

Preparation and Evaluation of Diclofenac Sodium Niosomes Using Round Bottom Flask Method

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Abstract

Aim: The main objective of this study is to prepare and evaluate niosomes as a carrier for diclofenac sodium. Niosomes are bilayer microparticles formed by self-association of non-ionic surfactant and cholesterol after hydration in the aqueous phase. **Materials and Methods:** Niosomes of SPAN-60 and cholesterol were prepared in three batches (A, B, and C) with different ratio (5:5, 6:4, and 7:3) using a round bottom flask method. Prepared niosomes were evaluated for entrapment efficiency, particle size analysis, and morphology and *in vitro* drug release study. **Results and Discussion:** The entrapment efficiency of all three formulations was found to be 91.43%, 92.53%, and 92.33%, respectively. All formulations were observed under motic microscope and particles were assumed to be spherical in shape. The average particle size of all the three batches was analyzed by Zetasizer (Delsa C Particle Analyzer), and it was found that the batch C produced the best nano-sized formulation with an average particle diameter of 311.6 nm. *In vitro* release study was done using a dialysis bag and after 300 min of studies, batch C (7:3), which was found to have $68.6 \pm 1.4\%$ cumulative drug release, which indicates controlled and steady drug release profiling of the formulation. **Conclusion:** After comparing all three batches of evaluation results on the basis of drug entrapment, particle size analysis, morphology, and *in vitro* drug release study, Batch C (7:3) formulation was considered as the best formulation.

Key words: Bilayer, cholesterol, diclofenac sodium, multilamellar vesicles, niosomes, sorbitan monostearate (Span-60)

INTRODUCTION

Niosomes are bilayer microparticles (10–10000 nm), which are formed by self-association of non-ionic surfactant and cholesterol after hydration in aqueous phase [Figure 1].^[1,2] Niosomes can deliver both hydrophilic and hydrophobic types of drugs.^[3] Hydrophilic drugs can be delivered by entrapping them in aqueous core of particle or by adsorbing on the surface of the bilayer.^[4] Hydrophobic drugs can be delivered by encapsulating them into the bilayer of non-ionic surfactants. On the basis of size, niosomes are categorized, namely, small unilamellar vesicles (10 nm–100 nm), large unilamellar vesicles (100 nm–500 nm), and multilamellar vesicles (0.5 μm –10 μm). Common techniques for size determination of niosomes are Zetasizer and Mastersizer.^[5]

Preparation techniques

Reverse phase evaporation

Reverse phase evaporation is a method in which surfactant and cholesterol are dissolved in a mixture of ether and chloroform,^[6] after that the aqueous solution of the drug is added into the organic solution of surfactant followed by homogenization then the organic solvent is evaporated under low pressure to produce a niosome suspension.^[7]

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Received: 23-01-2020

Revised: 29-02-2020

Accepted: 06-03-2020

Emulsion method

Surfactant and cholesterol are dissolved in an organic solvent and then transferred into an aqueous solution of the drug.^[4] This oil-in-water emulsion is then heated to evaporate the organic solvent, and niosome suspension is formed.^[5]

Lipid injection method

Lipid injection method is free from organic solvent in which surfactant and cholesterol are melted and injected into the heated aqueous solution containing drugs with high stirring.^[8] This process leads to the formation of niosome suspension.

Ether injection method

Both drug and surfactant are dissolved in diethyl ether and slowly injected in aqueous phase with continuous heating above the boiling point of diethyl ether. The prepared niosomes after evaporation are further treated for size reduction.^[9]

Bubble method

Bubble method does not require the use of organic solvent. Cholesterol and surfactant are dissolved into the buffer solution and heated up to 70°C. At 70°C temperature, the solution is homogenized using a high shear homogenizer for 15 s, and nitrogen gas is passed through the solution which leads to the formation of niosomes.^[10]

Round bottom flask (RBF) method

We have decided to use RBF method to prepare niosomes of diclofenac because it is a simple technique in which cholesterol, surfactant, and drug are dissolved in organic solvent like chloroform using a RBF. Then, the RBF is fixed on a rotatory evaporator and an organic solvent is evaporated leaving a dry thin film on the surface of RBF.^[11] Thin film is hydrated using aqueous medium with mild shaking or sonication which produce hydrated niosomes [Figure 2].

Niosomes are considered to be spherical in shape and their shape and bilayer characteristics are analyzed by transmission electron microscope (TEM), scanning electron microscope (SEM), negative staining TEM, freeze fractured TEM, dynamic light scattering (DLS), and bilayer small-angle X-ray scattering.^[12]

Niosomes are biocompatible, non-immunogenic microparticles and can easily escape the reticuloendothelial system.^[13] Niosomes are very useful to prepare depot and sustain release formulations of both lipophilic and hydrophilic drugs. They can protect the drug from the enzymatic degradation hence increases stability and bioavailability of the drug.^[14] When niosomes are incorporated in topical preparations, they enhance the skin permeability and absorption of the drug.^[15] Niosomes are easy to produce on a large scale due to their easy manufacturing and inexpensive

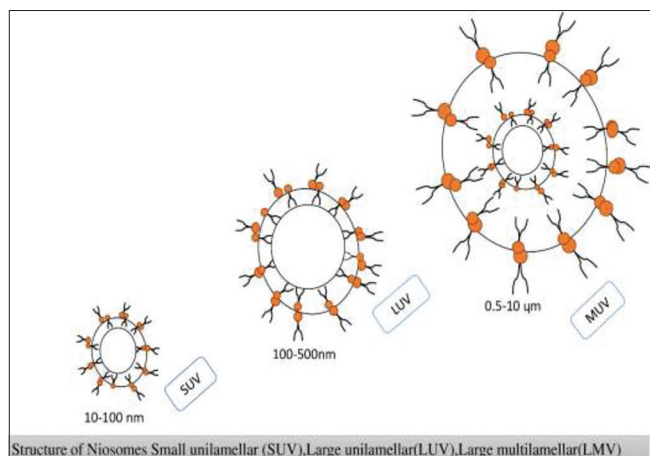


Figure 1: Different types of niosomes

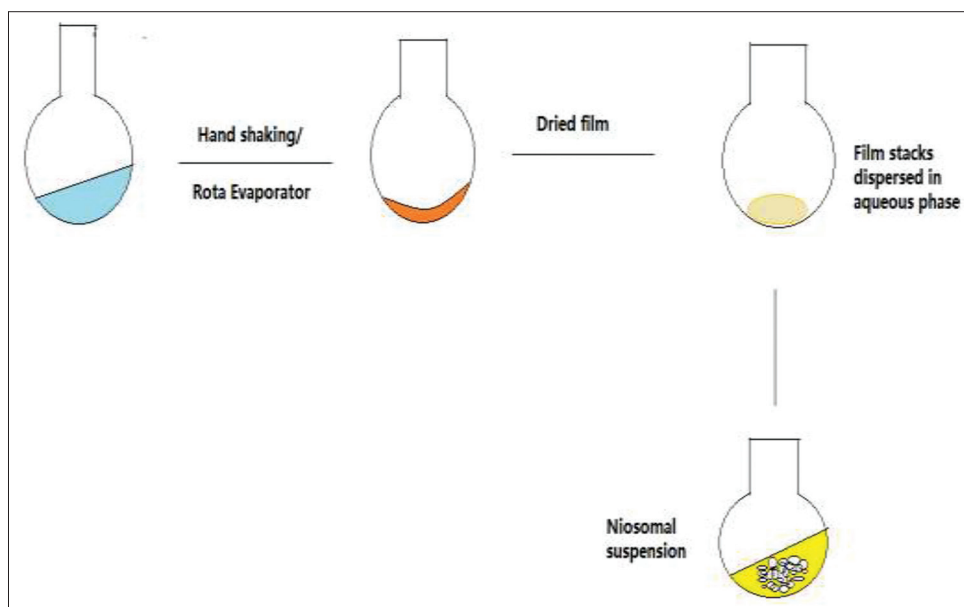


Figure 2: Round bottom flask method

raw materials used in production. They also have fewer stability problems compared to the liposome.

Niosomes have wide applicability as a novel drug delivery system for targeted drug delivery to specific organs such as brain, liver, lungs, ocular system, and skin.

Diclofenac sodium is a nonsteroidal anti-inflammatory drug with potent analgesic moderate anti-inflammatory action. Diclofenac sodium comes under Class-II of the Biopharmaceutical Classification System. It has poor solubility and absorption from the oral route of administration. It also causes gastric bleeding as a side effect.

We may prepare topical hydrogel niosomal preparation of diclofenac sodium. Niosomes can increase the penetration of drug through the stratum corneum hence increase local analgesic action of the drug. Niosomal preparation of diclofenac sodium overcomes its side effect of gastric bleeding and also increases its physical stability.

Sorbitan monostearate (SPAN-60)

Sorbitan monostearate is a non-ionic surfactant consists of a hydrophilic region and a lipophilic region in its structure.^[8] The hydrophilic-lipophilic balance value of SPAN-60 is 4.7. SPAN-60 is biocompatible and has low toxicity compared to the anionic and cationic surfactants.

Cholesterol

Cholesterol is used as a stabilizing agent with the non-ionic surfactant. Cholesterol makes hydrogen bond with the alkyl chain of surfactant which provides better stability and lowers the membrane fluidity hence overcomes the problem of drug leaking from niosomes.^[16]

MATERIALS AND METHODS

Diclofenac was obtained as a gift sample from Nestor Pharmaceuticals Limited, Faridabad, Haryana. The required materials, i.e., Span 60 was purchased from Central Drug House (P) Ltd., Bombay, cholesterol was purchased from Loba Chemie Pvt. Ltd., Mumbai, chloroform was purchased from Central Drug House (P) Ltd. Bombay, and methanol was purchased from SD Fine-Chem limited, Mumbai, phosphate buffer and water.

Methods of preparation

Niosomes of diclofenac sodium were prepared using RBF method by taking different ratios of surfactant and cholesterol.

RBF method

SPAN 60 and cholesterol were taken respectively in different ratios (5:5, 6:4, and 7:3) in RBF. Diclofenac sodium was dissolved in 10 ml methanol and 10 ml chloroform using

a separate beaker and transferred into the RBF containing SPAN-60 and cholesterol. Solvent mixture was evaporated using rotatory evaporator (Heidolph G3, Germany) for 15–30 min at 50 rpm and 40°C temperature. After evaporation, dry thin film was formed on sidewalls of RBF. Thin film was hydrated using phosphate buffer (pH = 7.4) and solution was sonicated (Labman Scientific Instrument, Chennai) for 5 min at 25°C temperature [Table 1].

Evaluation of niosome

Standard curve of diclofenac sodium

The dilution was made from stock solution of diclofenac (100 µg/ml) to get concentration of 0.5, 1, 2, 4, 6, and 8 µg/ml, respectively. Absorbance of diclofenac sodium was taken at 276 nm in ultraviolet (UV) visible spectrophotometer. The calibration curve was plotted between absorbance and concentration [Figure 3].^[17]

Entrapment efficiency

Entrapment efficiency of niosomal formulation^[18] was determined by centrifugation method. For this, 10 ml

Table 1: Formulation table

Ingredients	A (5:5) weight taken	B (6:4) Weight taken	C (7:3) weight taken
Span 60	43.06 (mg)	23.19 (mg)	54.13 (mg)
Cholesterol	38.66 (mg)	17.22 (mg)	25.83 (mg)
Drug (Diclofenac)	30 (mg)	30 (mg)	30 (mg)
Chloroform	10 (ml)	10 (ml)	10 (ml)
Methanol	10 (ml)	10 (ml)	10 (ml)
Water	Qs	Qs	Qs
Phosphate Buffer (7.4)	15 (ml)	15 (ml)	15 (ml)
Dye (Rhodamine)	Qs	Qs	Qs

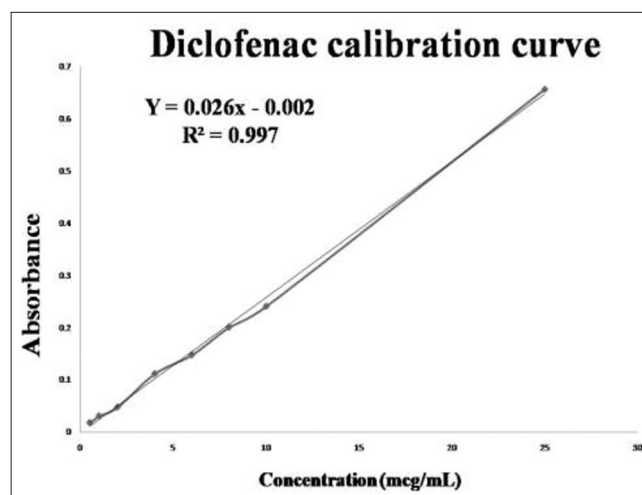


Figure 3: Standard curve of diclofenac sodium

niosomal suspension was poured into a centrifugation tube and centrifuged at 1000 rpm for 10 min. The clear fraction was further used for the determination of free drug using UV/visible spectrometer at 276 nm examined under the UV visible spectroscopy. The entrapment efficiency was calculated using the following formula:

$$\text{Entrapment efficiency \%} = \frac{C_t - C_f}{C_t} \times 100$$

Where, C_t the concentration of total drug and C_f is the concentration of entrapped drug.

Particle size analysis and zeta potential

For particle size measurement Zetasizer (Delsa C Particle Analyzer) was used.^[19] Sample was filled in cuvette carefully and there should be no bubble formation in cuvette. Then, the cuvette was inserted into the instrument by opening the lid of Zetasizer and sample was analyzed. Simultaneously, zeta potential was measured for the same sample using Zetasizer.

Morphology and particle shape

Shape and morphology of niosomal formulation were determined by microscope. Shape of niosomal vesicles is assumed to be spherical. Niosomes examined under the motic microscope. The suspension of niosomes was stained using sulforhodamine B solution (sulforhodamine B solution previously prepared using 0.5% solutions of acetic acid and water).

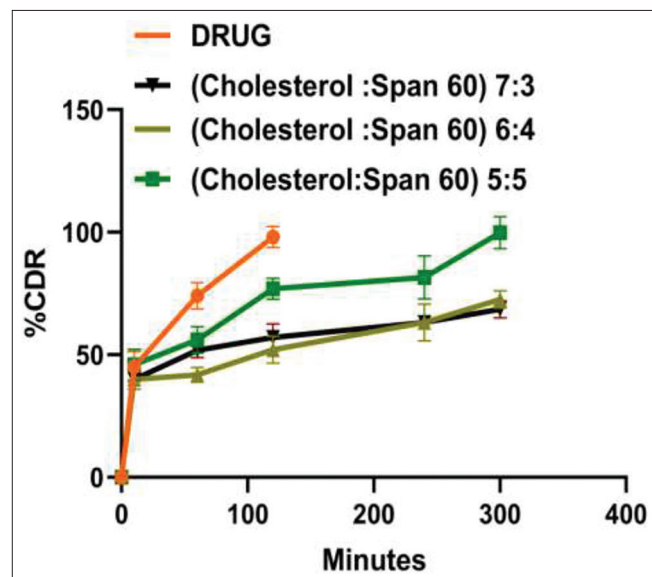


Figure 4: *In vitro* drug release studies of niosomes

SEM analysis

The morphological characteristics of the prepared niosomal formulation were examined under SEM (JSM-5610 LV Scanning microscope, Jeol, Tokyo, Japan). Before microscopy, niosomal formulation (7:3) was suspended in a phosphate buffer (pH 7) by vortex for 1 min, then one drop was separated on small clean slide cover and left to dry overnight maintaining temperature at below 10°C in a desiccator. Next day, they were mounted on carbon tap, it is also known as daily size adhesive tape, and sputter-coated using a thin gold-palladium layer under a argon atmosphere using a gold sputter module in a high-vacuum evaporator (JFC 1100 fine coat ion sputter, Jeol, Tokyo, Japan). The coated samples were then scanned in BSE mode and photomicrograph was taken at acceleration voltage of 20 KV in different manifestation and different location (100–1000 nm)

In vitro drug release study of niosome dispersion

For *in vitro* drug release study the formulation batches, namely, 5:5, 6:4, and 7:3 of cholesterol and Span 60 was incorporated in a dialysis bag (12000 d). The dialysis bag was tight by both the end submerged in 500 ml conical flask containing 400 ml of phosphate buffer solution (7.4) with 0.8% tween 20 as a medium. The flask was kept at 37°C in shaker incubator at different period of time. 5 ml of actuates amount was withdrawn and 5 ml buffer was incorporated to maintain sink condition. The amount of drug release from niosome suspension was measured by UV-visible spectroscopy at 276 nm [Figure 4].

RESULTS AND DISCUSSION

Preformulation studies of diclofenac sodium determined by absorption maxima and it was found to be at 276 nm and the method developed obeyed beer's law with linearity plot. Niosomes prepared with non-ionic surfactants of alkyl ester including Span-60 (sorbitan esters) were utilized to determine the encapsulation of associated diclofenac sodium. The Niosome formulation was prepared by RBF method. The formulation was studied for physical characteristics such as determination of vesicle morphology, size distribution, and encapsulation efficiency and was found to be within the acceptable limits, as shown in Table 2.

The niosomes were observed as spherical vesicles with a smooth surface. The vesicles were discrete and separate

Table 2: Encapsulation efficiency, size distribution, and vesicle morphology of various niosome formulations

Evaluation parameters	Batch-A (5:5)	Batch-B (6:4)	Batch-C (7:3)
Size distribution (Particle size in nm)	892.4	813.0	311.6
Drug entrapment (%)	91.43±1.2	92.53±1.2	92.33±1.2
<i>In vitro</i> drug release (After 300 min) (%)	99.8±1.2	72.6±1.2	68.6±1.4
Vesicle morphology study (Microscopy)	As reported in Figure 6	As reported in Figure 6	As reported in Figure 6

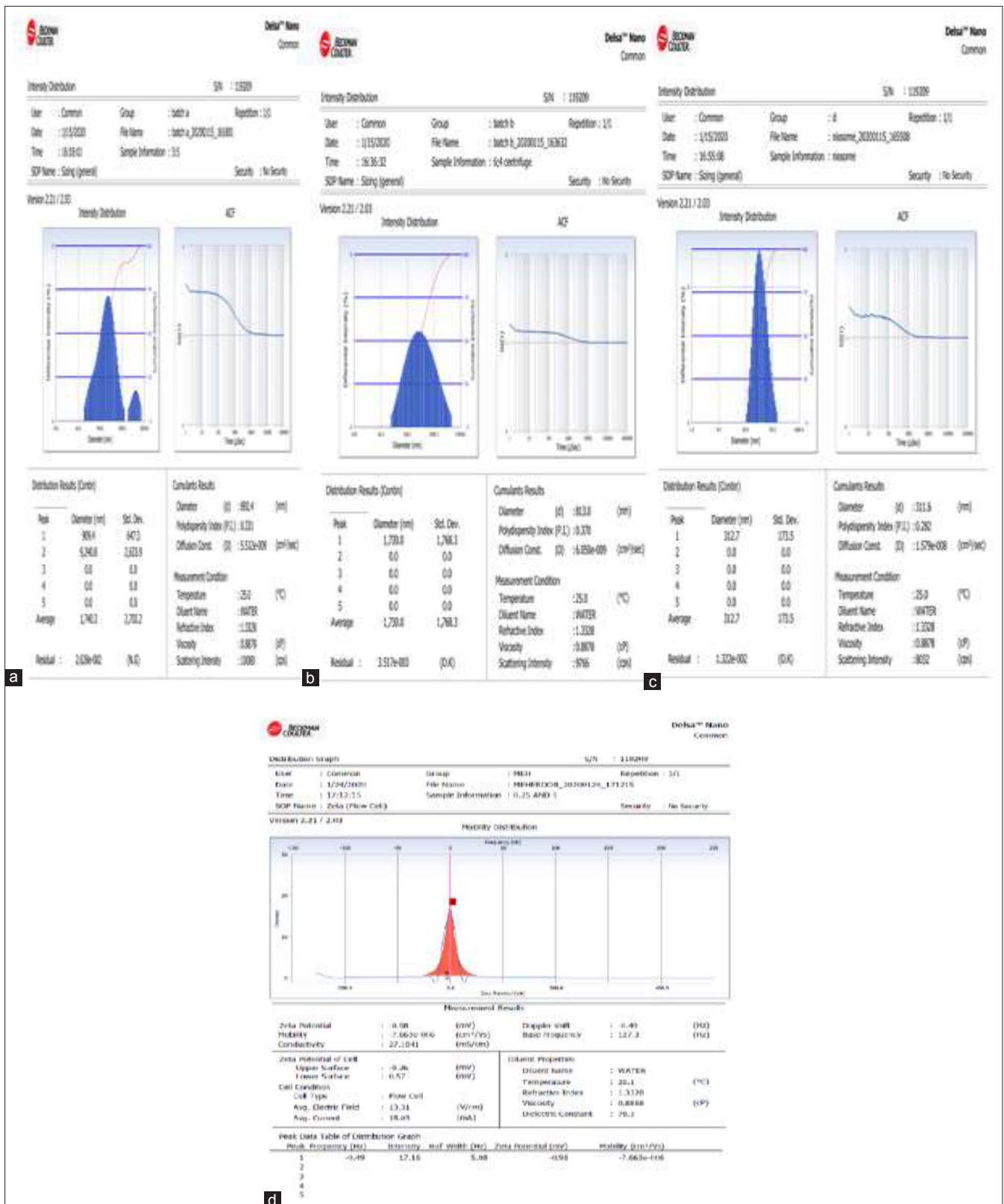


Figure 5: Particle size analysis using dynamic light scattering; (a) particle size of diclofenac sodium-containing niosomes at 5:5 ratio of cholesterol:Span 60 (b) particle size of diclofenac sodium-containing niosomes at 6:4 ratio of cholesterol:Span 60 (c) particle size of diclofenac sodium-containing niosomes at 7:3 ratio of cholesterol:Span 60 (d) zeta potential results of optimized 7:3 niosome batch

with no agglomeration or aggregation. The particle size of the niosome was determined using microscopy studies. The size of the vesicles was independent of surfactant and uniform, as vesicles of all the surfactants were sonicated to same size.

Standard curve of diclofenac sodium

The calibration curve was plotted between absorbance and concentration.

Drug release profile

The release of diclofenac was found to be $93.63 \pm 1.4\%$ after 120 min of studies. Where else three different combinations of cholesterol and span 60 (5:5, 6:4, and 7:3) produce $99.8 \pm 1.2\%$, $72.6 \pm 1.2\%$, and $68.6 \pm 1.4\%$ of cumulative drug release after 300 min of *in vitro* drug release studies. It was reported that Span 7:3 was found to have best control drug release profiling as after 300 min studies it was showing slow and controlled release profiling.

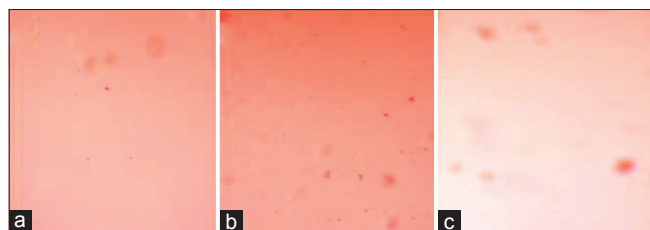


Figure 6: Morphological studies of niosomes containing diclofenac sodium using motic microscope (a) morphology of diclofenac sodium-containing niosomes at 5:5 ratio of cholesterol:Span 60 (b) morphology of diclofenac sodium-containing niosomes at 6:4 ratio of cholesterol:Span 60 (c) morphology of diclofenac sodium-containing niosomes at 7:3 ratio of cholesterol:Span 60

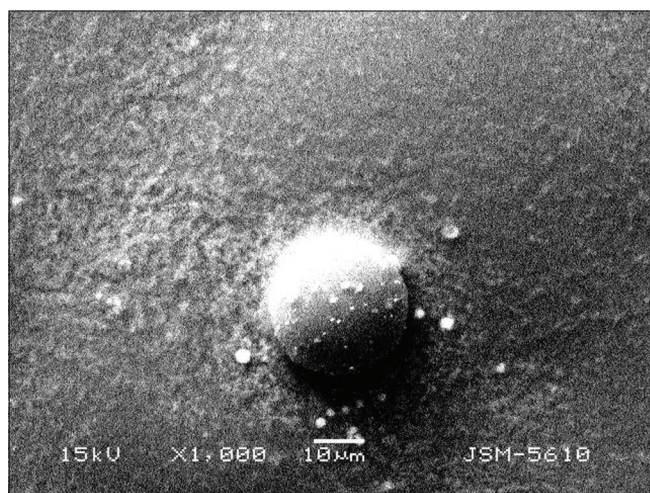


Figure 7: Scanning electron microscopy photographs for diclofenac niosome (7:3); in a field containing different particle sizes using 1000× magnification power

Size distribution studies and zeta potential

The niosomal dispersions were characterized for size distribution using DLS technology (Zetasizer). The sizes of the niosomes were between 311.6 and 892.4 nm. The higher value was observed for diclofenac coated niosomal formulation Batch-C (7:3) simultaneously, zeta potential was measured for niosomal formulation Batch-C (7:3) and was found to be -98mV . Therefore, the diclofenac coated niosomes showed better stability Figure 5.

Vesicle morphology study

The niosomes were observed under a motic microscope and the particle was observed spherical in shape and surrounded by the lipid layer. The images taken from the microscope are shown in and graph plots of particle size are mentioned in Figure 6.

SEM

Scanning electron microscopy of the niosome (7:3) indicates spherical shaped niosome and has good correlation with particle size results mentioned in dynamic light scattering [Figure 7].

CONCLUSION

Niosomes are novel drug carriers to design effective drug delivery systems. The concept of loading hydrophilic, lipophilic drugs, or both drugs together into or niosomes for better targeting of the drug at an appropriate site is widely accepted by researchers. Niosomes are mainly composed of non-ionic surfactants and cholesterol. Numbers of experiments have been performed with various types of niosomes in delivery of the anti-inflammatory agents, anti-infective agents, anticancer agents, and so forth.

Diclofenac sodium was successfully entrapped within the lipid bilayers of the vesicles with high efficiency. Niosome formulated with span 60 have shown the best entrapment efficiency as compared with niosomes prepared with other grades. Niosomes optimal Batch (C) was able to entrap drug where entrapment efficiency was found to be $93.63 \pm 1.22\%$.

The relevant studies demonstrated that niosomes improve the stability of the entrapped drug, reduce the dose, and enable targeted delivery to a specific type of tissue. The structural properties and characteristics of the niosomes can be enhanced using novel preparations, loading, and modification methods for particular routes of administration. Niosomal drug delivery proved to be one of the successful approaches to accomplish the goals successfully; niosomes present itself as promising tools in commercially available therapeutics.

ACKNOWLEDGMENT

The authors are thankful to ISF College of Pharmacy, Moga, for providing facilities to carry out research and also thankful to respected Director Sir, Professors of ISF College of Pharmacy, Moga, Punjab.

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Source of Support: Nil. **Conflicts of Interest:** None declared.