FORMULATION AND DEVELOPMENT OF RISPERIDONE LOADED NIOSOMES FOR IMPROVED BIOAVAILABILITY: IN VITRO AND IN VIVO STUDY

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Abstract: Risperidone is a well-known antipsychotic drug that is extensively employed in the treatment of schizophrenia and other psychotic disorders. Orally administered risperidone gets metabolized by cytochrome P-450 enzymes into equipotent 9-hydroxy risperidone which have limited access to blood brain barrier (BBB). To overcome this issue niosomes containing risperidone were formulated, optimized and evaluated based on the assumption that non-ionic surfactant prevents the metabolism of risperidone by cytochrome P-450 enzymes that catalyzes the conversion of risperidone to its metabolite. Niosomes were prepared by sonication method and span 60 formulations exhibited the highest entrapment efficiency (92.83%) followed by span 40, span 80, span 20 and tweens of different grades. Vesicle size ranging from 180 nm to 388.9 nm exhibited lower PDI (0.171 to 0.437) and high zeta potential values (-20.4 mV to -50.6 mV). TEM showed that vesicles are spherical and FTIR and DSC studies revealed the absence of any potential incompatibility between the formulation ingredients. Niosomes exhibited in-vitro sustained release profile and followed Fickian diffusion mechanism. No significant change in general appearance, vesicle size and entrapment efficiency was observed for the formulations stored at refrigerated conditions (4 ± 1°C) and at room temperature (25 ± 2°C) for a period of 90 days. The niosomes were stable against solubilization action of bile salts (sodium desoxycholate). Ex-vivo studies revealed that both the flux and permeability coefficient was increased for niosomes with and without bile salts and enhancement ratio was almost 2-fold for niosomes without bile salts and 1.33-folds for niosomes containing bile salts. The relative bioavailability of niosomes prepared with bile salts was observed to be 108% in comparison to standard, whereas niosomes prepared without bile salts exhibited a higher bioavailability of 111%. It can be concluded that the developed niosomes offer better bioavailability of risperidone and this can be used for effective drug delivery.

Keywords: risperidone, niosomes; antipsychotic niosomes, non-everted sac method, bile salt stability, sodium desoxycholate

Oral route is most popular for drug administration due to its conveniences, non-invasiveness, increased patient compliance, possibility of self-administration etc. 60-70% of drugs in the market are available for oral administration only (1). The absorption of orally administered drug depends on the aqueous solubility of drug and its permeability across the biological membranes. Most of the orally administered drugs often exhibit poor aqueous solubility, less permeability, undergo acidic or enzymatic degradation, show hepatic first pass metabolism, inter and intrasubject variability, lack of dose proportionality etc., which leads to poor and variable absorption (2). Complex formation (3, 4), micronization (5, 6), solid dispersions (7, 8), nanoparticles (9, 10), microspheres (11), vesicular systems are among various approaches that have been used successfully to overcome the problems of insolubility and poor permeability and resulted in increased bioavailability of many drugs.

Drug delivery via colloidal carrier system like liposomes and niosomes offer many advantages like increased solubility, high permeability, carry both hydrophilic and hydrophobic drugs. These systems also act as drug reservoirs, provide drug targeting and control release of the drug (12). Liposomes however suffer from certain drawbacks like variable purity, oxidation and hydrolysis of phospholipids, sedimentation, fusion and aggregation of liposomes on storage, difficulty in sterilization and in main-
taining cryogenic conditions during its preparation; scale up problems etc. (13).

In view of this, niosome drug delivery systems were selected for the present study. Moreover niosomes contain non-ionic surfactants instead of phospholipids that are inert, non-toxic, cheap and bio-compatible and are more stable than phospholipids. Also the niosome preparation does not require the use of toxic solvents (14).

Risperidone \( (C_{23}H_{27}FN_4O_2) \) is a benzisoxazole derivative used for the treatment of various psychiatric disorders. It regulates dopamine transmission in various areas of brain and precipitates minimal extrapyramidal symptoms than conventional antipsychotics. Although it has good permeability across the biological membrane, formulation of risperidone has been limited due to its poor aqueous solubility (15, 16). Orally administered risperidone undergoes hepatic first pass metabolism by cytochrome P450 2D6 enzymes and resulted in poor bioavailability (17, 18). Several novel formulations have been prepared by different researchers to improve the bioavailability of risperidone. Some of them are cyclodextrin complexes (19, 20), polymeric micelles (21), SAIB systems (22-25), nanoparticles (15, 17, 26), solid dispersions (27), transdermal patches (28) etc. Despite of all these attempts, only available novel formulations of risperidone in the market is Risperdal®Consta™ (USFDA approved) which is a microsphere preparation meant to be administered intramuscularly (IM). However, the treatment with this formulation requires both, an oral therapy that will provide a loading dose and an intramuscular therapy with Risperdal Consta that will provide the maintenance dose for six weeks and will release the drug only after a lag period of 3 weeks. The repeated administration of risperidone injection results in large number of side effects (29, 30).

It is well known that non-ionic surfactant increases the permeation and absorption of several drugs and it also have the tendency to significantly inhibit cytochrome P 450 enzymes (31, 32). So inclusion of risperidone within vesicular structure can be expected to prevent the first pass effect and will increase the concentration of parent compound risperidone which has more permeability through blood brain barrier to its metabolite 9-hydroxy risperidone. Keeping the facts in mind the present study was designed to formulate the niosome formulation of risperidone so as to improve the bioavailability thereby significant reduction in cytochrome P-450 mediated metabolism of risperidone.

EXPERIMENTAL

Materials

Risperidone was received as a gift sample from Torrent Research Centre (Ahmedabad, India). Span 20, 40, 60, 80, tween 20, 40, 80, cholesterol and stearylamine were purchased from SD Fine Chemicals (Mumbai, India). All other chemicals and solvents used were of analytical grade.

Methods

Preparation of niosomes

Niosomes were prepared by thin film hydration method and sonication method as described by Manconi et al. (33). In thin film hydration method, the surfactant, cholesterol, stearylamine (65.0 : 30.0 : 5.0 mM) and risperidone (2 mg) were dissolved in mixture of chloroform and ethanol (3 : 1). Organic solvent was evaporated in rotary evaporator under vacuum which resulted in the formation of thin film which was further dried in stream of nitrogen gas. The film was hydrated with 10 mL of phosphate buffer (pH 7.4) using mechanical stirring. In sonication method, all the above mentioned ingredients were taken in a beaker. 10 mL of phosphate buffer (pH 7.4) was added and probe sonicated at room temperature for 5-7 min till milky white dispersion (niosomes) was obtained. In ether injection method (12), ether solution containing surfactant mixture was introduced slowly through a fine needle to the preheated aqueous solution (60°C) which led to the evaporation of ether followed by milky white niosome formation. The formulations were packed in tightly closed container and stored at 4°C for further evaluation (33).

Evaluation of niosomes

Entrapment efficiency (%EE)

The percent entrapment efficiency of risperidone in niosomes was determined by centrifugation technique (34). Eppendorff tube containing niosomes was centrifuged at 14 000 rpm at 4°C for 15 min (Pico 21 centrifuge, Thermo Scientific Heraeus US). The supernatant containing unentrapped risperidone was withdrawn and analyzed at 280 nm using UV spectrophotometer against phosphate buffer (containing 30% ethanol) as blank (35). The pellet was also analyzed after breaking the vesicles with Triton X-100. The entrapment efficiency was calculated by the following formula:

\[
\% \text{ EE} = \frac{\text{Total amount of drug added} - \text{drug in supernatant}}{\text{Total amount of drug added}} \times 100
\]
Vesicle size, polydispersity index and zeta potential

Vesicle size analysis was carried out using Malvern Zetasizer Nano ZS (Malvern Instruments, UK). The niosomes were dispersed in double distilled water (DDW) and the size estimation was carried out in triplicates. Polydispersity index (PDI) was also determined as a measure of homogeneity. Zeta potential of the niosome formulations was also determined so as to confirm the stability of the formulations (36).

Morphology

The morphological characteristics of prepared niosomes were examined using transmission electron microscopy. Small amount of niosome formulation was mixed with 1% of phosphotungstic acid and one drop of this mixture was placed on carbon coated grid and excess sample was drawn out using filter paper. After 2 min of drying TEM was observed.

Fourier transform infra-red (FTIR) spectroscopy

Infrared spectroscopy of risperidone, span 60, physical mixtures (1:1), blank and risperidone loaded niosome samples were carried out by FTIR spectrophotometer (ALPHA-T, Brucker) and the spectra were recorded in the region of 3500 to 1000 cm⁻¹.

Differential scanning calorimetry (DSC)

DSC experiments were performed using DSC instrument (Netzsch, DSC-204 F1 Phoenix). About 5 mg of risperidone, span 60, physical mixtures, lyophilized blank and risperidone loaded niosome sample was weighed and individually capped in aluminium crucible. The crucible was then kept under a dynamic atmosphere of nitrogen (50 mL/min) and a heat flow rate of 10°C/min from 30 to 200°C and the corresponding spectra between heat flow (w/g) on Y-axis and temperature on X-axis were obtained.

In-vitro release study

In-vitro release study of the niosome formulation was performed using locally fabricated vertical Franz diffusion cells (37). The niosome formulation (equivalent to 400 µg of risperidone) was kept in the donor compartment which was separated by an egg membrane from the receptor compartment. The receptor compartment consisted of phosphate buffer pH 7.4 with 30% ethanol (to maintain sink condition) which was constantly stirred at 100 rpm on a magnetic stirrer and the temperature was maintained at 37 ± 1°C. 5 mL samples were withdrawn at different time intervals and were replaced with the fresh buffer. The samples were analyzed for risperidone content by UV-spectrophotometer at 280 nm using suitable blank. The cumulative percent drug released was plotted against time. The data were then fitted into various kinetic models to explain the mechanism of drug release from the formulations (38).

Stability studies

Stability at different temperatures

Stability studies for niosome formulations were performed at different temperatures as per the ICH guidelines. The purpose of stability testing was to provide evidence on how the quality of drug product varies with time under the influence of variety of environmental factors such as temperature, humidity and light. The niosome formulations were filled in tightly closed glass vials and were kept at refrigeration condition (4 ± 1°C) and at room temperature (25 ± 2°C) in a stability chamber for a period of 1, 2 and 3 months. The samples were analyzed for physical appearance, particle size and entrapment efficiency at different time intervals. The physical appearance (aggregation) of stored formulations was evaluated by visual observations, particle size by Zetasizer Nano ZS (Malvern Instruments, UK) and entrapment efficiency of niosomes was determined by centrifugation method (39).

Stability of niosomes in the presence of bile salts

Stability of the niosomes in the presence of bile salt solution was determined according to the method described by Pardakhty et al. (40) with minor modifications. A stock solution of sodium deoxycholate in double distilled water was prepared and diluted to obtain the concentrations of 2.5 mM, 5 mM, 7.5 mM, 10 mM, 15 mM and 20 mM. 5 mL of niosome formulation was added to 50 mL of diluted bile salt solutions and was incubated at 37°C for 1 h. The turbidity of the samples was measured using Systronics (s) µC turbidity meter 135 and was expressed in terms of nephelometric turbidity units (NTU).

Ex-vivo absorption study

The study protocol was approved by the University Animal Ethics Committee IAEC of Banasthali University, Banasthali, India (BU/BT/394/13-14). Ex-vivo absorption study was performed using non-everted rat sac method (41). The male Wistar rats were housed in temperature and humidity controlled room with free access to water.
and standard rat chow. The overnight fasted rats were sacrificed by spinal dislocation and small intestine was immediately removed by cutting across the upper end of duodenum and the lower end of ileum. The small intestine was washed carefully with cold normal oxygenated saline solution (0.9% w/v, NaCl) using a syringe. The clean intestinal tract was prepared into 8 ± 0.2 cm long sacs. Each intestinal sac was filled with niosome formulation (equivalent to 200 µg of risperidone) via syringe (blunt needle) and the two sides of the intestine were tied tightly with a thread and was suspended in an organ tube containing 70 mL phosphate buffer, pH 7.4 (with 30% v/v ethanol), which was maintained at 37 ± 1°C temperature and provided with continuous aeration. An aliquot of 3 mL sample was withdrawn and replaced with same amount of fresh phosphate buffer. The sample was centrifuged and supernatant was analyzed for risperidone content using UV spectrophotometer at 280 nm. The steady state flux (J_{ss}), permeability coefficient (K_{p}) and enhancement ratio (ER) were also calculated (42).

**In-vivo study**

The in-vivo study protocol was approved by the Animal Ethics Committee IAEC (1321/PO/ReBi/S/10/CPCSEA). The rabbits (n = 3) were housed in animal house of the Institute. All the animals used in the study were caged and maintained according to the guidelines of CPCSEA or principles established for care and use of laboratory animals. Overnight fasted rabbits were divided into 4 groups (n = 3). Group I: Control (risperidone API), Group II: treated with conventional liquid preparation, Group III: niosomes (without bile) given orally and Group IV: niosomes containing bile salts given orally. The formulations were given equivalent to suitable therapeutic dose of the drug. Blood samples were collected from the marginal ear vein in heparinized tube at different time intervals and were processed and analyzed for risperidone content using HPLC method (43).

**Extraction of risperidone from blood plasma**

Collected blood samples were centrifuged at 2800 rpm for 10 min and plasma was separated, which was extracted by liquid-liquid extraction method (44) to give the serum samples. To 100 µL of serum sample 100 µL of 1 M sodium carbonate and 6 mL of phosphate buffer, pH 7.4 mixture was added and vortex mixed. It was centrifuged at 2800 rpm and supernatant (organic) layer was transferred to the test tube for drying under nitrogen atmosphere. The residue obtained after drying was dissolved in 100 µL of acetonitrile. 20 µL of the prepared sample was injected into the HPLC system for analysis of different pharmacokinetic parameters like maximum plasma drug concentration (C_{max}), time to reach maximum plasma drug concentration (t_{max}) and area under curve (AUC).

**Statistical analysis**

ANOVA (p < 0.05) followed by Dunnet multiple comparison test was applied to compare niosomes with and without bile with risperidone API and marketed formulation.

**RESULTS AND DISCUSSION**

**Optimization of method of preparation**

Niosomes are the non-ionic surfactant vesicles that are capable of entrapping wide variety of drugs. They contain non-ionic surfactants, cholesterol and stearylamine, all of which are listed in FDA inactive ingredient databases and are GRAS approved (45). In the present study, niosomes were formulated by different methods i.e., ether injection method, thin film hydration method, sonication method and were evaluated for various parameters like vesicle size, polydispersity index, zeta potential and entrapment efficiency. From Table 1, it can be clearly observed that the niosomes prepared by thin film hydration (F2) and ether injection method (F1) are comparatively

<table>
<thead>
<tr>
<th>Code</th>
<th>Method of preparation</th>
<th>Vesicle size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>%EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Ether injection method</td>
<td>725.0 ± 6.4</td>
<td>0.367</td>
<td>-35 ± 4.7</td>
<td>82.3 ± 3.12</td>
</tr>
<tr>
<td>F2</td>
<td>Film hydration method</td>
<td>521.0 ± 3.8</td>
<td>0.345</td>
<td>-37 ± 9.3</td>
<td>85.1 ± 2.15</td>
</tr>
<tr>
<td>F3</td>
<td>Sonication method</td>
<td>299.5 ± 2.1</td>
<td>0.254</td>
<td>-45.5 ± 9.3</td>
<td>92.1 ± 3.4</td>
</tr>
</tbody>
</table>

Span 60 : Cholesterol : Stearylamine : 65.0 : 30.0 : 5.0 mM, Risperidone: 2 mg, Polydispersity Index: PDI, Entrapment efficiency: EE, Millivolts: mV
larger than those prepared by sonication method (F3), the latter of which exhibited the smallest vesicle size of 299.5 ± 2.1 nm. Manconi et al. (36) have demonstrated that sonication method generally yield smaller vesicles and also proposed that the vesicle size depends on method of niosome preparation, composition of bilayer and drug load. Polydispersity index (PDI) is also known as heterogeneity index which measures the width of particle size distribution. PDI value in all the formulations ranges from 0.254 to 0.367, which has clearly indicated that formulations were homogeneous (46). The zeta potential was high in all the formulations which showed that formulations were stable. Formulation prepared with sonication method (F3) showed highest entrapment efficiency of 92.1 ± 3.4% when compared to ether injection method (F1) and film hydration method (F2). On the basis of highest entrapment efficiency, sonication method was considered as an appropriate method for niosome preparation.

### Table 2. Optimization of niosome composition.

| Code | Concentration (mM) Risp Vesicle size (nm) PDI Zeta potential (mV) %EE |
|------|---------------------|---------------------|---------|---------------------|-----|
|      | Sp 60 | Ch | SA | 2 | 202.7 ± 6.7 | 0.309 | -37.3 ± 6.48 | 48.3 ± 3.01 |
| C1   | 30.0  | 65.0 | 5.0 | 2 | 269.4 ± 7.1 | 0.161 | -39.7 ± 5.35 | 65.70 ± 2.8 |
| C2   | 47.5  | 47.5 | 5.0 | 2 | 207.2 ± 5.7 | 0.243 | -39.5 ± 6.84 | 79.89 ± 3.4 |
| C3   | 55.0  | 40.0 | 5.0 | 2 | 238.1 ± 3.2 | 0.256 | -45.0 ± 8.35 | 92.83 ± 2.4 |
| C4   | 65.0  | 30.0 | 5.0 | 5 | 242.3 ± 4.5 | 0.234 | -46.0 ± 8.35 | 94.36 ± 5.7 |
| C5   | 65.0  | 30.0 | 5.0 | 10 | 245.7 ± 6.5 | 0.248 | -45.6 ± 8.35 | 95.83 ± 6.1 |

Span 60: Sp 60, Cholesterol: Ch, Stearylamine: SA, Millimoles: mM, Polydispersity index: PDI, Entrapment efficiency: EE, Millivolts: mV

### Table 3. Optimization of type of surfactant for niosomes.

| Formulation code | Composition (mM) Vesicle size (nm) PDI Zeta Potential (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               | Tw 20 : Ch:SA 388.9 ± 8.8 0.350 | 21.9 ± 4.62 | 62.31 ± 3.4 |
| T2               | Tw 40 : Ch:SA 258.7 ± 7.3 0.437 | 21.3 ± 3.83 | 70.87 ± 3.4 |
| T3               | Tw 60 : Ch:SA 248.2 ± 7.5 0.427 | 20.4 ± 4.31 | 72.47 ± 3.4 |
| T4               | Tw 80 : Ch:SA 180.0 ± 8.7 0.389 | 25.1 ± 3.74 | 68.62 ± 3.4 |

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

Optimization of niosome compositions

Niosome formulations were prepared by varying the concentration of non-ionic surfactants and cholesterol, shown in Table 2. The entrapment efficiency was increased with the increase in amount of surfactant i.e., from C1 to C4. Similar findings have been reported by Yoshioka et al. (47), who proposed that above 70% surfactant concentration there was decrease in texture, shape and vesicles formation. Hence 65.0 : 30.0 : 5.0 mM (highest concentration of surfactant) was selected as the optimum concentration. Stearylamine was used as charge inducer and was taken in permissible amount, as reported by Hu and Rhodes (48). Increased amount of cholesterol in niosome was known to influence vesicle stability and permeability. In addition cholesterol gets intercalated between the bilayers and resulted in increased rigidity, decreased vesicle permeability and increased entrapment efficiency of drug within the vesicles.
In contrast, higher amount of cholesterol competes with the drug for packing space and excludes the drug from bilayer which lead to the lower entrapment efficiency (49). So cholesterol in 30 mM concentration (C4) was chosen as optimum value. When the amount of risperidone was increased from 2 to 10 mg (C4 to C6), the entrapment efficiency was increased, which was due to its suitable log P value. The lipophilic drugs are found to be better entrapped within the vesicles due to their interactions with the hydrophobic bilayers.

Selection of type of surfactant

Niosomes containing different types of surfactant were also formulated by sonication method and were evaluated for vesicle size, polydispersity index, zeta potential and % entrapment efficiency, the result of which are listed in Table 3.

Entrapment efficiency

The entrapment efficiency for niosomes was higher with spans as compared to tweens which was due to the lipophilic nature of span (lower HLB) where the lipophilic risperidone get easily entrapped. Tweens are hydrophilic (high HLB) because of the presence of hydroxyl group and ether oxygen in its molecular structure (50). Formulation S3 showed maximum entrapment efficiency (92.83 ± 3.4%) and it increases in the order of Span 60 > Span 40 > Span 80 > Span 20 which was due to the chemical structure, orientation and packing behavior of the surfactants. Span 20, 40 and 60 had the same polar head group but different alkyl chain. Span 60 had longer alkyl chain length which leads to the higher entrapment efficiency whereas span 80 has an unsaturated alkyl chain which made the chain flexible to bend and rotate. This rotation increases the steric hindrance between the hydrophobic chains, so adjacent molecule cannot be tight, which makes the bilayer more permeable (51). Span 60 and 40 have high phase transition temperature and are solid at room temperature, which favors the high entrapment of drug within the niosomes (52). Spans 60 exhibited the highest entrapment efficiency due to their longest alkyl chain and lower HLB (4.7) than span 20 and 40 (HLB 8.6 and 6.7). Similar findings have been reported by Hao et al. (51) and Balakrishnan et al. (53) for entrapment of colchicine and minoxidil.

Vesicle size, zeta potential and polydispersity index

The vesicle diameter is highly influenced by HLB value and hydrophobicity of the surfactant. With the increase in alkyl chain length, the hydrophobicity of the surfactant increases that causes lowering of HLB value. It was found that niosomes prepared with span 60 have the smallest vesicle size that was attributed to its lowest HLB. The results were in agreement to Wan and Lee (54), who states that diameter of niosomes, was directly proportional to HLB. With decrease in HLB value, surfactant becomes more hydrophobic with significant reduction in surface free energy. High zeta potential (Fig. 1) in all the cases indicated that formulations were stable and the lower PDI showed that formulations were homogenous. Niosomes with span 60 has the highest entrapment efficiency (92.83 ± 3.4%) and has been selected for further evaluations, because the in-vivo performance of niosomes depends on the amount of drug loaded in them.

![Figure 1. Zeta potential of risperidone niosomes with span 60](image-url)
TEM photomicrographs of lyophilized niosomes are shown in Figure 2, which revealed the presence of well-defined, perfectly spherical shaped niosomes.

FTIR spectroscopy

FTIR spectra of risperidone, span 60, physical mixture (1:1), blank niosomes and risperidone loaded niosomes are given in Figure 3. FTIR spectra of risperidone showed the characteristic peak at 1646 cm\(^{-1}\) (C=O stretching), 1534 cm\(^{-1}\) (N-H bending), 1128 cm\(^{-1}\) (C-H stretching), 853 cm\(^{-1}\) (C-H bending, aromatic) and 3744 cm\(^{-1}\) (N-H stretching). Span 60 showed the characteristic peaks at 2400 cm\(^{-1}\) (aromatic ring), 2800 cm\(^{-1}\) (aromatic ring), 1734 cm\(^{-1}\) (cyclic 5-membered ring), 1400 cm\(^{-1}\) (-CH\(_2\)), 2928 cm\(^{-1}\) (O-H stretching), 2800 cm\(^{-1}\) (O-H stretching) and 3400 cm\(^{-1}\) (O-H group). When the physical mixture of risperidone and span 60 was analyzed, the characteristic peaks of risperidone were present in physical mixture similar to that of individual risperidone spectrum and there was no detectable changes in FTIR spectra which confirmed the absence of any chemical interactions between them. FTIR spectra of blank and risperidone loaded niosomes showed that in between 3600 cm\(^{-1}\) and 3200 cm\(^{-1}\) wave number region there were some weak interactions between the risperidone and span 60. There may be a formation of hydrogen bonds or some other weak bonds due to Vander Waals forces, dipole moment etc. amongst the functional groups N-H, C=O and -OH groups of drug and span 60. However, these interactions may favor the formation of vesicular shape, stabilizing the structures and slower the drug release. The risperidone loaded niosomes showed the disappearance of characteristics peaks of risperidone, which showed the entrapment of drug within the vesicular structure (55, 56).

Differential scanning calorimetry

The DSC thermograms for risperidone, blank and risperidone loaded niosomes were shown in

<table>
<thead>
<tr>
<th>Release Kinetics</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi model</th>
<th>Korsmeyer Peppas model</th>
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<tbody>
<tr>
<td>K</td>
<td>r(^2)</td>
<td>K</td>
<td>r(^2)</td>
<td>K</td>
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<tr>
<td>5.6782</td>
<td>0.9668</td>
<td>0.0406</td>
<td>0.9424</td>
<td>19.816</td>
</tr>
</tbody>
</table>

Table 4. Drug release kinetics of the optimized formulations.

Figure 2. Transmission electron photomicrograph of risperidone niosomes
Figure 4. The thermal transition of risperidone occurs at 174°C corresponding to its melting point which was characterized by sharp endothermic peak at that temperature. Shaker et al. (57) have showed that span 60 exhibits endothermic peak at 59.32°C, the disappearance of which in Figure 4 was due to their bilayer formation with cholesterol. At the same time incorporation of risperidone within the niosomes resulted in the diminished peak of risperidone. Similar peaks for niosome formulation with span 60 have been reported by Khan et al. (58).

**In-vitro release study**

Figure 5 clearly indicate that risperidone release in initial 1 hour was faster in niosomes, later on it was significantly slowed down when compared
Almost 98% of the drug was released in 6 h from risperidone drug suspension while only 29% was released from niosomes with bile salt followed by span 80 (51.28%), span 40 (62.49%), span 60 (77.47%) and maximum with span 20 (88.36%). The molecular weight and interaction between the drugs with the bilayers resulted in the retarded release of risperidone from niosomes. The presence of biles in niosomes significantly slowed the release of drug. Similar pattern of drug release from niosomes have been reported by Hao et al. (51).

Table 4 clearly indicates that the release of drug from niosomes followed the Fickian diffusion mechanism (Korsmeyer-Peppas model) i.e., the release of drug occurs by molecular diffusion from higher concentration to lower concentration due to its chemical potential gradient. This model also considered several processes that occur simultaneously like diffusion of water inside the drug reservoir, swelling, gel formation, diffusing out of drug etc. (59).

Stability studies

Effect of temperature

Stability of the niosomes was evaluated at different conditions of temperature (4 ± 1°C and 25 ± 2°C). The formulations were analyzed for a period of 3 months and no visual changes were observed in the niosome formulation, the results are shown in Table 5. The niosomes showed no significant changes in the entrapment efficiency at 4 ± 1°C and 25 ± 2°C. There was slight decrease in entrapment efficiency which may be due to the leakage of drug from the vesicles. From the stability studies it was clear that niosome formulations were more stable at 4 ± 1°C than 25 ± 2°C temperatures. Similar results have been reported by Jadon et al. (60).

Effect of bile salts on niosomes

It has been previously reported that drug carrier systems frequently interacts with bile salts presents in GIT which affects vesicle stability, so it becomes necessary to evaluate the stability of niosomes in the presence of bile salts. The niosome for-
mulations containing different types of spans were incubated with 0, 2.5, 5, 7.5, 10, 15 and 20 mM solutions of sodium deoxycholate at 37°C for 1 hour and the effect of which were analyzed using turbidity meter. Vesicle-micelle transition occurred in 3 steps which includes I) Initial attachment of the bile salts to outer bilayer of vesicles and saturate them without causing any significant change in turbidity, II) Outer bilayers solubilization and III) Solubilization of remaining bilayers. Figure 6 showed that above 10 mM, abrupt decrease in turbidity occurred due to the solubilization of bilayers. Span 60 formulation did not show any sharp decrease in turbidity which was assumed to be due to its high phase transition temperature. Even after exposure to 20 mM sodium deoxycholate, span 60 formulations had maximum turbidity which showed the structural rigidity of the niosome bilayers. Low turbidity of span 20 and 80 formulations were due to their liquid nature which allows an easy transition of bilayer to micellar structure. Similar findings have been reported by Pardakhty et al. (40).

Bile salts have been used as penetration enhancers in various formulations and it acts by its membrane destabilizing activity (61). Schubert et al. (63) have shown that inclusion of bile salts within the vesicle tends to stabilize it. So niosomes (span 60) containing sodium deoxycholate (2.5, 5, 7.5, 10 mM) as an integral component was formulated and exposed to the same environment of bile salts and were analyzed for change in turbidity after 1 hour incubation at 37°C. From Figure 7, it was concluded that the turbidity of span 60 formulations containing 7.5 mM bile salt was higher even after exposure to 20 mM sodium deoxycholate solutions, whereas turbidity was lowest in the formulation containing no bile salt, which showed that inclusion of bile salts in the vesicles leads to their improved stability. Higher concentration of bile salts resulted in slight decrease in turbidity due to destabilizing property of detergent beyond CMC. So from the above findings, it was concluded that inclusion of bile salt within the vesicular system provides the vesicles to withstand disruption caused by physiological bile salts present

Table 5. Effect of temperature on niosome formulations.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time interval (days)</th>
<th>Parameters</th>
<th>Visual appearance</th>
<th>Vesicle size (nm)</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refrigeration (4 ± 1°C)</td>
<td>0</td>
<td>No change</td>
<td>299 ± 0.1</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>No change</td>
<td>308 ± 1.2</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>No change</td>
<td>315 ± 3.6</td>
<td>79.8</td>
<td></td>
</tr>
<tr>
<td>Room temperature (25 ± 2°C)</td>
<td>0</td>
<td>No change</td>
<td>299 ± 0.1</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>No change</td>
<td>312 ± 2.8</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>No change</td>
<td>322 ± 2.1</td>
<td>78.97</td>
<td></td>
</tr>
</tbody>
</table>

Figure 5. In-vitro release study of risperidone from niosomes
in GIT and thus significantly affecting their biological fate after oral administration. These findings were in accordance with that proposed by Arzani et al. (64).

Ex-vivo intestinal permeation studies

Both non-everted and everted rat sac techniques were used to assess the drug transport mechanism across the intestine and help to predict the in-vivo absorption profile of formulations in humans. Non-everted sac model exhibit certain advantages over everted sac method like simplicity, requires less amount of test samples (formulations), ease of successive serosal sample collection, less intestinal morphological changes/damage while everting, very small closed serosal compartment. So, non-everted sac technique was used to assess ex-vivo intestinal permeation. The permeability coefficient, flux and enhancement ratio of the formulations are shown in Table 6, the enhancement ratio for niosomes (without biles) was higher to niosomes containing biles. Flux is the rate with which drug molecules move across the membrane per unit surface area per unit time. So higher flux value indicates rapid rate of movement of drug molecules across the intestine. Niosome formulation has improved the flux of drug i.e. almost 2-fold for niosomes without bile and 1.33 fold for niosomes containing bile salts, respectively, compared to control (Table 6). Higher values of flux and permeability coefficients for niosomes without

![Figure 6. Effect of bile salts on niosomes containing different types of span](image1)

![Figure 7. Effect of bile salts on niosomes containing bile salt as integral components](image2)
and with bile salts indicated that non-ionic surfactant facilitated the movement of risperidone across the intestine. Poor solubility of risperidone was responsible for poor permeation of drug across intestine. The improved permeation of risperidone in niosomal vesicles might be contributed by their nanosize and surface tension reducing phenomenon, which would have facilitated the absorption of drug across intestine through specialized transport mechanisms.

**In-vivo study in rabbits using HPLC**

The chromatograms for risperidone API, niosomes without and with bile salts and marketed risperidone oral solution are shown in Figure 8. The plasma drug concentration profiles of different formulations are shown in Figure 9 and the pharmacokinetic parameters for risperidone API, marketed formulation, and niosome with and without bile salts are shown in Table 7. Niosome containing bile salts exhibited much faster rate of drug absorption and peak plasma concentration ($C_{\text{max}}$) reached to 296.42 ng/mL in 4 h, niosomes without bile salt also showed good $C_{\text{max}}$ of 232.64 ng/mL. Both niosome formulations with and without bile have better $C_{\text{max}}$ than marketed formulation (214.5 ng/mL). $C_{\text{max}}$ depends on dose administered, rate of absorption and elimination and is often related to the intensity of pharmacological response. The dose was same for all the 4 groups, higher $C_{\text{max}}$ value indicated the higher rate of drug absorption. Arzani et al. (64) have showed that incorporation of bile within niosomes facilitates its absorption by increasing its penetration across the biological membrane due to the transcytosis by M-cells of Peyer’s patches at the intestinal lymphatic tissues and results in high $C_{\text{max}}$ value.

Area under curve (AUC) represents the extent of drug absorption (bioavailability) from its dosage form. In the present study, AUC$_{(0-24\ h)}$ was significantly ($p < 0.05$) increased in both the niosome formulations which indicates their higher bioavailability. The relative bioavailability was also increased in both the cases; it was higher for niosomes without bile (111%) followed by niosomes with bile salts (108%) which could be due to the membrane penetration ability of non-ionic surfactants and also the ability of niosomes to permeates the biological

### Table 6. Ex-vivo intestinal permeation study and the permeability parameters

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Flux (µg/cm²h⁻¹)</th>
<th>Permeability coefficient</th>
<th>Enhancement ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (aqueous drug suspension)</td>
<td>66.85</td>
<td>0.5571</td>
<td>1</td>
</tr>
<tr>
<td>S-60 niosomes</td>
<td>139.459</td>
<td>1.1607</td>
<td>2.08</td>
</tr>
<tr>
<td>S-60 niosomes with bile</td>
<td>89.078</td>
<td>0.7423</td>
<td>1.33</td>
</tr>
</tbody>
</table>

### Table 7. Mean pharmacokinetic parameters of risperidone after oral administration of risperidone API, marketed oral solution and niosomes with and without bile salts.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>API (plain risperidone)</th>
<th>Niosomes with bile salt</th>
<th>Niosomes without bile salt</th>
<th>Marketed risperidone oral solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$(ng/mL)</td>
<td>238.14 ± 9.1</td>
<td>296.42 ± 8.4</td>
<td>232.64 ± 13.5</td>
<td>214.51 ± 10.2</td>
</tr>
<tr>
<td>$T_{\text{max}}$(h)</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>AUC$_{(0-24h)}$(ng.h/mL)</td>
<td>2608.08</td>
<td>2666.52</td>
<td>2738.35</td>
<td>2464.09</td>
</tr>
<tr>
<td>AUC$_{(0-8h)}$* (ng.h/mL)</td>
<td>2836.25</td>
<td>3265.47*</td>
<td>3887.21*</td>
<td>2836.16</td>
</tr>
<tr>
<td>$t_{\text{1/2}}$(h)</td>
<td>0.658</td>
<td>0.532</td>
<td>0.759</td>
<td>0.563</td>
</tr>
<tr>
<td>$V_f$</td>
<td>9.92</td>
<td>10.66</td>
<td>10.97</td>
<td>10.75</td>
</tr>
<tr>
<td>$F%$</td>
<td>105</td>
<td>108</td>
<td>111</td>
<td>100</td>
</tr>
<tr>
<td>MRT last (h)*</td>
<td>9.45 ± 0.38</td>
<td>12.79 ± 0.11</td>
<td>23.46 ± 0.39</td>
<td>29.88 ± 0.51</td>
</tr>
<tr>
<td>MRT∞(h)</td>
<td>12.36</td>
<td>17.59</td>
<td>26.89</td>
<td>38.17</td>
</tr>
</tbody>
</table>

*Peak plasma concentration, *time of maximum plasma concentration, *area under curve for time 0 to 24h, *area under curve for time 0 to infinity, *elimination half-life, *volume of distribution, *relative bioavailability, *mean residence time,*p < 0.05, ANOVA followed by Dunnett multiple comparison test was applied to compare transdermal proniosomes with API and marketed formulation.
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membrane via several combination of mechanisms. Niosomes ability to prevent the conversion of drug to its metabolite by intestinal enzymes also contributes to the increased bioavailability of the drug (65).

$T_{\text{max}}$ was 4 hours in all the formulations except for niosomes without bile where it was significantly enhanced to 8 hours. $T_{\text{max}}$ is useful in estimating rate of absorption, onset time and onset of action and also important in assessing the efficacy of drug. $t_{\text{1/2}}$ of the niosome formulation without bile salt was enhanced when compared to risperidone API, oral solutions and niosomes containing bile salts, so it ensures the prolong residence time of niosomes within the body and maintenance of the therapeutic level over the long period of time. Similar results

Figure 8. Chromatograms for (a) risperidone, API (b) niosomes (c) niosomes containing bile salt and (d) marketed risperidone solution

Figure 9. In-vivo studies of niosomes
have been observed by Rogerson et al. (66) who reported increase in $t_{1/2}$ and prolonged residence time of drug encapsulated in niosomes. Mean residence time (MRT) is the average amount of time spent by the drug in the body before being eliminated. MRT for niosomes without and with bile was found to be higher than with risperidone API, i.e., risperidone loaded niosomes exhibit prolonged circulation in blood than aqueous drug suspension. Attia et al. (67) have also shown that niosomes loaded with acyclovir causes significant increase in mean residence time (MRT) and hence provide sustained release from formulation. Waddad et al. (68) by molecular modelling simulation have explained the binding of the morin hydrate drug to human serum albumin by hydrogen bonds which increases AUC to 1.3-2.7 fold and shows higher accumulation of drug in brain compartment than drug in solution. Volume of distribution ($V_d$) is a hypothetical parameter that indicates the distribution of drug in various tissues (69). A significant increase in $V_d$ was also observed in case of niosomal formulations to that of API that clearly indicate its better extent of distribution in body tissues.

**CONCLUSION**

The aforementioned study has explored the potentials of risperidone loaded niosomes for oral delivery to overcome the bioavailability issues in the treatment of various psychiatric disorders. The optimized span 60 formulation showed the highest entrapment efficiency, sustained release profile which followed Fickian diffusion kinetics and improved stability upon exposure to bile salt environment (when 7.5 milimole of bile salts added in the niosome formulations). In-vivo studies in rabbits showed improvement in $C_{max}$ and AUC values which ensures increased bioavailability of risperidone. So it can be concluded that niosomes prepared with span 60 could be used as a promising delivery system to improve the bioavailability of risperidone.

**REFERENCES**


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