter paper. TEM analysis after drying for 2 minutes.

f. Drug-excipients compatibility studies

The drug-excipients compatibility studies were performed using infrared spectroscopy and differential scanning calorimetry.

Drug-excipients compatibility study using FTIR spectroscopy

Infrared spectra of aripiprazole, delay 60, physical mixing, empty and aripiprazole loaded liposome sample were obtained using FTIR spectrophotometer (ALPHA-T, Bruker) to analyze any conflicts between them.

***Drug-excipients compatibility study using differential scanning calorimetry(DSC*)**

DSC experiments were performed using a DSC instrument (Netzsch, DSC-204 F1 Phoenix). Approximately 5 mg of aripiprazole, lyophilized empty and aripiprazole loaded vesicle samples were weighed and loaded into aluminum crucibles, respectively. The crucible was maintained in a strong nitrogen atmosphere (50 ml/min) with a heat flow of 10°C/min increasing from 30°C to 200°C, a heat flux in the Y-axis (w/g), and temperature. The Y axis of the spectrum corresponds to the center. x axis level.

g. Drug release kinetics

Fit data from in vitro release studies to various kinetic models such as zero order, first rate, Higuchi and Peppa models to elucidate drug release mechanisms.

h.* Stability studies (*effect of temperature)

Stability studies of vesicle formulations were performed at different temperatures according to ICH guidelines. The purpose of the stability analysis is to provide evidence of how the quality of the medicinal product changes under the influence of various environmental factors such as temperature, humidity and light. Liposome formulations were packaged in airtight glass vials and stored in a stable room for 1, 2, and 3 months under refrigeration (4 ± 1°C) and room temperature (25 ± 2°C). These models were analyzed at different times in terms of physical appearance, size and encapsulation performance. The physical appearance (aggregation) of the stored samples was evaluated by visual inspection, the size was evaluated by Zetasizer Nano ZS (Malvern Instruments, UK), and the encapsulation efficiency was determined by centrifugation.

i. *Ex-vivo* absorption study

Ex vivo absorption studies were performed using the unevered mouse capsule method.

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Male Wistar rats were housed in a temperature-controlled room with access to free water and standard rat chow. The overnight fasted mice were sacrificed by the spinal cord and immediately removed by dissecting the small intestine, duodenum, and lower ileum. Using a syringe, thoroughly flush the small intestine with cold oxygenated saline (0.9% w/v, NaCl).The cleaned intestines were prepared in 8 ± 0.2 cm long bags. Fill each intestine with a nobody preparation (equivalent to 200 µg aripiprazole) on a syringe (large needle), thread both sides of the intestine tightly and extract in 70 ml of phosphate buffer, pH 7.4 (with 30% v. /). v ethanol) are stored at 37 ± 1 °C with continuous aeration. Remove a 3 ml aliquot and replace it with an equal volume of fresh phosphate-buffered saline.The sample was centrifuged and the supernatant analyzed for aripiprazole content using a UV spectrophotometer at 280 nm. Plan the cumulative drug permeability area versus time and calculate different permeation parameters such as steady-state flux, permeation coefficient, and rate of increase.

RESULT AND DISCUSSIONS

FORMULATION AND EVALUATION OF NIOSOMES:_Nano bodies are vesicles of nonionic surfactants capable of holding many drugs. They contain GRAS-approved non-ionic surfactants, cholesterol and stearylamine, all listed in the FDA Inactive Ingredient Database.

Selection of preparation method of niosomes

In this study, liposomes were prepared by various methods such as ether injection, film hydration and sanitation, and vesicle sizes, polydispersity index, zeta potential and encapsulation efficiency were obtained.

Table 3: Optimization of a method for noisome preparation

Code	Method of Preparation	Vesicle size(nm)	PDI	Zeta potential (mV)	%EE
F1	Ether injection method	725.0 ± 6.4	0.367	-35 ± 4.7	82.3 ± 3.12
F2	Film hydration method	521.0 ± 3.8	0.345	-37 ± 9.3	85.1 ± 2.15

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F3	**Sonication method**	**299.5 ± 2.1**	**0.254**	**-45.5 ± 9.3**	**92.1 ± 3.4**

Span60: Cholesterol: Stearylamine: 65.0:30.0:5.0mM, Aripiprazole: 2mg, Polydispersity Index: PDI, Entrapment efficiency: EE, Millivolts: mV

It can be clearly seen from Table 3, which shows the smallest Vesicle size for the former, that the vesicles prepared by the thin film hydration method (F2) and ether injection (F1) are larger than those prepared by the sonication method (F3). 299.5 ± 2.1 nm. The sonication method usually produces small vesicles, and the vesicle size is also said to depend on the vesicle preparation method, bilayer composition, and drug loading. The polydispersity index (PDI), also known as the heterogeneity index, is used to measure the width of the particle size distribution.The PDI values in all formulations ranged from 0.254 to 0.367, clearly indicating that the formulations were homogeneous. Zeta potential is higher (-35 ± 4.7 to -45.5 ± 9.3) The formulations were found to be stable in all formulations. Compared with the ether injection method (F1) and the film hydration method (F2), the formulation prepared by the ultrasonic method (F3) showed the highest encapsulation efficiency with 92.1% ± 3.4%.Based on the encapsulation efficiency results, the sonication method is considered to be a suitable method for the preparation of nanocores..

3.2.1.1 Optimization of formulation ingredients

Noisome formulations were prepared by sonication method, different concentrations of nonionic surfactants, cholesterol, and aripiprazole, and then several parameters were evaluated, such as vesicle size, polydispersity index, zeta potential, and encapsulation efficiency, respectively, as shown in Table 4.

Table 4: Optimization of noisome composition

Code	Concentration(mM)			Risp (mg)	Vesicle Size (nm)	PDI	Zeta potential (mV)	%EE
	Sp60	Ch	SA					
C1	30.0	65.0	5.0	2	202.7 ± 6.7	0.309	-37.3 ± 6.48	48.3 ± 3.01
C2	47.5	47.5	5.0	2	269.4 ± 7.1	0.161	-39.7 ± 5.35	65.70 ± 2.8
C3	55.0	40.0	5.0	2	207.2 ± 5.7	0.243	-39.5 ± 6.84	79.89 ± 3.4
C4	**65.0**	**30.0**	**5.0**	**2**	**238.1 ± 3.2**	**0.256**	**-45.0 ± 8.35**	**92.83 ± 2.4**
C5	65.0	30.0	5.0	5	242.3 ± 4.5	0.234	-46.0 ± 8.35	94.36 ± 5.7
C6	65.0	30.0	5.0	10	245.7 ±	0.248	-45.6 ± 8.35	95.83 ±

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					6.5			6.1

Span60:Sp60,Cholesterol:Ch,Stearylamine:SA,Millimoles:mM,PolydispersityIndex:PDI,Entrapment efficiency:EE, Millivolts: mV

The encapsulation efficiency increases with increasing surfactant dose as from C1 to C4. It has also been shown that tissue, shape, and vesicle formation reduce the amount of surfactant by more than 70%. Choose 0: 30.0: 5.0 mM (the maximum amount of surfactant) as the best concentration. Stearylamine was used as a rate inducer and was administered in the allowable amount. An increase in cholesterol from C4 to C1 leads to a decrease in encapsulation activity.The incorporation of cholesterol into vesicles is known to affect vesicle stability and permeability. In addition, cholesterol intercalates between bilayers, resulting in increased stiffness, decreased vesicle permeability, and increased encapsulation efficiency of drugs in vesicles. Conversely, more cholesterol competes with the drug for storage space and excludes the drug from the bilayer, causing negative interactions. Therefore, 30 mM (C4) cholesterol level was chosen as a positive value. When the amount of aripiprazole was increased from 2 mg to 10 mg (C4 to C6), the encapsulation efficiency increased due to the favorable log P value.Lipophilic drugs have been found to be better in blood vessels due to their interaction with the hydrophobic bilayer vesicles containing different types of surfactants were also produced by sonication and analyzed for vesicle size, polydispersity index, zeta potential and percent encapsulation efficiency, the results are listed in Table 5.

Table5: Selection of surfactant for niosomes

Formulation Code	**Composition (mM)** 65.0: 30.0:5.0	**Vesicle size (nm)**	**PDI**	**Zeta Potenial (mV)**	**% EE**
S1	Sp20: Ch:SA	232.0 ± 7.5	0.171	-24.0 ± 3.76	74.5 ± 5.3
S2	Sp40: Ch:SA	219.1 ± 3.4	0.277	-40.1 ± 7.79	90.01 ± 4.2
S3	**Sp60: Ch: SA**	**213.9 ± 3.1**	**0.269**	**-50.6 ± 9.31**	**92.83 ± 3.4**
S4	Sp80: Ch:SA	290.2 ± 4.3	0.209	-35.4 ± 3.96	78.5 ± 2.35
T1	Tw20: Ch: SA	388.9 ± 8.8	0.350	21.9 ± 4.62	62.31 ± 3.9
T2	Tw40: Ch: SA	258.7 ± 7.3	0.437	21.3 ± 3.83	70.87 ± 4.2
T3	Tw60: Ch: SA	248.2 ± 7.5	0.427	20.4 ± 4.31	72.47 ± 5.3

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T4	Tw80: Ch: SA	180.0 ± 8.7	0.389	25.1 ± 3.74	68.62 ± 3.1

Span: Sp, Cholesterol: Ch, Stearylamine: SA, Millimoles: mM, Polydispersity Index: PDI, Entrapment efficiency:EE,Millivolts: mV,Tween: Tw

Evaluation of noisome

a. Entrapment efficiency (%EE)

The encapsulation efficiency of liposomes is higher for apertures compared to Tween, which may be due to diffusion (low HLB) and lipophilicity of aripiprazole. In contrast, Tween is hydrophilic (high HLB) due to the presence of hydroxyl and ether oxygen in its molecular structure. Formulation S3 showed the best encapsulation efficiency (92.83 ± 3.4%) and was found in the order Span60 > Span40 > Span80 > Span20, which could be due to the chemical formula, direction and volume characteristics of the surfactants.Essentially, Span 20, 40 and 60 have the same head but different alkyl chains. Due to the long alkyl chain of Span 60, it has higher encapsulation efficiency for lipophilic drugs. Span 80, on the other hand, has an alkyl chain that allows the chain to bend and turn easily. This rotation increases the steric barrier between the hydrophobic chains so that adjacent molecules cannot bind tightly, making the bilayer more permeable. Span 60 and 40 have high temperature variation and are strong at room temperature; this is also necessary to maintain high levels of medication in the bloodstream.As a result, Span 60 ((HLB 4.7 low) appeared to be the best carrier with better performance than Span 20 and 40 (HLB 8.6 and 6.7).

b. Vesicle size, zeta potential, and polydispersity index

Are strongly influenced by the vesicle diameter, HLB value, and surfactant hydrophobicity. As the alkyl chain length increases, the hydrophobicity of the surfactant increases, resulting in a lower HLB value. Liposomes prepared with Span 60 were found to have the smallest vesicle size attributable to its low HLB. The diameter of the liposomes is directly proportional to the HLB. As the HLB value decreases, the surfactant becomes more hydrophobic and the inertia force decreases.In all cases, high zeta potentials (Figure 1) indicate stable formulations and low PDIs indicate homogeneous formulations.

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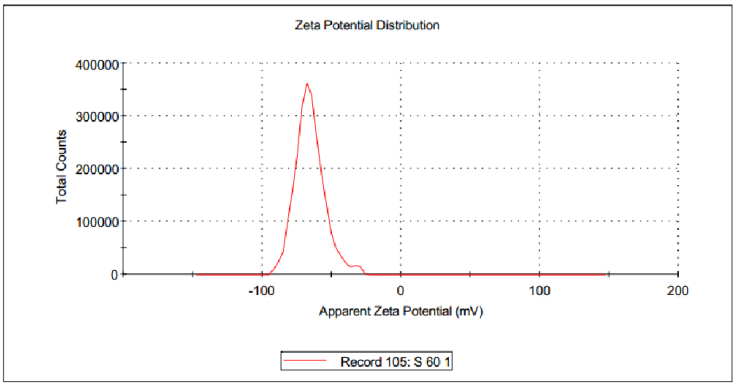

Fig1: Zeta potential of Aripiprazole niosomes with span 60

60 aperture liposomes have the highest encapsulation efficiency (92.83 ± 3.4%). Because the in vivo efficacy of liposomes depends on the amount of drug loaded into them, the formulations were chosen for further evaluation.

b. *In-vitro* release study

Figure 2 presents' drug release data from vesicle formulations of aripiprazole, clearly showing that aripiprazole is released more rapidly during the first hour of vesicles and slowly after release with aripiprazole API (stop solution). Approximately 98% of the drug is released from the aripiprazole suspension within 6 hours, while only 29% is released from the bile salts containing cysts, followed by Span 80 (51.28%), Span 40 (62.49%), Span 60 (77.47% and above 20). (88.36%). The interaction between the components of the vesicles creates a dense bilayer, resulting in delayed release of aripiprazole from the vesicles. The presence of bile in the vesicles significantly reduces drug release.

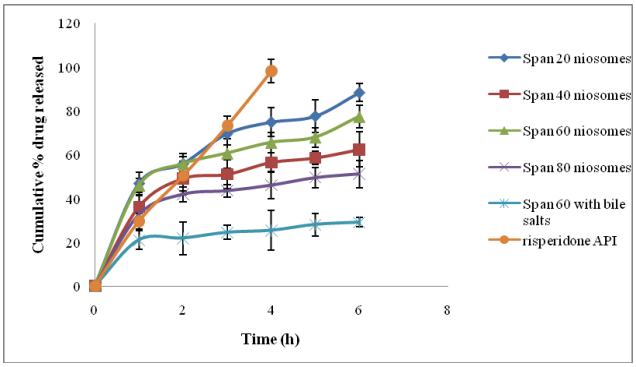

Fig2: *In-vitro* release study of Aripiprazole from niosomes

c. Stability studies of niosomes in presence of bile salts

In the past, it has been reported that drug release is often associated with bile salts in the gut, thus affecting vesicle stability, and therefore vesicle stability should be evaluated in the presence of bile salts. The liposome containing different apertures

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was incubated with 0, 2.5, 5, 7.5, 10, 15 and 20 mM sodium deoxycholate solutions for 1 hour at 37 °C and their effects were analyzed using a nephelometer (Figure 3). Vesicle-micelle exchange occurs in 3 stages; I) the initial binding of bile salts to the outer surface of blood vessels and saturating them without causing major changes, II) breaking of the outer bilayers, and III) the remaining bilayer .Figure 3 clearly shows a reduction in turbidity beyond 10 mM due to degradation of the bilayer. The Span 60 formulation did not show a significant reduction in turbidity, believed to be due to the high transition temperature (Table 6). Span 60 formulations exhibited the highest turbidity even after exposure to 20 mM sodium deoxycholate, indicating the structural rigidity of the liposomal bilayer. The low turbidity of Span 20 and 80 formulations is due to their fluidity, which can transform the bilayer into a micelle structure.

Table 6: Effect of bile salts on niosomes containing different types of span

Sodium desoxycholate (mM)	Turbidity(NTU)			
	Span20	Span40	Span60	Span80
0	500	500	500	500
2.5	482	490	492	411
5	441	465	484	372
7.5	423	452	471	331
10	407	431	460	309
15	258	339	433	181
20	159	283	363	103

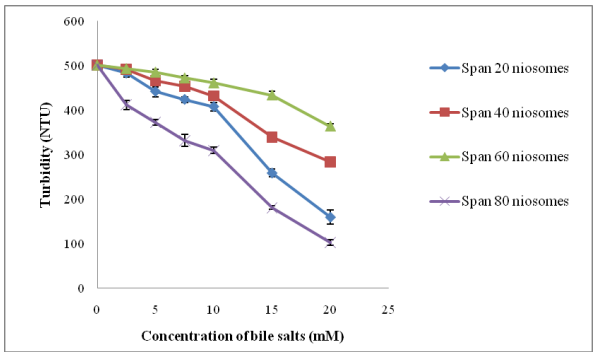

Fig3: Effect of bile salts on niosomes containing different types of span

The incorporation of bile salts into the vesicle tends to stabilize it, so bile salts are used in various formulations as permeability enhancers made of unstable properties to the membrane. Liposomes (span 60) were formulated with sodium deoxycholate (2.5, 5, 7.5, 10 mM) as bulk material and exposed to the same bile salt medium and analyzed for turbidity on most things after 1 hour incubation at 37°C. There are 7 for the turbidity of the Span 60 formulation.More than 5 mM of bile salts were observed even after exposure to 20 mM sodium deoxycholate solution, while the turbidity was

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lowest in the samples without bile salts, indicating that the inclusion of bile salts in the cysts contained the cysts (Table 7 and Figure 4). The higher concentration of bile salts causes a slight reduction in turbidity due to the labile properties of detergents other than CMC. Therefore, it can be concluded from the above findings that the incorporation of bile salts into the vesicle system makes the blood vessels resistant to damage caused by the bile body salts present in the gastrointestinal tract, thereby affecting the biological fate after oral administration.

Table 7: Effect of bile salts on niosomes containing bile salts as an integral component

Sodium desoxycholate (mM)	Turbidity(NTU)				
	Span60 (0mM)	Span60 (2.5mM)	Span60 (5mM)	Span60 (7.5mM)	Span60 (10mM)
0	500	500	500	500	500
2.5	485	494	496	498	495
5	474	487	488	490	486
7.5	455	478	480	486	476
10	420	462	467	470	445
15	400	430	440	460	419
20	363	410	435	452	380

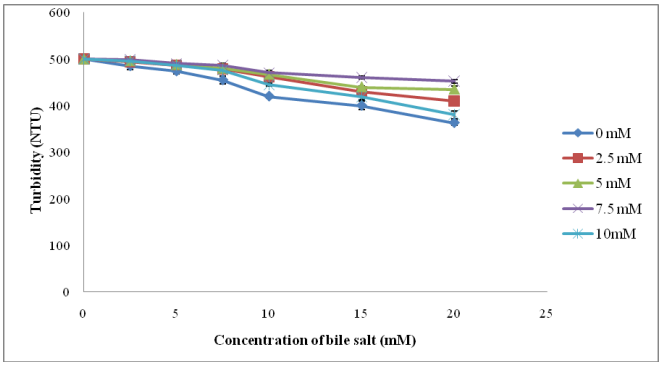

Fig 4: Effect of bile salts on niosomes containing bile salt as integral components

After these initial scans, nanocores with a range of 60 were selected and analyzed for morphology, DSC, FTIR, drug release kinetics, ex vivo absorption studies, etc. further analyzes were made..

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d. Morphology

Morphological features of the prepared liposomes (S3) were analyzed by transmission electron microscopy. The TEM micrograph of lyophilized liposomes is shown in Figure 5, which perfectly demonstrates the presence of well-defined liposomes..

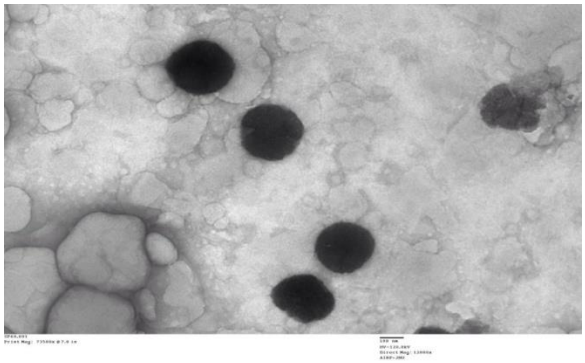
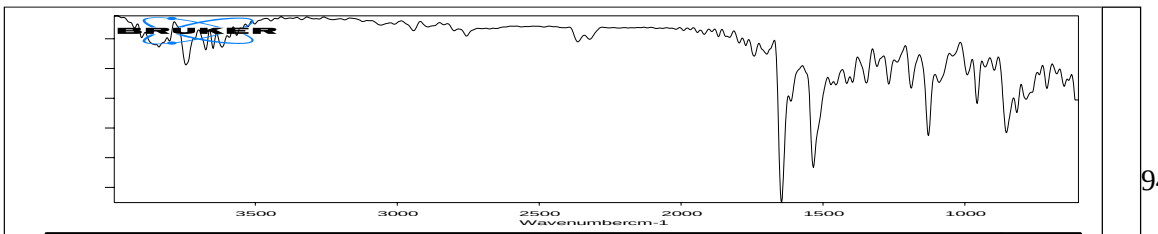


Fig5: Transmission electron photomicrograph of Aripiprazole niosomes

e. Drug-excipient compatibility studies

The drug-excipient compatibility studies were performed using infrared spectroscopy and differential scanning calorimetry.Drug-excipient compatibility study using FTIR spectroscopy. The FTIR spectra of aripiprazole, spread 60, body mix (1:1), empty liposomes and aripiprazole loaded liposomes are shown in Figure 6 . The FTIR spectrum of aripiprazole shows a characteristic peak at 1646cm-1 (C=O stretch), 1534cm. -1 (N-H bending), 1128cm-1 (C-H stretching), 853cm-1 (C-H bending, aromatic) and 3744cm-1 (N-H) stretching). Span 60, 1200cm-1 (aliphatic), 1734cm-1 (cyclic five-member ring), 1400cm-1 (-CH3), 2928cm-1 (aliphatic C-H stretch, asymmetrical), 2800cm-1 (C-H with aliphatic characteristic peaks) stretched, symmetrical) and 3400 cm-1 (O-H stretched). When analyzing the physical mixture of aripiprazole and delay 60, the peak properties of aripiprazole in the physical mixture are similar to the individual aripiprazole spectra and do not change. FTIR spectroscopy confirmed that there was no chemical interaction between them. FTIR spectra of empty and aripiprazole loaded liposomes showed some weak cholesterol interference and spanned 60 between the 3600cm-1 and 3200cm-1 wave number region.

N-H, C=O, -OH and other functional groups, van der Waals forces, dipole moments etc. may form hydrogen bonds or other weak bonds. However, these interactions can improve vesicle formation, stabilize the structure, and slow drug release.



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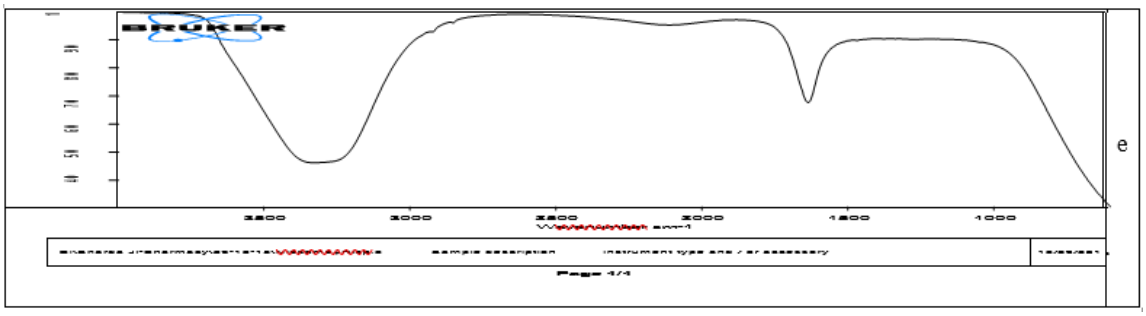
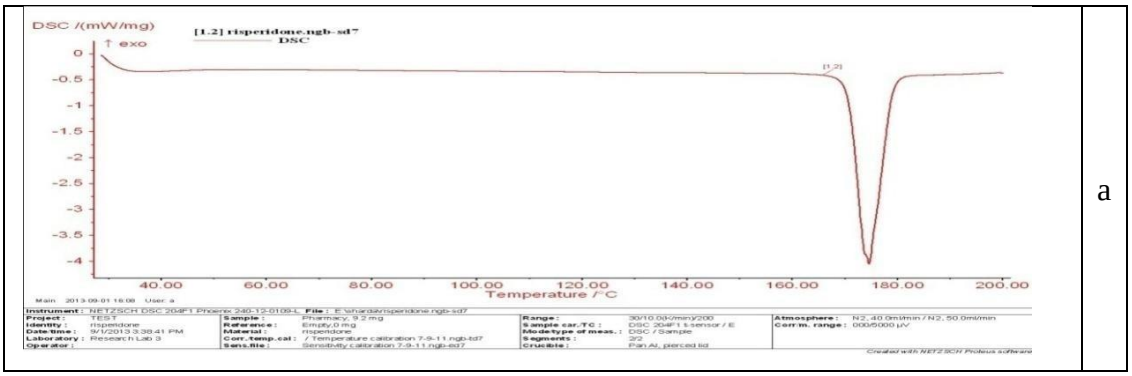


Fig 6: IR spectra (a)Aripiprazole (b)span60 (c)physicalmixtures (d) blankniosomes (e) Aripiprazole loaded niosomes

Drug-excipient compatibility study using differential scanning calorimetry (DSC)

The DSC thermograms for Aripiprazole, blank and Aripiprazole loaded niosomes are shownin Fig 7.



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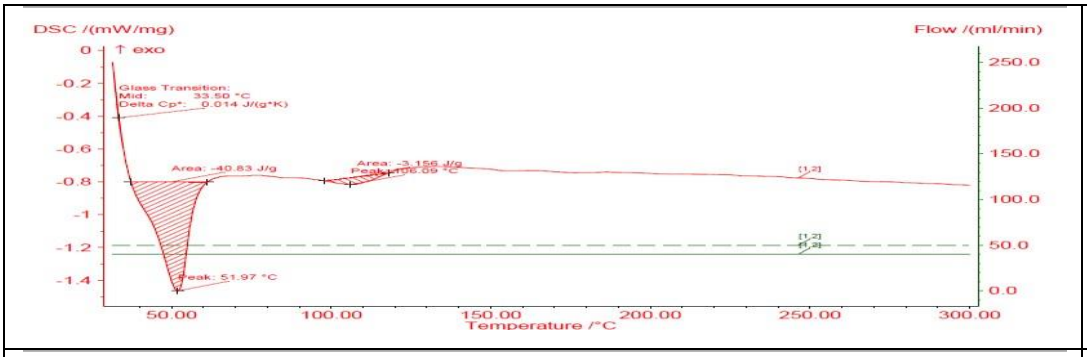

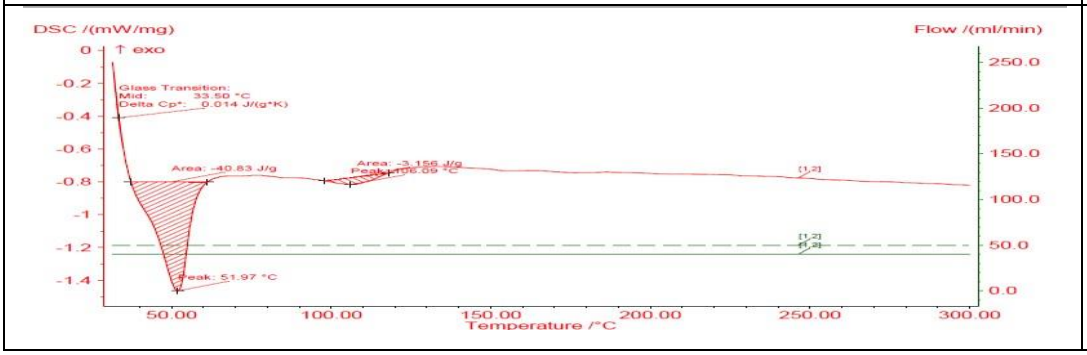

Fig7: DSC curves of (a)Aripiprazole (b)blank niosomes (c)Aripiprazole loaded niosomes

The thermal change of aripiprazole occurs at 174 °C, which corresponds to its melting point, with a sharp endothermic peak at this temperature. Aperture 60 presents a peak at 59.32°C and in Figures 7b and 7c the peak disappeared because they formed a double layer with cholesterol. Similar peaks were reported for 60 aperture liposome formulations. Using molecular modeling studies and thermal analysis, cholesterol intercalates with surfactant molecules and provides vesicle stiffness during liposome bilayer formation.

f. Drug release kinetics

The release of aripiprazole from the lipoids followed the Korsmeyer-Peppas pattern (Table 8). Since the n value is less than 0.5, the release of aripiprazole follows the Fickian diffusion mechanism, i.e. the release of the drug occurs from a higher concentration to a lower concentration by molecular diffusion due to the chemical potential gradient.

The Korsmeyer-Peppas model also describes several simultaneous processes in the drug during drug absorption, such as diffusion of water, swelling, gel formation, and drug leaching..

Table 8: Drug release kinetics of the optimized formulation

Release	Zero order	First order	Higuchi Model	Korsmeyer Peppa's model

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Kinetics	K	r^2	K	r^2	K	r^2	n	r^2
	5.6782	0.9668	0.0406	0.9424	19.816	0.9725	0.2679	**0.9759**

g. Stability studies (at different temperatures)

The stability of the liposomes was evaluated at different temperatures (4 ± 1 °C and 25 ± 2 °C). The formulation was evaluated for 3 months, no changes were found in the liposome formulation, the results are shown in Table 9.

Table 9: Effect of temperature on niosome formulations

Temperature	**Time interval (days)**	**Parameters**		
		Visual appearance	**Vesicle size (nm)**	**Entrapment efficiency(%)**
Refrigeration (4 ± 1ºC)	0	Nochange	299 ± 0.1	85
	45	Nochange	308 ± 1.2	80
	90	Nochange	315 ± 3.6	79.8
Roomtemperature(25 ± 2ºC)	0	Nochange	299 ± 0.1	85
	45	Nochange	312 ± 2.8	83
	90	Nochange	322 ± 2.1	78.97

The encapsulation efficiency of the vesicles does not change significantly between 4 ± 1°C and 25 ± 2°C. Encapsulation efficiency is slightly reduced, which may be due to drug leakage from the vesicles. It is clear from stability studies that liposomal formulations are more stable at 4 ± 1 °C than at 25 ± 2 °C.

h. *Ex-vivo* absorption studies

Both non-inverted and inverted rat capsule techniques are used to evaluate drug transport mechanisms in the gut and can help predict the in vivo absorption of samples in humans. Compared with the protruding pouch technique, the non-extroverted pouch technique has some advantages such as simplicity, less sample testing (preparation), easy serial serosal specimen collection, and less intestinal

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morphology changes/injuries. Oxygenated tissue culture medium and a special preparation method ensure tissue viability of the intestinal bursa for up to 2 hours. Therefore, the non-inverted pouch method was used to measure ex vivo intestinal permeability. See Table 10 for a comparison of the permeation coefficient, flux, and reliability of each formulation.Vesicles (non-biliary) have a higher volume than bile.

Table10: *Ex-vivo* intestinal permeation study and the permeability parameters

Formulation	Flux ($\mu gcm^{-2}h^{-1}$)	Permeability coefficient	Enhancement Ratio
Control	66.85	0.5571	1
S-60niosomes	139.459	1.1607	2.08
S-60 niosomes with bile	89.087	0.7423	1.33

Summary and conclusion

The vesicles were prepared by diethyl ether injection process, thin layer hydration and sonication process, resulting in a homogeneous preparation with high encapsulation performance. At 65:30:5 mM (Span60:cholesterol:stearylamine) the Span60 formulation achieved the highest yield with 92.83%. All liposome formulations have low polydispersity indices and high zeta values, providing stability and homogeneity. Flux is the speed of chemical molecules passing through a membrane per unit area per unit time. Therefore, higher flux values indicate faster movement of drug molecules through the stomach. Niosomal formulations (bile and non-biliary) showed an almost 2-fold and 1.33-fold improvement in aripiprazole efflux, respectively, compared to control.FTIR studies confirm that there is no combination of chemicals. Aripiprazole and formulation additives. This promotes good vesicle formation, stabilizes the structure and delays the release of the active ingredient. DSC data also support these results. This model has proven to be stable at low temperature (4 ± 1 °C) and without significant changes in cup size and performance of the encapsulation material.

Incorporation of bile salts (sodium deoxycholate) up to 7.5 mM into vesicles improves vesicle stability against bile salt dissolution.

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