FORMULATION DEVELOPMENT, OPTIMIZATION, EVALUATION, AND STABILITY STUDIES OF NIOSOMES

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Running Title: Formulation and Evaluation Niosomes.

Abstract

The niosomes are organized using thin-film hydration and sonication techniques. For the film hydration method, dissolve surfactant, cholesterol, stearylamine (65.0:30.0:5.0 mM) and captopril (2 mg) in aggregates containing chloroform:ethanol (3: 1).

The solvent was evaporated under vacuum on a rotary evaporator to form a thin film. Using a stirrer, replace the membrane then hydrate with 10 mL of phosphate buffer (pH 7.4). For ultrasonic techniques, surfactants, cholesterol, stearylamine (65.0:30.

0:5.0 mM) and captopril (2 mg) were mixed in a beaker, and 10 ml of phosphate buffer (pH 7.4) was added. I left it for five hours. White dispersions (niosomes) were synthesized 7 min before sonication as needed.

In the ether injection technique, a solution containing a mixture of ether and surfactant is introduced slowly into an aqueous solution (preheated to 60°C) to evaporate the ether and synthesize a milky paste. The products were thus analyzed for a number of parameters, including percent encapsulation efficiency, vesicle length, polydispersity index, and zeta capacity, and were determined to be state-of-the-art. technology for making niosomes based on this specification.

Introduction

The transdermal drug delivery process offers many advantages for adjacent drug delivery and systemic therapy. However, skin is often misdiagnosed for its strong barrier properties compared to other biofilms due to the impermeable nature of the stratum corneum. Due to the low permeability of the skin, the drug entry point is small. Various drug delivery modules are available to increase the permeability of molecules. b.

Energetic and passive devices including iontophoresis, phonophoresis, penetration enhancers and other drug delivery modules. Vesicular delivery of the drug may be beneficial because the vesicles tend to fuse together and adhere to the moving bed, primarily due to the excellent permeability of the drug at the interface of the pores and the stratum corneum of the skin. It is believed to increase thermodynamic activity. These vesicles act as drug depots. Surface charges on the vesicles can modulate the rate of drug release at the target site.

The surface charge of vesicles is usually assessed by measuring the zeta potential. It provides data on the general grounding rate of particles and the consequences of environmental changes, and through the steady efforts of formulators, increasingly complex drug delivery structures are designed. The scientific goals are to broaden structure, minimize side effects, provide long-term therapeutic benefits at lower doses, reduce frequency of administration, and improve patient compliance.

Optimization of formulation ingredients

Generation of niosomes in different batches using current and type switching of nonionic surfactants. Therefore, after selecting the optimized formulation, the obtained products were analyzed for various indicators such as polydispersity index, zeta strength, vesicle length and encapsulation efficiency.

Materials and Method

Formulation and evaluation of niosomes

Niosomes are capable of entangling a wide variety of medicine. They include non-ionic stearylamine, LDL cholesterol, and surfactants.

Selection of nio somes method of preparation

Nio someswere synthesized by different methodologies

1. Etherinjection Technique
2. Thin film hydration Technique
3. Sonication Technique

The formulation was analyzed for the vesicle size, polydispersity index, zeta potential, and entrapment efficiency, the result of which are listed in Table1.

Table1: Optimization of method for niosomepreparation

Code	Method of preparation	Vesicle size in (nm)	PDI	Zeta potential(mV)	%EE
F1	Ether injection method	715.0 ± 6.4	0.357	-34 ± 4.7	83.3 ± 3.14
F2	Film hydration method	524.0 ± 3.8	0.345	-37 ± 9.3	85.1 ± 2.15
F3	Sonication method	299.5 ± 2.1	0.254	-45.5 ± 9.3	92.1 ± 3.4

Span60: Cholesterol:Stearylami ne:65.0:30.0:5.0mM, Captopril:2mg, Polydispersity Index:PDI, Entrapment efficiency: EE, Millivolts:mV

Optimization of formulation ingredients

Niosome preparations were prepared by the sonication method with varying concentrations of nonionic surfactant, cholesterol, and captopril, as shown in Table 2, and analyzed for vesicle size, polydispersity index, zeta potential, trapping efficiency, etc. were evaluated for various parameters.

Table2: Optimization of niosome composition

Code	Concentration(mM)			Risp (mg)	Vesicle size(nm)	PDI	Zeta potential (mV)	%EE
	Sp60	Ch	SA					
C1	30.0	65.0	5.0	2	202.7 ± 6.7	0.309	-37.3 ± 6.48	48.3 ± 3.01
C2	47.5	47.5	5.0	2	269.4 ± 7.1	0.161	-39.7 ± 5.35	65.70 ± 2.8
C3	55.0	40.0	5.0	2	207.2 ± 5.7	0.243	-39.5 ± 6.84	79.89 ± 3.4
C4	65.0	30.0	5.0	2	238.1 ± 3.2	0.256	-45.0 ± 8.35	92.83 ± 2.4
C5	65.0	30.0	5.0	5	242.3 ± 4.5	0.234	-46.0 ± 8.35	94.36 ± 5.7
C6	65.0	30.0	5.0	10	245.7 ± 6.5	0.248	-45.6 ± 8.35	95.83 ± 6.1

Span60:Sp60,Cholesterol:Ch,Stearylamine:SA,Millimoles:mM,PolydispersityIndex:PDI,

Entrapmentefficiency:EE, Millivolts:mV

Continuous efforts are developing increasingly sophisticated drug delivery systems. Niosomes containing different kinds of surfactants were also synthesized by sonication technique and analyzed for vesicle size, zeta potential, polydispersity index and trapping efficiency. These are listed in Table 3.

Table3:Selection ofsurfactantfor niosomes

Formulation Code	Composition (mM) 65.0:30.0:5.0	Vesiclesize (nm)	PDI	ZetaPotential (mV)	%EE
S1	Sp20:Ch:SA	232.0 ± 7.5	0.171	-24.0 ± 3.76	74.5 ± 5.3
S2	Sp40:Ch:SA	219.1 ± 3.4	0.277	-40.1 ± 7.79	90.01 ± 4.2
S3	Sp60:Ch:SA	213.9 ± 3.1	0.269	-50.6 ± 9.31	92.83 ± 3.4
S4	Sp80:Ch:SA	290.2 ± 4.3	0.209	-35.4 ± 3.96	78.5 ± 2.35
T1	Tw20:Ch:SA	388.9 ± 8.8	0.350	21.9 ± 4.62	62.31 ± 3.9
T2	Tw40:Ch:SA	258.7 ± 7.3	0.437	21.3 ± 3.83	70.87 ± 4.2

T3	Tw60:Ch:SA	248.2 ± 7.5	0.427	20.4 ± 4.31	72.47 ± 5.3
T4	Tw80:Ch:SA	180.0 ± 8.7	0.389	25.1 ± 3.74	68.62 ± 3.1

Span:Sp, Cholesterol:Ch, Stearylamine:SA, Millimoles:mM, Polydispersity Index:PDI, Entrapmentefficiency:EE,Millivolts:mV,Tween:Tw

Result and Discussion

a. Entrapment efficiency(%EE)

Formulation number S3showed the highest entrapment efficiency (92.83 ± 3.4%) and was found in the order ofSpan60>40 > 80> 20 which could be due to its structure,3-dimensional orientation, and behavior of its surfactants

Span60 ((lower HLB 4.7) seems to be the best carrier with good efficiency of entrapmentthanSpan20andSpan40(HLB8.6and6.7).

b. Vesicle size, zeta potential, and polydispersity index

Niosomes synthesized using Span60 have the smallest vesicle size. This may be due to the low HLB. Excessive zeta potentials in all cases (Figure 1) reflected that the formulations were potent, and the reduction in PDI confirmed that the formulations were homogenous.

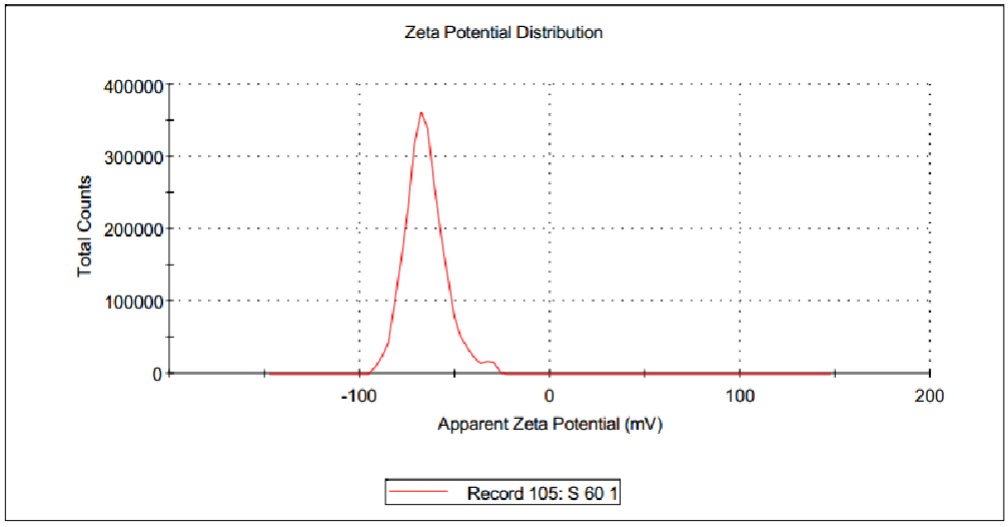

Fig1: Zeta potential of Captopril niosomeswithSpan60

c. Niosomes with an interval of 60 had the best adhesion performance (92.83 ± 3.4%). Selected formulations were chosen for further evaluation because the in vivo performance of niosomes depends on the amount of drug loaded into the niosomes.

***In-vitro* release study**

Figure 2 shows the cumulative drug release profile of captopril from the niosome formulation. This indicates that the release of captopril from the niosomes is rapid and slow during the first hour compared to the release rate of captopril API (suspension). About 98% of the drug was released from the captopril drug suspension within 6 hours, while only 29% was released from the niosomes. Top 20 team (88.36%).

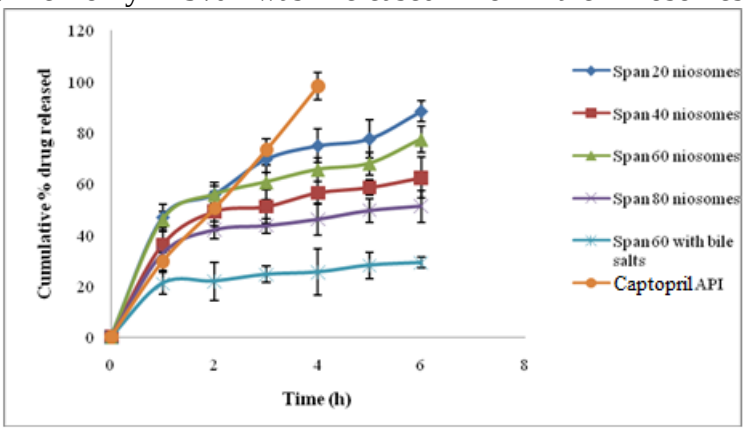

Fig2:*In-vitro*releasestudy ofCaptopril from niosomes

d. Stability studies of niosomes in presence of bile salts

Preparations of niosomes containing different groups of species were incubated with 0, 2.5, 5, 7.5, 10, 15 and 20 mM sodium deoxycholate solutions at 37°C for 1 h, and the effect was measured using a turbidimeter. Analysis and evaluation (Figure 3).

From Figure 16, it can be seen that the turbidity above 10 mM drops sharply with kilometers due to the solubility of the bilayer. The Span60 formulation did not significantly reduce haze. This is due to the high cutting transition temperature (Table 4). The Span60 formulation exhibited the highest turbidity even after the release of 20 mM sodium deoxycholate, confirming the structural tension of the niosome bilayer. The low turbidity of the Span20 and Eighty formulations is due to their fluidity, which allows a smooth transition from bilayer to micellar form.

Table4:Effect ofbilesaltson niosomes containingdifferent typesof span

Sodium desoxycholate (mM)	Turbidity(NTU)			
	Span20	Span40	Span60	Span80
0	500	500	500	500
2.5	482	490	492	411
5	441	465	484	372
7.5	423	452	471	331
10	407	431	460	309
15	258	339	433	181
20	159	283	363	103

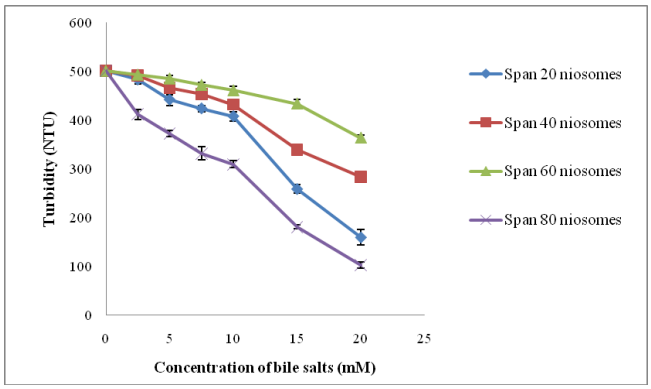

Fig 3: Effect of bile salts on niosomes containing different types of span

Table 5: Effect of bile salts on niosomes containing bile salts as an integral component

Sodiumdes oxycholate (mM)	Turbidity (NTU)				
	Span60 (0mM)	Span60 (2.5mM)	Span60 (5mM)	Span60 (7.5mM)	Span60 (10mM)
0	500	500	500	500	500
2.5	485	494	496	498	495
5	474	487	488	490	486
7.5	455	478	480	486	476
10	420	462	467	470	445

15	400	430	440	460	419
20	363	410	435	452	380

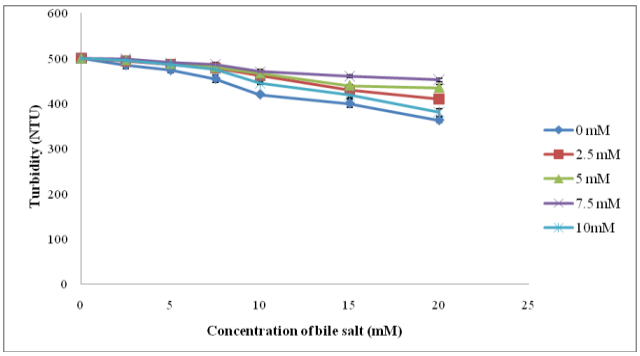

Fig 4: Effect of bile salts on niosomes containing bile salt as integral components

e. Morphology

The morphological properties of niosomes (S3) were observed by transmission electron microscopy. TEM photomicrograph of lyophilized niosomes is shown in Fig 5,

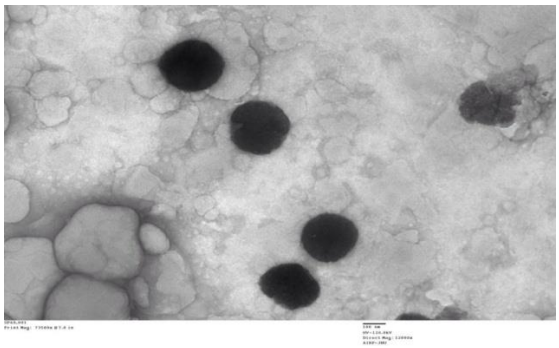

Fig 5: Transmission electron photomicrograph of Captopril niosomes

f. Drug-excipient compatibility studies

Drug-excipient compatibility studies were performed using infrared spectroscopy and differential scanning calorimetry.

Drug-excipient compatibility study using FTIR spectroscopy

FTIR spectra of captopril, Span60, aggregates (1:1), empty niosome and charged niosome

of captopril are shown in Figure 2. N-H bent), 1128 cm -1 (C-H stretched), 853 cm -1 (C-H bent, aromatic) and 3744 cm -1 (N-H stretched). Span60 is 1200 cm-1 (aliphatic), 1734 cm-1 (five-membered cyclic ring), 1400 cm-1 (-CH3), 2928 cm-1 (aliphatic C-H stretch, not equal), 2800 cm-1 (aliphatic relative ring) C-H-root). , symmetric) and 3400 cm-1 (O-H stretch).

Analysis of the physical mixture of captopril and Span60 showed the presence of characteristic captopril peaks in the physical mixture similar to the individual captopril spectra, no detectable changes in the FTIR spectra, and no chemical differences between them. Indicates that no significant interaction was found. His FTIR spectra of empty and captopril-loaded niosomes showed weak interactions between cholesterol and Span60 in the 3600 cm-1 and 3200 cm-1 wavenumber regions. Between N-H, C=O, -OH and other functional groups, van der Waals force, dipole moment, etc. Leads to the formation of hydrogen bonds and other weak bonds.

However, these interactions may promote the formation of the vesicle shape, thereby stabilizing the structure and delaying drug release. Formation of hydrogen bonds between membrane components of niosomes and its impact on encapsulation efficiency and slowed drug release.

Fig 6: IR spectra(a) Captopril (b) Span 60 (c) physical mixtures (d) blank niosomes (e) Captopril loaded niosomes

Drug-excipient compatibility take a look at the usage of differential scanning calorimetry (DSC)

The thermal transition of captopril occurs at 174°C, which corresponds to its melting point, and it exhibits a sharp endothermic peak at this temperature. Span 60 exhibits an endothermic peak at 59.32°C, and the disappearance of this peak is shown in Figure 3. 20b and 20c are due to cholesterol forming a bilayer.

Wilke et al. Using molecular modeling studies and thermal analyses, we have shown that during the formation of the niosome bilayer, cholesterol is embedded between surfactant molecules, resulting in rigidity of the vesicles.

g. Drug release kinetics

The release of captopril from the niosomes followed the Korsmeyer-Peppas model (Table 6). For n values less than 0.5, the release of captopril follows a Fick diffusion mechanism. H. Drug release occurs through a chemical potential gradient from high to low concentration due to molecular diffusion.

The Korsmeyer-Peppas model also indicates several concurrent processes such as B. Diffusion of water into the drug reservoir, drug swelling, gelation, and leaching during absorption.

Table 6: Drug release kinetics of the optimized formulation

Release-Kinetics	**Zero order**		**First order**		**Higuchi Model**		**Korsmeyer Peppa's model**	
	K	r^2	K	r^2	K	r^2	n	r^2
	5.6782	0.9668	0.0406	0.9424	19.816	0.9725	0.2679	0.9759

h. Stability studies (at different temperatures)

The stability of the Niosome was evaluated under different temperature conditions (4 ± 1 ºC and 25 ± 2 ºC). He analyzed the formulation for 3 months and observed no visual changes in the niosome formulation. The results are shown in Table 7.

Table7: Effect of temperature on niosome formulations

Temperature	**Time-interval (days)**	**Parameters**		
		Appearance	**Vesicle-Size (nm)**	**Entrapment-efficiency(%)**
Refrigeration (4 ± 1ºC)	0	No-change	299 ± 0.1	85
	45	No-change	308 ± 1.2	80
	90	No-change	315 ± 3.6	79.8
Room temperature (25 ± 2ºC)	0	No-change	299 ± 0.1	85
	45	No-change	312 ± 2.8	83
	90	No-change	322 ± 2.1	78.97

The niosome capture efficiency did not change significantly between 4 ± 1°C and 25 ± 2°C. Encapsulation efficiency was slightly reduced, possibly due to drug leakage from the vesicles. Stability studies showed that the niosome formulation was more stable at 4 ±

1°C than at 25 ± 2°C.

***Ex-vivo* absorption studies**

Non-inverse and inverse Ratsack techniques have been used to assess mechanisms of drug transport in the gut. This helps predict the in vivo absorption profile of the formulation in humans. Compared to the inverted sac method, the non-inverted sac model has the following advantages: Therefore, we used the non-inverted sac method to assess isolated intestinal permeability. Table 8 shows the hydraulic conductivity, flux and amplification of the formulations. The expansion rate of niosomes (without bile) was greater than that of niosomes containing bile.

Table8: *Ex-vivo* intestinal permeation study and the permeability details

Formulation	**Flux ($\mu gcm^{-2}h^{-1}$)**	**Permeability coefficient**	**Enhancement ratio**
Control	66.85	0.5571	1
S-60niosomes	139.459	1.1607	2.08
S-60niosomeswith bile	89.078	0.7423	1.33

Summary and conclusion

The niosomes were prepared using the technique of ether injection, thin layer hydration and sonication, and sonication, which resulted in a homogeneous formulation with high entrapment efficiency. At a ratio of 65:30:5 mM (Span60: cholesterol: stearylamine), its Span60 formulation achieved the highest encapsulation efficiency of 92.83%. All niosome formulations show low polydispersity indices and high zeta potential values, which guarantee the stability and homogeneity of the formulation. FTIR studies ensure that there are no drug incompatibilities.

H. Captopril and formulation additives. It promotes the formation of vesicles, stabilizes the structure and slows the release of active ingredients. DSC data also supports these findings. The formulation was more stable at low temperature (4 ± 1°C), and no

significant change in vesicle size and encapsulation efficiency was observed.

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