SPER Publications and Solutions Pvt. Ltd.

Original Article

Oral nanobilosomes of ropinirole: Preparation, compatibility and Ex-vivo intestinal absorption study

Samer Khalid Ali[1*], Entidhar Jasim Muhammed Al-Akkam[1]

[1]Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Correspondence: Samer Khalid Ali, Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq. samerkhalidali@gmail.com

ABSTRACT

Bilosomes are vesicular systems composed of bile salts and non-ionic surfactants with cholesterol. Bilosomal vesicles have numerous benefits compared to liposomes and niosomes. The study aimed to evaluate the compatibility of ropinirole with excipients in bilosomal formulation and compare the intestinal permeation of ropinirole when administered orally in the form of a bilosomal dispersion versus an oral solution. Ropinirole bilosomal dispersion was prepared, optimized, and lyophilized using a freeze dryer to Ropinirole-loading bilosomal powder (Rop-bp) by using mannitol as cryoprotectants. The characteristics of the bilosomal formulations (Fourier transform infrared spectroscopy, differential scanning calorimetry, X-ray powder diffraction, field emission scanning electron microscope, and Ex-vivo intestinal permeation study), were evaluated. Fourier Transform Infrared Spectroscopy analysis provided evidence that there was no chemical interaction observed between Ropinirole and the other excipients present in the formulation. Differential scanning calorimetry and X-ray powder diffraction revealed the encapsulation of the amorphous form of ropinirole into bilosomal vesicles, and a scanning electron microscope indicated the spherical shape of bilosomes within the nanometer range (≈155-170nm). The *ex-vivo* study on intestinal permeation demonstrated that the bilosomes exhibited a notable improvement in intestinal permeation, approximately three times higher than that of the drug solution. In conclusion, results demonstrated no chemical interaction between ropinirole with other excipients in bilosomal formulation. The bilosomes are good carriers for enhancing intestinal permeation of ropinirole.

Keywords: Ropinirole, Bilosomal vesicles, Liposomes, X-ray powder diffraction, Intestinal permeation

Introduction

Non-ionic surfactant-based vesicles have become a significant topic of interest in the pharmaceutical industry because of their impressive ability to encapsulate hydrophilic and hydrophobic drugs. Recent research has revealed that these vesicles can enhance the effectiveness of drugs by increasing their bioavailability [1].

Bilosomes are vesicular systems composed of bile salts and non-ionic surfactants with cholesterol [2]. Bilosomal vesicles offer numerous benefits compared to traditional vesicular-type systems such as liposomes and niosomes [3]. The nanosized vesicle and stability of bilosomes make them a promising choice for delivering drugs orally, many research has confirmed their safety and effectiveness in this application [4].

Bile salts in bilosomes are believed to enhance the movement of hydrophilic drugs through the paracellular route of the epithelium. Bile salts may accomplish this by linking calcium ions, which can loosen the tight junctions between cells and facilitate drug penetration.

The enhanced oral bioavailability observed in drugs delivered through bilosomes is believed to be attributed to their improved uptake as intact vesicles by M-cells located in the Peyer patches. Additionally, bilosomes are thought to facilitate increased transportation of drugs through the lymphatic pathway, further contributing to their improved oral bioavailability [4-6].

Ropinirole (Rop) is a nonergoline antiparkinson drug primarily used for the treatment of Parkinson's disease. It is also indicated for the management of moderate-to-severe idiopathic Restless Leg-Syndrome [7]. Ropinirole is rapidly absorbed after oral

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Khalid Ali S, Al-Akkam EJM. Oral nanobilosomes of ropinirole: Preparation, compatibility and Ex-vivo intestinal absorption study. J Adv Pharm Educ Res. 2023;13(4):8-15. https://doi.org/10.51847/B7uaDLOWfq

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

administration. Ropinirole has relatively low bioavailability, with an absolute oral bioavailability of approximately 50%. The low oral bioavailability of ropinirole is primarily attributed to its significant hepatic first-pass metabolism [8]; additionally, due to its hydrophilic nature, Rop may have difficulty crossing biological membranes, which may limit its permeation in the body tissue. Currently available Rop, as traditional tablet dosage forms, cannot achieve acceptable oral bioavailability [9, 10].

The aim is to study the compatibility of ropinirole with excipients in bilosomal formulation and compare the intestinal permeation of ropinirole when administered orally in the form of a bilosomal dispersion versus an oral solution.

Materials and Methods

Materials

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

Preparation of bilosomes dispersion of rop

Ropinirole bilosomal dispersion was prepared, optimized, and lyophilized using a freeze dryer (Labconco, Canada) to Rop-loading bilosomal powder (Rop-bp) by using mannitol as cryoprotectants, the lyophilization process involved subjecting the formulation to a primary freezing temperature of -20°C for a duration of 24 h. Subsequently, the formulation was lyophilized for 72 h under a pressure of 0.4 bar [11], and the obtained powder was stored in a tightly closed container for further investigations. The constitution of the optimized bilosomes formula is shown in **Table 1**.

Table 1. Constitution of Optimized Bilosomes Formula of Rop

Ingredients	Amount
Ropinirole Hcl	50 mg
Sodium deoxycholate (SDC)	5 mg
Span®60	0.90 w/v %
Tween®60	1.80 w/v %
Cholesterol	0.90 w/v %
Deionized water	10 ml

Preparation of physical mixture (PM)

The PM was prepared by uniform mixing of drug, surfactant, cholesterol, and bile salt in the same ratios of the optimized bilosomes formula. The resulting mixture was passed through a 60-mesh sieve to obtain particles of uniform size [12].

Characterization of Rop-loading bilosomal powder

Compatibility study

Fourier transform infra–red spectroscopy (FTIR)

FTIR spectroscopy was employed to assess potential interactions between Ropinirole (Rop) and other excipients, as well as to verify the identity of the drug. The spectra were recorded using an FTIR spectrophotometer (FTIR 43000, Shimadzu, Japan) for the pure drug, physical mixture (PM), and Rop-bp. Samples were accurately weighed and prepared in KBr disks, and the spectra were recorded over a frequency range of 4,000-400 cm^{-1} with a specific spectral resolution [13].

Differential scanning calorimetry (DSC)

The DSC was utilized to investigate the thermal behavior of Ropinirole (Rop), physical mixture (PM), and Rop-bp. The samples were subjected to DSC analysis using a DSC-60 plus instrument (Shimadzu, Japan) at a scanning rate of 10°C/min. The temperature range examined was 25–300°C, and the measurements were conducted under a constant nitrogen purge at a rate of 50 ml/min. This technique allows for the detection of any thermal changes in the samples, which can indicate the potential occurrence of interactions between Rop and other excipients [14, 15].

X-ray powder diffraction(XRD)

X-ray diffractometry (Shimadzu, Japan) was used to obtain XRD spectra of the Rop-bp, PM, mannitol, and Rop. To obtain the spectra, the samples were exposed to X-ray radiation. The scanning angle was set between 5 to 80 of 2ɵ, the voltage and current were set at -40kV and -40mA, respectively [16].

Field emission scanning electron microscope (FESEM)

Rop-loading bilosomal powder was analyzed by FESEM (Tescan MIRA3 French). It is apparatus used for an image surface roughness analysis, used to explain the shape, and size of vesicles, the resulting photograph were uploaded to a computer for further analysis [17, 18].

Ex-vivo intestinal permeation study

Ex-vivo permeation studies of pure Rop solution and Rop-bp were carried out using a non-everted rat gut sac method with modification [19].

Male Wistar rats weighing approximately 275-300 g were obtained from the animal house at the College of Pharmacy, University of Baghdad. The experimental procedure was approved by the Search Ethics Committee to ensure compliance with ethical guidelines. All rats involved in the study received humane care following the Guideline for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No 85-23, revised 1996).

The experimental procedure involved fasting Wistar rats overnight while allowing them free access to water. The rats were then anesthetized with diethyl ether, and a longitudinal abdominal incision was made. The small intestine was carefully removed, and the mesentery was manually stripped off. The intestine was thoroughly washed with a normal saline solution using a cannula equipped with a needle. the intestine was cut into sacs measuring 10 cm in length and having a diameter of 0.280 cm. After tying one end, the ileum sac was filled with (1ml) of pure Rop solution and Rop-loading bilosomal powder after reconstitution with 1ml phosphate buffer saline (pH 7.4) containing approximately (5mg) of Rop, then tying the other end of the sac and Each intestinal sac was immersed in a 150 ml of the permeation media (phosphate buffer saline pH 7.4) at 37°C in a dissolution apparatus USP II (Mingsheng PM, China) which operated at 50 rpm and continuously gassed with oxygen (approximately~ 20 bubbles/ minute) (5ml) samples were withdrawing at 5, 10, 15, 30, 90, and 120 min. and the sample was analyzed by UV spectrophotometry (model UV-19001 PC, Shimadzu, Kyoto, Japan) at 249 nm and the cumulative amount of Rop permeated was calculated and plotted against time [20, 21]. The experiment was made in triplicate.

Ex-vivo ropinirole permeation data analysis

The area of the intestinal sac was determined using Eq.1 and the apparent permeability coefficients were determined using Eq. 2 [11, 20]:

$$SA = 2\pi r h \quad (1)$$

Where, SA (cm^2) is the area of the intestinal sac, and r (cm) is the intestinal radius.

$$P_{app} = F/SA \times C_0 \quad (2)$$

Where: P_{app} (cm/min) is the apparent permeability, **F** ($\mu g/min.cm^2$) is the flux and C_0 ($\mu g/ml$) is the initial drug concentration.

The enhancement ratio (ER) of the Rop-bp was calculated using Eq. 3 [22]:

$$ER = \frac{\text{Permeability coefficient of Rop} - \text{bp}}{\text{Permeability coefficient of pure Rop}} \quad (3)$$

Results and Discussion

Compatibility study

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the pure Rop exhibit spectra at 3413cm^{-1} (N-H stretching), 1612cm^{-1} (C=C stretching), 3074cm^{-1} (aromatic, C-H stretching), 2935cm-1 and 2880cm-1(aliphatic C-H stretching), 1311cm-1 and 1346cm^{-1} (C-N stretching), 1759cm^{-1} (C=O stretching) **(Figure 1a)** [23, 24], which were also evident in the spectra of physical mixtures of the Rop with an excipient. But, with simple shifting in position and intensity of characteristic peaks especially for NH stretching of physical mixture which is due to H-bond formation, these outcomes indicate the compatibility of the drug with the excipient used in the formulation of bilosomes **(Figure 1b)**. Furthermore, Lyophilized Rip-loading bilosomes showed no significant changes in their functional group regions, with only slight variations in intensity and amplitude observed **(Figure 1c)**. This confirms the absence of any chemical interactions between Rop and the other excipients present in the formulation [25, 26].

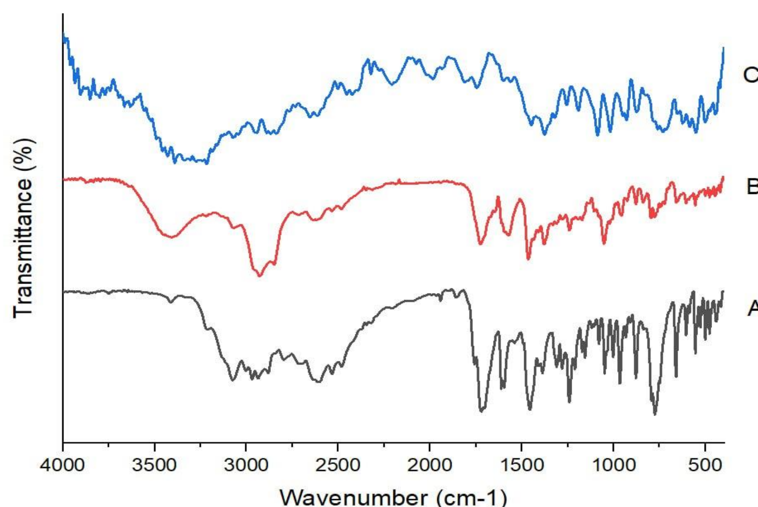

Figure 1. FTIR of Ropinirole hydrochloride (a), Physical mixture (b) and Lyophilized optimized formula (c)

Differential scanning calorimetry (DSC)

Thermal analysis is a crucial method for studying various aspects of a drug, including its polymorphic state, stability, and purity. Additionally, it helps in detecting potential interactions between the drug and excipients used in the formulation. One sharp endothermic peak was achieved with Rop at 251.6°C, as also shown in other studies [27] **(Figure 2a)**, which indicated no change in its melting temperature and the drug had a crystalline nature with high purity [28].

The observed decrease in the intensity of the endothermic peak of Rop in the physical mixture **(Figure 2b)** may be attributed to its dilution with the excipients present. This indicates that there is no significant interaction between the drug and the formulation excipients. Similar findings were reported by Khalil *et al*. in their study [29].

The Rop peak was absent in lyophilized formulations **(Figure 2c)**. Only the single peak of mannitol was observed at a melting point of 170.10 °C [13]. This indicated the encapsulation of an amorphous form of ropinirole into bilosomal vesicles [30, 31].

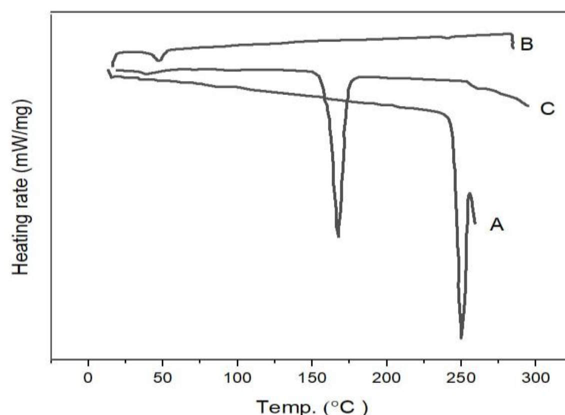

Figure 2. DSC of Ropinirole hydrochloride (a), Physical mixture (b) and Lyophilized optimized formula (c)

X-ray powder diffraction(XRD)

The XRD diffractogram of pure Rop, PM, Mannitol and Rop-bp are shown in **Figures 3a-3d** respectively.

The diffraction pattern of the pure Rop exhibited distinct and intense peaks at 16.47°, 18.23°, 19.23°, 22.34°, 24.72°, 26.98°, 29.91°, 35.39°, 40.60°, and 46.10°. These peaks signify the crystalline nature of the drug **(Figure 3a)**, which was further confirmed by the results obtained from DSC. The pattern of the physical mixture exhibited characteristic peaks of Rop but with lower intensity compared to the pure drug **(Figure 3b)**. This observation can be attributed to the dominating effect of the excipients present in the mixture, which may have influenced the intensity of the drug peaks [32]. The pattern of the lyophilized Rop-bilosomal dispersion exhibited broad peaks, which can be attributed to the presence of a significant amount of cryoprotectant mannitol. Additionally, the peaks corresponding to mannitol were observed with a reduction in intensity or absence of numerous sharp peaks seen in the crystalline Rop diffractogram pattern **(Figures 3c and 3d)**. These findings indicate that the drug (Rop) was successfully encapsulated into the formed bilosomal vesicles [33-35].

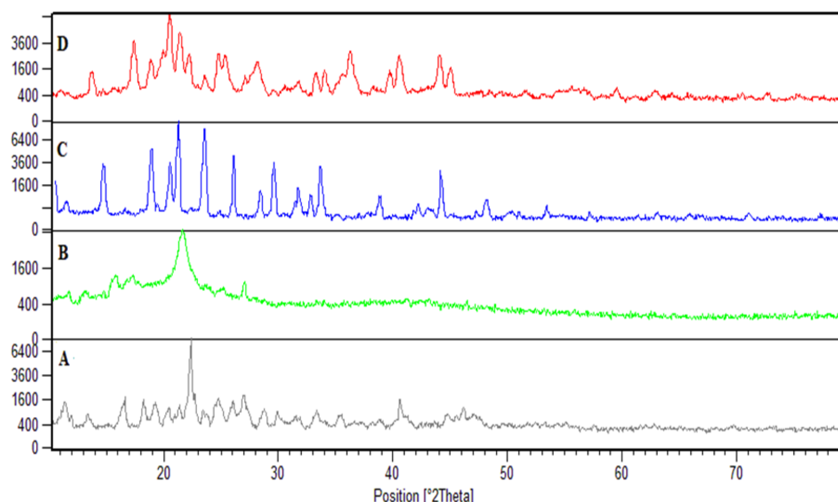

Figure 3. XRD of Ropinirole (a), Physical mixture (b), Mannitol (c) and lyophilized optimized formula (d)

Field emission scanning electron microscope (FESEM)

The FESEM scans revealed the spherical shape of bilosomes with uniform distribution [36], the same result was recorded by Islam *et al*. [37]. The size of Rop- bilosomal vesicles was within the nanometer range (≈. 155-170 nm) as shown in the **Figure 4**. The vesicle size was determined by Image J software [38].

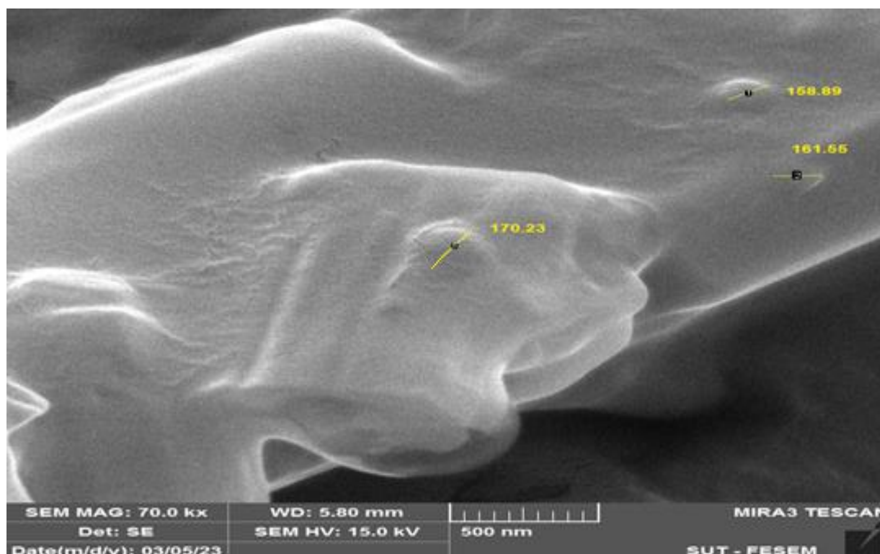

Figure 4. The FESEM of lyophilized optimized formula

Ex-vivo intestinal permeation study

The non-everted intestinal sac method was employed to evaluate the intestinal permeation of Rop from the Rop-bp compared to their corresponding pure drug solution.

Figure 5 illustrates the plot of the cumulative amount of Ropinirole (Rop) permeated from Rop-bp and the pure drug solution in the ileum. The steady-state flux (F) was determined from the slope of the linear equations, and the corresponding P_{app} data are presented in **Table 2**.

The cumulative amount permeated from the lyophilized optimized formula was found to be significantly higher ($p < 0.05$) than pure drug solution.

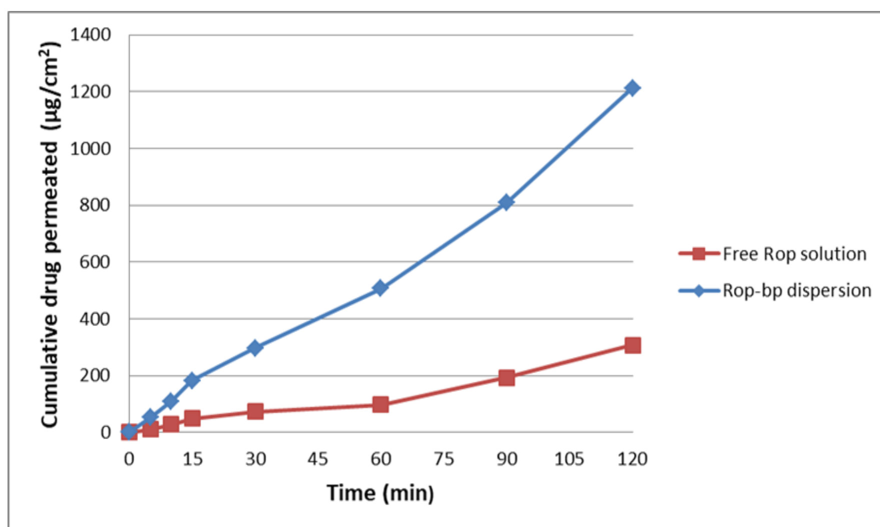

Figure 5. The permeation of Rop from pure Rop solution and Rop-bp dispersion through non–everted rat ileum, values of mean ±SD (n=3)

Table 2. The ex-vivo absorption parameters of Rop from Rop-bp dispersion and pure Rop solution

Sample	Flux (µg/min.cm²)	Permeability coefficient $P_{app}*10^{-5}$ (cm/min)
Rop-bp dispersion	10.329	23.475
Pure Rop solution	3.4848	7.92

Results were determined as mean ±SD (n=3)

From the above **(Table 2)**, it is deduced that the the lyophilized optimized formula showed a permeation enhancement ratio of 3 fold, as compared with the pure drug solution. Moreover, it was found that after 120 min, about 71± 0.53 % of the initial amount of Rop was permeated from lyophilized optimized formula (Rop-bp), compared to only 30± 0.34 % from pure drug solution.

The obtained data came in agreement with previous studies, concerned with the impact of bilosomal formulation on intestinal permeation enhancement [11, 39]. The significant improvement in permeation observed with bilosomal formulations can be attributed to their small vesicle size and the complete internalization of Rop into these vesicles. Small vesicles are known to diffuse more rapidly across the intestinal membrane, resulting in enhanced permeation [22, 40].

Conclusion

The use of FTIR confirmed that there were no chemical interactions between Ropinirole and other excipients in the bilosomal formulation. DSC and XRD analysis indicated that the amorphous form of Ropinirole was successfully encapsulated within the bilosomal vesicles. FESEM images revealed that the bilosomes exhibited a spherical shape with a size range of approximately 155-170nm. Furthermore, ex-vivo gut permeation studies demonstrated a significant enhancement in the permeation of Ropinirole with bilosomal formulations compared to the drug solution, showing a three-fold increase in permeation. Based on these findings, it can be concluded that bilosomes are promising carriers for the oral delivery of Ropinirole, as they effectively enhance its intestinal permeation.

Acknowledgments: The authors thank the College of Pharmacy - University of Baghdad for providing the necessary research resources.

Conflict of interest: None

Financial support: None

Ethics statement: The committee protocol in the College of Pharmacy/University of Baghdad approved this study (No: REACUBCPS32023A), which complied guideline for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No 85-23, revised 1996).

References

1. Ge X, Wei M, He S, Yuan WE. Advances of non-ionic surfactant vesicles (niosomes) and their application in drug delivery. Pharmaceutics. 2019;11(2):1-16. doi:10.3390/pharmaceutics11020055
2. Waglewska E, Pucek-Kaczmare A, Urszula Bazylińska. Self-assembled bilosomes with stimuli-responsive properties as bioinspired dual-tunable nanoplatform for pH/temperature-triggered release of hybrid cargo. Colloids Surf B Biointerfaces. 2022;215(6):1-11. doi:10.1016/j.colsurfb.2022.112524
3. Abdelbary AA, Abd-Elsalam WH, Al-Mahallawi AM. Fabrication of novel ultra deformable bilosomes for enhanced ocular delivery of terconazole: IN vitro characterization, ex vivo permeation and in vivo safety assessment. Int J Pharm. 2016;513:688-96. doi:10.1016/j.ijpharm.2016.10.006
4. Nemr AA, El-Mahrouk GM, Badie HA. Hyaluronic acid-enriched bilosomes: an approach to enhance ocular delivery of agomelatine via D-optimal design: Formulation, in vitro characterization, and in vivo pharmacodynamic evaluation in rabbits. Drug Deliv. 2022;29(1):2343-56. doi:10.1080/10717544.2022.2100513
5. Bhairy S, Pitchika S, Maurya S, Patil J. Stability and in-vivo efficacy of bile salts containing vesicles (bilosomes) for oral delivery of vaccines and poorly soluble active drug molecules. Indo Am J Pharm Res. 2020;10(10):1326-34. doi:10.5281/zenodo.4066495
6. Gupta DK, Ahad A, Waheed A, Aqil M, Al-Jenoobi FI, Al-Mohizea AM. Bilosomes: A novel platform for drug delivery. In: Nayak AK, editor. Systems of nanovesicular drug delivery. 1st ed. Academic Press; 2022. p. 293-309. doi:10.1016/B978-0-323-91864-0.00004-8
7. Zesiewicz TA, Chriscoe S, Jimenez T, Upward J, VanMeter S. A fixed-dose, dose–response study of ropinirole prolonged release in early stage Parkinson's disease. Neurodegener Dis Manag. 2017;7(1):49-59. doi:10.2217/nmt-2016-0039
8. Pardeshi CV, Belgamwar VS. Ropinirole-dextran sulfate nanoplex for nasal administration against Parkinson's disease: In silico molecular modeling and in vitro-ex vivo evaluation. Artif Cells Nanomed Biotechnol. 2017;45(3):635-48. doi:10.3109/21691401.2016.1167703
9. Dudhipala N, Gorre T. Neuroprotective effect of ropinirole lipid nanoparticles enriched hydrogel for parkinson' s disease: In vitro, ex vivo, pharmacokinetic and pharmacodynamic evaluation. Pharmaceutics. 2020;12(5):448. doi:10.3390/pharmaceutics12050448
10. Di Prima G, Campisi G, De Caro V. Amorphous ropinirole-loaded mucoadhesive buccal film: A potential patient-friendly tool to improve drug pharmacokinetic profile and effectiveness. J Pers Med. 2020;10(4):242. doi:10.3390%2Fjpm10040242
11. Saifi Z, Rizwanullah M, Mir SR, Amin S. Bilosomes nanocarriers for improved oral bioavailability of acyclovir: A complete characterization through in vitro, ex-vivo and in vivo assessment. J Drug Deliv Sci Tech. 2020;57:101634. doi:10.1016/j.jddst.2020.101634
12. Ali SK, Al-khedairy EBH. Solubility and dissolution enhancement of atorvastatin calcium using solid dispersion adsorbate technique. Iraqi J Pharm Sci. 2019;28(2):105-14. doi:10.31351/vol28iss2pp105-114

13. Ali H, Ghareeb MM, Al-Remawi M, Al-Akayleh FT. New insight into single phase formation of capric acid / menthol eutectic mixtures by Fourier-transform infrared spectroscopy and differential scanning calorimetry. Trop J Pharm Res. 2020;19(2):361-9. doi:10.4314/tjpr.v19i2.19
14. Jassim ZE, Al-Kinani KK, Alwan ZS. Preparation and evaluation of pharmaceutical cocrystals for solubility enhancement of dextromethorphan HBr. IJDDT. 2021;11(4):1342-9.
15. Kumar B, Sahoo PK, Manchanda S. Formulation, characterization and ex vivo study of curcumin nano-invasomal gel for enhanced transdermal delivery. OpenNano. 2022;7(100058):1-10. doi:10.1016/j.onano. 2022.100058
16. Jabar HE, Abd-Alhammid SN. Improvement of the solubility and dissolution characteristics of risperidone via nanosuspension formulations. Iraqi J Pharm Sci. 2022;31(1):43-56. doi:10.31351/vol31iss1pp43-56
17. Muhammed SA, Al-Kinani KK. Formulation and in vitro evaluation of meloxicam as a self-microemulsifying drug delivery system. F1000Res. 2023;12:315. doi:10.12688/f1000research.130749.2
18. Alwadei M, Kazi M, Alanazi FK. Novel oral dosage regimen based on self nanoemulsifying drug delivery systems for codelivery of phytochemicals-Curcumin and thymoquinone. Saudi Pharm J. 2019;27(6):866-76. doi:10.1016/j.jsps.2019.05.008
19. Boseila AA, Abdel-Reheem AY, Basalious EB. Design of bile-based vesicles (BBVs) for hepatocytes specifc delivery of daclatasvir: comparison of ex-vivo transenterocytic transport, in-vitro protein adsorption resistance and HepG2 cellular uptake of charged and β-sitosterol decorated vesicles. PLoS ONE. 2019;14(7):1-19. doi:10.1371/journal.pone.0219752
20. Sabr LA, Hussein AA. Comparison between conventional and supersaturable self-nanoemulsion loaded with nebivolol: Preparation and In-vitro/Ex-vivo evaluation. Iraqi J Pharm Sci. 2020;29(1):216-25. doi:10.31351/vol29iss1pp216-225
21. Illendula S, Knv R. Method development and validation of ropinirole by using uv method development and validation of ropinirole by using UV spectroscopy method. World J Pharm Pharm Sci. 2022;11(8):1418-27. doi:10.20959/wjpps20228-22808
22. Ismail A, Teiama M, Magdy B, Sakran W. Development of a novel bilosomal system for improved oral bioavailability of sertraline hydrochloride: Formulation design, in vitro characterization, and ex vivo and in vivo studies. AAPS Pharm Sci Tech. 2022;23(188):1-18. doi:10.1208/s12249-022-02339-0
23. Silverstein RM, Bassler GC, Morrill TC. Spectrometric identification of organic compounds, 3rd ed.; John Wiley & Sons: New York; 2005. p. 72-172.
24. Kar K, Pal RN, Bala NN. Preparation, characterisation and evaluation of ropinirole hydrochloride loaded controlled release microspheres using solvent evaporation technique. Int J Pharm Pharm Sci. 2018;10(6):57-67. doi:10.22159/ijpps.2018v10i6.26070
25. Kadhim ZM, Mahmood HS, Alaayedi M, Ghareeb MM. Formulation of flurbiprofen as microsponge drug delivery system. Int J Pharm Res. 2020;12(3):748-53. doi:10.31838/jpr/2020.12.03.141
26. Varma MM, Kumar AMS. Formulation and evaluation of matrix tablets of ropinirole hydrochloride for oral controlled release. Indian J Pharm Pharmacol. 2015;2(1):27-42. doi:10.31850/Ijpp/2015. 2.01.134
27. Moffat A, Osselton M, Widdop B. Clarke's analysis of drugs and poisons. 4th edition. The Pharmaceutical Press; 2011. p. 2030.
28. Basher TA, Muhammad EJ. Formulation and In-vitro evaluation of itraconazole floating microparticles. Iraqi J Pharm Sci. 2020;29(1):236-46. doi:10.31351/vol29iss1pp236-246
29. Khalil RM, Abdelbary A, Kocova El-Arini S, Basha M, El-Hashemy HA. Evaluation of bilosomes as nanocarriers for transdermal delivery of tizanidine hydrochloride: In vitro and ex vivo optimization. J Liposome Res. 2019;29(2):171-82. doi:10.1080/08982104.2018.1524482
30. Taweel MM El, Aboul-einien MH, Kassem MA, Elkasabgy NA. Intranasal zolmitriptan-loaded bilosomes with extended nasal mucociliary transit time for direct nose to brain delivery. Pharmaceutics. 2021;13(11):1-28. doi:10.3390%2Fpharmaceutics13111828
31. Salem HF, Nafady MM, Ali AA, Khalil NM, Elsisi AA. Evaluation of metformin hydrochloride tailoring bilosomes as an effective transdermal nanocarrier. Int J Nanomed. 2022;17:1185-201. doi:10.2147/ijn.s345505
32. Avadhani KS, Manikkath J, Tiwari M, Godavarthi A, Vidya SM, Raghu C, et al. Skin delivery of epigallocatechin-3-gallate (EGCG) and hyaluronic acid loaded nano-transfersomes for antioxidant and anti-aging effects in UV radiation induced skin damage. Drug Deliv. 2017;24(1):61-74. doi:10.1080/10717544.2016.1228718
33. Alhalmi A, Amin S, Khan Z, Beg S, Al O, Saleh A, et al. Nanostructured lipid carrier-based codelivery of raloxifene and naringin: Formulation, optimization, in vitro, ex vivo, in vivo assessment, and acute toxicity studies. Pharm Artic. 2022;14:1-28. doi:10.3390/pharmaceutics14091771
34. Ahmed EM, Nabil SR, Faten F, Mohamed NS. Bilosomes as a novel carrier for the cutaneous delivery for dapsone as a potential treatment of acne: Preparation, characterization and in-vivo skin deposition. J Liposome Res. 2020;30(1):1-11. doi:10.1080/08982104.2019.1577256
35. Ahmed S, Kassem MA, Sayed S. Bilosomes as promising nanovesicular carriers for improved transdermal delivery: Construction, in vitro optimization, ex vivo permeation and in vivo evaluation. Int J Nanomed. 2020;15:9783-98. doi:10.2147%2FIJN.S278688
36. Mondal D, Mandal RP, De S. Addressing the superior drug delivery performance of bilosomes─A microscopy and fluorescence study. ACS Appl Bio Mater. 2022;5(8):3896-911. doi:10.1021/acsabm.2c00435

37. Islam N, Zahoor AF, Syed HK, Iqbal MS, Khan IU, Abbas G, et al. Improvement of solubility and dissolution of ebastine by fabricating phosphatidylcholine / bile salt bilosomes. Pak J Pharm Sci. 2020;33(5):2301-6. doi:10.36721/PJPS.2020.33.5.SUP.2301-2306.1
38. Rana N. Particle size and shape analysis using imagej with customized tools for segmentation of particles. Int J Eng Res Technol. 2015;4(11):247-50. doi:10.17577/IJERTV4IS110211
39. Aburahma MH. Bile salts-containing vesicles: Promising pharmaceutical carriers for oral delivery of poorly water-soluble drugs and peptide/protein-based therapeutics or vaccines. Drug Deliv. 2016;23(6):1847-67. doi:10.3109/10717544.2014.976892
40. Zafar A, Alruwaili NK, Imam SS, Hadal Alotaibi N, Alharbi KS, Afzal M, et al. Bioactive apigenin loaded oral nano bilosomes: formulation optimization to preclinical assessment. Saudi Pharm J. 2021;29(3):269-79. doi:10.1016%2Fj.jsps.2021.02.003