

Original Research Article

Development of niosomal formulations loaded with cyclosporine A and evaluation of its compatibility

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Sent for review: 4 May 2018

Revised accepted: 28 July 2018

Abstract

Purpose: To formulate niosomes of cyclosporine A using nonionic surfactants, and to use the attenuated total reflectance/Fourier transform infrared (ATR-FTIR) technique to explore solid/liquid interfacial phenomena as well as compatibility between active drug and pharmaceutical excipients.

Methods: Niosomes of cyclosporine A were prepared using the thin-film hydration method. Cholesterol and non-ionic surfactants, including polyethylene glycol sorbitan monostearate (Tween 60) and sorbitan monostearate (Span 60), were used as excipients. The ATR-FTIR spectra of all the ingredients, their physical mixtures, and niosomal formulations were studied. The niosomes were characterized for size, polydispersity index (PDI), zeta potential, and entrapment efficiency.

Results: Six niosomal formulations (F₁ – F₆) were successfully developed. Niosomal formulation F₂ prepared at the ratio of 6:4 surfactant to cholesterol, presented the highest entrapment efficiency of 77.28 %. The ATR-FTIR spectra of niosomal formulations did not show incompatibility. The size of the selected formulation (F₂) was 1049 nm while its SEM image displayed a spherical nature of the niosomes.

Conclusion: The results show that cyclosporine A can be entrapped in niosomes using non-ionic surfactants and cholesterol. Furthermore, there is no significant interaction between the ingredients of niosomes and cyclosporine A.

Keywords: Cyclosporine A, Attenuated total reflectance Fourier transform infrared spectroscopy, Niosomes, Compatibility, Non-ionic surfactants

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INTRODUCTION

In the last few decades, scientists have developed an interest in the formulations of

surfactant vesicles to improve drug delivery systems. Nanocarriers of drugs such as niosomes are formulated, which can act as “magic bullets” to target affected cells and organs [1].

Among different immunosuppressant drugs available, cyclosporine A is of unique importance. The structure of cyclosporine A contains cyclic peptide comprising of 11 amino acids (cyclic endecapeptide). Cyclosporine A is a strong immunosuppressive drug used primarily to decrease rejection after kidney, heart, lungs, liver, pancreas, and bone marrow transplants. It is also used for the prevention and cure of graft-versus-host-disease, aplastic anemia, severe psoriasis and eczema, and is used in rheumatoid arthritis of a severe nature, when second-line therapy is not available. Its usual dose in organ transplantation is from 10 mg/kg to 15 mg/kg per day for seven to fourteen days after the operation, and then the dose is reduced slowly from 2 mg/kg to 6 mg/kg per day as a maintenance dose. The therapeutic index of cyclosporine A is low, and it has many toxic effects which include gastrointestinal intolerance, hepatotoxicity, nephrotoxicity, and certain neurological issues [2,3].

Cyclosporine A is a white powder. It is not soluble in water, but it is soluble in ethyl alcohol, methyl alcohol or methanol, acetone, chloroform, ether, and dichloromethane. In saturated hydrocarbons, cyclosporine A is slightly soluble [4]. After oral administration, its absolute bioavailability and pharmacokinetics are variable, due to its less absorption. To increase the solubility of less water-soluble drugs and to increase the *in vivo* absorption of drugs, the microemulsion system was developed [5]. The oil-based formulation of cyclosporine A (Sandimmune) results in poor bioavailability and variable results in different patients. The bioavailability of cyclosporine A is also reduced due to its pre-systemic metabolism in the liver and gut wall. Sandimmune Neoral is a microemulsion-based formulation, and it provides high bioavailability through the oral route. Research is being conducted to further improve its bioavailability. Liposomes with sodium deoxycholate provide high entrapment efficiency to cyclosporine A and increased bioavailability in comparison to conventional liposomes and microemulsion-based formulations [6]. Furthermore, if we prepare niosomes of cyclosporine A, the problems associated with liposomal formulation of cyclosporine A can be resolved, and the bioavailability and dissolution profile of cyclosporine A can be improved with reduced side effects. Therefore, various niosomal formulations have been prepared to increase the solubility profile of cyclosporine A and to increase its availability at the site of absorption.

To study the chemical interactions in biological studies at solid/liquid interfaces, the ATR-FTIR technique is widely used. It evaluates the compatibility and possible interactions within ingredients of niosomal formulations [8].

EXPERIMENTAL

Materials

Cyclosporine A was obtained from Xi'an Lyphar Biotech Co., Ltd. Polyethylene glycol sorbitan monostearate (Tween 60), Sorbitan monostearate (Span 60), Polyethylene glycol sorbitan monooleate (Tween 80), and Sorbitone monooleate (Span 80) were purchased from Daejung Chemicals & Metals Co., Ltd. Cholesterol, and 1-hexadecyl pyridinium chloride monohydrate was obtained from Alfa Aesar GmbH & Co. KG, Germany. Phosphate buffered saline tablets were obtained from Oxoid Ltd, England. In the laboratory, fresh distilled water was prepared by employing a distillation apparatus. Chloroform and methyl alcohol were obtained from Daejung Chemicals & Metals Co., Ltd, Korea.

Preparation of physical mixtures

A mortar and pestle was used to prepare physical mixtures by mixing equimolar amounts of cyclosporine A, nonionic surfactants, and cholesterol. For physical mixture 1 (PM1) cholesterol, Polyethylene glycol sorbitan monostearate also known as Tween 60, cyclosporine A, and Sorbitan monostearate also known as Span 60 were mixed together, while, for physical mixture 2 (PM2), cholesterol, Polyethylene glycol sorbitan monooleate also known as Tween 80, cyclosporine A, and Sorbitone monooleate also known as Span 80 were mixed together in equimolar ratio (1:1:1:1).

The ingredients of the mixture were mixed in a mortar for approximately ten to fifteen minutes so that a homogenous mixture was achieved.

Preparation of niosomal formulation

Niosomal formulations of cyclosporine A were prepared along with non-ionic amphiphiles such as Polyethylene glycol sorbitan monostearate, also known as Tween 60, and Sorbitone monooleate, also known as Span 80, and so on, and cholesterol using thin-film hydration method. The composition of niosomal formulations is given in Table 1. In thin-film hydration method the drug/non-ionic surfactants and cholesterol were mixed in chloroform/methanol (2:1). When these ingredients were mixed in the solvent, the

Table 1: Composition of niosomal formulations

Formulation code	Surfactants (mg)		Cholesterol (mg)	Molar ratio (Surfactant: Cholesterol)	Total μ mol of lipid contents
	Tween 60	Span 60			
F1	229	75.36	57.99	7:3	500
F2	196.35	64.59	77.33	6:4	500
F3	163.62	53.83	96.66	5:5	500
	<u>Tween 80</u>	<u>Span 80</u>			
F4	229.25	75	57.99	7:3	500
F5	196.50	64.29	77.33	6:4	500
F6	163.75	53.57	96.66	5:5	500

Each formulation contained 25 mg (21 μ moles) of the drug

solution was put in the rotary evaporator at 57 °C, and it was allowed to evaporate until a thin film was formed on the wall of flask. Then for one hour, this thin film was hydrated with 20 mL of phosphate buffer saline (pH 7.4) in a water bath using gentle stirring at 60 °C. Consequently, the niosomal preparations were stored in a refrigerator at 4 – 8 °C overnight, so that the niosomes matured [9].

ATR-FTIR spectroscopic studies

The spectra of cyclosporine A, non-ionic amphiphiles, cholesterol, physical mixture 1 and 2, and formulations of niosomes were taken from FTIR Spectrophotometer (Thermo Nicolet 6700, USA) in the wave number range of 4000 – 500 cm^{-1} . For scanning pike, the single bounce attenuated total reflectance (ATR) method was used.

Characterization of niosomes

Size and zeta potential

The size, polydispersity index (PDI), and zeta potential were determined by using Malvern zetasizer, version 7.11. It gives size distribution report by intensity.

Morphological features

The shape and morphology were studied using the field emission, scanning electron microscope. For the niosomal formulation, a drop is taken and placed on an aluminum stub using adhesive silver tape. Aluminum stubs were placed in a vacuum overnight and then sputter coated, in the process of which gold was used [10].

Drug entrapment studies

The niosomes loaded with cyclosporine A were separated by ultracentrifugation at 12000 \times g for thirty minutes at 4 °C. The supernatant was separated, and niosomes were disrupted using methanol [11]. High pressure liquid

chromatography was used to determine the amount of cyclosporine A in niosomal formulations. [12,13]. Nucleosil C18 column was used. Water and acetonitrile in ratio 3:7 and v/v were used in the mobile phase. The mobile phase was filtered through a 0.45- μ m membrane filter, and a syringe filter of nylon 0.45 μ m was used to filter samples. Acetonitrile can dissolve the cellulose filters, consequently, nylon filters were used instead. The flow rate 1.0 ml/min was taken, and at 210 nm, the effluents were monitored [14].

Statistical analysis

One-way analysis of variance (ANOVA) was conducted at 95% confidence interval using GraphPad Prism 6 software. The significance level was set to $p < 0.05$. It is used to compare the mean values of niosomes size and entrapment efficiencies of six niosomal formulations, and it is used to determine statistical significance.

RESULTS

ATR-FTIR spectra of components of niosomal formulation are shown in Figure 1. The components are (A) Cyclosporine A, (B) Cholesterol, (C) Sorbitan monostearate (Span 60), (D) Sorbitone monooleate (Span 80), (E) Polyethylene glycol sorbitan monostearate (Tween 60), (F) Polyethylene glycol sorbitan monooleate (Tween 80), and (G) Hexa decyl pyridinium chloride. Characteristic peaks of individual components are shown in Table 2. The characteristic peaks in the FTIR spectrum of cyclosporine A, cholesterol, Span 60, Span 80, Tween 60, and Tween 80 and are similar to peaks as given in literature, which indicates the purity of samples and the fact that they are suitable for niosomal formulations.

ATR-FTIR spectra of physical mixtures (PM1 and PM2) are shown in Figure 2. It shows similar peaks in the spectra of physical mixtures as in the individual spectrum of pure ingredients.

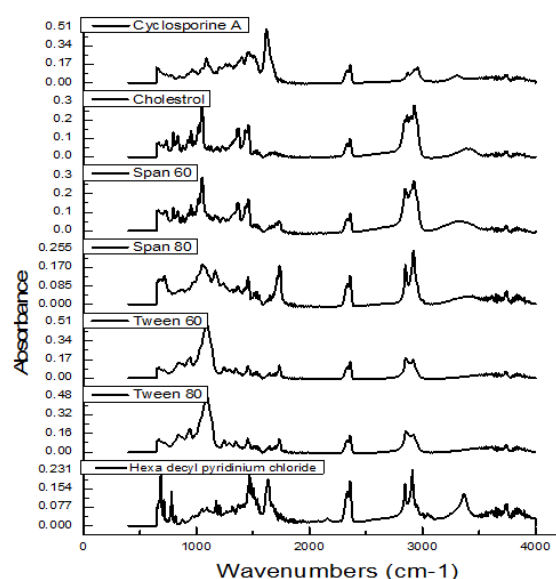


Figure 1: ATR-FTIR spectra of individual components

Characteristic peaks of physical mixtures are depicted in Table 3. No significant shifts of peaks were observed. Consequently, it was observed that there were no interactions between the ingredients.

The ATR-FTIR spectra of niosomal formulations (F1, F2, F3, F4, F5, and F6) are shown in Figure 3. In the spectra of niosomal formulation, most of the peaks are diffused, demonstrating a strong physical interaction between cholesterol, cyclosporine A, and surfactants. Characteristic peaks of niosomal formulations are given in Table 4. No significant shifts of new peaks were observed which indicates that no chemical interaction is present.

FTIR spectrum of cyclosporine A

Briefly, the characteristic peaks in spectra of cyclosporine A were at 3310 cm^{-1} , 2957 cm^{-1} , 1621 cm^{-1} , and 1091 cm^{-1} , which indicates amines, carboxylic acid, amides, and the alkyl substituted ether functional group respectively.

The characteristic peaks of 2360 cm^{-1} which indicate the primary and secondary amines functional group were found in the FTIR spectrum of cholesterol, cyclosporine A, Span 60, Span 80, Tween 60, Tween 80, hexa decyl pyridinium chloride, physical mixture 1 and 2, and also in formulation 1, 4, and 5.

The characteristic peaks of 1457 cm^{-1} , 1458 cm^{-1} , and 1459 cm^{-1} which indicate alkanes and the aromatic ring stretch functional group were found in Tween 80, cholesterol, Span 80, cyclosporine A, Span 60, physical mixture 1 and 2, and in formulation 1 and 5.

Table 2: Characteristic peaks of individual components

Component	Characteristic peak (cm^{-1})	Functional group
Cyclosporine A	3310	Amines or hydroxyl group
	2957	Carboxylic acids
	2360	Primary and secondary amines
	1621	Amides, Conjugated ketone
	1457	Alkanes, Aromatic ring stretch
	1091	Amines, Alkyl substituted ether
Cholesterol	967	Alkenes
	3420	Hydroxy group
	2929	Carboxylic acids
	2360	Primary and secondary amines
Span 60	1459	Alkanes, Aromatic ring stretch
	3310	Amines or hydroxyl group
	2926	Carboxylic acids
	2360	Primary and secondary amines
	1733	Aldehyde
Span 80	1052	Cyclohexane ring vibrations, Alkyl substituted ether
	2921	Carboxylic acids
	2360	Primary and secondary amines
	1736	Esters
Tween 60	1457	Alkanes, Aromatic ring stretch
	1054	Cyclohexane ring vibrations, Alkyl substituted ether
	2920	Carboxylic acids
	2360	Primary and secondary amines
Tween 80	1734	Aldehyde
	1092	Amines, Alkyl substituted ether
	2856	Carboxylic acids
Hexa decyl pyridinium chloride	2360	Primary and secondary amines
	1734	Aldehyde
	1457	Alkanes, Aromatic ring stretch
	1091	Amines, Alkyl substituted ether
	3367	Amines or hydroxyl group
	2912	Carboxylic acids
	2360	Primary and secondary amines
	1635	Secondary amine, Conjugated ketone
	1471	Methylene C—H bend, carbonate ion
	1177	Secondary amine

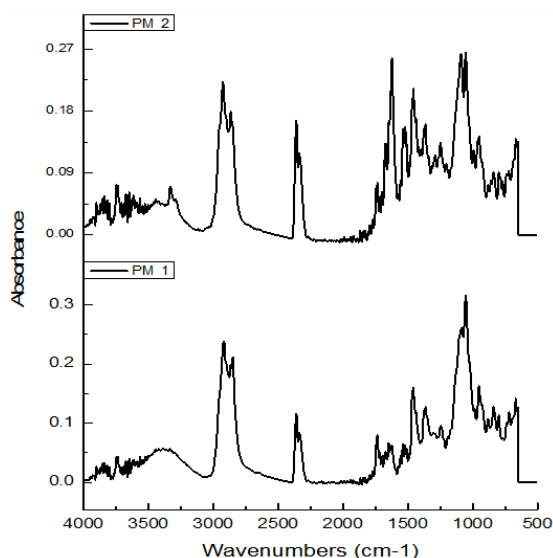


Figure 2: ATR-FTIR spectra of physical mixture 1 (PM1) and physical mixture 2 (PM2)

Table 3: Characteristic peaks of physical mixtures

Physical mixture	Characteristic peak (cm ⁻¹)	Functional group
PM1	3420	Hydroxy group
	2919	Carboxylic acids
	2360	Primary and secondary amines
	1734	Aldehyde
	1458	Alkanes, Aromatic ring stretch
	1054	Cyclohexane ring vibrations, Alkyl substituted ether
PM 2	2927	Carboxylic acids
	2360	Primary and secondary amines
	1622	Secondary amine, Conjugated ketone
	1457	Alkanes, Aromatic ring stretch
	1091	Amines, Alkyl substituted ether
	1054	Cyclohexane ring vibrations, Alkyl substituted ether
	951	Alkenes

The characteristic peaks of 1091 cm⁻¹ and 1092 cm⁻¹ which indicate amines and alkyl substituted ether functional groups were found in cyclosporine A, Tween 60, and 80 and physical mixture 2.

FTIR spectra of excipients

In short, the characteristic peaks in spectra of cholesterol were at 3420 cm⁻¹ and 2929 cm⁻¹, which indicates the hydroxy group and the carboxylic acid functional group respectively.

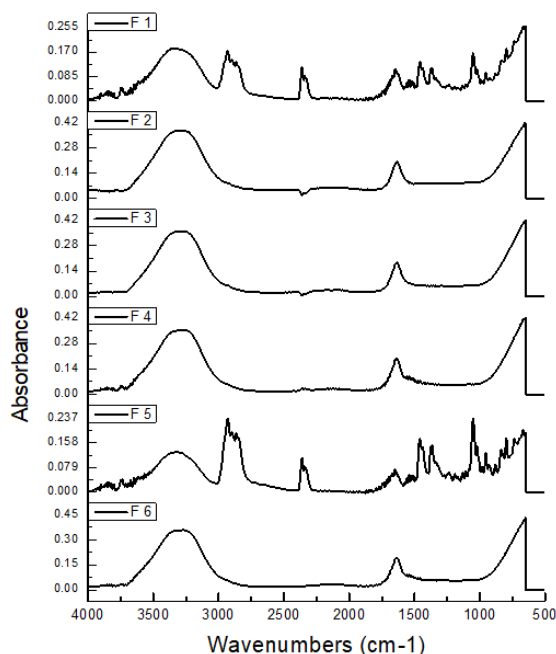


Figure 3: ATR-FTIR spectra of niosomal formulations (F1, F2, F3, F4, F5, and F6).

Table 4: Characteristic peaks of niosomal formulation

Formulations	Characteristic peak	Functional group
F1	3336	Hydroxy group, Carboxylic acids
	2930	Carboxylic acids
	2360	Primary and secondary amines
	1649	Secondary amine
	1457	Alkanes, Aromatic ring stretch
	1052	Cyclohexane ring vibrations, Alkyl substituted ether
F2	3299	Hydroxy group, Carboxylic acids
	1631	Secondary amine, Conjugated ketone
F3	3259	Hydroxy group, Carboxylic acids
	1635	Secondary amine, Conjugated ketone
4	3262	Hydroxy group, Carboxylic acids
	2359	Primary and secondary amines
	1636	Secondary amine, Conjugated ketone
F5	3322	Hydroxy group
	2929	Carboxylic acids
	2360	Primary and secondary amines
	1649	Secondary amine, Conjugated ketone
	1458	Alkanes, Aromatic ring stretch
	1021	Cyclohexane ring vibrations, Alkyl substituted ether
F6	3273	Hydroxy group, Carboxylic acids
	1636	Secondary amine, Conjugated ketone

The characteristic peaks of 2920 cm⁻¹, 2921 cm⁻¹, 2926 cm⁻¹, and 2929 cm⁻¹, which indicate the carboxylic acid functional group were found in Tween 60, Span 80, Span 60, and cholesterol

respectively. The physical mixture 1 and 2 shows characteristics peaks 2919 cm^{-1} and 2927 cm^{-1} , and the formulations 1 and 5 shows characteristics peak of 2930 cm^{-1} and 2929 cm^{-1} , which indicate the carboxylic acid functional group. The characteristic peaks in spectra of Span 60 were at 3310 cm^{-1} and 2926 cm^{-1} which indicate amines or the hydroxyl group and the carboxylic acids functional group respectively.

The characteristic peak in spectra of Span 60 and Formulation 1 was at 1052 cm^{-1} , and the characteristic peak in spectra of Span 80, physical mixture 1 and 2 was at 1054 , both of these peaks indicate the alkyl substituted ether functional group. Also, the characteristic peak in spectra of Span 60 was at 1733 cm^{-1} , the characteristic peak in spectra of Span 80 was at 1736 cm^{-1} , and the characteristic peak in spectra of Tween 60, Tween 80 and physical mixture 1 was at 1734 cm^{-1} . All these peaks indicate the aldehyde functional group respectively.

Span 80 showed the characteristic peak at 1736 cm^{-1} which indicates the esters functional group. Tween 80 showed the characteristic peak at 2856 cm^{-1} which indicates the Carboxylic acids functional group. Hexa decyl pyridinium chloride showed the characteristic peaks at 3367 cm^{-1} , 1635 cm^{-1} , 1471 cm^{-1} , and at 1177 cm^{-1} . These peaks indicate Amines or hydroxyl group, Secondary amine or conjugated ketone, carbonate ion, and secondary amine functional group respectively [15,16].

Size and zeta potential

The mean size and PDI of niosomal formulations is given in Table 5. It indicates that by changing the surfactant to cholesterol level in different formulations, the size of niosomes is also affected significantly (ANOVA, $p < 0.05$). The mean size of niosomes of the selected formulation (F_2) was $1049.3 \times 10^{-3}\text{ }\mu\text{m}$.

The zeta potential of the selected formulation (F_2) was $9.40 \pm 0.18\text{ (mV)}$.

Morphology of niosomes

Scanning electron microscopy (SEM) was employed in the morphological study. In figure 4, the SEM image of the optimized niosomal formulation F_2 is demonstrated. The spherical shape of the niosomes is depicted in Figure 4.

Drug entrapment

Niosomes were separated using ultracentrifuga-

tion, and the amount of Cyclosporine A in different formulations is provided in Table 6.

Table 5: Mean size and polydispersity index of niosomal formulations

Formulation code	Size (μm)	Polydispersity index
F ₁	$436.1 \times 10^{-3} \pm 0.141$	0.451 ± 0.04
F ₂	$1049.3 \times 10^{-3} \pm 0.117$	0.435 ± 0.15
F ₃	$534.7 \times 10^{-3} \pm 0.215$	0.471 ± 0.06
F ₄	$415.2 \times 10^{-3} \pm 0.311$	0.356 ± 0.11
F ₅	$424.1 \times 10^{-3} \pm 0.185$	0.481 ± 0.17
F ₆	$673.8 \times 10^{-3} \pm 0.135$	0.572 ± 0.21

Data are mean \pm standard deviation; in all formulations, size of the niosomes was significantly different from each other $p < 0.05$

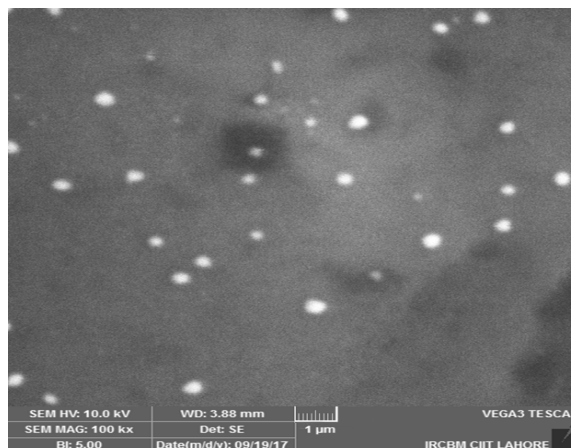


Figure 4: SEM image of optimized formulation F_2

Table 6: Mean entrapment efficiency of niosomal formulations

Formulation code	Surfactant: cholesterol ratio	Entrapment efficiency (%)
Tween 60+Span 60: cholesterol		
F ₁	7:3	26.88 ± 0.43
F ₂	6:4	77.28 ± 0.35
F ₃	5:5	52.08 ± 0.38
Tween 80 + Span 80: cholesterol		
F ₄	7:3	63.84 ± 0.41
F ₅	6:4	56.12 ± 0.52
F ₆	5:5	43.68 ± 0.39

Data are mean \pm standard deviation; in all niosomal formulations, entrapment efficiency was significantly different from each other $p < 0.05$

DISCUSSION

Niosomal formulations were successfully developed at various ratios of surfactant and

cholesterol (Table 1) using thin film hydration technique.

ATR-FTIR spectra of individual components are shown in Figure 1. Characteristic peaks in the FTIR spectrum of cyclosporine A are given in Table 2. These are similar to peaks as given in literature, which indicates the purity of this sample and indicates that impurities are not present [17].

ATR-FTIR spectra of physical mixture 1 (PM1) and physical mixture 2 (PM2) are shown in Figure 2. Physical mixtures of cyclosporine A with cholesterol and nonionic surfactants were prepared by simple mixing in equimolar ratios. Characteristic peaks of physical mixtures 1 & 2 are given in Table 3. The FTIR spectrum shows similar peaks in spectra of physical mixtures as in an individual spectrum of pure ingredients. The spectrum of physical mixtures did not show a significant shift of peaks which indicates that there were no interactions.

The ATR-FTIR spectrum of niosomal formulations are shown in Figure 3. It showed similar peaks and the marked reduction of IR bands of drugs as well as the intensity of peaks. The characteristic peaks with corresponding functional groups are given in Table 4. Due to the formation of niosomes, most of the peaks are diffused, demonstrating a strong physical interaction between cholesterol, drugs, and surfactants. The lack of a significant shift of a new peak indicates a lack of chemical interaction. So, it depicts that cyclosporine A is intact and can show its therapeutic effect.

The size and polydispersity index of the niosomes of different formulations are given in Table 5. The size of the niosomes were significantly different from each other ($p < 0.05$). In formulation F₂, Span 60 was used. It had a critical packing parameter in the range of 0.5 to 1 and can be used in niosomal formulations [18]. The SEM image of optimized formulation F₂ shows the spherical shape of niosomes in our study as shown in Figure 4, which is also similar to the naltrexone niosomes made by using Span 60 [19].

Different studies of niosomes showed that the cholesterol concentration is increased up to some extent, i.e. entrapment efficiency increases from 0 to 50 % [20, 21]. In formulations F₁ to F₃, the surfactants used were Span 60 and Tween 60, and the concentration of cholesterol was gradually increased from 30 to 50 % as shown in Table 1. The maximum entrapment of 77.28 % was achieved in formulation F₂ as shown in Table 6. In which surfactants and cholesterol were in the ratio of 6:4. Therefore, 40 %

cholesterol shows maximum entrapment efficiency. Statistical analysis of data given in Table 6 indicated that the percentage of entrapment efficiency was dependent significantly on surfactant to cholesterol ratio in the niosomal formulations (ANOVA; $p < 0.05$).

The minimum amount of cholesterol necessary to form vesicles increases as the HLB of surfactants increases above 10. In F₂, the mixed surfactant system is used, i.e. Tween 60 + Span 60 is used instead of a single surfactant. This combination helps in increasing the entrapment efficiency of cyclosporine A. This mixed surfactant system was also used in another study to increase the entrapment efficiency of the poorly soluble drug named diacerein. They used sorbitan monolaurate and poloxamer 184 as a mixed surfactant, and this resulted in the increased entrapment efficiency of diacerein [22].

In formulations F₄ to F₆, the surfactants used were Tween 80 and Span 80, while in F₄ the cholesterol concentration was 30 % as shown in Table 1. Among these formulations F₄ to F₆, maximum entrapment 63.84 % was achieved in F₄ as shown in Table 6. Consequently, it has been shown with regard to this surfactant system that further increasing the cholesterol concentration up to 50 % does not increase the entrapment efficiency.

In F₃ and F₆, the cholesterol concentration was the highest: up to 50 %, but the entrapment efficiency was less. The reason for this fact may be that the higher concentration of cholesterol causes the disruption of niosomes, leading to the leakage of the drug [23]. The data given in Table 6 shows that the percentage of entrapment efficiency is highly dependent on the amount of surfactant and amount of cholesterol which is used in appropriate ratios in different formulations.

CONCLUSION

The niosomal formulation of cyclosporine A has successfully been developed with the aid of cholesterol and non-ionic surfactants. Niosomal formulation F₂ based on the nonionic surfactant to cholesterol ratio (6:4) indicates the maximum entrapment of cyclosporine A (77.28 %). There is no incompatibility or interaction between the ingredients of the niosomal formulation. Thus, these niosomes can be used to enhance the bioavailability of cyclosporine A.

DECLARATIONS

Acknowledgement

The authors thank Dr Muhammad Sher, who is in-charge of the Hi-Tech Lab, University of Sargodha for his kind support and guidance.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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