PHARMACIA

Pharmacia 71: 1–7
DOI 10.3897/pharmacia.71.e116917

Research Article

Evaluation of types and concentration of bile salts impact on physical properties of nisoldipine-loaded bilosomes

Ghada Hamid Naji[1], Fatima J. Al Gawhari[2]

1 *Department of Pharmaceutics, College of Pharmacy, University of Babylon, Hillah, Babylon Governorate, Iraq*
2 *Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Iraq*

Corresponding author: Ghada Hamid Naji (ghada.hamed1100p@copharm.uobaghdad.edu.iq)

Received 5 December 2023 ♦ **Accepted** 19 January 2024 ♦ **Published** 9 February 2024

Citation: Naji GH, Al Gawhari FJ (2024) Evaluation of types and concentration of bile salts impact on physical properties of nisoldipine-loaded bilosomes. Pharmacia 71: 1–7. https://doi.org/10.3897/pharmacia.71.e116917

Abstract

Background: Bilosomes are lipid vesicles that exhibit flexibility and deformability. They consist of phospholipids and amphiphilic bile salts. Compared to the normal vesicular systems such as liposomes and niosomes, bilosomes provide several notable advantages, including simplified manufacturing, cost-effectiveness, and enhanced stability.

Aim: The main objective of the present work was to evaluate the effect of different bile salts on the physical properties that include entrapment efficiency, vesicle size, and polydispersity index(PDI). In addition, *in vitro* drug release for nisoldipine (NSD) loaded bilosomes was evaluated.

Methods: Nisoldipine-loaded bilosomes were made using a thin film hydration technique. Cholesterol along with surfactant (span 60) was employed, and the formulation also contained several different bile salts, including sodium deoxycholate (SDC), sodium glycocholate (SGC), and sodium taurocholate (STC).

Results: The developed NSD bilosomes exhibited an entrapment efficiency ranging from 44.2 ± 0.3 to 82.36 ± 0.80% and a vesicle size diameter in the nanometric dimensions (166 ± 1.83 to 237.8 ± 3.3 nm). An in-vitro release study revealed that formulas prepared with SDC bile salts showed higher drug release than SGC and STC formulas.

Increasing the bile salt amount from 5 mg to 10 mg increases entrapment efficiency with increasing vesicle size. Further increase in bile salt led to decreased entrapment efficiency with increased vesicle size. SDC gives the best result in terms of entrapment efficiency and acceptable size. STC provides the largest particle size due to its high molecular weight compared to SGC and SDC.

Conclusion: The SDC bile salt component is better suited for manufacturing NSD bilosomes. This is because this component yields the best results regarding high entrapment efficiency, nano size, and prolonged drug release.

Keywords

Bilosomes, entrapment efficiency, sodium deoxycholate, sodium taurocholate, thin film hydration

Introduction

Drugs are ineffective until a safe method of delivering them to the circulatory system or a site-specific delivery system is discovered, despite the many advances in pharmaceutical research (Drais and Hussein 2022).

Due to their diverse advantages, lipid-based vesicular systems play a crucial role in pharmaceutical delivery. The advantages encompass the ability to encapsulate both lipophilic and hydrophilic molecules, along with the capability to overcome various challenges such as drug insolubility and drug loading, so bilosomes are preferred over another lipid-based vesicular system (Kharouba et al. 2022).

Bilosomes refer to lipid vesicles that exhibit flexibility and deformability, mostly comprised of phospholipids and amphiphilic bile salts. In contrast to typical vesicular systems such as liposomes and niosomes, bilosomes provide several notable advantages, including simplified manufacturing processes, cost-effectiveness, and enhanced stability (Khafagy et al. 2023).

They resist degradation by enzymatic GIT due to the presence of bile salt in the vesicle membrane, which enhances penetration and makes oral delivery more effective (Anon 2020; Waglewska et al. 2020).

Bilosome formulations frequently use non-ionic surfactants, such as span series, bile salts, and cholesterol. These materials are widely regarded as harmless and have been demonstrated to be fully biocompatible and biodegradable when used to create bilosomes. (Binsuwaidan et al. 2022).

Bile salts are chemicals that can solubilize and enhance the penetration of substances. They are utilized extensively because they are not toxic and are therefore compatible with biological systems (Zafar et al. 2021).

Bile salt gives the vesicles a negative charge, increasing their storage stability and encouraging drug absorption through the M-cells in the Peyer's patch. After that, cellular activities continue into the underlying lymphoid tissue, preventing hepatic first-pass metabolism and facilitating drug transport into the intestinal lymphatic system (Ismail et al. 2022). Patient compliance and adherence improved due to reduced frequent doses (Kadhim and Rajab 2022).

Different types of bile salts can be used to prepare bilosomes. Some examples include sodium glycocholate (SGC), sodium deoxycholate (SDC), sodium taurocholate (STC), and sodium taurodeoxycholate (STDC) (Palekar-Shanbhag et al. 2020).

Nisoldipine (NSD) (C20H24N2O6) is a calcium channel blocker belonging to the dihydropyridine family that stops calcium from entering cardiac and vascular smooth muscle transmembrane. When calcium ions pass through certain ion channels, the heart contracts. So if this channel (Ca^{++} channel) is blocked this leads to arterioles dilation (Chaudhari et al. 2019) and decreases the contraction of cardiac muscles. For that reason it is primarily employed in the management of cardiovascular disorders, such as the treatment of hypertension, cardiovascular disease, and several types of angina, such as prinzmetal or variant angina. The half-life is seen to be long, between 7 and 12 hours.

The average duration required to reach the highest levels of the drug in the bloodstream has been documented as 9.2 ± 5.1 hours (Nekkanti et al. 2016).

The drug Nisoldipine belongs to Biopharmaceutical classification system (BCS) as (class II drug;that means that it has low solubility and high permeability properties. Furthermore, nisoldipine undergoes first-pass metabolism, before reaching the site of drug action and only 5% of it is used by the body (Al-Edhari and Al Gawhari 2023). Furthermore, NSD's low water solubility (5.77 × 10−3 g/L) led to poor dissolution and low bioavailability (Chavan et al. 2020; Gasmi et al. 2023).

The present work was designed to develop and formulate NSD bilosomes using different types of bile salts and assess the effect of these salts on bilosomes properties, including entrapment efficiency, particle size, PDI, and drug release.

Methods and methods

Materials

We bought nisodipine from Lee Chemicals in India (patch number 293). We bought cholesterol, bile salts (SDC, SGC, and STC), and surfactants (Span 60) from Hyperchem Limited company Chemicals in India. Analytical grade solvents and other compounds were all used in the current experiment.

Preparation of nisoldipine nano-bilosomes

In order to create NSD bilosomes, the NSD was dissolved in an appropriate size about (ten ml) of chloroform in a flask. Additionally, cholesterol and a specified amount of surfactant (span 60) were also dissolved during this process (Al-Hussaniy et al. 2023).

To ensure that the organic solvent was completely eliminated, it was evaporated under a vacuum at 60 °C using a rotary evaporator (Buchi R-210 Rotavapor System, Germany) set to 90 rpm for 30 minutes. After that, 10 mL of phosphate buffer (pH 7.4) containing bile salts was used to hydrate the thin, dry film that had developed on the inner wall of the flask.

To create Nisoldipine loaded bilosomes, the resulting hydrated dispersion of BS was sonicated in a bath sonicator (Powersonic 410, Hwashin Technology, Korea). While the completed combination was being kept in the refrigerator, it was observed for a number of criteria (Ahad et al. 2018) see Table 1.

Table 1. Composition of various NSD bilosomes formulations.

Formula No.	NSD (mg)	Cholesterol (mg)	Span 60 (mg)	SDC (mg)	SGC (mg)	STC (mg)	Sonication time (min)
F1	10	80	240	5	/	/	10
F2	10	80	240	10	/	/	10
F3	10	80	240	20	/	/	10
F4	10	80	240	/	5	/	10
F5	10	80	240	/	10	/	10
F6	10	80	240	/	20	/	10
F7	10	80	240	/	/	5	10
F8	10	80	240	/	/	10	10
F9	10	80	240	/	/	20	10

Characterization and evaluation of NSD loaded bilosomes

Particle size and Poly Dispersity Index (PDI)

The Zetasizer device from Malvern (Malvern UK) was utilized to perform the particle size measurement. This device is capable of performing dynamic light scattering, which involves estimating the intensity of light scattered by the particles in the tested sample as a meaning of time while maintaining a temperature of 25 °C and a scattering angle of 90° (Al-Mahmood and Abd Alhammid 2022; Al-Sawaf and Jalal 2023).

The real refractive index and the imaginary refractive index were set at 1.456 and 0.01, respectively. It was determined that a homogenous distribution of vesicles existed when the PDI value was less than 0.1, but a larger globule dispersion was indicated by large values that approached 1 (Rajab 2022).

Entrapment efficiency

As an indirect technique, the %EE of NSD in the bilosomes may be resolute by measuring un-entrapped NSD in the dispersion media. One milliliter of bilosomes was put in a centrifugation tube and spun at 16,000 rpm for an hour at 4 °C using a cold centrifuge. The drug was quantitively analyzed with a UV-VIS spectrophotometer set at wave length about 237 nm after the resultant supernatant was separated from the rest of the sample and diluted with PBS. Therefore, %EE was considered since we used this equation (Al-Mahallawi et al. 2015):

$$EE\% = \frac{Total\ amount\ of\ drug - Amount\ of\ free\ drug}{Total\ amount\ of\ drug} \times 100$$

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was conducted in order to validate the purity of the medicine and ascertain its compatibility with other excipients. The sample was combined with dry potassium bromide (KBr) in a fixed ratio of 1:10, and subsequently, the resulting mixture was compacted into pellets. The range of samples was measured from 4000 cm^{-1} to 400 cm^{-1}. The analysis was conducted on the pure drug, a physical mixture of the drug, cholesterol, span 60, and bile salt, as well as the selected bilosome formulation (Jassim et al. 2021).

In vitro drug release

Different bilosmoe formulations, each made with a different type of bile salts, were subjected to *in vitro* drug release testing. A volume of two milliliters was extracted from the formulae, which were subsequently transferred into dialysis baggage that had been soaked overnight. Subsequently, the dialysis bags were introduced into a type two dissolving apparatus, specifically the paddle type, operating at a temperature of 37 ± 0.2 °C and a rotation speed of 50 rpm.

The release medium employed consisted of 250 ml of a phosphate-buffered saline (PBS) solution, including 0.75% w/v sodium lauryl sulfate (SLS), with the intention of establishing a sink condition.

Samples of three milliliters were removed at predefined time intervals (1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hours). These were then changed with the same volume with fresh Buffer solution to ensure the maintenance of sink condition. The UV/VIS spectrophotometer was utilized to measure the cumulative release of NSD at a wavelength of 237 nm (Saifi et al. 2020).

Statistical analysis

The results of the experimental study were presented as the mean of triplicate models ± standard deviation (SD). These results were analyzed using a one-way ANOVA test to assess the statistical significance of the changes in the applied factors (Altalebi et al. 2023).

A significance level of $p < 0.05$ was used to determine if the observed changes were statistically significant, while a significance level of $p > 0.05$ indicated that the changes were not statistically significant (Noor and Ghareeb 2021; Shaaban et al. 2023).

Results and discussion

The NSD loaded bilosomes that had already been made were analyzed for their entrapment efficiency. Results in Table 2 revealed that entrapment efficiency increases significantly from 60.68% in F5 (SGC) into 73.22% and 82.36% in F8 (STC) and F2 (SDC) respectively.

Table 2. Physical properties of NSD bilosomes.

Formula No.	Vesicle size (VS)	PDI	Entrapment Efficiency (EE%)
F1	166 ± 1.83	0.33 ± 0.3	71 ± 0.42%
F2	175.73 ± 0.39	0.19 ± 0.03	82.36 ± 0.80%
F3	211.5 ± 0.74	0.19 ± 0.081	60.13 ± 0.78%
F4	177.9 ± 1.24	0.19 ± 0.01	49.37 ± 1.26%
F5	185.7 ± 0.96	0.27 ± 0.07	60.68 ± 0.78%
F6	230.1 ± 3.5	0.26 ± 0.01	44.2 ± 0.3%
F7	186.96 ± 0.32	0.29 ± 0.028	60.13 ± 0.35%
F8	208.36 ± 0.9	0.16 ± 0.037	73.22 ± 0.59%
F9	263 ± 2.4	0.27 ± 0.03	52.55 ± 1.01%

This could be due to lower HLB value (higher hydrophobicity) of SDC (HLB=16) compared to that of SGC and STC possessing higher HLB values (23.1) and (22.1) respectively. The higher hydrophobicity made it easier for the hydrophobic drug NSD to get into the core of the bilayer, which led to a higher EE (El-Nabarawi et al. 2020), as seen in Fig. 1.

Increasing bile salts from 5 to 10 mg Fig. 2, entrapment efficiency increase significantly from 71% to 82% in F1 and F2 respectively. SDC has surface active property and is integrated into bilayer membrane surfaces, enhancing lipid membrane flexibility and drug solubility, increasing entrapment efficiency (Zafar et al. 2021).

Further increase of bile salt concentration leads to significant decrease in EE from 82.36% in F2 into 60.13% in F3 because high bile salt content enhances the possibility of acting as a solubilizing surfactant and lowers vesicle

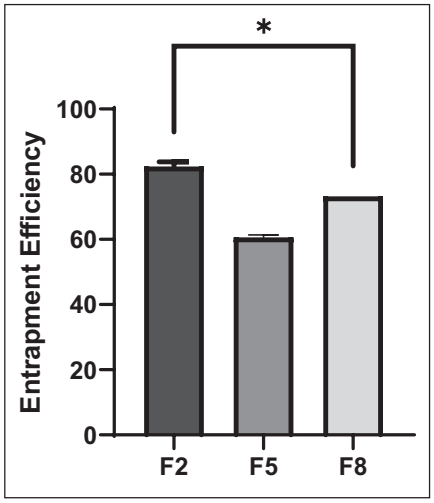

Figure 1. Effect of type of bile salts on entrapment efficiency ($p < 0.05$).

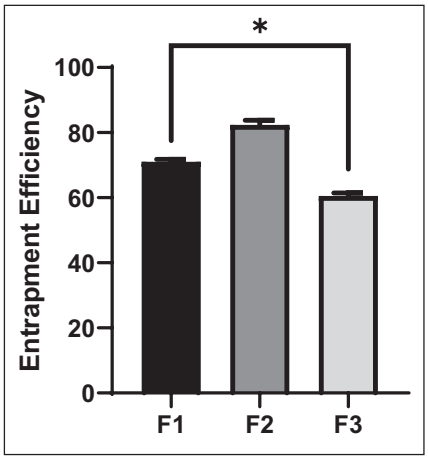

Figure 2. Effect of Bile salt concentration on Entrapment Efficiency ($p < 0.05$).

compactness, facilitating drug leakage and decreasing EE% (Zakaria et al. 2021).

According to the data in Table 2, the bilosomes' particle size distribution index (PDI) ranged from 0.16 ± 0.037 to 0.33 ± 0.3 (for formulations F8 and F1), indicating a limited size distribution. According to the results of an ANOVA test, the PDI of the produced vesicles was not substantially ($p > 0.05$) affected by the conditions that were investigated.

Increasing the concentration of bile salt from 5 mg to 10 mg and to 20 mg leads to a significant increase in vesicle size for all types of bile salts ($p < 0.05$) as seen in Fig. 3.

That's because bile salt, when concentrated to a high level, tends to agglomerate, and that's exactly what happened here. Anionic bile salts and their steroid structure increase vesicle size in two ways: (i) by increasing the volume of the aqueous core and (ii) by increasing the steric repulsive power among bilosome bilayers, which may increase bilosome bulkiness (Mahmoud et al. 2022).

The increase in vesicle size was determined to be as follows, based on the bile salts that were investigated:

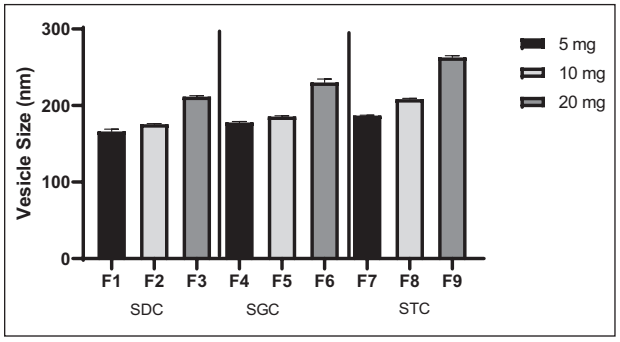

Figure 3. Effect of different concentrations of different bile salts on vesicle size ($p < 0.05$).

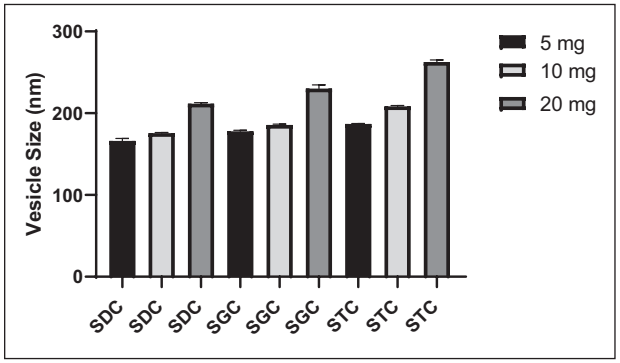

Figure 4. Effect of bile salts kinds on vesicle dimensions ($p < 0.05$).

STC > SGC > SDC. It's possible that the variation in vesicle size is caused by a difference in the structure of the bile salt that was used (Abdelbary et al. 2016). SGC (487.6 g/mol) and SDC (414.56 g/mol) based bilosomal vesicles may have created smaller vesicle size when compared to the bulkier STC (537.69 g/mol), which may have resulted in a larger size (Mohsen et al. 2020), Fig. 4 showed that (F7–F9) prepared with STC give significantly larger vesicle size as compared with (F4–F6) and (F1–F3) prepared with SGC and SDC respectively.

Three formulas, F2, F5, and F8 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 NSD from bilosomes.

The bilosome formulas prepared using SDC F3 showed a higher % drug release (97%) compared to the corresponding bilosome formulas F5 (93.5%) and F8 (87.45%) prepared with SGC and STC, respectively at the end of 24 hr, Fig. 5.

Release studies show biphasic drug efflux from bilosomal preparations. The initial phase exhibited a prompt and intense release of the drug, primarily resulting from the detachment of the drug molecules that were adsorbed onto the outer surface of the bilosome vesicles. This was followed by a subsequent phase characterized by a gradual and prolonged release of the drug over an extended duration. This sustained release is attributed to the bilosomes' ability to function as a reservoir for the drug, facilitating its controlled and continuous release (Salim et al. 2022; Kumar et al. 2023).

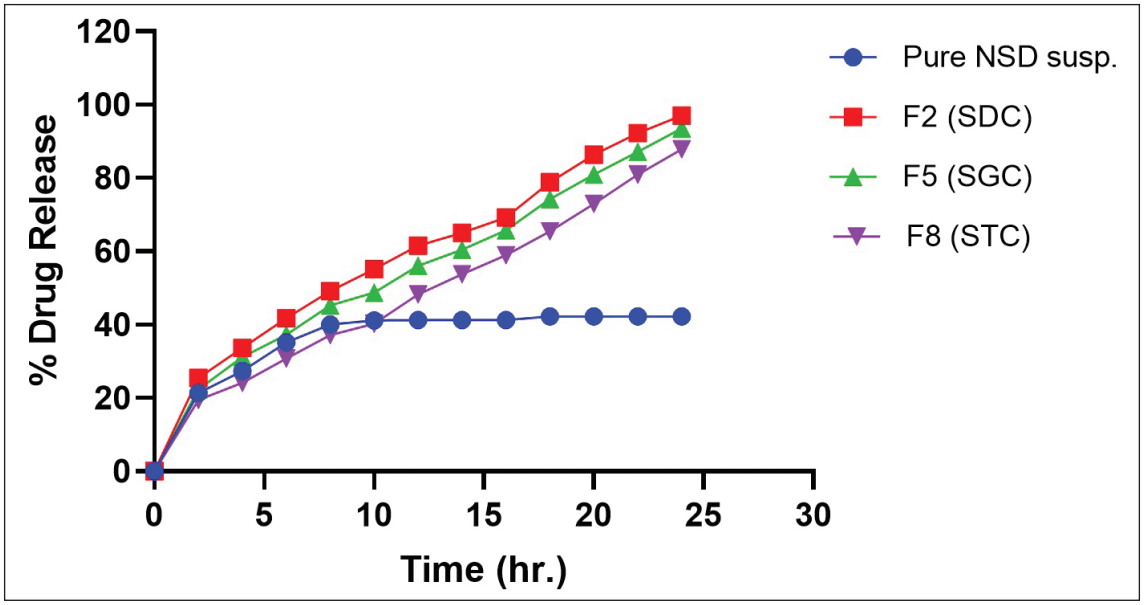

Figure 5. In vitro release profile of NSD from different formulas (F2, F5 and F8) along with pure drug suspension.

In addition to improving penetration by integrating itself into the bilayer membrane, SDC is able to increase the fluidity and flexibility of the vesicle bilayer, which in turn makes drug seepage simpler (Salem et al. 2022).

The drug release from bilosomes containing STC was lower than that of SGC-containing bilosomes. The numerous hydrophobic methylene groups on the bilosome side chains during the STC's conjugation with taurine account for this action, delaying medication release (Ali and Al-Akkam 2023).

Since NSD is highly lipophilic, it would prefer to remain within the lipophilic system than release medium. Increased bile salt concentration had a positive effect on drug release which means increased drug release because it could enhance the fluidity of the bilayer and penetration enhancer effect (Ahmed et al. 2020; Rana et al. 2023).

From the above results, F2 was the optimum formula in terms of small vesicle size with the higher entrapment efficiency along with best drug release, so FTIR was done to determine if there is a chemical interaction between their components.

Fig. 6A displayed the FTIR for a physical combination, a pure medication, and a chosen formula. In the pure NSD spectra, the N-H stretching peak was located at 3318.40 $cm^{-1,}$ the C-O stretching peak at 1650.01 cm^{-1}, the N-O stretching peak at 1526.25 cm^{-1}, and the C=O stretching peak at 1211.37 $cm^{-1.}$ (Fu et al. 2017) .

The physical mixture's FTIR spectrum shows that the (C-O stretching vibration) has slightly shifted from 1650.01 cm^{-1} to 1648.71 cm^{-1}, while the other primary peaks have not altered. This suggests that NSD and the other ingredients needed to create bilosomes do not interact. There is no superimposition between fingerprint regions, which is evidence that there have been modifications in the physical character (Fig. 6) (Kadhim et al. 2020).

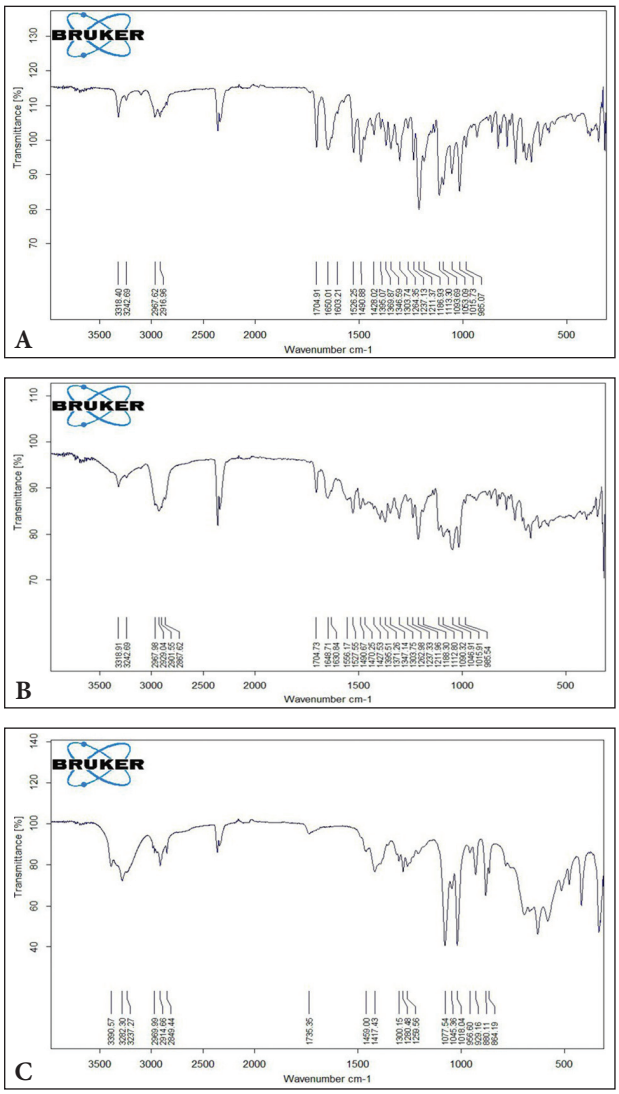

Figure 6. FTIR for A. Pure NSD; **B.** Physical Mixture; **C.** F2 NSD bilosome formula.

Conclusion

The bilosomal formulations effectively entrapped NSD. The physical features of NSD loading bilosomes were affected by the type and amount of bile salts used, including entrapment efficiency, vesicle size, polydispersity index, and in vitro drug release. SDC was found to be better suited for the preparation of NSD-loading bilosomes. The FTIR analyses revealed no incompatibility between the medication and the other excipients in the mixture.

The study's results are encouraging since the NSD-BS results were in the nanosize range, allowing for optimal NSD delivery through excellent trapping and prolonged drug release.

Funding source

The authors did not receive any source of funds.

Author contribution

Ghada Hamid Naji: conduct the research, draft writing, and experimental analysis; Fatima J. Al_Gawhari: supervision, revision, and proofreading.

Acknowledgments

The authors express their gratitude to the College of Pharmacy at the University of Baghdad for supporting the project.

References

Abdelbary AA, Abd-Elsalam WH, Al-Mahallawi AM (2016) Fabrication of novel ultradeformable bilosomes for enhanced ocular delivery of terconazole: In vitro characterization, ex vivo permeation and in vivo safety assessment. International Journal of Pharmaceutics 513: 688–696. https://doi.org/10.1016/j.ijpharm.2016.10.006

Ahad A, Raish M, Ahmad A, Al-Jenoobi FI, Al-Mohizea AM (2018) Eprosartan mesylate loaded bilosomes as potential nano-carriers against diabetic nephropathy in streptozotocin-induced diabetic rats. European journal of pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences 111: 409–417. https://doi.org/10.1016/j.ejps.2017.10.012

Ahmed S, Kassem MA, Sayed S (2020) Bilosomes as Promising Nanovesicular Carriers for Improved Transdermal Delivery: Construction, in vitro Optimization, ex vivo Permeation and in vivo Evaluation. International Journal of Nanomedicine 15: 9783–9798. https://doi.org/10.2147/IJN.S278688

Al-Edhari GH, Gawhari A (2023) Study the effect of formulation variables on preparation of nisoldipine loaded nano bilosomes. Iraqi Journal of Pharmaceutical Sciences 32: 271–282. https://doi.org/10.31351/vol32issSuppl.pp271-282

Al-Hussaniy HA, Almajidi YQ, Oraibi AI, Alkarawi AH (2023) Nanoemulsions as medicinal components in in soluble medicines. Pharmacia 70: 537–547. https://doi.org/10.3897/pharmacia.70.e107131

Ali SK, Ej A-A (2023) Effects of different types of bile salts on the physical properties of ropinirole-loaded. Al-Rafidain Journal of Medical Sciences 5: 134–142. https://doi.org/10.54133/ajms.v5i.176

Al-Mahallawi AM, Abdelbary AA, Aburahma MH (2015) Investigating the potential of employing bilosomes as a novel vesicular carrier for transdermal delivery of tenoxicam. International Journal of Pharmaceutics 485: 329–340. https://doi.org/10.1016/j.ijpharm.2015.03.033

Al-Mahmood AA, Alhammid A (2022) Preparation and Ex-Vivo Evaluation of Stabilized Cefdinir Nanosuspension. Pakistan Journal of Medical & Health Sciences 16: 1–6. https://doi.org/10.53350/pjmhs20221612789

Al-Sawaf OF, Jalal F (2023) Novel probe sonication method for the preparation of meloxicam bilosomes for transdermal delivery: Part one. Journal of Research in Medical and Dental Science 11: 5–12.

Altalebi RR, Al-Hussaniy HA, Al-Tameemi ZS, Al-Zobaidy MA-H, Albu-Rghaif AH, Alkuraishy HM, Hedeab GM, Azam F, Al-Samydai AM, Naji MA (2023) Non-alcoholic fatty liver disease: relation to juvenile obesity, lipid profile, and hepatic enzymes. Journal of Medicine and Life 16: 42–47. https://doi.org/10.25122/jml-2022-0091

Anon (2020) Formulation of flurbiprofen as microsponge drug delivery system. International Journal of Pharmaceutical Research 12. https://doi.org/10.31838/ijpr/2020.12.03.141

Binsuwaidan R, Sultan AA, Negm WA, Attallah NGM, Alqahtani MJ, Hussein IA, Shaldam MA, El-Sherbeni SA, Elekhnawy E (2022) Bilosomes as nanoplatform for oral delivery and modulated in vivo antimicrobial activity of lycopene. Pharmaceuticals (Basel, Switzerland) 15: 1043. https://doi.org/10.3390/ph15091043

Chaudhari GD, Ghodgaonkar SA, Vilegave KV (2019) Design, formulation and evaluation of extended release tablet containing nisoldipine. Journal of Pharmacy and Pharmaceutics 6: 1–9.

Chavan RB, Lodagekar A, Yadav B, Shastri NR (2020) Amorphous solid dispersion of nisoldipine by solvent evaporation technique: preparation, characterization, in vitro, in vivo evaluation, and scale up feasibility study. Drug Delivery and Translational Research 10: 903–918. https://doi.org/10.1007/s13346-020-00775-8

Drais HK, Hussein AA (2022) Investigation of lipid polymer hybrid nanocarriers for oral felodipine delivery: Formulation, method, in-vitro and ex-vivo evaluation. Iraqi Journal of Pharmaceutical Sciences 31: 119–129. https://doi.org/10.31351/vol31iss1pp119-129

El-Nabarawi MA, Shamma RN, Farouk F, Nasralla SM (2020) Bilosomes as a novel carrier for the cutaneous delivery for dapsone as a potential treatment of acne: preparation, characterization and in vivo skin deposition assay. Journal of Liposome Research 30: 1–11. https://doi.org/10.1080/08982104.2019.1577256

Fu Q, Ma M, Li M, Wang G, Guo M, Li J, Hou Y, Fang M (2017) Improvement of oral bioavailability for nisoldipine using nanocrystals. Powder Technology 305: 757–763. https://doi.org/10.1016/j.powtec.2016.10.068

Gasmi M, Rahma Z, Williams-Hooker RW, Sharma A (2023) Research Article Production and Characterization of Recombinant Multi-Epitope G Glycoprotein of Rabies Virus. Medical and Pharmaceutical Journal 2: 134–148.

Ismail A, Teiama M, Magdy B, Sakran W (2022) 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 PharmSciTech 23: 188. https://doi.org/10.1208/s12249-022-02339-0

Jassim ZE, Al-Kinani KK, Alwan ZS (2021) Preparation and Evaluation of Pharmaceutical Cocrystals for Solubility Enhancement of Dextromethorphan HBr. International Journal of Drug Delivery Technology 11(4): 1342–1349.

Kadhim ZJ, Rajab NA (2022) Formulation and Characterization of Glibenclamide Nanoparticles as an Oral Film. International Journal of Drug Delivery Technology 12(1): 387–394.

Khafagy ES, Almutairy BK, Abu Lila AS (2023) Tailoring of novel bile salt stabilized vesicles for enhanced transdermal delivery of simvastatin: A new therapeutic approach against inflammation. Polymers 15(3): 677. https://doi.org/10.3390/polym15030677

Kharouba M, El-Kamel A, Mehanna R, Thabet E, Heikal L (2022) Pitavastatin-loaded bilosomes for oral treatment of hepatocellular carcinoma: a repurposing approach. Drug Delivery 29: 2925–2944. https://doi.org/10.1080/10717544.2022.2120925

Kumar KV, Vidyadhara S, Babu JR (2023) Favipiravir: Emergent antiviral drug during Covid-19. Medical and Pharmaceutical Journal 2: 175–180. https://doi.org/10.55940/medphar202344

Mahmoud TM, Nafady MM, Farouk HO, Mahmoud DM, Ahmed YM, Zaki RM, Hamad DS (2022) Novel bile salt stabilized vesicles-mediated effective topical delivery of diclofenac sodium: A new therapeutic approach for pain and inflammation. Pharmaceuticals (Basel, Switzerland) 15: 1106. https://doi.org/10.3390/ph15091106

Mohsen AM, Salama A, Kassem AA (2020) Development of acetazolamide loaded bilosomes for improved ocular delivery: Preparation, characterization and in vivo evaluation. Journal of Drug Delivery Science and Technology 59: 101910. https://doi.org/10.1016/j.jddst.2020.101910

Nekkanti V, Rueda J, Wang Z, Betageri GV (2016) Comparative evaluation of proliposomes and self micro-emulsifying drug delivery system for improved oral bioavailability of nisoldipine. International Journal of Pharmaceutics 505: 79–88. https://doi.org/10.1016/j.ijpharm.2016.03.065

Noor AH, Ghareeb MM (2021) Transdermal dissolvable microneedle-mediated delivery of controlled release ondansetron hydrogen chloride nanoparticles. International Journal of Drug Delivery Technology 11(3): 858–863.

Palekar-Shanbhag P, Lande S, Chandra R, Rane D (2020) Bilosomes: Superior vesicular carriers. Current Drug Therapy 15: 312–320. https://doi.org/10.2174/1574885514666190917145510

Rajab NA (2022) Formulation and characterization of niosomes for controlled delivery of tolmetin. Journal of Pharmaceutical Negative Results 13: 159–169. https://doi.org/10.47750/pnr.2022.13.04.021

Rana S, Sharma N, Dutt S, Patil RD (2023) Hepatoprotective effects of *Carissa spinarum* extract on carbon tetra chloride induced liver damage in zebrafish (*Danio rerio*). Medical and Pharmaceutical Journal 2: 206–220. https://doi.org/10.55940/medphar202362

Saifi Z, Rizwanullah M, Mir SR, Amin S (2020) Bilosomes nanocarriers for improved oral bioavailability of acyclovir: A complete characterization through in vitro, ex-vivo and in vivo assessment. Journal of Drug Delivery Science and Technology 57: 101634. https://doi.org/10.1016/j.jddst.2020.101634

Salem HF, Nafady MM, Ali AA, Khalil NM, Elsisi AA (2022) Evaluation of metformin hydrochloride tailoring bilosomes as an effective transdermal nanocarrier. International Journal of Nanomedicine 17: 1185–1201. https://doi.org/10.2147/IJN.S345505

Salim Mahmood A, Ammoo AM, Ali MHM, Hameed TM, Al-Hussaniy HA, Aljumaili AAA, Al-Fallooji MHA, Kadhim AH (2022) Antiepileptic effect of neuroaid on strychnine-induced convulsions in mice. Pharmaceuticals (Basel, Switzerland) 15: 1468. https://doi.org/10.3390/ph15121468

Shaaban SM, Gaber Z, Semary S, Dewidar AM (2023) Impact of vitamin B12 on outcome of early stage luminal A and B breast cancer, single center experience. Medical and Pharmaceutical Journal 2: 17–27.

Waglewska E, Pucek-Kaczmarek A, Bazylińska U (2020) Novel surface-modified bilosomes as functional and biocompatible nanocarriers of hybrid compounds. Nanomaterials (Basel, Switzerland) 10: 2472. https://doi.org/10.3390/nano10122472

Zafar A, Alruwaili NK, Imam SS, Hadal Alotaibi N, Alharbi KS, Afzal M, Ali R, Alshehri S, Alzarea SI, Elmowafy M, Alhakamy NA, Ibrahim MF (2021) Bioactive Apigenin loaded oral nano bilosomes: Formulation optimization to preclinical assessment. Saudi pharmaceutical journal: SPJ: the official publication of the Saudi Pharmaceutical Society 29: 269–279. https://doi.org/10.1016/j.jsps.2021.02.003

Zakaria MY, Fayad E, Althobaiti F, Zaki I (2021) Abu Almaaty AH. Statistical optimization of bile salt deployed nanovesicles as a potential platform for oral delivery of piperine: Accentuated antiviral and anti-inflammatory activity in MERS-CoV challenged mice. Drug Delivery 28: 1150–1165. https://doi.org/10.1080/10717544.2021.1934190