



Research Article

Effects of Different Types of Bile Salts on the Physical Properties of Ropinirole-Loaded Bilosomes

Samer Khalid Ali ^{1*} , Entidhar Jasim Al-Akkam ¹ 

¹ Department of pharmaceuticals, College of Pharmacy, University of Baghdad, Baghdad, Iraq

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Abstract

Background: Bilosomes are vesicular nanocarriers that contain bile salts, making them more flexible and resistant to degradation in the gastrointestinal tract. **Objective:** To evaluate the effect of two bile salts on the physical properties and stability of the ropinirole-loading bilosome. **Methods:** Sixteen bilosomal formulations were prepared by a reverse-phase evaporation method. Each formula includes a mixture of non-ionic surfactants (Span[®]60 and Tween[®]60), along with cholesterol and bile salts (either sodium taurocholate (STC) or sodium glycocholate (SGC)). The characteristics of the bilosomal formulations (drug content, entrapment efficiency, vesicle size, polydispersity index, zeta potential, *in-vitro* drug release, and fourier transform infrared spectroscopy) were evaluated. **Results:** The entrapment efficiency of ropinirole was reduced by using sodium glycocholate instead of sodium taurocholate. The vesicle size and zeta potential were also affected by the type of bile salt and its amount. Drug release profiles were sustained, indicating a good entrapment of ropinirole. The STC-containing bilosomes are more stable than the SGC-containing bilosomes. Bilosomal formula F5 showed the highest entrapment efficiency (64.82%), suitable vesicle size (179.8 nm), zeta potential (-9.162 mV), polydispersity index (0.5116), and *in vitro* drug release (62.33%) after 24 hr. **Conclusion:** Sodium taurocholate was more suitable for the preparation of ropinirole-loading bilosomes, with more stability of bilosomes in bile salt solution.

Keywords: Bilosomes, Ropinirole, Sodium glycocholate, Sodium taurocholate, Reverse-phase evaporation method.

تأثير أنواع مختلفة من أملاح الصفراء على الخواص الفيزيائية للبيوسومات المحملة بالروبينيرون

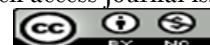
الخلاصة

الخلفية: البيوسومات هي نوع من الناقلات النانوية الحويصلية التي تحتوي على أملاح صفراوية داخل هيكلها ثنائي الطبقات، مما يجعلها أكثر مرونة ومقاومة للتحطم في الجهاز الهضمي. **الهدف:** تقييم تأثير أملاح صفراوية على الخواص الفيزيائية واستقرار البيوسومات المحملة بالروبينيرون. **الطريقة:** تم تحضير ستة عشر صيغة بايوسوم بطريقة التبخير ذات الطور العكسي، تتكون كل صيغة من خليط من المواد الخافضة للتوتر السطحي غير الأيونية جنباً إلى جنب مع الكوليسترول والأملاح الصفراوية. تم تقييم محتوى الدواء، كفاءة الحصر، حجم الحويصلة، مؤشر التشتت المتعدد، جهد الزيت، إطلاق الدواء في المختبر، والتحليل الطيفي بالأشعة تحت الحمراء. **النتائج:** تم تقليل كفاءة انحباس روبينيرون باستخدام جليكولات الصوديوم بدلا من توروكولات الصوديوم. تأثر حجم الحويصلة وإمكانات زيتنا أيضا بنوع الملح الصفراوي وكميته. تم الحفاظ على ملامح إطلاق الدواء، مما يشير إلى حصر جيد للروبينيرون. البيوسومات المحتوية على STC أكثر استقرارا من البيوسومات المحتوية على SGC. أظهرت الصيغة البيوسومية F5 أعلى كفاءة انحباس (64.82%)، وحجم حويصلة مناسب (179.8 نانومتر)، وجهد زيتنا (-9.162 مللي فولت)، ومؤشر تعدد التشتت (0.5116)، وإطلاق الدواء في المختبر (62.33%) بعد 24 ساعة. **الاستنتاج:** كان توروكولات الصوديوم أكثر ملاءمة لإعداد البيوسومات المحملة بروبينيرون، مع مزيد من الاستقرار للبيوسومات في محلول الملح الصفراوي.

* **Corresponding author:** Samer K. Ali, Department of Pharmaceuticals, College of Pharmacy, University of Baghdad, Baghdad, Iraq; Email: samer.khaled1100p@copharm.uobaghdad.edu.iq

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INTRODUCTION

Ropinirole hydrochloride (ROH) is a medication that belongs to the nonergoline class and is considered a dopamine agonist. It works by binding and activating dopamine receptors D2 and D3 in the brain, which improves motor function [1]. Ropinirole hydrochloride is marketed as oral tablets and is approved for the treatment of Parkinson's disease (PD) and Restless Leg Syndrome (RLS) [2]. Ropinirole hydrochloride is chemically known as "4-[2 (dipropylamino) ethyl]-1,3-dihydro-2H-indol-2-one". ROH is highly hydrophilic, BCS class III, has a molecular weight of 296.84, and can be dissolved in methanol and water with a solubility of 133 mg/ml, a low Log P value of 2.7, and a pKa value of 9.5. Its oral bioavailability is around 50% because it is extensively metabolized in the liver. Additionally, due to its hydrophilic nature, ROH may have difficulty crossing biological membranes, which may limit its permeation in the body. It has a half-life of 6 hours, and its recommended dose ranges from 0.75–24 mg/day orally [3–5]. Bilosomes are vesicular nanocarriers that incorporate bile salts into their bilayer of vesicles, making them more flexible and resistant to degradation in the gastrointestinal tract [6]. Conventional nano-vesicular carriers such as liposomes and niosomes can only protect drugs to a certain extent from enzymatic degradation in the GIT. However, bilosomes overcome this limitation [7]. Bile salts are solubilizing and permeation-enhancing agents. They are popularly used because of their biological compatibility and lack of toxicity. Bile salts are added to the lipid bilayers of bilosomes to make them more stable and easier to take by mouth. Bilosomes are a good choice for giving drugs by mouth because they are stable and have nanosized vesicles. Research has also shown that they are safe and effective for this purpose [8]. Different types of bile salts can be used to prepare bilosomes. Some examples include sodium glycocholate (SGC), sodium deoxycholate (SDC), and sodium taurocholate (STC). The choice of bile salt will depend on factors such as toxicity and permeation effects. Each bile salt has unique properties and may be better suited for certain applications [9]. Nonionic

surfactants (span and tween) are frequently used in making bilosomes due to their stability and compatibility in comparison to other surfactants, causing minimal irritation to the cells of the body. They are less affected by changes in pH and ionic strength [10,11]. The aim of this study was to prepare ROH-loaded bilosomes using two different types of bile salts (STC and SGC) to study their effect on the physical properties and stability of bilosomes, which is designed to improve oral delivery of ROH.

METHODS

Materials

Ropinirole hydrochloride (Wuhan Hanweishi Pharmchem Co., China), Cholesterol (Avonchem, UK), Sodium glycocholate and taurocholate (Leyan Co., China), Span@60, Tween@60 and methanol (Loba Chemie Pvt., India), Phosphate buffered saline (PBS) pH 7.4, Chloroform and Diethyl ether (Hi-Media Laboratories., India).

Preparation of ROH-loaded bilosomes

The reverse-phase evaporation method was used to prepare sixteen different formulations (F1–F16) of ROH-loading bilosomes [12]. A mixture of surfactants tween@ 60 and span@ 60 and a weighted amount of cholesterol were combined in a round-bottomed flask with an adapter containing 10 ml of a mixture of chloroform and diethyl ether at a 1:1 ratio. An aqueous phase was created by dissolving 50mg of ROH and a weighted amount of STC or SGC in 2 ml of deionized water. The two phases were mixed using ultrasonic baths (LiebeWh, China) to form a stable white emulsion. The emulsion was then dried in a rotary evaporator (Copley, UK) at 150 rpm and rehydrated with 10 ml of deionized water. The resulting bilosome suspension (5 mg/ml) was heated in a water bath at 60°C for one hour, then sonicated to reduce the vesicle size, and then stored at 4°C for further analysis [13]. The constituents of prepared bilosome formulas are shown in Table 1.

Table 1: Constituents of ROH-Loaded Bilosomes Formulas

Formula No.	ROH (mg)	Types of bile salts	Bile salt (mg)	Span 60@ (w/v %)	Tween@0 (w/v %)	Cholesterol (w/v %)
F1	50	STC	5	0.90	1.80	0.60
F2	50	STC	10	0.90	1.80	0.60
F3	50	STC	5	1.46	2.94	0.60
F4	50	STC	10	1.46	2.94	0.60
F5	50	STC	5	0.90	1.80	0.90
F6	50	STC	10	0.90	1.80	0.90
F7	50	STC	5	1.46	2.94	0.90
F8	50	STC	10	1.46	2.94	0.90
F9	50	SGC	5	0.90	1.80	0.60
F10	50	SGC	10	0.90	1.80	0.60
F11	50	SGC	5	1.46	2.94	0.60
F12	50	SGC	10	1.46	2.94	0.60
F13	50	SGC	5	0.90	1.80	0.90
F14	50	SGC	10	0.90	1.80	0.90
F15	50	SGC	5	1.46	2.94	0.90
F16	50	SGC	10	1.46	2.94	0.90

Determination of drug content of ROH-loaded bilosomes

The accurate volume of each ROH-loaded bilosome formula (1 ml), which is equivalent to 5 mg of ROH, was dissolved in 9 ml of methanol and then sonicated for 5 min. Then, 1 ml of the solution was diluted with methanol. Finally, the solution was assayed for drug content using UV spectrophotometry by measuring the absorbance at 249 nm using a UV/VIS spectrophotometer (model UV-1900I PC, Shimadzu, Kyoto, Japan) [14,15]. The percentage of drug content in the bilosomes was calculated using equation 1 [16].

$$\text{Drug content \%} = \frac{A_a}{A_t} \times 100 \dots \text{Equation (1)}$$

Where A_a = Practical determined amount of ROH and A_t = Theoretical amount of ROH.

Determination of percent entrapment efficiency (%)

The ultrafiltration technique was used to separate the free drug from bilosome dispersion. A sample reservoir of an Amicon® Ultra-4 centrifugal filter (Merck Millipore, Italy) with a molecular weight cutoff of 10 kDa was filled with 4 mL of the bilosome dispersion. The sample was centrifuged at 6000 rpm for 30 min. at room temperature using a Hettich type centrifuge, Germany. The filtrate was withdrawn from the bilosomal preparation, which contains the free ROH that was not successfully entrapped within the bilosomes. This filtrate was analyzed using a UV spectrophotometer (model UV-19001 PC, Shimadzu, Kyoto, Japan) at 249 nm to determine the amount of free drug that was not entrapped. The measurements were likely performed in triplicate. The entrapment efficiency (EE%) was determined using equation 2 [17].

$$\text{EE(\%)} = \frac{A_t - A_f}{A_t} \times 100 \dots \text{Equation (2)}$$

Where A_t = Amount of total drug and A_f = Amount of free drug.

Measurement of vesicles size (VS), polydispersity index (PDI), and zeta potential (ZP) of ROH-loaded bilosomes

Using a Zetasizer (a Nano ZS instrument by Malvern Instruments, Worcestershire, UK), dynamic light scattering (DLS) was a technique for determining the VS and PDI of ROH-loading bilosomes. The measurements were conducted at a temperature of 25 ± 2 °C. Additionally, the ZP of the bilosomes was measured by observing how bilosomes moved 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. The ZP measurement allowed an understanding of the surface charge of the bilosomes, which was important for the stability of the bilosomes [18,19].

In vitro drug release study

The dialysis bag method used a dialysis membrane (MWCO 12,000–14,000; USA) to measure how much drug was released from a pure drug solution and ROH-loaded bilosomes. A dialysis bag containing 1 ml of the preparation was placed in a medium of 500 ml of phosphate buffer (pH 6.8) and maintained at 37 ± 0.5 °C and 100 rpm using USP apparatus II (paddle). Samples (5 ml) were taken after regular intervals (30 min, 1, 2, 4, 6, 8, 12, and 24 h) and measured using UV spectrophotometry (model UV-1601 PC, Shimadzu, Kyoto, Japan) at 249nm. The dialysis bag method allows the drug to be released from the bilosomes into the buffer medium. The samples were then taken and measured for drug concentration over time to determine the release profile of the drug from the bilosomes [20,21]. The similarity factor (f_2) was utilized to assess whether the release profiles of pure drug solution and ROH-loaded bilosomes were similar (equation 3).

$$f_2 = 50 \times \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^n |R_t - T_t|^2 \right]^{-0.5} \times 100 \right\} \dots \dots \text{Equation (3)}$$

The similarity factor (f_2) takes into account the number of release time points (n), as well as the reference (R_t) and test (T_t) dissolution values. The release profiles were considered similar if the f_2 value was greater than 50 (50-100). However, if the f_2 value was less than 50, the profiles were considered dissimilar [22,23]

Stability of Bilosomes in Bile Salt Solution

The stability of bilosome formulas against bile salt (SDC) solution formulations was assessed using a method that had been previously reported [24]. Several concentrations, 4, 8, 12, 16, and 20 mM of SDC, were dissolved in pH 7.4 phosphate-buffered saline (PBS) to create a series of solutions. A volume of 40 μ L of bilosome dispersion was mixed with 760 μ L of each diluted SDC solution and incubated at 37°C. After 1 h, the turbidity% of the formulas was analyzed using a spectrophotometer at 400 nm, with SDC solutions without bilosomes as a blank for each SDC concentration. The percentage turbidity was calculated using Equation 4 and graphed against the concentration of SDC [24].

$$\text{Turbidity \%} = A / B \times 100 \dots \dots \text{Equation (4)}$$

The absorbance of bilosomes formulas after incubation in SDC solution was represented by (A), and the absorbance of bilosomes formulas after incubation in pH 7.4 PBS was represented by (B).

Fourier Transform Infrared (FTIR)

This method was for obtaining the Fourier transform infrared (FTIR) spectra of various samples, including the drug (ROH), surfactant, bile salt, physical mixture, and an optimized bilosome formula using Shimadzu (FTIR 43000, Japan). To prepare the samples for examination, they were dispersed in KBr powder and compressed into transparent discs [25]. The FTIR spectra were recorded in the spectra region from 4000 to 400 cm^{-1} , with an instrument resolution of 4 cm^{-1} [26].

Statistical analysis

The results were presented as the mean of triplicate samples along with the standard deviation. Statistical analysis was conducted using one-way analysis of variance (ANOVA). The significance level was set at a p-value < 0.05.

RESULTS AND DISCUSSION

The ROH-loaded bilosomes that had already been made were analyzed for their drug content. This was the amount of drug in the bilosomes as a percentage of their total weight. The percent drug content values ranged from 96.3±0.5% to 101.1±0.5% (Table 2). These values indicate that the ROH was uniformly distributed in the aqueous core of the bilosomes. This result suggests no significant drug loss during the development of bilosomes, and bilosomes are a good drug carrier system. This is an important parameter to be determined in the development of bilosomes, as it indicates the efficiency of bilosomes in encapsulating the drug.

Table 2: Drug Content, Entrapment Efficiency, Vesicles Size, Polydispersity Index, and Zeta Potential of ROH- loading Bilosomes

Formula No.	Drug Content (%)	EE (%)	VS (nm)	PDI	ZP (mV)
F1	98.3±0.5	55.13±0.31	147.95±6.65	0.43±0.01	-12.54±0.48
F2	98.3±0.8	53.47±1.41	134.05±0.95	0.38±0.03	-13.04±7.89
F3	99.7±0.6	52.98±1.53	229.00±0.50	0.52±0.01	-10.64±0.02
F4	97.9±0.5	50.88±1.67	223.35±13.25	0.41±0.11	-12.17±1.15
F5	100.7±0.3	64.82±1.80	179.8±2.45	0.51±0.06	-9.16±2.31
F6	99.8±0.3	62.91±3.47	157.1±25.45	0.40±0.01	-11.07±1.08
F7	98.4±0.8	62.47±0.99	372.65±52.05	0.52±0.04	-8.12±4.03
F8	99.2±0.4	60.52±1.33	344.80±85.0	0.43±0.15	-8.99±4.11
F9	97.4±0.6	50.6±0.70	146.75±6.55	0.39±0.03	-8.87±4.81
F10	99.5±0.2	48.30±1.79	133.21±0.79	0.36±0.02	-9.72±3.91
F11	98.2±0.6	47.21±1.43	228.95±33.65	0.49±0.01	-8.43±2.93
F12	98.5±0.8	45.32±1.89	223.2±0.10	0.39±0.11	-8.59±0.00
F13	101.1±0.5	60.56±1.64	178.5±13.30	0.48±0.07	-8.02±0.57
F14	99.5±0.2	58.89±1.10	155.35±4.25	0.38±0.02	-9.03±3.61
F15	98.5±0.6	56.41±2.68	334.9±82.70	0.50±0.11	-4.87±0.43
F16	98.5±0.4	54.58±1.64	306.5±10.70	0.41±0.10	-5.09±0.29

Values were expressed as mean±SD; n= 3.

The data presented in Table 2 shows that bilosome vesicles formed by using lipophilic surfactants (Span®60) and hydrophilic surfactants (Tween®60) successfully entrapped the ROH. The entrapment efficiency (EE%) ranges from 45.32±1.89 % to 64.82±1.80%, which is considered relatively high. Hydrophilic drugs had been shown to exert higher affinity to entrap inside vesicle cores formed by using a span®60 and tween®60 mixture. Also, the high transition temperatures of Span® 60 and Tween®60 provide high levels of drug encapsulation [27]. These results agreed with the results obtained by Saifi *et al.* [28]. The results of this study show that increasing the quantity of bile salt (STC or SGC) leads to a decrease in entrapment efficiency. but this decrease is not statistically significant ($p>0.05$) when comparing F1, F3, F5, F7, F9, F11, F13, and F15 with the corresponding formulas F2, F4, F6, F8, F10, F12, F14, and F16, respectively. Additionally, the increased

quantity of Span®60 and tween® resulted in a non-significant decrease in the entrapment efficiency of the bilosomes ($p>0.05$). This effect was observed in the formulations containing higher amounts of surfactant, such as F3, F4, F7, F8, F11, F12, F15, and F16, in contrast to the corresponding formulas F1, F2, F5, F6, F9, F10, F13, and F14, respectively, which had a lower amount of surfactant. On the other hand, increasing the quantity of cholesterol causes a statistically significant increase ($p< 0.05$) in entrapment efficiency (%) as in F5, F6, F7, F8, F13, F14, F15, and F16 against the corresponding formulas F1, F2, F3, F4, F9, F10, F11, and F12, respectively. This suggests that cholesterol plays a key role in determining the EE% and that varying the quantity of bile salts or surfactants may not significantly impact the EE% of the bilosomes [29]. The bilosome formulas (F1–F8) that were prepared using STC showed a higher EE% ($p<0.05$) compared to the corresponding bilosome formulas (F9–F16) used by

SGC. This can be attributed to the lower HLB value of STC (22.1) compared to SGC (23.1). The higher hydrophobicity of STC behaved as a barrier, which slowed down the drug leakage from the vesicles, resulting in a higher EE%, as shown in Figure 1 [30,31]. Vesicular particle size can impact the absorption and bioavailability of drugs taken orally. Smaller vesicular sizes up to 400 nm can increase the surface area for absorption and improve the ability of the drug to pass through the intestinal barrier, resulting in higher levels of the drug in the bloodstream and improved bioavailability [32].

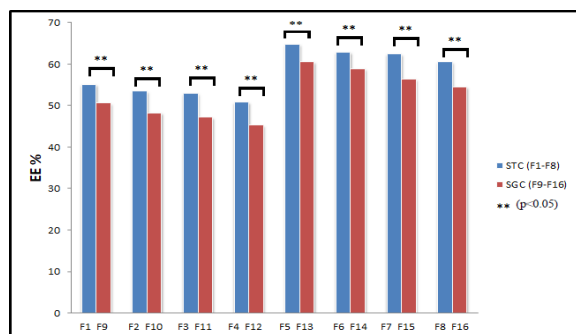


Figure 1: Effect of the types of bile salts on EE% of ROH-loading bilosomes

Table 2 shows that the vesicular size (VS) ranged from 133.21 ± 0.79 nm to 372.65 ± 52.05 nm. The results of this study show that increasing the quantity of bile salt (STC or SGC) leads to a decrease in vesicle size. but this decrease is not statistically significant ($p > 0.05$) when comparing F1, F3, F5, F7, F9, F11, F13, and F15 with the corresponding formulas F2, F4, F6, F8, F10, F12, F14, and F16, respectively. On the other hand, increasing the quantity of surfactant caused a statistically significant increase ($p < 0.05$) in vesicle size as in F1, F2, F5, F6, F9, F10, F13, and F14 against corresponding formulas F3, F4, F7, F8, F11, F12, F15, and F16. Increasing the quantity of cholesterol also exhibited an increase in vesicle size, but this increase was not statistically significant ($p > 0.05$) when comparing F1, F2, F9, and F10 against the corresponding formulas F5, F6, F13, and F14. However, there was a statistically significant increase ($p < 0.05$) in vesicle size when comparing F3, F4, F11, and F12 against the corresponding formulas F7, F8, F15, and F16. According to the data presented in Figure 2, among the types of bile salts studied, the VS increment was a little higher for bilosome formulas containing STC than SGC. The difference in VS might be due to the difference in the structure of the bile salts used. STC, which has a larger structure (537.69 g/mol), may have resulted in a larger VS than SGC-based bilosomal vesicles (487.6 g/mol).

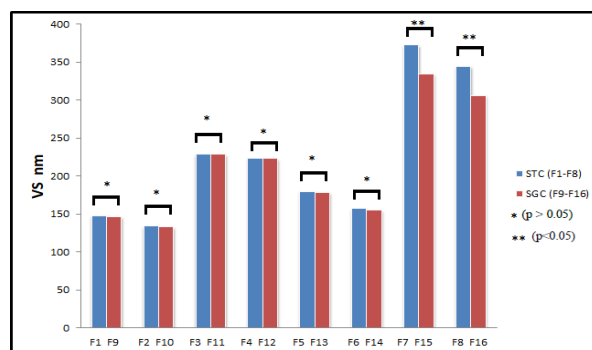


Figure 2: Effect of the types of bile salts on vesicle size of ROH-loading bilosomes.

These results are consistent with previous studies [6, 33]. The polydispersity index (PDI) measures the homogeneity of a formulation. A PDI value close to 0 indicates that all the vesicles in the sample are the same size, while a value close to 1 indicates that the particles are very different in size or highly polydisperse. This study's PDI values ranged from 0.3586 ± 0.02 to 0.5233 ± 0.04 , indicating a relatively monodisperse system [34]. According to an ANOVA test, the amount of bile salt, surfactant, and cholesterol does not significantly affect the PDI for ROH-loading bilosomes. This result is consistent with previous findings in the article, which also showed that formulation variables do not significantly impact PDI values for lornoxicam bilosomes [18]. Although the types of bile salts used in the bilosome formulation resulted in an increase in the polydispersity index (PDI), the ANOVA test indicated that the effect was not statistically significant ($p > 0.05$). The sequence of increasing PDI was observed as STC > SGC, as shown in Figure 3.

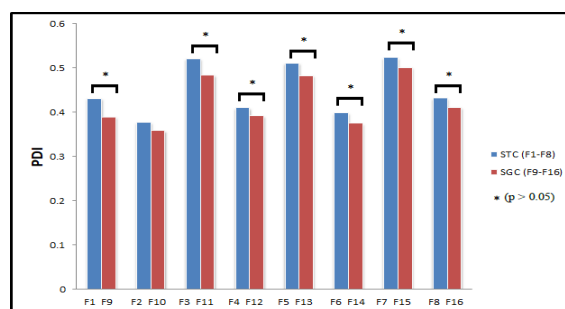


Figure 3: Effect of the types of bile salts on PDI of ROH-loading bilosomes.

The Zeta Potential (ZP) value is an important parameter used to determine the stability of colloidal dispersions. It measures the electrostatic potential difference between the dispersed particles and the surrounding medium. A high magnitude of ZP indicates high stability due to the strong repulsive forces between the particles. Conversely, a low ZP

value indicates instability, and the particles tend to aggregate due to attractive forces [29]. The values of ZP ranged from -4.873 ± 0.43 to -13.038 ± 7.89 . According to an ANOVA test, it was observed that increasing the quantity of bile salts (STC or SGC) exhibited a non-significant increase in the zeta potential of the bilosomes ($p > 0.05$). Increasing the amount of SDC from (5 mg) as in formulas F1, F3, F5, F7, F9, F11, F13, and F15 to (10 mg) as in corresponding formulas F2, F4, F6, F8, F10, F12, F14, and F16, respectively, resulted in a corresponding increase in the zeta potential of the bilosomes. Additionally, increasing the quantity of Span®60 and tween®60 resulted in a non-significant decrease in the zeta potential of the bilosomes ($p > 0.05$). This effect was observed in the formulations containing higher amounts of surfactant, such as F3, F4, F7, F8, F11, F12, F15, and F16, in contrast to the corresponding formulas F1, F2, F5, F6, F9, F10, F13, and F14, respectively, which had a lower amount of surfactant. Finally, it was found that increasing the quantity of cholesterol in the bilosome formulas F5, F6, F7, F8, F13, F14, F15, and F16 caused a non-significant decrease in the zeta potential ($p > 0.05$) as compared with the corresponding formulas F1, F2, F3, F4, F9, F10, F11, and F12, respectively. As shown in Figure 4, the amount of bile salts (STC or SGC) added to the vesicles correlates with an increased negative charge. This increase in negative charge is due to the negative groups present in the bile salts [35]. The type of bile salt showed a significant difference ($p < 0.05$); SGC gave the lowest ZP values (Figure 4).

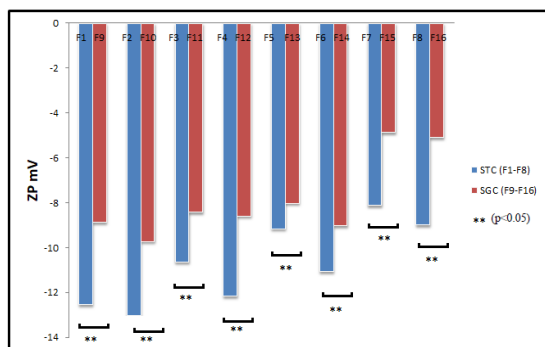


Figure 4: Effect of the types of bile salts on Zeta potential of ROH-loading bilosomes.

Due to zeta potential being proportional to the hydrophobicity of bile salts, STC had more hydrophobicity than SGC; due to this, STC and SGC had HLP of 22.1 and 23.1, respectively [36]. Four formulas, F5, F6, F13, and F14, were selected to do *in vitro* drug release according to EE% and types of bile salt, to determine the effect of types of bile salt on the drug release percentage of ROH from bilosomes. The release of ROH from various bilosome formulas (F5, F6, F13, and F14) was slow and extended per day

compared with the pure drug solution. The formula F5 showed the least drug release (62.33%) compared to F6 (67.65%), F13 (71.28%), and F14 (74.78%) at 24 h and pure drug solution (100%) at 4 hr (Figure 5).

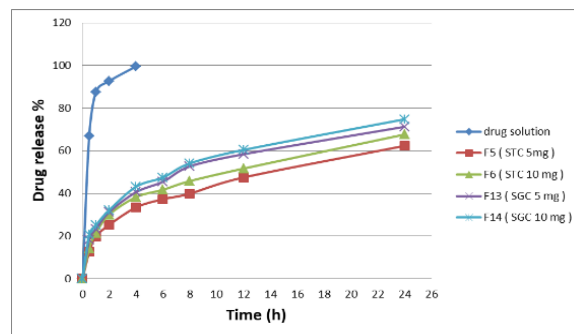


Figure 5: *In vitro* drug release (%) of ROH solution, formulas F5, F6, F13, and F14 in phosphate buffer pH 6.8 at 37 °C.

The slow and extended release of ROH 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 [10]. The drug release profile from the bilosomal formulas (F5, F6, F13, and F14) investigated showed a biphasic pattern. The first phase, which lasted for the first 2 hours, showed a rapid burst release of the drug, which may be due to the detachment of the ROH present on the external surface of the vesicles. The second phase, which was sustained, showed a slower release of the drug, which was attributed to the gradual release of ROH from the vesicular bilayer into the release medium [21,37]. Similar findings were reported by Salem *et al.* for releasing the hydrophilic drug, metformin from bilosomes [30]. The bilosomes containing STC showed less drug release than those containing SGC. This effect was because STC lowers the critical micelle concentration (CMC) due to the many bilosome side-chain hydrophobic methylene groups throughout its conjugation with taurine, thereby delaying the release of the drug [38]. Although the least drug release was observed in F5, F5 had similar release profiles ($f_2 = 68.69$), ($f_2 = 54.93$), and ($f_2 = 50.379$), compared to F6, F13, and F14, respectively. The study focused on four bilosome formulas (F5, F6, F13, and F14) with higher EE% and tested their stability in SDC solutions with varying concentrations for an hour. SDC is a type of bile salt that forms micellar structures in water [39]. The stability was determined by measuring turbidity, and the results were plotted in a graph showing the relationship between turbidity and SDC concentration (Figure 6).

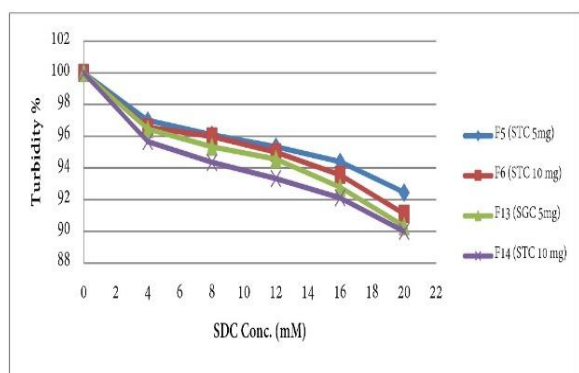


Figure 6: Stability of bilosomes formulations in sodium deoxycholate solutions.

As the concentration of bile salt increased, the level of turbidity decreased. It is possible that an interaction between the composition of the bilayer and bile salts resulted in the creation of mixed vesicles at higher concentrations of bile salts. However, all tested formulas exhibited a lower decrease in turbidity, indicating high stability against bile salts. In particular, F5 and F6 were the most stable and exhibited a more rigid bilayer structure, indicating that they can protect the encapsulated drug in gastrointestinal conditions [40]. From the obtained results, it was found that both F5, F6, F13, and F14 had the highest EE% of ROH ($64.82 \pm 1.80\%$), ($62.91 \pm 3.47\%$), ($60.56 \pm 1.64\%$), and ($58.89 \pm 1.10\%$), respectively. Also, F5 had a non-significant difference in EE% ($p > 0.05$) with F6, but a significant difference in EE% ($p < 0.05$) with both F13 and F14. However, F5 and F6 had suitable vesicle sizes (179.8) nm and (157.05) nm, zeta potential (-9.162) mV and (-11.066) mV, and polydispersity index (0.5116) and (0.3989), respectively (Figure 7).

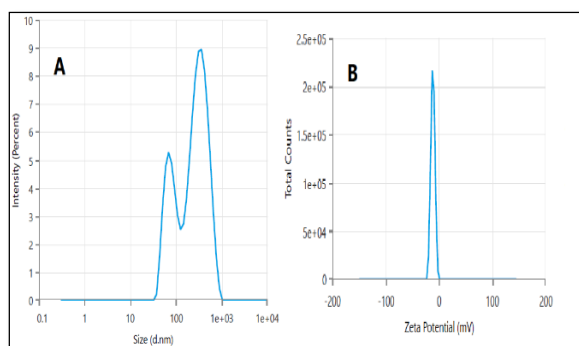


Figure 7: Vesicle size (A), Zeta potential of F5 formula (B).

However, from an economical point of view, F5 with 5mg of STC is preferred over F6 with 10mg of STC, so F5 was subjected to further analysis. The characteristic peaks in the spectra of ropinirole hydrochloride were at 3413cm^{-1} , 1612cm^{-1} , 3074cm^{-1} , 2935cm^{-1} , 2880cm^{-1} , 1311cm^{-1} , 1346cm^{-1} , and 1759cm^{-1} , which indicate (N-H stretching), (C=C stretching), (aromatic, C-H

stretching), (aliphatic C-H stretching), (C-N stretching), and (C=O stretching), respectively (41, 42). FTIR spectra of ropinirole hydrochloride, physical mixture, and F5 formula exhibited no change in their function group regions, confirming the lack of chemical interaction between the drug and other excipients of the formulation. On the other hand, the fingerprint regions are not superimposed, which validates physical character changes (Figure 8) [43].

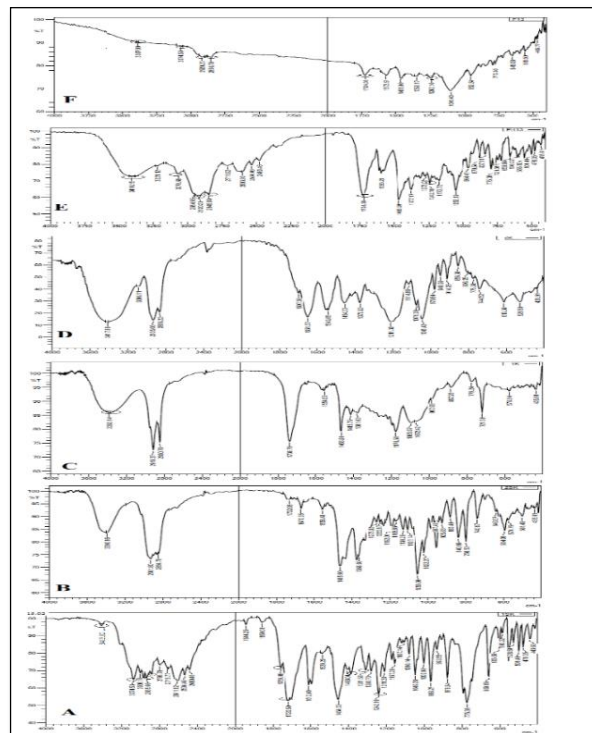


Figure 8: FTIR spectrum of ROH (A), Cholesterol (B), Span@60 (C), Sodium taurocholate (D), Physical mixture (E), and F5 bilosomes formula (F).

Conclusion

Ropinirole was successfully entrapped in bilosomal formulations. The physical features of ropinirole-loading bilosomes (entrapment efficiency, vesicle size, polydispersity index, in vitro drug release, and zeta potential) were affected by the type of bile salts used. Furthermore, sodium taurocholate was found to be better suited for the synthesis of ropinirole-loading bilosomes, with greater bilosome stability in bile salt solution. The FTIR analyses revealed no incompatibility between the medication and the other excipients in the mixture.

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Conflicts of interest

There are no conflicts of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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