Bilosomes as Soft Nanovesicular Carriers for Ropinirole Hydrochloride: Preparation and In-vitro Characterization

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Abstract

Bilosomes are nanovesicular carriers that contain bile salts and non-ionic surfactants in a bilayer with cholesterol. These are more ultra-deformable, elastic, and flexible than other nanovesicular carriers. Ropinirole hydrochloride (RH) is a non-ergot dopamine D2-agonist. It is used to manage Parkinson’s disease and to minimize “off” variations in levodopa responsiveness. The oral bioavailability is about 50% due to it being extensively metabolized in the liver, with an elimination half-life of about 6 h and BCS class III. The aim of this study was to provide the sustained release of RH by preparing it as soft nanovesicular carriers (bilosomes). Eight formulas of RH bilosomes were prepared by a reverse-phase evaporation method using mixed surfactant (span®60: tween®60 at 1:2 ratio), cholesterol and sodium deoxycholate as bile salt. All formulas were evaluated for their drug content, entrapment efficiency, vesicle size, polydispersity index, Zeta potential, drug release, transmission electron microscopy and Fourier transform infrared spectroscopy. Results showed all prepared RH bilosomes were in nano-range with vesicle size ranging (from 124.40±10.60 to 294.60±11.50) nm, Zeta potential ranged (from -16,955±7.372 to -23.22±0.880) mV and with entrapment efficiency % ranged (57.30±6.48% to 77.77±3.78%).

The best formula F5 showed vesicle size (160.60±15.82) nm, Zeta potential (-20.75±0.930) mV, and entrapment efficiency (%77.77±3.78%) with 84.39% in-vitro release of RH after 24 h. It is concluded that bilosomes are good nanovesicular carriers for enhancing oral drug delivery and providing the sustained release of RH and all that may enhance oral bioavailability for RH.

Keywords: Ropinirole, Soft Nanovesicular Carriers, Bilosomes, Reverse-Phase Evaporation Method

Introduction

Most drugs are administered orally. It is the preferred method due to its low complication risk, high patient compliance, and easy accessibility.

Oral formulation development faces numerous obstacles, most of which stem from medicine’s poor water solubility and membrane permeability. Instability, pH, efflux transporters,
In addition to solubility and permeability, drug metabolism impacts oral bioavailability. These factors limit the bioavailability of the medicine (3).

New generations of "soft" nanocarriers have many advantages over conventional vesicular-type systems (4). Between them, Bilosomes are nanovesicular carriers that contain bile salts and non-ionic surfactants in a bilayer with cholesterol. These are more ultra-deformable, elastic, and flexible than other nanovesicular carrier systems (5).

The bile salt is biologically compatible and has no toxicity (6). Bile salt may enhance the permeability, bioavailability, and half-life of several medications.

In recent times, it has been shown that bilosomes offer superior defences versus harsh conditions of the digestive tract, such as acidity, bile salts, and digestive enzymes (4). Bilosomes were formed using several bile salts, including sodium deoxycholate (SDC), Sodium glycocholate (SGC), and sodium taurocholate (STC). Due to its low toxicity and excellent permeation-enhancing potential, SDC is the most prevalent of these agents (6).

Ropinirole (RH) is a non-ergot dopamine D2-agonist like bromocriptine, but it also binds to D3 receptors. It is used similarly in managing Parkinson's disease and to minimize "on-off" variations in levodopa responsiveness. Ropinirole treats moderate-to-severe idiopathic RLS (8).

The RH is quickly absorbed from the gastrointestinal system, and peak plasma concentrations are reached within 1.5 h after oral dosing; meals may decrease the absorption rate, but not the amount. The estimated bioavailability is around 50% due to it being extensively metabolized in the liver. It is distributed broadly throughout the body, and plasma protein binding is minimal (10 to 40%). Less than 10% of an oral dosage of ropinirole is excreted unaltered. RH has been found to have a mean elimination half-life of around 6 h (8, 9).

Currently available RH traditional Tablet dosage forms cannot achieve acceptable oral bioavailability (11).

The aim of this study was to prepare and evaluate in-vitro the bilosomes as an oral drug delivery system and to provide the sustained release of RH by preparing it as soft nanovesicular carriers (bilosomes).

**Materials and methods**

**Materials**

Ropinirole hydrochloride (Wuhan Hanweishi Pharmchem Co., Ltd., China), Span 60 and Tween 60 (Loba Chemie Pvt., Ltd., India), Cholesterol and sodium deoxycholate (Avonchem Ltd., UK).

**Preparation of bilosomes of RH**

Eight formulas of RH-bilosomes were prepared by reverse-phase evaporation method (11). A mixture of surfactants (tween® 60 : span® 60 1:2) and weighted amount of cholesterol was placed in a round-bottomed flask with an adapter containing a 10 ml of mixture of (chloroform : diethyl ether 1:1). The aqueous phase was made by dissolving 50 mg of RH and weighted amount of sodium deoxycholate in 2 ml of deionized water, the two phases were mixed by sonication to create a white emulsion. The emulsion was dried in a rotary evaporator at 150 rpm, then hydrated with 10 ml of deionized water. The resulting bilosomes suspension was kept at 60°C for one hour, then sonicated to reduce the size of the vesicles before being stored at 4°C for further analysis (13). The composition of bilosomes formulas are shown in Table 1.

**Table 1. Composition of Bilosomes Formulas of RH**

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>Drug (mg)</th>
<th>sodium deoxycholate (mg)</th>
<th>tween® 60 : span® 60 1:2 (mg)</th>
<th>cholesterol (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>50</td>
<td>5</td>
<td>270</td>
<td>60</td>
</tr>
<tr>
<td>F2</td>
<td>50</td>
<td>10</td>
<td>270</td>
<td>60</td>
</tr>
<tr>
<td>F3</td>
<td>50</td>
<td>5</td>
<td>440</td>
<td>60</td>
</tr>
<tr>
<td>F4</td>
<td>50</td>
<td>10</td>
<td>440</td>
<td>60</td>
</tr>
<tr>
<td>F5</td>
<td>50</td>
<td>5</td>
<td>270</td>
<td>90</td>
</tr>
<tr>
<td>F6</td>
<td>50</td>
<td>10</td>
<td>270</td>
<td>90</td>
</tr>
<tr>
<td>F7</td>
<td>50</td>
<td>5</td>
<td>440</td>
<td>90</td>
</tr>
<tr>
<td>F8</td>
<td>50</td>
<td>10</td>
<td>440</td>
<td>90</td>
</tr>
</tbody>
</table>
Characterization of bilosomes of RH

Determination of Drug content of bilosomes of RH

An accurate volume (1 ml) of each formula equivalent to 5 mg of RH was measured and mixed in 9 ml of methanol and was sonicated in a sonication bath for 5 min. From this, 1 ml of the solution was taken and further diluted with methanol[14]. The solution was assayed for drug content using a UV/VIS spectrophotometer (model UV-1900I PC, Shimadzu, Kyoto, Japan) at 249 nm [15]. The percentage of drug content in the bilosomes was calculated by using equation 1[16].

\[
\text{Drug content \( \% \) = } \frac{\text{The actual amount of RH}}{\text{The theoretical amount of RH}} \times 100
\]

Eq. 1

Determination of percent entrapment efficiency (EE%)

The unentrapped drug was separated from the bilosomes dispersion using the ultrafiltration technique. A measured volume of 4 mL of the bilosomes dispersion was added to the sample reservoir of the Amicon® Ultra-4 centrifugal filter (Molecular weight cutoff of 10 kilo Daltons) (Merck Millipore Ltd.) and was centrifuged (Hettich centrifuge) at 6000 revolutions per minute (rpm), and the sample was spun for 30 min at room temperature. The filtrate that was withdrawn from the bilosomes preparation containing free RH that was not successfully entrapped within the bilosomes. To determine the amount of drug that was not entrapped, this filtrate was analyzed by the ultrafiltration technique. A measured volume of 4 mL of the filtrate that was withdrawn from the bilosomes dispersion was added to the sample reservoir of the Amicon® Ultra-4 centrifugal filter (Molecular weight cutoff of 10 kilo Daltons) (Merck Millipore Ltd.) and was centrifuged (Hettich centrifuge) at 6000 revolutions per minute (rpm), and the sample was spun for 30 min at room temperature. The filtrate that was withdrawn from the bilosomes preparation containing free RH that was not successfully entrapped within the bilosomes. To determine the amount of drug that was not entrapped, this filtrate was analyzed by using a UV spectrophotometer (model UV-1900I PC, Shimadzu, Kyoto, Japan) at 249 nm [15]. The measurements were performed in triplicate. The EE% was determined using an equation 2 [18].

\[
\text{EE}(\%) = \frac{\text{Amount of total drug}}{\text{Amount of total drug} - \text{Amount of unentrapped drug}} \times 100
\]

Eq. 2

Measurement of vesicles size (VS), polydispersity index (PDI) and Zeta potential (ZP)

The VS and PDI of the bilosomes loaded with the RH were measured using dynamic light scattering (DLS) with a Zetasizer Nano (ZS) instrument made by Malvern (Worcestershire, UK). The measurements were conducted at a temperature of 25±2 °C. Additionally, the ZP was also measured by observing movement of vesicles in an electrical field in distilled water with the same instrument. To ensure accurate measurements, the samples were diluted 15 fold with distilled water before being analyzed [19, 20].

In-vitro drug release study

The drug release from RH solution and RH-loaded bilosomes was assessed using the dialysis bag method (MD34 1M, 34 nm, MWCO 12,000–14,000, USA). A dialysis bag containing 1 ml of the bilosomes dispersion was placed in a release medium consisting of 500 ml of phosphate buffer (pH 6.8), due to the drug is absorbed in the intestine [21], and maintained at 37 ± 0.5 °C with 100 rpm using USP apparatus II. Samples (5 ml) were taken at regular intervals (30 minutes, 1, 2, 4, 6, 8, 12, and 24 hours) and measured spectrophotometrically (model UV-1900I PC, Shimadzu, Kyoto, Japan) at 249 nm [22, 23].

Comparative in-vitro release of the pure drug, F5 and F6 was studied:-

The similarity factor (f2) was applied for study as shown in equation 3 [20].

\[
f2 = 50 \cdot \log \left\{ 100 \cdot \left[ 1 + \frac{1}{n} \sum_{i=1}^{n} \left( R_t - T_0 \right)^2 \right]^{-0.5} \right\}
\]

Equation (3)

The f2 value compares the similarity of two drug release profiles. It is calculated based on the number of release time points (n), the reference dissolution values (Rt), and the test dissolution values (Tt) at each time point. When the f2 value is higher than 50, the two release profiles are considered similar, while values lower than 50 indicating that the profiles are not similar. The range between 50 and 100 is considered similar [25].

Determination of Shape of Bilosomes by Transmission electron microscopy (TEM)

To determine the shape of the optimized RH bilosomes formula, a high-resolution transmission electron microscope (TEM) was used. The sample was prepared by diluting a drop of the selected formula with distilled water, allowing it to dry on a carbon-coated copper grid then examining it with the TEM (Zeiss Supra 40vp, Germany). The TEM allowed researchers to visualize the shape and structure of the bilosomes and any other features present in the sample [26].

Fourier Transform Infrared (FTIR) Analysis

Fourier Transform Infrared spectra of RH, span60, SDC, physical mixture and the optimized RH bilosomes formula were obtained using Shimadzu, FTIR Spectroscopy (FTIR 43000, Japan). Each sample was dispersed in KBr powder, blended well in mortar and pestle, and compressed into transparent discs for FTIR examination.

The FTIR spectra were recorded in spectra region 4000 to 400 cm−1 at an instrument resolution of 4 cm−1 [27].

Results and Discussion

Characterization of bilosomes of RH

Drug content of bilosomes

The prepared RH-loaded bilosomes were characterized for percent drug content. It was found to be in the range of 97.3±0.5% to 100.5±0.9% (Table 2). These results indicated that the RH was uniformly distributed in the aqueous core of the bilosomes dispersions of all formulas and there was no loss of RH during preparation of bilosomes.
Table 2. Drug Content, Entrapment Efficiency, Vesicles Size, Polydispersity Index and Zeta Potential of RH loading Bilosomes

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>Drug Content* (%)</th>
<th>EE* (%)</th>
<th>VS* (nm)</th>
<th>PDI*</th>
<th>ZP* (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>98.2±0.7</td>
<td>63.99±4.36</td>
<td>136.80±20.00</td>
<td>0.3871±0.039</td>
<td>-21.655±2.565</td>
</tr>
<tr>
<td>F2</td>
<td>98.9±0.7</td>
<td>60.44±2.49</td>
<td>124.40±10.60</td>
<td>0.3563±0.022</td>
<td>-23.22±0.880</td>
</tr>
<tr>
<td>F3</td>
<td>99.6±0.5</td>
<td>59.50±3.23</td>
<td>225.61±25.51</td>
<td>0.4501±0.073</td>
<td>-19.20±0.685</td>
</tr>
<tr>
<td>F4</td>
<td>97.5±0.8</td>
<td>57.30±6.48</td>
<td>193.85±15.25</td>
<td>0.3800±0.064</td>
<td>-20.94±0.175</td>
</tr>
<tr>
<td>F5</td>
<td>100.1±0.7</td>
<td>77.77±3.78</td>
<td>160.60±15.82</td>
<td>0.3901±0.1206</td>
<td>-20.75±0.930</td>
</tr>
<tr>
<td>F6</td>
<td>100.5±0.9</td>
<td>71.37±1.76</td>
<td>155.10±6.900</td>
<td>0.3457±0.048</td>
<td>-21.54±1.025</td>
</tr>
<tr>
<td>F7</td>
<td>97.3±0.5</td>
<td>70.39±6.04</td>
<td>294.60±11.50</td>
<td>0.4553±0.122</td>
<td>-16.95±7.372</td>
</tr>
<tr>
<td>F8</td>
<td>97.8±0.3</td>
<td>66.58±2.98</td>
<td>230.15±42.85</td>
<td>0.4064±0.016</td>
<td>-17.62±2.705</td>
</tr>
</tbody>
</table>

*Results as mean ± SD, n=3

Entrapment efficiency of RH bilosomes

The EE% of RH-loaded bilosomes are presented in Table 2. These results indicate that bilosomes were successful in entrapping the RH. The EE% for the RH bilosomes was ranged from 57.3% - 77.77%, which was considered to be a good entrapment efficiency, but it was not very high due to hydrophilic nature of RH (9). Hydrophilic drugs had been shown to exert good affinity to trap inside vesicle cores formed by using lipophilic surfactants (span®60) and a hydrophilic surfactant(tween®60) mixture. Also, high transition temperatures of Span® 60 and Tween®60 provide high levels of drug encapsulation (28). These results were in agreement with results that obtained by Saifi Et el. (29).

The SDC could affect the drug entrapment efficiency (EE%) of bilosomes, However, the effects are not statistically significant when the quantity of bile salts is increased (p>0.05). The fluidizing effect of SDC on lipid bilayers could cause the release of the drug, resulting in a decrease in EE%. On the other hand, increasing the SDC content could also increase the solubility of the drug in the dispersion medium due to the formation of mixed micelles, which could compromise EE% (Figure 2, A) (30).

Surfactants can also affect EE% (Figure 2, B), but the effect is not statistically significant when the quantity is increased (p>0.05). However, the difference between two specific conditions (F5 and F7) is statistically significant (p<0.05). The formation of micelles at higher surfactant concentrations could contribute to this effect, as well as the rearrangement of surfactant molecules within the lipid bilayer structure, which could increase the permeability and fluidity of the vesicle membrane, leading to drug leakage (31).

In contrast, increasing the quantity of cholesterol significantly increased EE% (p < 0.05) (Figure 2, C). Cholesterol could improve the lipophilicity and rigidity of the lipid bilayer membrane, leading to increased stability and drug encapsulation of bilosomes vesicles(32).

Figure 2. Effect of increase the quantities of Sodium deoxycholate (A), Span®60: tween®60 1:2 (B) and cholesterol (C) on EE% of RH-bilosomes.
Vesicle size of bilosomes of RH

The results of this study show that increasing the quantity of SDC leading to a decrease in vesicle size (Figure 3, A), but, this decrease is not statistically significant (p>0.05) when comparing F1, F3 and F5, with corresponding formula F2, F4 and F6 respectively. However, there was a significant decrease (p<0.05) in vesicle size when comparing F7 and F8. This decrease in vesicle size was likely due to the reduced surface tension and increased flexibility of bilosomes caused by the increased SDC content (29,33).

On the other hand, increasing the quantity of surfactant causing a statistically significant increase (p<0.05) in vesicle size as in F1, F2, F5 and F6 against corresponding formula F3, F4, F7 and F8 as shown in (Figure 3, B). This was thought to be due to the structure of the surfactant, as vesicle size is dependent on the length of the alkyl chain. Surfactants with longer alkyl chains, such as Span®60 and tween®60, tend to create larger vesicles. Therefore, increasing the amount of surfactant caused an increase in the vesicle size of prepared RH-loaded bilosomes (34).

Increasing the quantity of cholesterol also exhibited an increase in vesicle size (Figure 3, C), but this increase was not statistically significant (p>0.05) when comparing F1 against F5, F2 against F6. However, there was a statistically significant increase (p<0.05) in vesicle size when comparing F3 against F7 and F4 against F8. This increase in vesicle size was likely due to the high cholesterol concentration hindering the close packing of lipids in the vesicles and resulting in a higher distribution of aqueous phase within the vesicle, as well as the increased drug entrapment in the vesicles caused by the increased cholesterol concentration (29,35).

Polydispersity Index of bilosomes of RH

The polydispersity index (PDI) is a degree of homogeneity of the formulations. Formulations with a PDI value close to zero indicate that all the vesicles in the sample are the same size, while those with a PDI value close to one indicate that the particles are very different in size or highly polydisperse. For all formulas (F1-F8), the PDI values ranged from (0.3457±0.048 to 0.4553±0.122) indicating a relatively monodisperse system (36).

The quantities of SDC can affect the PDI of bilosomes dispersion. Increasing the quantity of SDC from 5mg as in F1, F3, F5 and F7 causing a decrease in the PDI as in corresponding formulas F2, F4, F6 and F8 respectively (Figure 4, A). however, Surfactants and cholesterol can also affect PDI (Figure 4, B, C); an increase in the quantity of surfactant and cholesterol leads to an increase the PDI.

According to an ANOVA test, the variables, bile salt (SDC), surfactants, and cholesterol did not had a significant effect on the values of PDI for RH bilosomes. These results were in consistent with previous studies, which also showed that formulation variables do not significantly impact PDI values for bilosomes of lornoxicam (35).
soft nanovesicular carriers (bilosomes)

**Zeta potential of bilosomes of RH**

It was observed that increased the quantity of SDC exhibited a small non-significant increase in the zeta potential of the bilosomes (p>0.05) (Figure 5, A). The anionic nature of SDC likely caused the negative charge in the formulations. Increasing the amount of SDC from (5 mg) as in formulas F1, F3, F5 and F7 to (10 mg) as in corresponding formulas F2, F4, F6 and F8 respectively resulted in a corresponding increase in the zeta potential of the bilosomes (37,38).

Additionally, increased the quantity of Span®60: tween®60 1:2 resulted in a small non-significant decreased in the zeta potential of the bilosomes (p>0.05) (Figure 5, B). The non-ionic surfactant, which was hydrophilic in nature, may have exerted a shielding effect on the surface of the vesicular bilayer, leading to a less negative zeta potential (38,39). This effect was observed in the formulations containing higher amounts of surfactant, such as F3, F4, F7, and F8 in contrast to corresponding formulas F1, F2, F5 and F6, which had lower amount of surfactant.

Finally, it was found that increasing the quantity of cholesterol in the bilosomes (F5-F8) caused a small but non-significant decrease in the zeta potential (p>0.05) as compared with corresponding formulas (F1-F4) (Figure 5, C). This may have been due to competition between cholesterol and bile salts for space in the bilayer, leading to a lower concentration of bile salt in the bilayer and on the surface of the bilosomes, and, subsequently, a less negative zeta potential (40).

The negative charge on vesicles is thought to promote drug uptake through M-cells in the Peyer’s patch and enhanced drug absorption through the intestinal lymphatic transport pathway as well as oral bioavailability. Additionally, the negative charge can also increase the fluidity of the intestinal membrane, making it easier for the vesicles to be transported through it (41).

**Selection of the best formula**

From the obtained results, it was found that both F5 and F6 had the highest EE% of RH (77.77±3.78% and 71.37±1.76%), respectively, also F5 and F6 had suitable vesicle size (160.60) nm and (155.10) nm, zeta potential (−20.75) mV and (−21.545) mV and polydispersity Index (0.3901) and (0.3457), respectively (Figure 7).
In vitro drug release (%)

Two formula F5 and F6 was selected to do in vitro drug release (%). The RH drug release from bilosomal formulations F5 and F6 was found to be slow, gradual and extended per day when compared with pure drug solution.

The slow and extended release of RH observed in the studied formulations was attributed to the benefits of bilosomes as colloidal nanocarriers. These vesicles can act as drug reservoirs, allowing for a sustained release of the drug encapsulated within them. Furthermore, incorporating cholesterol into the vesicles reduces the permeability and release of the drug trapped inside by decreasing the fluidity of the vesicular membrane of bilosomes (42). These results were in agreement with results that obtained by Liu Et al. (43).

The bilosomal formulation, F5, showed less drug release (84.39%) compared to F6 (96.12%) and pure drug solution (100%) at 4h. (figure 6), the highest entrapment efficiency (EE%) of RH in formulas F5 and F6 may causes sustained release of the drug from carriers. The discrepancy in release properties may be attributed to increases in SDC concentration from 5mg (F5) and 10 mg (F6) leading to increased drug release and the different membrane rigidity of bilosomes (7).

From both bilosomal formulations F5 and F6, the drug release studies reveal that a typical biphasic release pattern was observed with bilosomal formulations, with an initial rapid burst release (for 2 h), followed by a sustained release for a period of 24 h. These result were probably because of the fact that these formulations had optimized amount of surfactant, cholesterol and bile salt. Both formulas (F5 and F6) having a higher concentration of cholesterol exhibited a decreased rate of drug release (7, 41).

Although, the highest drug release was observed in F6, but both of them had similar release profile (f2=56). But, from an economical point of view F5 with 5mg of SDC is preferred over F6 with 10mg of SDC, so F5 was subjected to further analysis.

Transmission electron microscopy (TEM) image

Figure 8 display TEM image of F5. This image depicted spherical vesicle that was within the nanometer range, which is a characteristic of bilosomes vesicular structure. The size of the vesicles in the optimized formulation was in consistent with the results obtained from the dynamic light scattering method (44).
The FTIR spectrum of the pure drug showed the characteristic FTIR peaks at 3413 cm\(^{-1}\) (N-H stretching), 1612 cm\(^{-1}\) (C=C stretching), 3074 cm\(^{-1}\) (aromatic, C-H stretching), 2935 cm\(^{-1}\) and 2880 cm\(^{-1}\) (aliphatic C-H stretching), 1311 cm\(^{-1}\) and 1346 cm\(^{-1}\) (C-N stretching), 1759 cm\(^{-1}\) (C=O stretching). The above-mentioned peaks at specific wave numbers were also observed in physical mixtures and F5. It was concluded, based on the FTIR results (Figure 9), that there were no drug excipient interactions. 

Figure 9. FTIR spectrum of RH (A), Cholesterol (B), Span\(^{60}\) (C), Sodium deoxycholate (D), Physical mixture (E) and F5 bilosomes formula (F).
Conclusion
The sustained release of ropinirole hydrochloride was obtained by preparing it as soft nanovesicular carriers (bilosomes) by a reverse-phase evaporation method using mixed surfactant (span®60:tween®60 1:2), cholesterol and SDC as bile salt. The good entrapment efficiency of bilosomes was obtained, in spite of hydrophilic nature of ropinirole, so the bilosomes is considered as good carriers for the ropinirole.

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Ethics Statements
None

Conflict of interest
The author(s) declare no conflicts of interest.

Author contributions
Samer K. Ali : PhD student Entidhar J. Al-Akam: Supervisor

References


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