Sys Rev Pharm 2020;11(12):636-643
A multifaceted review journal in the field of pharmacy

In-vitro; ex-vivo assessment of anti-inflammatory Tapentadol loaded non-ionic surfactant vesicular systems for effective transdermal delivery

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Abstract
Tapentadol is an anti-inflammatory drug and mostly used rheumatoid arthritis commonly at 200mg an oral dose twice daily. Because of its first-pass metabolism, its bioavailability is low (32%).To overcome this problem, the transdermal route appear to be the optimal route for tapentadol administration. The present work focuses on developing niosomes for transdermal delivery of tapentadol for relieving pain and exploring possible mechanism of better skin permeation of niosomes. Niosomes were prepared by ethanol injection technique and characterized for various physicochemical parameters. Niosomes were in size range of (268±1.02 to 750±1.02nm). The lowest particles size (268 nm), highest entrapment efficiency (75.25%±1.32) and zeta potential -38.8 mV found for N8 niosomal batch. The stability profile of niosomal suspension after 8 weeks showed that 68±0.25% of drug was retained in the system. *In vitro* skin permeation studies of niosomes showed 98.92±1.2% of permeation over 24 hrs. N8 niosomal batch was incorporated in 1.5% Carbopol 931NF and carried out the *ex vivo* rat skin permeated studies states that niosomal suspension shows maximum flux (30µg/cm^2/hr) comparatively niosomal gel (26.29µg/cm^2/hr) and plain gel (20.84 µg/cm^2/hr). These improvements in tapentadol formulation may be useful in developing a more effective NSAIDS therapy.

Keywords: Tapentadol, Span 60, Flux, permeation, TDDS and skin

INTRODUCTION

Transdermal drug delivery system (TDDS) is a frequently exercised drug delivery design to transport the drugs through skin into the body. It is effort delivery strategy that can overcome the limitations of conventional route of drug administration [1]. The major advantages of TDDS is to effectively avoid the gastrointestinal tract (GIT) problems, hepatic metabolism, provide the steady state plasma drug concentration, reduced dosage frequency by controlled release tendency and improves the patient compliance [2]. Since many decades, various dosage forms are used for local indications by skin. Scopolamine transdermal film (innovator TRANSDERM SCOP®) is developed by GlaxoSmithKline Consumer Healthcare Ltd and is therapeutic indicated for motion sickness, approved for USFDA in the United States in 1982. Various conventional dosage forms are available such as semisolid dosage forms (ointments, pastes, creams and gels) are the most common. However, foams, sprays, medicated powders, solutions, lotions and medicated adhesive plasters also available [3]. They may be either rubbed on or sprayed on to the skin. These formulations are applied in the treatment of various skin disorders. These dosage forms are failed to improve the systemic availability of the drug for over period of the time due to poor permeation across the skin. To overcome these drawbacks, various colloidal dispersions are available to improve to improve the systemic availability of drugs, minimize the toxic manifestations of the drugs and improve the patient compliance. Generally colloidal dispersions are various types such as liposomes, niosomes, ethosomes, emulsions etc [4]. Among these, we have selected niosomes as dosage for the current study. Development of niosomes is associated with specific requirements mainly to accomplish suitability to patient, to get proper targeting of drug to its site of action and to design an appropriate dose of the drug. Since few decades, there has been huge research investigation to enhance the drug permeation through sub cutaneous using non-ionic surfactant based vesicular systems [5]. Niosomes water filled colloidal particles, made by non-ionic surfactants such Tweens and Spans etc. These niosomes are alternative to liposomes for various pharmaceutical applications due to their superior stability [6].

MATERIALS

Tapentadol purchased from sigma Aldrich pvt Ltd. Span 60 procured from Loba chemicals Pvt Ltd, cholesterol and stearic acid procured from Alfa easer Pvt Ltd. All the remaining analytical grade excipients are procured from Merck Pvt Ltd.

Development of Tapentadol noisome for transdermal

delivery

Tapentadol (TP) is an opioid analgesic with inhibition both of noradrenalin (norepinephrine) reuptake activity and μ-opioid receptor agonist with minimal inhibition reuptake of serotonin. This double mode of action makes it particularly effective in the nociceptive and neuropathic pains treatment. TP has a half-life four hours. It belongs to BCS- Class-I drug (high solubility and high permeability) [7].

Oral route of administration, TP has poor bioavailability (32%) due to the extensive first pass metabolism. The parent compound is metabolized at about 97 %. None of metabolites contribute to an activity of analgesic. The intended action is eventually only accomplished with a high dosage. The usual oral dose in treating musculoskeletal pain is 600 mg of maximum dose per every 12 hrs interval by controlled release. Alternatively, it may be given as a 50, 75 and 100 mg immediate release tablet once 4 to 6 hrs to control of pain. TP is used as to treat severe pain due to being an opioid analgesic, such as pain of cancer and postoperative, etc. Vomiting and nausea are a frequently associated problem in such cases, and poor patient compliance with oral administration is therefore seen. Some disadvantages of an oral administration are that a patient can change the unit dosage incorrectly; leading to the patient may not be able to swallow the drug, or the dangerous overdose. Compared to other opioids, Tapentadol has comparatively less capacity for tolerance that making this the drug of choice to be formulated for prolonged delivery among the other opioids. To avoid associated side effects with oral delivery, the current study aims to develop TP niosome for transdermal delivery using Span 60 respectively [7].

The objective of the present work is to develop niosomes for transdermal delivery that provide TP prolonged release. Since a TP analgesic effect remains for short period, the prolongation of drugs action would significantly benefits a patient through continuously maintaining the herapeutic pain relief level. Prolonged TP release in also overcomes a problem of inadequate relief of pain because fluctuation in frequency of dosing through oral therapy. The prepared of Niosomes by ethanol injection method using Span60 and cholesterol was found to be critical in the preparation and stabilization of niosomes. Amount of stearic acid (10 mg) and drug TPHCL (50mg) were kept constant. Formulations codes and variables values are shown in the table 1.

Table 1. Composition of TP Niosome

Formulation codes	Span 60 (mg)	Cholesterol (mg)	Stearic acid mg	TPHCL (mg)	Phosphate buffer pH 6.8 (mL)
N1	30	25	10	50	10 mL
N2	30	50			
N3	30	75			
N4	60	25			
N5	60	50			
N6	60	75			
N7	90	25			
N8	90	50			
N9	90	75			

Preparation of niosome

Niosome was prepared by ethanol injection method. Drug, stearic acid, cholesterol, Span60 are correctly weighed in a beaker and dissolved in 1mL of ethanol. The surfactant mixture in ethanol was injected slowly through 14-gauge needle into a beaker containing 10 mL of pH 6.8 phosphate buffer adjusted at a temperature of ≥50°C under stirring at 500 rpm (Remi magnetic stirrer) using a Teflon-coated bead. The system was subjected to evaporation for 40 minutes to remove ethanol. Due to formation of noisome, the aqueous phase became milky immediately. To fit a final niosomal suspension volume to 10 mL, buffer was added. Furthermore, niosomes were filled & stoppered in 10 mL vials and stored at 2-8°C refrigerated conditions [8-9].

Characterization of niosome

Drug content

Niosomal suspension equivalent to 2mg of TP was taken and lysed with methanol. Drug content was determined by subsequent dilutions with the buffer and measuring the absorbance at a λ_{max} of 275 nm [10].

Particle size distribution and zeta potential & polydispersity index measurement of size

A niosomal dispersion mean particle size was determined by photon correlation spectroscopy using Zetasizer 300 HSA (Malvern Instruments). The analysis was done at a temperature of 25 ± 2°C, keeping the detection angle at 90 °. The mean size of the vesicle was given in *d* (0.9) nm. The zeta potential of a niosomes was measured with a laser Doppler electrophoretic mobility measurement using Zetasizer 300HSA (Malvern Instruments) at a temperature of 25±2 °C [10].

Percentage of entrapment efficiency (% EE)

Using the centrifugation process, the unentrapped drug was separated from the niosome. At a temperature of 4°C (Remi cooling centrifuge), the niosomal dispersion was centrifuged for 90minutes at 20000rpm. Unentrapped drug-containing in supernatant was removed and UV spectrophotometrically measured at 275nm against phosphate buffer saline pH6.8. All a determination was made in triplicate [11]. A drug-trapped amount in niosomes was determined as following

$EE (\%) = [(C_d - C)/C_d] * 100$

Where C_d is a total drug concentration and C is an unentrapped drug concentration

***In vitro* release studies**

Vertical Franz diffusion cells with an effective diffusion area of 4.52cm^2 were used for *in-vitro* permeation studies. In the donor compartment, 2mL of volume of different formulations was placed. 25mL of phosphate buffer pH6.8 as receptor medium used to ensure sink condition. At 37 ° C, a receptor compartment was maintained and stirred at 100rpm by a magnetic bar. The donor compartment was discrete by the cellulose dialyzing membrane (Membra-Cel

MD 34-14, cut-off 14kD) from the receptor compartment that was soaked overnight in a receptor medium. At predetermined time intervals (0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24h), In order to maintain a constant volume, 1mL aliquots were pulled from a sampling port and substituted with an equal volume of fresh buffer. In reference to a constructed calibration curve, a sample were analysed spectrophotometrically at 275nm [11].

Drug release kinetics

The result of an *in-vitro* drug release study of the niosomes optimized batch was fitted with various kinetic equations such as zero order (cumulative% released vs. time) to understand the mechanism and kinetics of drug release, first order (log% drug remaining vs. time), Higuchi's model (cumulative% drug released vs. square root of time), Peppas (log% drug released vs. log time) and erosion $((1-Q)^{1/3}$ vs. time). For the linear curve obtained by regression analysis, K and r values were calculated [11].

Stability study

For this study, niosomal suspension optimized batch was kept for a period of 2months at 2 to 8°C and 25 ± 2°C /60% RH. The stability of niosomes was investigated in terms of changes in size, assay and %EE [12].

Visualization by transmission electron microscopy

On a carbon film-covered copper grid, a drop of vesicle dispersion was applied. To form a thin-film specimen, excess dispersion was blotted from the grid with filter paper. The sample stained with uranyl acetate 2%, air dried and examined under scanning electron microscope (HITACHI S3400 SEM instrument) at a magnification of 60000 X [10].

FTIR analysis

Appropriate amounts of TP, Span60, and cholesterol and niosomal dispersions by using ATR technique. The IR spectra of the resultant mixtures were recorded on a Bruker FTIR spectrophotometer equipped with Opus software [12].

Preparation of niosomal gel

An optimized batch of niosomal dispersion N 8 based on maximum drug release, highest entrapment efficiency and optimum particle size was used for a niosomal gel preparation. Carbopol 931NF was selected as the gel base. Based on the earlier work done in our laboratory, 1.5 % concentration of the Carbopol 931NF was used for the preparation of gel. Selected batch of the suspension was centrifuged to separate the unentrapped drug. A pellet obtained was resuspended in the buffer and then added Carbopol 931NF at required amount and kept overnight for complete polymer chains hydration. Triethanolamine was used to neutralize the pH of the gel at 6 to 7 and induce gelling [10].

Preparation of plain drug gel

Pure TP was incorporated in 1.5 % Carbopol 931NF base by trituration and stirred by using a glass rod to get 0.5 %w/w of smooth homogenous TP plain gel.

Drug content and content uniformity of gels

The gel samples (equivalent to 5mg of drug) from niosomal gel and plain gel were withdrawn and the content of drug was determined using a UV spectrophotometer. Similarly, the uniformity of a content was determined by analyzing the concentration of drugs in gel taken from three different points in the container. Gel was shaken with sufficient quantity of buffer to extract the drug from plain gel and with methanol for niosomal gel and then analyzed by using UV spectrophotometer at a λ_{max} of 275 nm [12].

***Ex vivo* permeation studies**

Ex-vivo permeation studies have been performed using sacrificed rat skin. A hand razor was used to shave the abdominal hair of albino rats (Wistar strain). The abdominal skin was surgically removed from the animal and carefully cleaned by attaching subcutaneous fat. Prior to the experiment, the membrane was soaked in the buffer. By using Franz diffusion cell, drug permeation from all the formulations was determined. On the receptor compartment with the stratum corneum side facing upward into the donor compartment, the excised rat skin was mounted. The experiment was run for an optimised niosomal dispersion, niosomal gel and plain gel. To compare the results, the permeation of drug from plain gel was also tested. An amount of dispersion or gel equal to 5mg of TP was applied to the skin in the donor compartment in all the experiments. 25 mL of pH 6.8 phosphate buffer was used as receptor medium to maintain sink condition. The available diffusion area of cell was 4.52 cm^2. At 37 ° C with magnetic stirring at 100rpm, the receptor compartment was maintained. Samples were pulled from the receptor compartment at predetermined time intervals and substituted immediately with an equal volume of fresh buffer solution. The samples were then analyzed using UV spectrophotometer at a λ_{max} of 275nm [13].

RESULT AND DISCUSSION

The present work focuses on developing novel niosomal carriers for transdermal delivery of TP for relieving pain and exploring possible mechanism of better skin penetration and permeation of niosomal carrier. Transdermal drug delivery using niosomes enhances the flux and decreases the drug retention in the skin to give systemic effect. Hence a transdermal niosomal gel was formulated using gum carbopol 931NF as gel base. The drug content precentage of the niosomal formulations varied between 99.04 to 100.1%. Hence all a formulas were within the standard limits. In each niosomal formulation, this reveals uniform drug distribution. A mean vesicle size was in a range 750-268nm respectively. It was observed that the particle size was decreased with increased concentration of span 60. It indicated that weighed amount of the drug going to be load in sufficient quantity of span 60 leads to decrease the size. Apart from the span 60, the cholesterol concentration is also impact on particles size and % of EE. TP, being as a BCS Class-1 drug, loading into the internal aqueous core of the niosomal vesicles, so the cholesterol at lower level allowed the maximum internals aqueous cores leads to increased size and % EE of TP whereas the beyond certain limit cholesterol restrict the entry of aqueous medium in to the niosomal internal core leads to decrease the size. The polydispersity index (PDI) was in a range of 0.223 to 0.545, which present a narrow distribution of vesicle size. The %EE of tapentadol loaded niosomes was found in the range of entrapment efficiency of 32.1% to 75.25% respectively. The highest entrapment efficiency of 75.25 % was observed for N8 formulation. The values are shown in the table 2.

It was observed that %EE of TP was increased with increased concentration of the span 60 whereas on other hand,

increasing the cholesterol concentration, the % EE of TP is decreasing. The PDI and vesicle size of all nine batches is shown in table 2.

Table 2. Physico-chemical characterization of TP niosomes

Formula code	Drug content ± s.d (%)	Particle size (nm)	PDI	Zeta potential (mV)	% of EE ± s.d. (%)
N1	99.4±0.90	750±1.02	0.358	-22.8	32.12±0.81
N2	100.1±0.17	656±1.10	0.286	-28.5	55.25±0.75
N3	99.37±0.10	720±1.24	0.354	-24.8	39.12±0.01
N4	99.10±0.32	523±1.02	0.252	-23.7	51.23±0.65
N5	98.32±0.85	412±0.20	0.230	-19.6	59.63±0.88
N6	99.25±0.87	446±0.24	0.223	-32.2	48.25±0.52
N7	99.89±0.70	301±0.21	0.358	-28.1	66.75±0.06
N8	99.98±0.85	268±1.02	0.545	-38.8	75.25±1.32
N9	99.89±0.8	289±1.01	0.386	-23.6	59.24±0.86

Each value represents mean±s.d. (n=3)

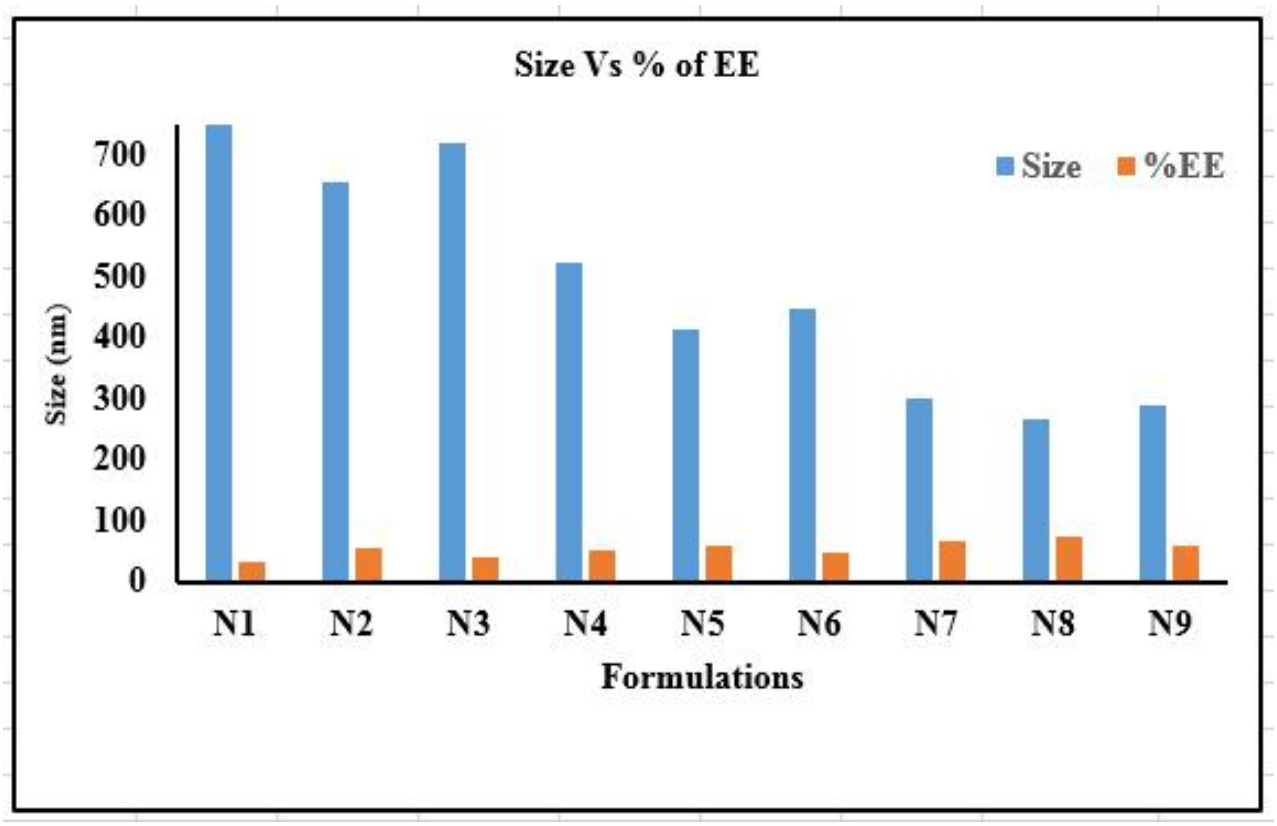


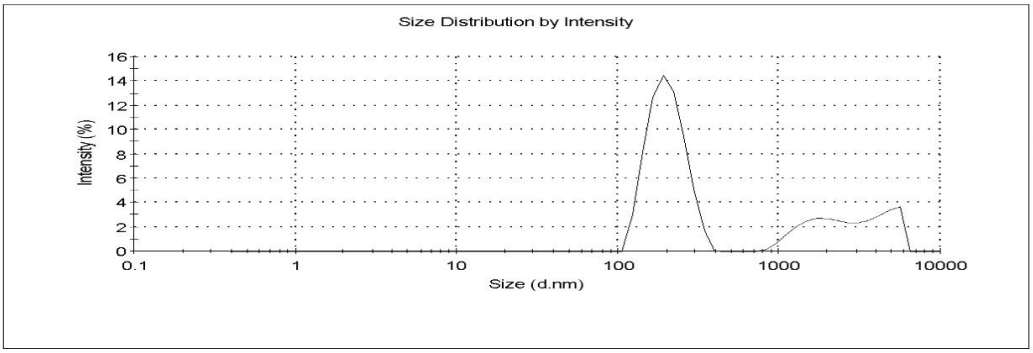
Figure 1. Comparative particle size and % of EE of niosomes

The impact of particle size on % of EE of tapentadol shown in figure 1.

Prepared niosomal dispersions Zeta potential values ranged from -19.6 to-38.8mV (Figure2). The prepared noisome has sufficient surface charge to avoid fusion or aggression of vesicles because a presence of stearic acid, which induce a sufficient surface charge. A surface charge is necessary for its stability in any liquid dosage form. When contrast to other colloidal dosage forms, niosomes have been reported to have greater stability. Zeta potential value > ± 30mV is important for effective stabilization and aggregation inhibition. Niosomal dispersion exhibited a maximum value of zeta potential -38.8mV because a charge of surface imparting nature of stearic acid. The zeta potential values appearance which prepared niosomes have sufficient charge because electric repulsion to inhibit vesicle aggregation.

a)

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 298.7	Peak 1:	203.3	66.3	51.58
PdI: 0.545	Peak 2:	1956	19.1	646.3
Intercept: 0.948	Peak 3:	4378	14.6	876.5
Result quality : Good				



b)

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -38.8	Peak 1:	-38.8	100.0	8.00
Zeta Deviation (mV): 8.00	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.266	Peak 3:	0.00	0.0	0.00
Result quality :Good				

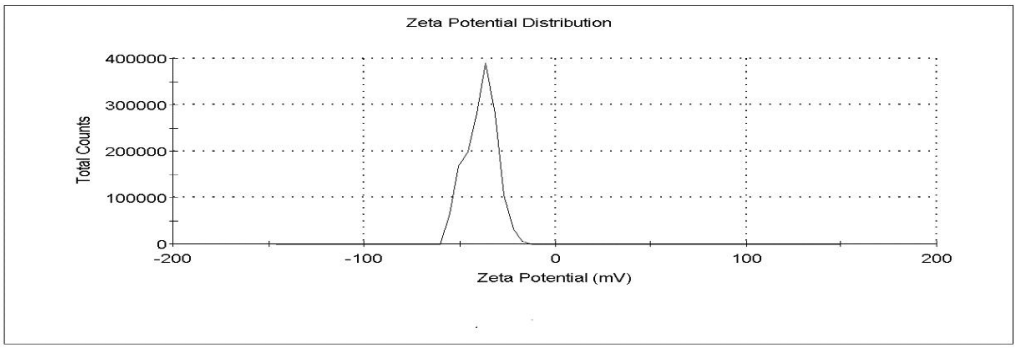


Figure 2. Particle size distribution and zeta potential of selected N8 Niosomal batch

Figure 3. shows transmission electron microscopy of the vesicle. Spherical large unilamellar vesicles were the niosomes obtained.

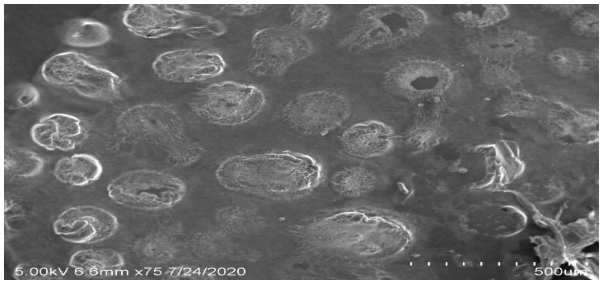


Figure 3. Surface morphology of N8 Niosomes Suspension by SEM

***In vitro* release studies**

The *in vitro* release profile of all formulations was studied using cellulose membrane as semi permeable membrane. The research detected that the released drug from the formulations depends on the relative quantity of surfactant and cholesterol present. These studies states that N8 showed a maximum drug release of 99.29 % after 24 hrs. The percentage release of the drug raised with increased concentration of surfactant and at certain limit of surfactant, the percentage release reduced at higher levels of cholesterol. This is because of cholesterol, at higher levels makes the surfactant bilayers more rigid and retards the release of the drug. The *in vitro* release profile of all the formulations is shown in figure 4.

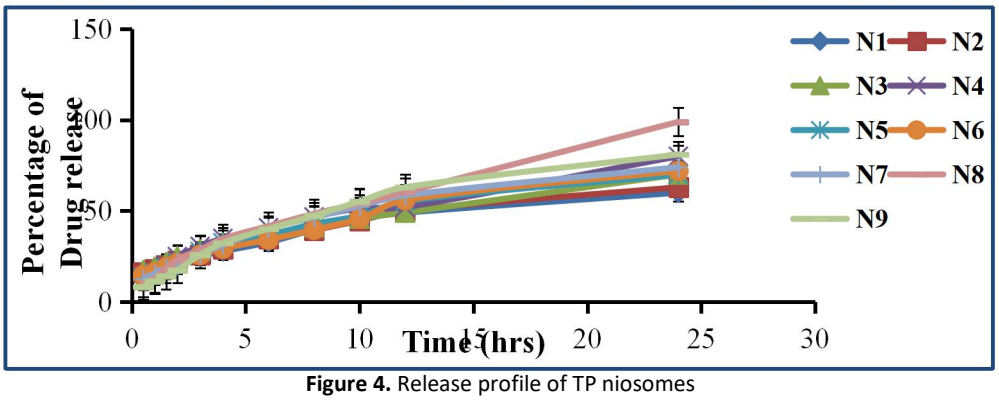


Figure 4. Release profile of TP niosomes

The highest entrapment efficiency and maximum drug release was found in N8 formulation, so it was optimized

and selected for further studies.

Release kinetics

The data obtained from the *in vitro* release of optimised formulation was fitted to various kinetic equations like zero order, first order, Higuchi, Peppas and erosion to determine the mechanism of drug release. The release kinetics is shown in the table 3. As indicated by its higher 'r' value (0.98) than first order (0.94), the drug release followed zero order kinetics. The mechanism of drug release was by diffusion as appeared from the higher 'r' value (0.99) of Higuchi plot than erosion (0.98) and the type of diffusion was non-Fickian as observed by 'n' value of Peppas plot (0.56).

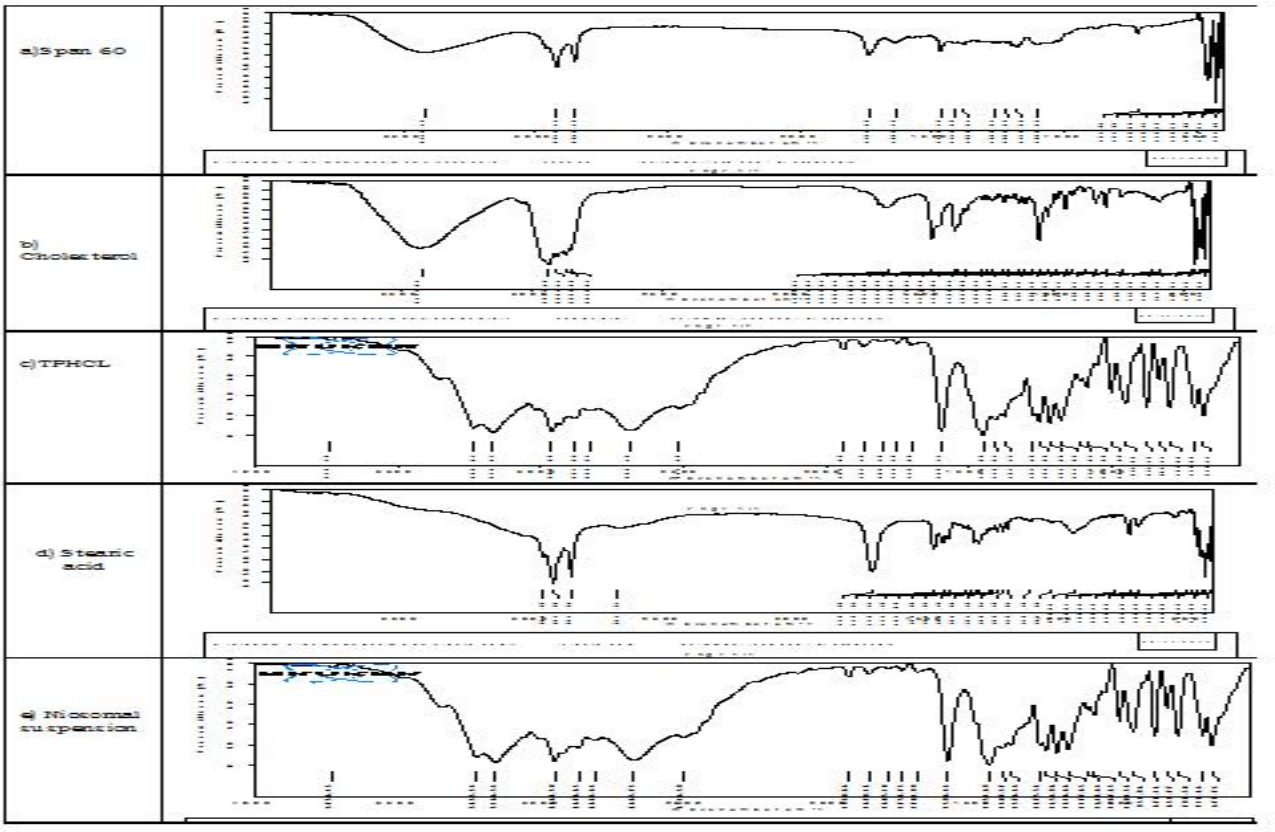
Table 3. Release kinetics of optimized formulation

Batch Code	Zero order		First order		Higuchi	Erosion	Peppas	
	K_o	r	K_1	r	r	r	r	n
N8	3.68	**0.98**	0.16	0.94	**0.99**	0.98	0.99	0.56

The stability studies indicated that the results of the stability study indicated that the % entrapment efficiency of the niosomal dispersion decreased from 75 to 68% when stored at 2-8°C and to 63 % when stored at 25°C after 2 months. The drug drain from the vesicles was less at 2-8°C. This is because of the phase transition of the surfactant at higher temperature causing vesicle leakage during storage. So that the proffered storage condition for the niosome was 2-8°C.

FTIR studies (Figure 5) were done to observe the prospective interactions between TP, and excipients. The FTIR spectrum of TP shown N-H stretching observed at 1996 cm^{-1} and 2682 cm^{-1} may be due to N-H stretching. The strong multiband absorptions of tertiary amine hydrochloride salts in the 2700 to 2330 cm^{-1} regions. Asymmetric stretching vibrations of the methyl groups are assigned as a strong band at 2961cm^{-1}. Cholesterol FTIR spectrum display C-O alcoholic stretching vibration at 1022.59cm^{-1}, O-H stretching vibration at 3417.9cm^{-1}, C-H (aliphatic) stretching vibration at 2932cm^{-1} and C=C stretching vibration at 1465cm^{-1}. The FTIR spectrum of stearic acid shows C-H aliphatic stretching vibration at 2955cm^{-1} and C=O (acid) stretching vibration at 1703 cm^{-1}. Span 60 (sorbiton monostearate) FTIR spectrum present –O- (cyclic ether) stretching at 1468cm^{-1}, O-H stretching vibration at 3418cm^{-1}, C=O (ester) stretching at 1737cm^{-1}, and the FTIR spectrum of niosomal suspension present N–H stretching vibration at 3344.66 cm^{-1} , aliphatic C–H stretching vibration at 2928cm^{-1}-2849.88cm^{-1} and aromatic C=O stretching vibration at 1744.23 and C=C aromatic stretching vibration at 1467.02-1413.85 cm^{-1}. As results from FTIR spectra of TP, excipients and niosomal suspension, there was no interaction of TP with excipients used in the formulations

Figure 5. FTIR spectrum of (a) Span60 (b) Cholesterol (c)TPHCL (d) Stearic acid (e) Niosomal suspension.



Characterization of niosomal and plain gels

Drug content for niosomal gel and plain gel was found to be 98.75 % and 98.39 % respectively and the content uniformity obtained was 98.60±1.04 % and 99.41±0.96 % (mean±s.d., n=3) for niosomal gel and plain gel respectively.

Comparative *ex vivo* permeation

Comparative *ex vivo* permeation studies were conducted for optimized batch of niosomal suspension, niosomal gel prepared using optimized batch and for plain gel. The results are given in the figure 6. Cumulative amount of drug permeated through skin was 770.07µg/cm^2, 667.97µg/cm^2 and 503.41µg/cm^2 for niosomal suspension, niosomal gel and plain gel respectively. The permeation of niosomal gel was reduced when compared to niosomal suspension, which is elucidating by increasing formulation viscosity due to gum karaya that delay vesicles release from its consistent mass. The permeation of drug from niosomal gel is more compared to plain gel as encapsulation of TP in niosomes produced an enhancement of permeation compared to plain gel. Transdermal flux (J) for the three formulations across the rat skin was calculated from the slope of the cumulative drug permeated per unit area verses time plot. The permeability coefficient (Kp) was calculated from the transdermal flux and the applied concentration in the donor compartment (C_{donor}) as per the following equation.

$Kp = J / C_{donor}$

Transdermal flux and Kp of niosomal suspension were found to be higher compared to niosomal gel and plain gel. Table 4 display results.

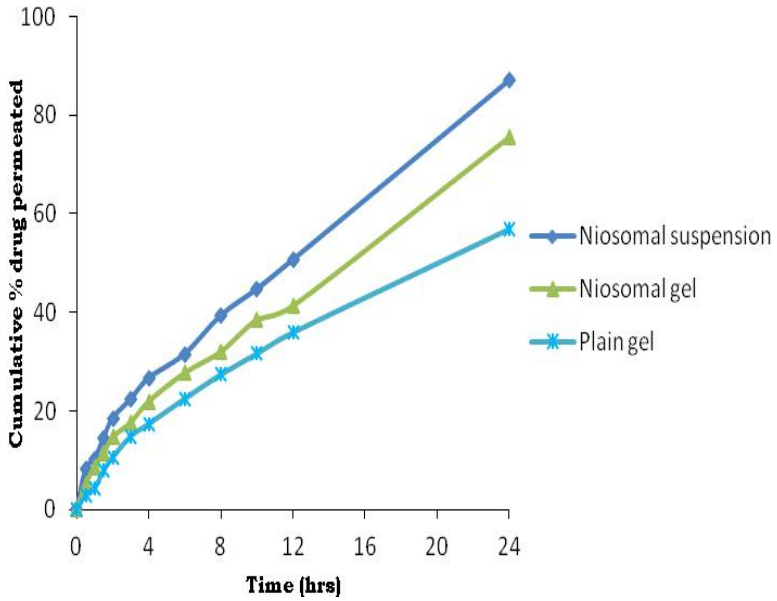


Figure 6. Comparative *ex vivo* permeation

Table 4. *Ex vivo* permeation profile of test products

Formulation	Cumulative amount permeated (µg/cm^2)	Transdermal flux (µg/cm^{2}h^{-1})	Permeability coefficient (cm/hr)
Niosomal suspension	770.07	30.10	7.52 X 10^{-3}
Niosomal gel	667.97	26.29	6.5 X 10^{-3}
Plain gel	503.41	20.84	5.2 X 10^{-3}

Ex-vivo permeation studies confirm the ability of niosomes to alter the therapeutic effect of TP due to the synergetic effect of surfactant and cholesterol. Niosomal vesicles increase the fluidity of skin barrier by interacting with lipid portion of the stratum corneum. It helps the vesicles to partition easily and to penetrate deeper into the skin from niosomal suspension. Higher cumulative amount of drug permeated and steady state transdermal flux from the niosomal gel formulation can be explained by solubilisation, penetration and permeation enhancement effect of amphiphiles of the niosomes bilayer.

CONCLUSION

Tapentadol loaded niosome were successfully developed by ethanol injection technique. The highest entrapment efficiency and zeta potential was found to be 75.25%±1.32 and -38.8mV respectively. The *In vitro* skin permeation studies of niosomes showed 98.92±1.2% of permeation over 24 hrs. The FTIR confirms the drug and excipients compatibility. The *ex vivo* rat skin permeated studies states that niosomal suspension shows maximum flux (30µg/cm^2/hr) comparatively niosomal gel (26.29µg/cm^2/hr) and plain gel (20.84 µg/cm^2/hr). These improvements in TP formulation may be useful in developing a more effective therapy.

REFERENCES

1. Barry BW (1983) Dermatological formulations: percutaneous absorption. New York and Basel: Marcel Dekker.
2. El Maghraby GM, Williams AC (2009) Vesicular systems for delivering conventional small organic molecules and larger macromolecules to and through human skin. Expert Opin Drug Deliv 6:149-163.
3. Barry BW (1988) Action of skin penetration enhancers-the Lipid Protein Partitioning theory. Int J Cosmet Sci 10(6):281-293.
4. Madhavi N, Sudhakar B and Ratna JV Colloidal dispersions (liposomes and ethosomes) for skin drug delivery and their role on rheumatoid arthritis. Asian J. Pharm. 2016; 10(3):208-221.
5. Aranya Manosroi, Charinya Chankhampan, Worapaka Manosroi, Jiradej Manosroi, Transdermal absorption enhancement of papain loaded in elastic niosomes

incorporated in gel for scar treatment, Eur. J. Pharmaceut. Sci.2013; 48; 474–483.
6. Gannu P. Kumar, Pogaku Rajeshwar rao, Nonionic surfactant vesicular systems for effective drug delivery—an overview, Acta Pharm. Sin. B.2011;1(4): 208–219.
7. https://go.drugbank.com/drugs/DB06204 accessed on 23/07/2020
8. Mali N, Darandale S, Vavia P. Niosomes as a vesicular carrier for topical administration of minoxidil: formulation and in vitro assessment. Drug Deliv Transl Res. 2013;3(6):587-92.
9. Ghanbarzadeh S, Arash K, Sanam A. Nonionic surfactant-based vesicular system for transdermal drug delivery. Drug Deliv. 2014; 22:8, 1071-1077.
10. Madhavi N, Sudhakar B, Reddy KVNS, Ratna JV. Design by optimization and comparative evaluation of vesicular gels of etodolac for transdermal delivery. Drug Dev Ind Pharm. 2019;45(4):611-628.
11. Sudhakar B, Varma JN, Murthy KV. Formulation, characterization and ex vivo studies of terbinafine HCl liposomes for cutaneous delivery. Curr Drug Deliv. 2014;11(4):521-30.
12. Saurabh Bansal, Geeta Aggarwal, Pankaj Chandel, S. L. Harikumar Design and development of cefdinir niosomes for oral delivery.J Pharm Bioallied Sci. 2013; 5(4):318–325.
13. Ketul K. Patel, Praveen Kumar, Hetal P. Thakkar Formulation of Niosomal Gel for Enhanced Transdermal Lopinavir Delivery and Its Comparative Evaluation with Ethosomal Gel. AAPS PharmSciTech. 2012;13(4):1502–1510.