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Study the Effect of Formulation Variables on Preparation of Nisoldipine Loaded Nano Bilosomes [#]

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Abstract

Nisoldipine (NSD) is a dihydropyridine class of calcium channel blockers used for hypertension treatment. It belongs to class II BCS (low solubility with high permeability). Its absolute bioavailability is only 5% due to presystemic metabolism in the gut wall. It is also a substrate for a CYP3A and P-glycoprotein. Bilosomes (BS) are lipid bilayer vesicles incorporating bile salts in their walls to prevent degradation by GIT bile salts. The aim of this study is to prepare nisoldipine bilosomes as vesicular carrier and assess the effect of different formulation variables such as type and amount of surfactant, amount of cholesterol, amount of bile salts and sonication time on particle size, entrapment efficiency and poly dispersity index of the prepared bilosomes. The bilosomes were prepared by thin film hydration method and they were optimized using different types of non-ionic surfactants span20, 40 and 60 along with different amount of cholesterol and different sonication time.The prepared formulas have particle size between 138.3 -356.43 nm, polydispersity index 0.19- 0.38 and entrapment efficiency equal to 45.46-82.36%. Results showed that increasing cholesterol amount led to increase entrapment efficiency with increase particle size. Further increase in cholesterol led to decrease entrapment efficiency with further increase in particle size. While increase amount of surfactant (span 60) give higher entrapment of drug with decrease particle size. While increasing sonication time is associated with decrease in particle size and entrapment efficiency. The obtained results indicated that F4, composed of span 60 (240 mg), cholesterol (80 mg), SDC (10mg) and sonication time for (10 min) considered as optimum formula due to its high entrapment efficiency (82.36%), small particle size (175.7 ± 0.68 nm), low PDI (0.19 ± 0.03). This study concluded that NSD loaded nanobilosoms were prepared successfully using thin film hydration method with good entrapment, nanosize range and with sustained release of drug so they can be considered as a promising nan-carrier for drug delivery.

Key words: Bilosomes, Cholesterol, Entrapment efficiency, Hydrophilic-lipophilic balance, Polydispersity index.

دراسة تأثيير متغيرات التحضير على عقار النيزولدبين المحضر بشكل نانو بايلوسومات [#]

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الخلاصة

عقار النيزولدبين ينتمي الى مجموعة ديهيدروبيريدين من حاصرات قنوات الكالسيوم المستخدمة لعلاج ارتفاع ضغط الدم ، وهي تنتمي الى النوع الثاني من الادوية (قليلة الذوبان في الماء مع نفاذية عالية) ، توافرها الحيوي المطلق هو ٥ ٪ فقط بسبب التمثيل الغذائي المسبق في جدار الأمعاء. وهو أيضًا ركيزة لانزيم السايتوكروم و كلايكوبروتين. البايلوسومات عبارة عن حويصلات ثنائية الطبقة تحتوي على أملاح صفراوية في جدرانها لمنع تكسر هذه الحويصلات في الجهاز الهضمي بوساطة الاملاح الصفراوية .الهدف من هذه الدراسة هو تحضير بايلوسومات النيزولدبين كحامل حويصلي وتقييم تأثير متغيرات التحضير المختلفة مثل نوع و كمية المواد الخافضة للتوتر السطحي ، كمية الكوليسترول ، و مدة السونيكشن على حجم الجزيئات و نسبة كفاءة الحصر في الحويصلات ومؤشر التشتت المتعدد للبيليوسومات المحضرة .تم تحضير البيلوسومات المحضرة بطريقة الترطيب بالغشاء الرقيق وتم تحسينها باستخدام أنواع مختلفة من المواد الخافضة للتوتر السطحي غير الأيونية من سبان ٢٠ و ٤٠ و ٦٠ بالاضافة الى كميات مختلفة من الكوليسترول و مدة اهتزاز مختلفة .تحتوي الصيغ المحضرة على متوسط حجم يتراوح بين ١٣٨,٣ – 356.43 نانومتر ، مؤشر التشتت المتعدد 0.19- 0.38 ونسبة كفاءة حصر تعادل 45.46-82.36٪.

أظهرت النتائج أن زيادة كمية الكوليسترول تؤدي إلى زيادة الاحتباس مع زيادة حجم الجسيمات لكن مع زيادة اكثر في كمية الكوليسترول تؤدي إلى تقليل الاحتباس مع زيادة أخرى في حجم الجسيمات. بينما تؤدي زيادة كمية المواد الخافضة للتوتر السطحي (سبان ٦٠) إلى زيادة احتباس الدواء مع تقليل حجم الجسيمات يرتبط زيادة السونيكشن او الاهتزاز بالموجات فوق الصوتية بانخفاض حجم الجسيمات وكفاءة الحصر .أشارت النتائج التي تم الحصول عليها إلى أن الفورملا F4 ، المتكونة من سبان ٦٠ (٢٤٠ مجم) والكوليسترول (٨٠ مجم) و صوديوم ديوكسي كوليت (١٠ مجم) و مدة اهتزاز لمدة (١٠ دقائق) تعتبر افضل فورملا بسبب كفاءته العالية في احتواء الدواء (82.36٪). حجم الجسيمات النانوي (١٧٥,٧ ± ٠,٦٨ نانومتر) ، و مؤشر التشتت المتعدد منخفض (٠,١٩ ± ٠,٠٣). استنتجت هذه الدراسة أن نيزولدبين بايلوسومات تم تحضيرها بنجاح باستخدام طريقة ترطيب الأغشية الرقيقة مع كفاءة حصرجيدة للدواء و حجم جسيمات نانوي مع تحريرمستمر للدواء بحيث يمكن اعتبارها كناقل نانوي واعد لتوصيل الدواء.

الكلمات المفتاحية: البايلوسومات, كوليسترول, كفاءة الحصر, التوازن المحبة للماء, مؤشر التشتت المتعدد.

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Introduction

Recently, "Nanotechnology" has become very essential and extensively applied in the modern pharmaceutical industry to solve the bioavailability problem of poorly water-soluble drugs, which has long been a challenging in the pharmaceutical industry [(1)]. Nanotechnology has entered into the development of most areas of life, including the development and manufacture of medicines [(2)]. Typically, vesicular systems showed good results in this area of drug delivery, for example liposomes, niosomes, transferosomes, pharmacosomes, and provesicular systems, like proliposomes and proniosomes [(3)].

Both hydrophilic and lipophilic drugs can be incorporated successfully into lipid-based vesicular systems, including liposomes and conventional niosomes. The main challenges in these systems are payload leakage, gastrointestinal instability, and scaling-up difficulties [(4)].

Conventional vesicles suffer from GIT instability owing to the existence of bile salts that cause rapid discharge of the enclosed drug before reaching to the site of action due to lysis of the membrane of the vesicles [(5)]. So bilosomes (BS) (bile salt stabilized nano-vesicle) were introduced in order to overcome these problems that occurred with other vesicles [(6)].

BS are sealed, bilayered vesicular carriers of lipids that contain bile salts and nonionic surfactants. Their size ranges from 5 to 500 nm, and their vesicles can be spherical, unilamellar, or multilamellar [(7)]. They were first described by Conacher et al. from the University of Glasgow in 2001 [(8)]. Due to its excellent stability against the pH, enzymes, and bile salts present in the gastro-intestinal tract, BS has been developed for orally administered poorly water soluble medications and vaccinations [(9)].

Nisoldipine (NSD) ($C_{20}H_{24}N_2O_6$) is type of dihydropyridine class of calcium channel blockers that prevents the transmembrane influx of calcium into vascular smooth muscle and cardiac muscle. The heart contracts as calcium ions moves through specific ion channels. Blockage Ca channels causes arterioles dilatation [(10)].

NSD is a BCS class II drug, it undergoes extensive presystemic metabolism that makes its absolute bioavailability only 5%. It is also a CYP3A and P-gp substrate [(11)]. In addition to that poor bioavailability resulted from NSD poor aqueous solubility (5.77×10^{-3} g/L) which results in poor dissolution [(12)].

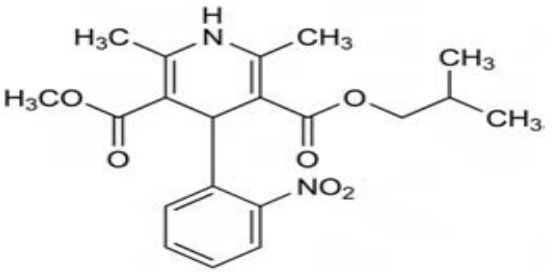


Figure 1. Structure of Nisoldipine.

This study aimed to demonstrate the influence of formulation variables like type of surfactant, cholesterol:surfactant ratio, concentration of bile salt and sonication time on NSD BS properties like particle size, entrapment efficiency and polydispersity index.

Materials and Methods

Nisoldipine was purchased from Lee for chemicals, India. Cholesterol, surfactants (span 20, span40 and span 60) and sodium deoxycholate (SDC) was purchased from Hyperchem for chemicals, India. All other chemicals and solvents employed in the present work were of analytical grade.

Preparation of nisoldipine nano-bilosomes

NSD BS were formulated by dissolving NSD along with cholesterol and certain amount of surfactant (span 20, span40 and span 60) in sufficient volume (10 ml) of chloroform in round bottom flask. By utilizing a rotary evaporator to remove the organic phase, a thin, dry film of the component parts was created. The film was then thoroughly cleaned of any remaining organic phase residue before being rehydrated with 10 ml of phosphate buffer saline (pH 7.4) containing SDC.

To prepare NSD-loaded nano-bilosomes, the hydrated dispersion of BS that resulted was sonicated in a bath sonicator. The finished mixture was monitored for several parameters while being stored in the refrigerator [(13)].

Table 1. The composition of various NSD prepared formulas.

Formula No.	NSD (mg)	Cholesterol (mg)	Span 20 (mg)	Span 40 (mg)	Span 60 (mg)	SDC (mg)	Sonication Time (min)
F1	10	80	80	/	/	10	10
F2	10	80	/	80	/	10	10
F3	10	80	/	/	80	10	10
F4	10	80	/	/	240	10	10
F5	10	80	/	/	320	10	10
F6	10	240	/	/	80	10	10
F7	10	320	/	/	80	10	10
F8	10	80	/	/	240	5	10
F9	10	80	/	/	240	20	10
F10	10	80	/	/	240	10	20
F11	10	80	/	/	240	10	30

Characterization and Evaluation of NSD Loaded Nanobilosomes

Particle size and Poly Dispersity Index (PDI)

Using photo-correlation spectroscopy (PCS), Zetasizer-ZS, Malvern Instrument (Malvern, UK), the particle size and PDI of NSD BS were assessed. Then, PDI-based standard deviations and mean particle sizes were reported. In order to confirm that there is no aggregation of particles occurred measurements were repeated [14,15].

Entrapment Efficiency

By quantifying un-entrapped NSD in the dispersion media, it was possible to determine the %EE of NSD in the bilosomes. Using a cooling centrifuge, 1 mL of bilosomes was placed in a centrifugation tube and rotated at 16,000 rpm for 1 hour at 4 °C (cold centrifuge were used for all formulas to separate free drug from bilosomes, the procedure was repeated using amicon tubes for only three formulas, results showed there was a correlation between two methods so continue with cold centrifuge). After separating and diluting the resulting supernatant with PBS, the drug concentration was determined using a UV-VIS spectrophotometer at 237 nm. Consequently, %EE was calculated from the following equation [16]:

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

Microscopic examination of NSD-loaded nanobilosomes

To identify the morphology of the NSD-loaded nanobilosomes, one drop of bilosomal dispersion was taken on the glass slide and observed under projection microscope with 40X magnification and 100X.

Transmission electron microscope (TEM)

To determine the shape of the prepared NSD-loaded nanobilosomes, a high-resolution transmission electron microscope (TEM) was used. A drop of prepared formulas was taken with suitable dilution with distilled water and allowed to dry at room temperature for 10 minutes while placing it on carbon-coated copper grids, finally the sample was examined for the shape [17].

Differential scanning clorimetry (DSC)

Thermal behavior of NSD and its compatibility with other formulation ingredients was estimated using differential scanning calorimeter equipped with an intracooler. Samples 2–4 mg was weighed accurately placed in aluminum pans and heated at 10°C per min rate in the range of 30-300°C in a nitrogen purging gas environment [18].

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR is used to confirm drug purity and to detect drug-excipient compatibility. FTIR was done for NSD pure drug, physical mixture (1:1 molar ratio of drug, cholesterol, span 60 and SDC). Also FTIR was done for the selected formula F4. The samples were made by KBr pellet technique and were studied at transmission mode over wave number from 4000 to 400 cm^{-1} [19].

In vitro drug release

In vitro drug release was performed for the selected bilosomal formulation (F4) in comparison with that of pure drug suspension. Two milliliters was taken from the selected formula F4 and pure drug suspension they were poured in dialysis bags (which were soaked overnight in the release media). Then the dialysis bags were placed in type two dissolution apparatus (Paddle type) at 37±0.2 ºC and rotation speed 50 rpm, the release media was 250 ml PBS solution (containing 0.75% w/v SLS) to achieve sink condition.

At predetermined time (1, 2, 4, 6, 8, 10,12, 14, 16, 18, 20, 22 and 24 hours) three milliliters samples were withdrawn and replaced by fresh PBS solution to maintain sink condition. The cumulative amount release of NSD was measured by UV/VIS spectrophotometer at 237 nm [20].

Statistical analysis

The outcomes of the experiments were expressed as a mean of triplicate samples ± standard deviation. They were investigated through the use of a one-way analysis of variance, where a P-value of

0.05 was regarded statistically significant and a P-value of greater than 0.05 was declared non-significant [21,22].

Variables affecting formulation

Different types of surfactants, different surfactant: cholesterol ratio, different concentration of bile salts along with different sonication time were used for preparation of NSD-loaded nanobilosomes.

The effect of a different surfactant type was studied in the formulas F1, F2, and F3. In each of these formulations, the amount of NSD (10 mg) and the quantity of SDC (10 mg), sonication time 10 min were kept constant.

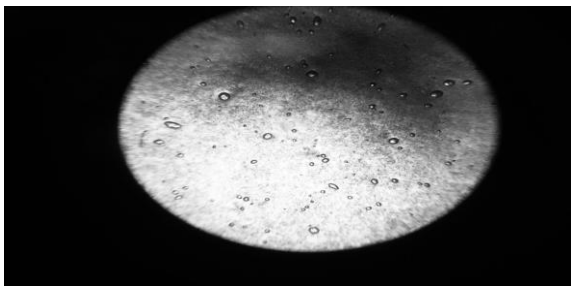
The effect of the surfactant: cholesterol ratio on the formulations of NSD-loaded nanbilosomes was evaluated in formulas F3, F4, and F5 at ratio of (1:1), (1:2.5) and (1:3.5) respectively. In each of these formulas, the concentration of drug (10 mg), bile salt (10 mg) and sonication time (10 min) all kept constant. Also the effect of cholesterol: surfactant ratio in formulas F6 and F7 in a ratio of 2.5:1 and 3.5:1 were studied.

The effect of different bile salt concentration in formulas F6 and F7 was evaluated. The ratio of surfactant: cholesterol was 1:2.5 and the sonication time was 10 min. Sonication time effect in formulas F8 and F9 was evaluated keeping all other variables constant.

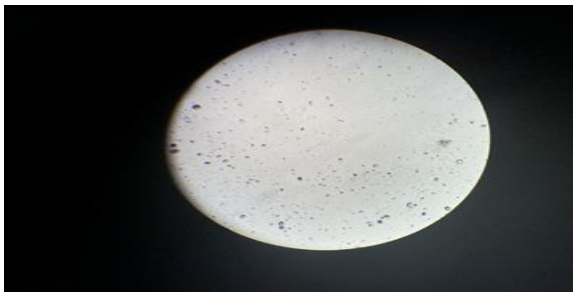
Results and Discussion

Optical microscope morphology

Results of light microscope for formula (F4) under oil immersion at 100 X and 40X shown in (Figure. 1) indicate the formation of vesicles.



A



B

Figure 2. optical microscopic image A) at 100 X B) at 40 X

TEM was done for some of the NSD-BS formulation and the results showed the presence of bilayer vesicles with different particle size as shown in (Figure. 3).

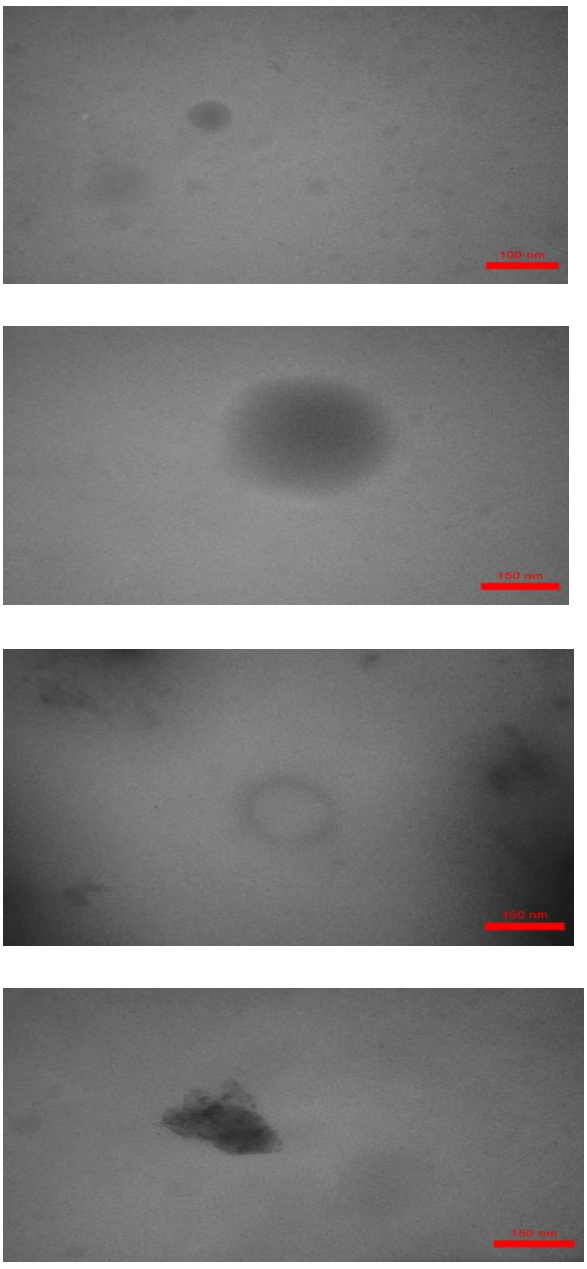


Figure 3. TEM images from different NSD-loaded nanobilosomes

Vesicle size

Table (2) showed the vesicle size of the NSD-loaded nanobilosomes formulations which were found in the range of 138.36 ± 2.79 – 356.43 ± 3.37nm.

In comparison with BS based on span 40 and span 60 (F2 and F3), those based on span 20 (F1) had larger vesicles. This may be due to decrease in surface energy with the use of hydrophobic surfactant (span 60) which had lower HLB value (4.7) leads to formation of small vesicles as seen in (Figure. 4). The findings agree with those of Yusuf et al., who found that utilizing less HLB surfactant led to smaller-sized vesicles. Additionally, when the HLB values shift toward the hydrophilic area, surfactant's water uptake increases, resulting in bigger vesicles [23].

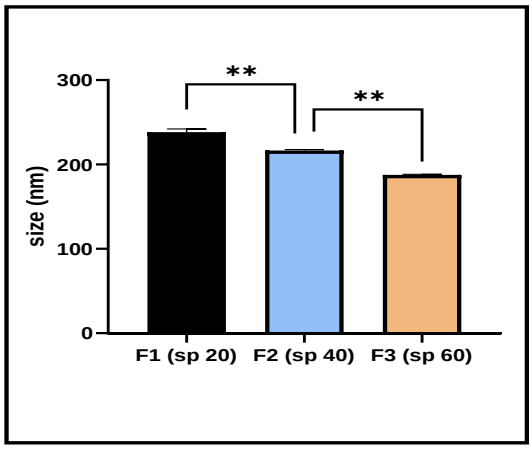


Figure 4. Effect of type of surfactant on vesicle size (indicate significant at p. value less than 0.05).**

The vesicle size was decreased significantly ($p<0.05$) from 187.6 nm into 175.7 nm when the concentration of surfactant (span 60) was increased from 80 mg in (F3) into 240 mg in (F4) (Figure. 5A), this is because of decreased the interfacial tension (energy) between the lipid and aqueous phase [24]. The same result was established in BS delivery of dapsone [25].

(Figure. 5B) showed that particle size increase significantly from 187.6 nm in F3 into 303.3 nm and 356 nm in F6 and F7 respectively, this due to the fact that cholesterol hindered the close packing of lipid vesicle and consequently greater aqueous phases inside the BS structure leads to larger vesicle size [26].

In addition to that as the concentration of cholesterol rises, this leads to increase the bilayer membrane's hydrophobicity causes the radius of the vesicles to increase, creating a more thermodynamically stable form. Due to the bilayer membrane's rigidity brought on by the presence of cholesterol, sonication cannot diminish the size of the vesicles, resulting in larger vesicles [27].

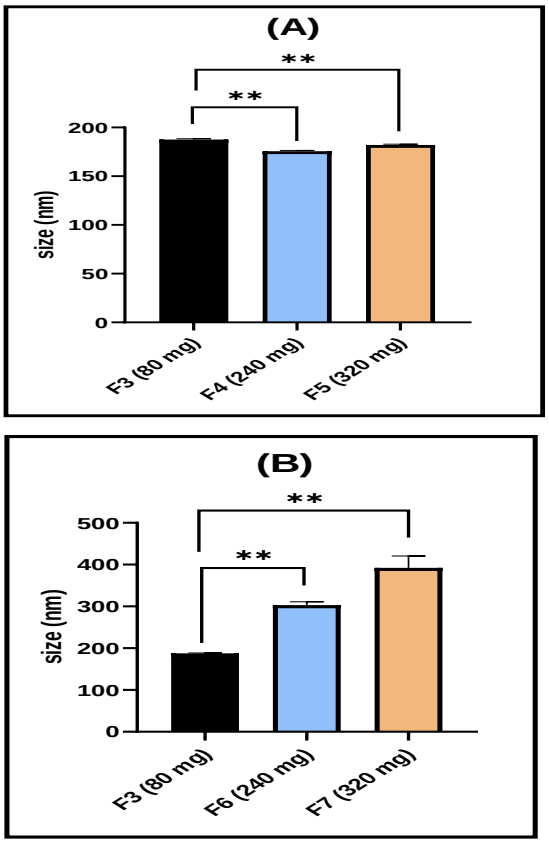


Figure 5. (A) Effect of surfactant conc. on vesicle size, (B) Effect of cholesterol conc. on vesicle size.

At a modest level of SDC (10 mg), bilosomes showed the smallest vesicle size F8. This could be related to the SDC's surface activity and molecular aggregation. While a high level of SDC F9 (20 mg) be likely to cause molecular accumulation, a low level of SDC has inferior ability to lessen the surface tension for membrane curvature, (Figure. 6A) [28].

Sonication tend to reduce particle size so increasing sonication time from 10 min in F4 to 20 min in F10 and 30 min in F11 accompanied by significant ($p<0.05$) size reduction from 175.7±0.68 into 161.8 and 138.3±4.84 nm respectively, as seen in (Figure. 6B). It is predicated on the hypothesis that ultrasonic waves are responsible for cavitation, or the mechanical cavitation of formed bubbles in liquids.

Generation of extremely high temperature, shock waves and high pressure as a result of final collapse of bubbles so, larger vesicles are then randomly but evenly broken down by the ultrasonic high energy to small discoid fragments which fold up to form thermodynamically stable vesicles. A significant decrease in the mean hydrodynamic diameter (p-value<0.05) is associated with the use of size reduction technique represented by bath sonicator [29].

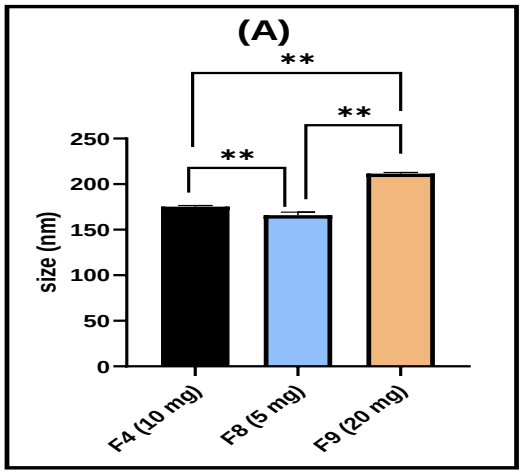


Figure 6. (A) Effect of SDC conc. on vesicle size,

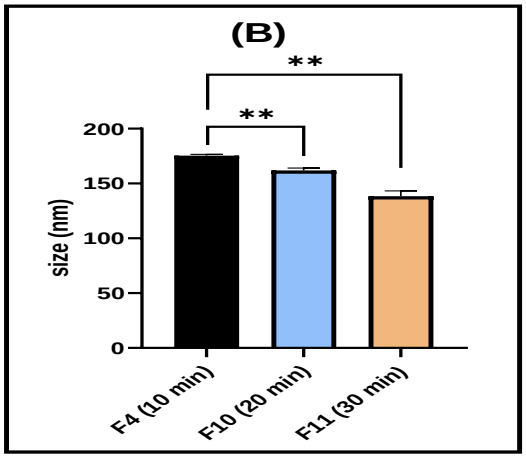


Figure 6. (B) Effect of sonication time on vesicle size.

Table 2. Mean Particle Size (PS), Polydispersity Index (PDI) and Entrapment Efficiency of Different NSD BS formulations, mean ± SD, (n=3)

Formula No.	Particle Size	PDI	Entrapment Efficiency (EE%)
F1	238.23 ± 2.30	0.3 ± 0.04	45.46±1.01%
F2	216.63 ± 0.53	0.26 ± 0.13	58.58±0.17%
F3	187.6 ± 0.45	0.28 ± 0.025	65.1±0.46%
F4	175.73 ± 0.39	0.19 ± 0.03	82.36±0.80%
F5	182.1 ± 0.47	0.25 ± 0.13	80.13±1.13%
F6	303.3 ± 4.55	0.36 ± 0.035	77.2±1.10%
F7	356.43 ± 3.37	0.26 ± 0.058	58.42±0.39%
F8	166 ± 1.83	0.33 ± 0.3	71±0.42%
F9	211.5 ± 0.74	0.19 ± 0.081	60.13±0.78%
F10	161.8 ± 1.28	0.24 ± 0.15	66.13±0.64%
F11	138.36 ± 2.79	0.38 ± 0.082	50.25±0.1%

Entrapment Efficiency

The impact of the length of the alkyl chain on the BS vesicles can be explained by the fact that the BS created with span 60 (F3) generally displayed greater entrapment efficiency (65%) than those formulated with the span 20 (F1) and span 40 (F2) that showed entrapment efficiency 54% and 58% respectively, as seen in (Figure. 7A).As span 60 has the longest saturated alkyl chain (C18), it produces more firm membrane bilayers followed by span 40 (C16) and span 20 (C12) [30]. Additionally, the alkyl chain length affects the surfactant's HLB value, which in turn may have an impact on the effectiveness of drug entrapment; the higher the drug entrapment efficiency, the lower the surfactant's HLB value. This effect was documented in the literature for both span 20 (HLB = 8.6) and sp60 (HLB = 4.7) niosome preparations, with the latter yielding a higher entrapment efficiency [31].

By increasing the span 60 concentration from 80 mg in (F3) into 240 mg in (F4) entrapment efficiency was increased significantly ($p<0.05$) from 65% into 82% due to the fact that increase span 60 concentration reduces the interfacial energy as well as increases the viscosity of the dispersion, which is prime to avoid the leak of drug from vesicles. Additionally, span 60 extended alkyl chain (C18) demonstrated the lipid bilayer's high stability and enhanced EE by reducing drug leakage [24].

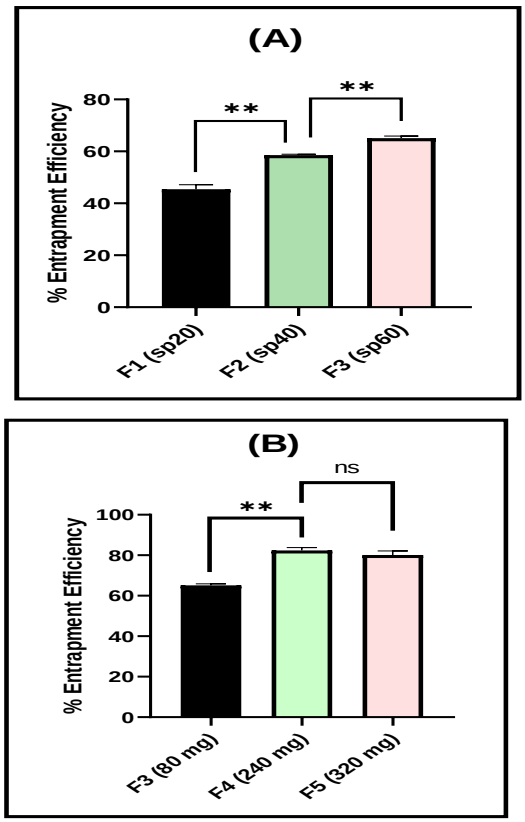


Figure 7. (A) Effect of type of surfactant on entrapment efficiency (B) Effect of increase concentration of span 60 on entrapment efficiency.

It was clearly depicted from the data shown in table (2) that entrapment efficiency was increased significantly ($p<0.05$) on increasing the cholesterol amount from 80mg in F3 into 240 mg in F6 whereas on further increase in cholesterol amount from 240 to 320 the entrapment efficiency was decreased from 77.2% in F6 into 58.42% in F7.

Two mechanisms may have contributed to this outcome. First, when the cholesterol ratio rises, bilayer vesicles' hydrophobicity and stability increase while their permeability decreases, which may effectively trap hydrophobic drugs in bilayers as the vesicles form. A second possibility is that a larger concentration of cholesterol would displace the drug from the bilayer, preventing the amphiphiles from assembling into drugs [(32)].

Increasing the amount of bile salt (SDC) from 10 to 20 mg leads to decrease EE% this due to the fact that increasing bile salt amount will increase possibility of the existence of mixed micelles that, in turn, will raise solubility of NSD in the aqueous phase. Additionally, higher bile salt levels improve the fluidity of the vesicular membranes, making them more permeable and decreasing the EE% of NSD [(33)].

Sonication time showed a significant effect (p-value<0.05) on entrapment efficiency, increase sonication time from 10 min in F3 to 20 min in F10 showed decrease in entrapment efficiency from 82% to 66% further increase in sonication time to 30 min in F11 leads to further decrease in entrapment efficiency to 50% as seen in (Figure. 9), this may be due to breaking the vesicles of BS and escape out the drug.

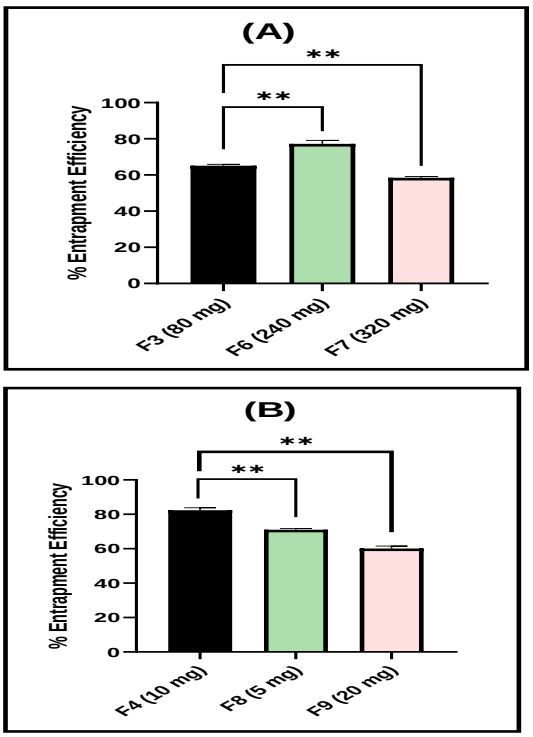


Figure 8. (A) Effect of cholesterol conc. on EE% (B) effect of conc. of SDC on EE%

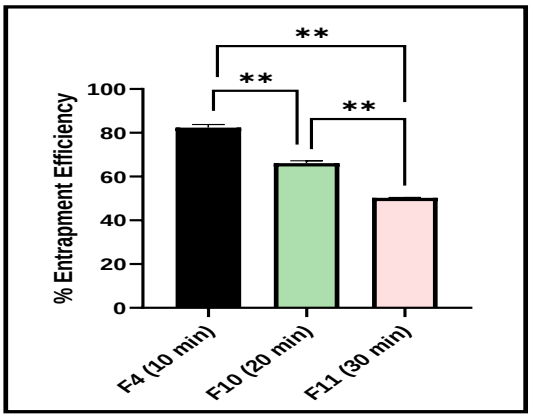


Figure 9. Effect of increasing sonication time on entrapment efficiency.

Poly dispersity index (PDI)

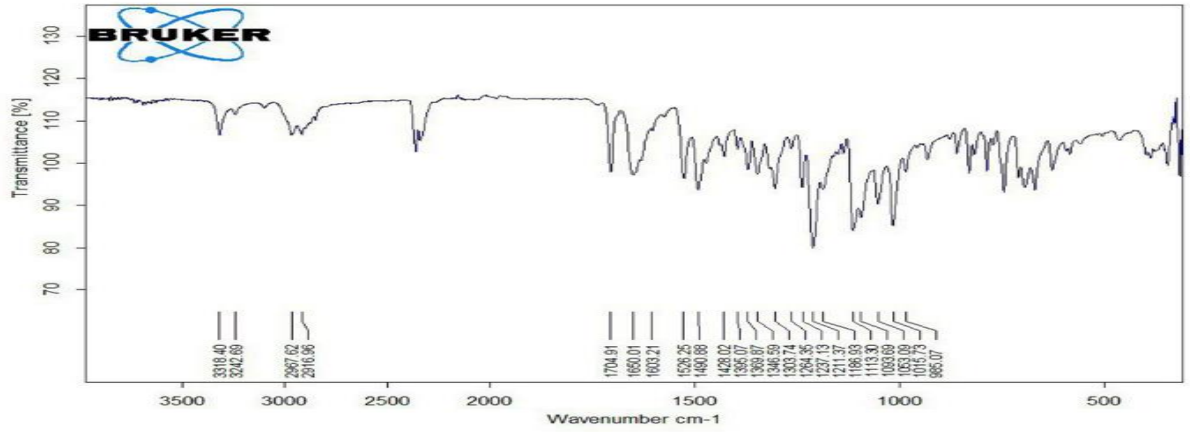
All NSD-BS formulations showed PDI between 0.1- 0.4 which indicate homogenous formulations. PDI values (Table 2) of ≤ 0.3 indicates homogenous monodispersed formulation with good stability and uniformity in droplet size distribution upon dilution [(34)].

Fourier transform infrared spectroscopy

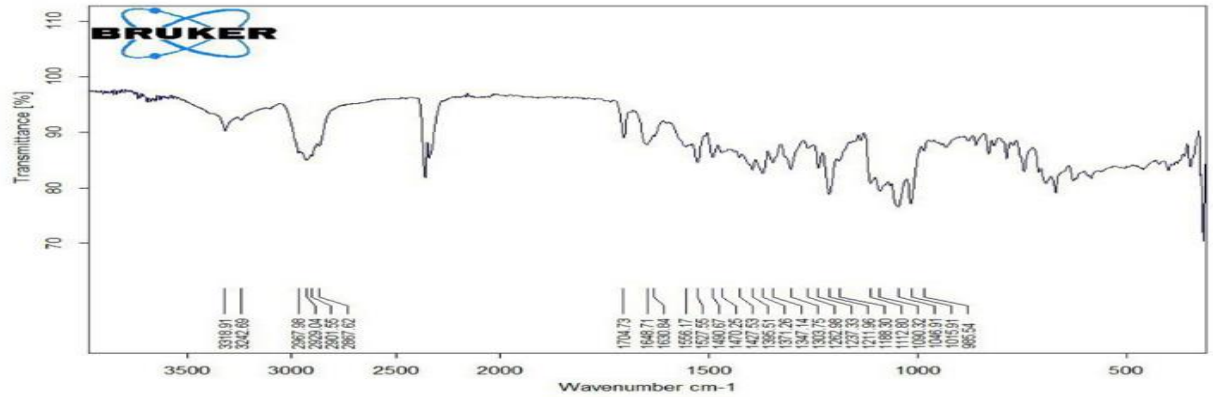
FTIR for pure drug, physical mixture and selected formula were shown in (Figure. 10). The spectrum of pure NSD showed a characteristic peak at 3318.40 cm^{-1} (N-H stretching), 1650.01 cm^{-1} (C-

O stretching), 1526.25 cm^{-1} (N-O stretching), and 1211.37 cm^{-1} (C=O stretching).

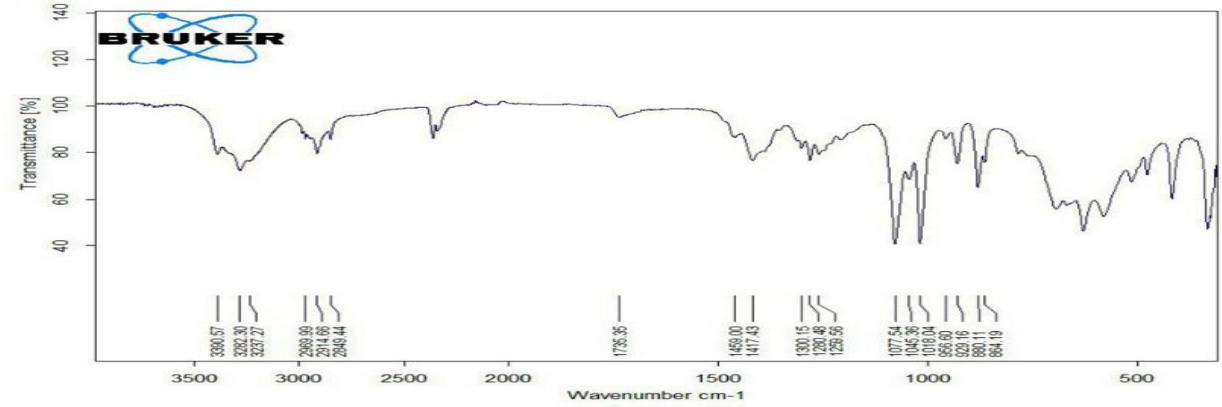
FTIR spectrum of physical mixture demonstrates a slight shift of the (C-O stretching vibration) from 1650.01 cm^{-1} to 1648.71 cm^{-1}, while the other principal peaks remain unchanged without any shifts. This indicates that no interaction between NSD and other materials used for bilosomes preparation.FTIR spectrum of the F4 NSD-loaded nanbilosomes formulation (Figure. 10C) lacks all the principal peaks of NSD which is due to the entrapment of drug in the bilosomes vesicles.



(A)



(B)



(C)

Figure 10. FTIR spectrum (A) pure NSD (B) physical mixture (C) F4 NSD-loaded nanobilosomes.

Differential scanning calorimetry

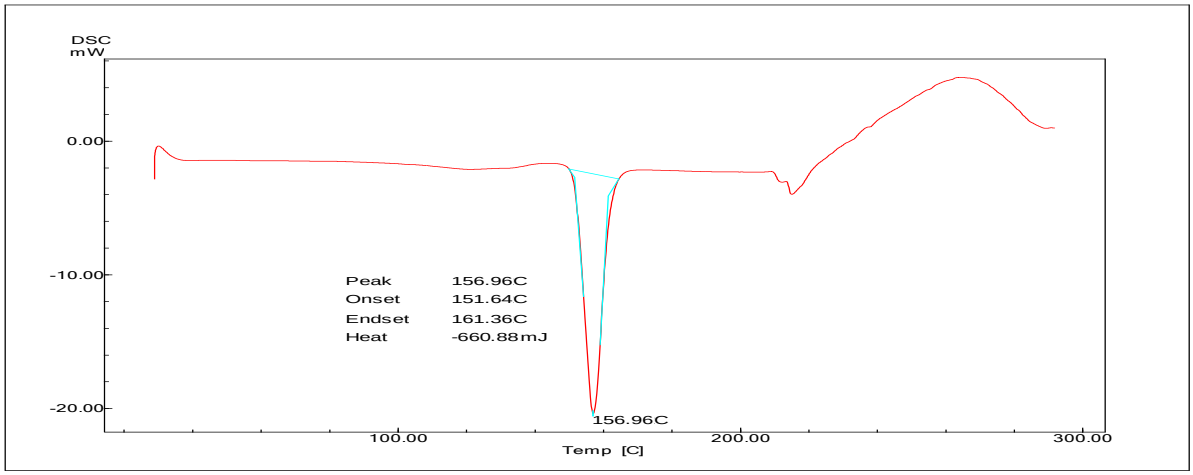
DSC thermograms of pure drug, physical mixture of drug and other components and lyophilized bilosome formulation (F4) are shown in (Figure. 11).

The DSC thermogram of pure ND showed a sharp endothermic peak at 156.96 ºC Figure. 9A which is corresponding to its melting range (152-157ºC) [35].

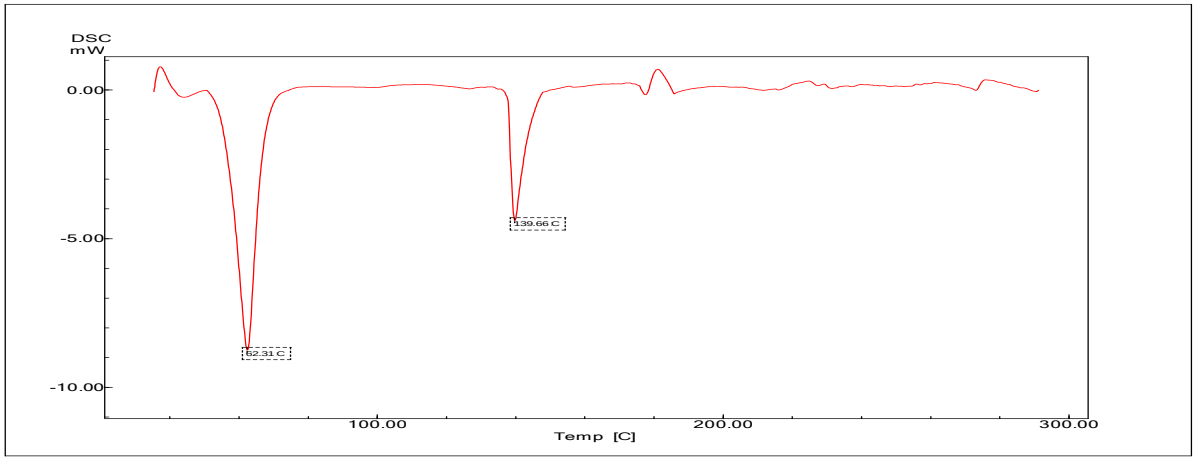
In the physical mixture (Figure. 11B), melting endotherm of drug was shifted into 139.6°C, it is known that the quantity of material used, especially in drug-excipient mixtures, could influence the peak shape and enthalpy.

Thus, these minor changes in the melting endotherm of drug could be due to the mixing of drug and excipient, which lowered the purity of each component in the mixture and this, might not necessarily indicate potential incompatibility.

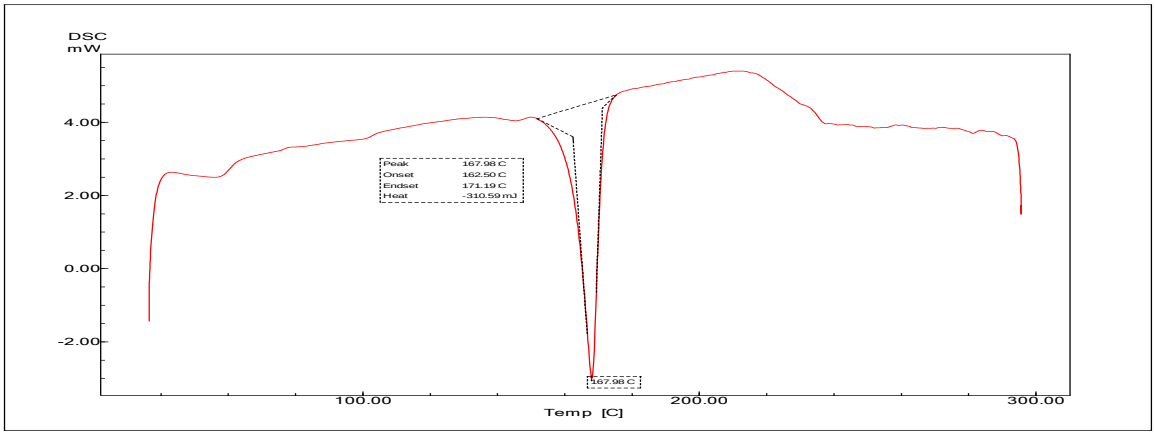
The absence of endotherm peak of drug in lyophilized formula (F4) due to the conversion of native crystalline state of the drug to amorphous state and the appeared sharp peak at 167.9°C is due to mannitol that is used in lyophilization process, as seen in (Figure. 11C) [20][36].



(A)



(B) **)**



(c)

Figure 11. DSC thermogram (A) pure drug (B) physical mixture (C) optimum formula F4.

In vitro release

In vitro release profile of NSD from F4 BS formulation showed that 96.61% of the drug released within 24 hour but with biphasic release profile with initial burst release in the first 2 h (25.43%) after that a sustained release for 24 h (96.61) was observed.

The initial burst release of NSD from the bilosomes was because of the desorption of NSD from the surface of bilosome vesicle in the initial 2 h whereas the sustained release of NSD from the optimized bilosomes was due to the high affinity of NSD for the hydrophobic counterpart of the vesicles.

The quick initial release of NSD, followed by a slow release over 24 h, would provide rapid drug onset while also allowing the patient to continue on therapy with fewer doses throughout the day [37].

While the in vitro release profile for NSD suspension showed only 41.13% drug release over a period of 8 h. The bilosomes showed significantly higher release ($p < 0.05$) of the drug than pure drug suspension [38].

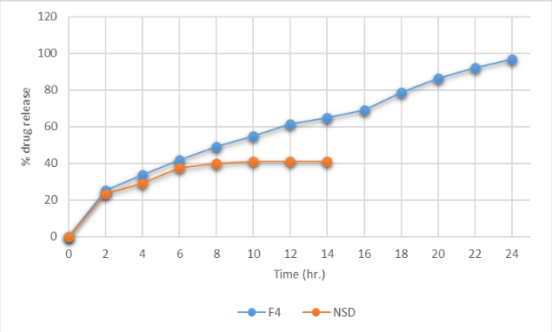


Figure 12. *In Vitro* release profile of optimum bilosome formula F4 in comparison with NSD suspension.

Conclusion

A promising strategy to address the limitations of antihypertensives is to use nanotechnology-based delivery methods. By altering both the drug's bioavailability and its release pattern, nanotechnology can successfully regulate high blood pressure. The NSD-BS formulation was prepared with a different type of surfactant and amount of bile salt along with different sonication time. The results obtained from the study are encouraging, as NSD-BS resulted were in nanosize range with good entrapment along with sustained release of drug which enable best delivery of NSD. In addition bilosomes prevent degradation of drug by the bile salts of the intestine resulting in increased its bioavailability.

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Conflict of Interest

There is no conflict of interest in this work.

Funding

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Ethics Statements

There is no need for ethical approval in this work.

Author Contribution

The authors responsibilities are described as follows: Preparation, collecting and analyzing data: Ghada Hamid. Designing, reviewing and supervising the project: Fatima Jalal.

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