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In-vivo evaluation of Topical Niosomal Gel containing Loxapine Succinate for the Treatment of Schizophrenia

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Abstract
The utilization of the antipsychotic medication Loxapine succinate on a daily basis is prevalent. Currently, there exist two distinct categories of dosage forms that are readily accessible in the market: capsules and injectables, specifically those administered intramuscularly. Nevertheless, individuals with psychosis encounter difficulties in adhering to the prescribed dosage forms on a daily basis, and at times, the administration of these forms proves to be challenging. The objective of the present study was to develop a niosomal gel formulation containing Loxapine succinate, with the aim of utilizing it for the management of schizophrenia. Niosomes were prepared through the implementation of a thin film hydration technique, utilizing different proportions of surface-active agents (Span 60, Span 80). The assessment of the vesicle size, entrapment efficiency, in-vivo release study, stability analysis, pH, viscosity, drug content, and homogeneity of the gel were conducted for the Loxapine succinate topical niosomal gel formulations.These benefits include enhanced patient compliance, improved skin retention in terms of both quantity and duration, heightened therapeutic efficacy, and reduced drug toxicity. This research aims to investigate the potential of the niosomal drug delivery system in providing a consistent and prolonged release of Loxapine succinate while mitigating the adverse effects associated with its oral administration. The sustained activation of the entrapped drug will lead to a reduction in the adverse effects associated with frequent oral administration of the drug. This implies that the utilization of topical niosomal gel-based drug delivery systems may serve as a valuable method for administering Loxapine succinate.

Keywords: Niosomal gels, Loxapine succinate, gel formulation, topical gel.

INTRODUCTION

Transdermal drug delivery systems represent a self-sustaining and convenient approach to medication administration, specifically designed to facilitate the controlled and appropriate delivery of drugs through a patient's skin. The mode of delivery mentioned above demonstrates significant advancements in the treatment of various medical conditions. The aforementioned system offers numerous advantages, including its user-friendly nature, minimal discomfort, and convenient usage. Additionally, it enables self-administration, allows for multi-day dosing, mitigates adverse effects, prevents fluctuations in drug levels, and importantly, enhances patient adherence. The adoption of this pharmaceutical administration technique has witnessed significant expansion in the discipline, attributable to its remarkable advantages.

Niosomes, lipid-based vesicles consisting of non-ionic surfactants, have been utilized for the purpose of encapsulating diverse pharmaceutical compounds, with the objective of augmenting their stability and extending their duration of storage. The non-ionic properties exhibited by these vesicles present an opportunity to alleviate drug toxicity and its accompanying adverse effects. The main aim of this drug delivery system, which is designed for a particular location, is to improve the precision of medication and therapeutic index, while also achieving drug targeting. Niosomes have demonstrated potential as a feasible carrier for pharmaceutical delivery. Niosomes can be generated using several methods, including manual agitation, ether injection, thin film hydration, and the pH gradient method. The epidermal tissue is subjected to drugs containing niosomes for dermal application without causing any systemic effects.

Niosomes can be generated using a range of methodologies, including manual agitation, ether injection, thin film hydration, and the pH gradient technique[1,2].

The advantages of employing niosomes[3]

- L'Oreal has been at the forefront of incorporating niosomes into topical pharmaceutical applications due to their advantageous characteristics. These include the use of aqueous-based vesicle suspension, which has been

shown to improve patient adherence compared to existing oil-based solutions. Niosomes possess the ability to encapsulate drug substances that are hydrophilic, lipophilic, and amphiphilic, making them valuable in the development of a wide range of pharmaceutical products.

- The morphological attributes of the vesicle can be adjusted to align with our specific needs.
- The utilization of niosomal vesicles enables the achievement of a regulated drug release, thereby guaranteeing a gradual and sustained pattern of release.

Further advantages of niosomes can be observed [4]

- Under normal circumstances, these entities demonstrate osmotic activity and stability. Moreover, they contribute to the stability of the entrapped medication.
- Surface active agents do not require specific storage conditions.
- The cutaneous absorption pathway has been discovered to increase the oral bioavailability of pharmaceutical substances.
- These substances possess the capacity to be administered via oral, parenteral, or topical routes of delivery.
- The surfactants employed in the formulation of niosomes exhibit favorable attributes such as biodegradability, biocompatibility, and non-toxicity.

There are several disadvantages that are commonly associated with the use of niosomes[5]

- The occurrence of physical instability is observed in specific systems.
- Aggregation refers to the phenomenon in which individual particles or molecules undergo a process of coalescence, resulting in the formation of larger structures.
- Fusion pertains to the amalgamation of multiple entities, resulting in the formation of a singular entity.The process of hydrolysis of drugs that are encapsulated has the potential to result in a decrease in the shelf life of the dispersion.

Schizophrenia

The term "schizophrenia" is derived from the Greek words "skhizein," denoting the act of dividing or splitting, and "phren," referring to the brain, intellect, and heart. Schizophrenia is a highly debilitating psychiatric disorder that can manifest at any stage of life, although it commonly emerges during late adolescence or in later adulthood. The aforementioned neurological disorder is distinguished by the presence of delusions, a loss of personality or the manifestation of a dual personality, cognitive disarray, restlessness, social isolation, and psychosis. According to psychologists, the early phase in the development of schizophrenia involves a disturbance in the neurotransmitter dopamine. The genetic predisposition towards mental illness is undeniably a contributing factor to this state of imbalance. Changes in the levels of additional neurotransmitters, such as serotonin, have the potential to be causative factors in the development of the disease[6,7].

In 2013, Shivhare et al. developed a niosomal gel with topical application for the transdermal delivery of an antihypertensive medication. The technique of thin film hydration was employed in the production of niosomal gels[8]. The stability, microscopic quality, and entrapment effectiveness of the prepared niosomes were evaluated. In 2014, Ashmoony et al. developed a formulation of clomipramine encapsulated in niosomes, with the aim of achieving transdermal controlled release. The enhancement of clomipramine (CLM) bioavailability can be achieved through the development and optimization of a niosomal transdermal formulation. This approach aims to reduce the extent of first-pass metabolism in the liver[9]. In 2016, Vijaya Rajendran developed niosomes as a means to enhance bioavailability by prolonging the maintenance of therapeutic plasma concentration. The diffusion of the hydrophilic sertraline HCl across the lipid-rich environment may occur at a slow rate due to the presence of lipophilic surfactants and cholesterol in the niosome[10]. This phenomenon leads to the formation of a depot effect. In 2019, Sayeda Salma et al. developed niosomes containing mirtazapine specifically for nasal administration. The approval for the treatment of depression has been granted by the United States Food and Drug Administration (FDA) for the tetracyclic antidepressant known as mirtazapine. The medication in question belongs to the BCS class II category and exhibits a limited oral bioavailability of 50% as a result of first-pass metabolism. The objective of this study was to enhance the solubility of the drug by encapsulating it within niosomal vesicles that were subsequently incorporated into an in-situ gel[11]. This approach was intended to improve the drug's permeability across biological membranes, thereby increasing its bioavailability. In the year 2020, Carrithers et al. conducted a study. The United States has recently granted approval for a transdermal formulation designed for the treatment of schizophrenia. The plasma concentrations and receptor binding levels observed in individuals using the transdermal formulation are similar to those observed in individuals using the sublingual formulation. When considering both efficacy and potential negative outcomes, it can be observed that transdermal asenapine exhibits a clinical response that is similar to that of sublingual asenapine. The administration of asenapine, a distinctive second-generation antipsychotic, necessitates methods that bypass the gastrointestinal tract due to its extensive hepatic first-pass metabolism, resulting in near-complete degradation. A sublingual formulation has been made available. In 2021, a novel formulation for intranasal delivery was developed by Raghavendra Kumar Dwivedi et al., utilizing an antidepressant agent. A niosomal in-situ gel containing desvenlafaxine succinate was prepared

using the thin film hydration method, commonly referred to as hand shaking. The scope of this study encompasses the conceptualization, advancement, and characterization of a niosomal in-situ gel[12]. The study conducted by Salem et al. (2022) The research conducted an investigation on the development of DXH-loaded glycerosomal (DXH-GLYS) in situ gel for rectal administration with the aim of enhancing DXH permeability and bioavailability[13].

Relevant work

Materials and method: The thin film hydration method was adapted in order to generate niosomes. The experiment involved dissolving accurately weighed quantities of surfactants (span 60, span 80) and CHO in a mixture of chloroform and methanol (2:1 ratio) containing 10 mg of loxapine succinate in a 10 ml volume. The solvent was subsequently evaporated at ambient temperature and reduced pressure, with continuous rotation, resulting in the formation of a thin lipid film. The film that had formed was hydrated using 10 mL of pH 7.4 phosphate-buffered saline (PBS). In order to generate niosomal dispersions encompassing both unbound and encapsulated pharmaceutical agents of diverse dimensions, it is necessary to employ appropriate techniques., the hydrated niosomes were subjected to a 20-minute sonication process in a bath sonicator as documented by Barakat et al., 2009[14].

Table.1 Optimization of molar ratios of span 60: cholesterol and Span 80: CHO with respect to vesicle size and entrapment efficiency.

S.no	Formulation Code	Span 20: CHO	Vesicle size (µm)	Entrapment efficiency (%)
	SF_1	1:1	1.8± 0.5	18.23±4.61
	SF_2	1:2	2.0±0.3	**69.10±4.06**
	SF_3	1:3	1.6 ±0.1	20.52±2.1
	SF_4	1:4	1.4 ±0.3	25.65±2.5
	SF_5	1:5	1.54 ±0.3	21554 ±1.5
Span 80 formulations				
	SF_6	1:1	1.6 ±0.1	30.23±3.5
	SF_7	2:1	1.7 ±0.14	67.80±4.01
	SF_8	3:1	2.0 ±0.06	27.35±2.2
	SF_9	4:1	1.5 ±0.02	19.56±4.04
	SF_{10}	5:1	1.44 ±0.45	4 ±1.5

Note: The SF2 Formulations exhibit a maximum drug entrapment efficiency of 69.10% and have been selected for subsequent investigation. The optimal formulation (SF2) mentioned earlier should be utilized for integration with a gel base.

Characterization of niosomes:

Microscopy &vesicle size analysis of niosomes: The utilization of optical microscopy was employed to confirm the formation of vesicles in niosomes, utilizing a resolution of 45x. The niosomal suspension was administered onto a glass slide and subsequently secured in place. Subsequently, the niosomal suspension was subjected to examination to assess the characteristics of the resulting film, which exhibited dryness and thinness. The process of obtaining microphotographs of niosomes involved the use of a digital camera attached to a microscope.The determination of vesicle dimensions was conducted using ocular and stage micrometers..The provided illustration portrays a microscopy image of niosomes that have been effectively loaded with a pharmaceutical compound.

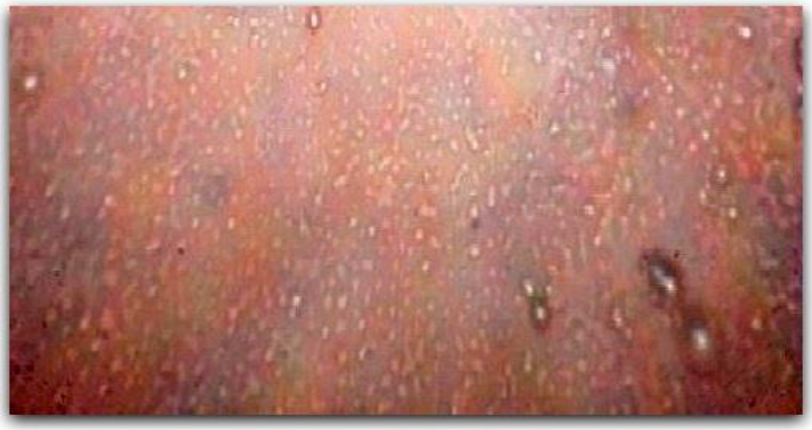

Fig. 1 Niosomes under optical microscope.

Entrapment Efficacy

The assessment of entrapment efficiency was conducted using the micro centrifuge RM-12CDX. The niosomal suspension was subjected to centrifugation at a speed of 12000 revolutions per minute for a period of 20 minutes. The concentration of the drug in the supernatant was determined through the utilization of UV spectroscopy, specifically at a wavelength of 252nm. A comparison was made between the absorbance of the drug and the standard calibration curve. Furthermore, the dilution factor was computed.

% of drug encapsulated was calculated by following equation –

EE% = [(Ct-Cf)/Ct)] × 100

Where,

Ct is the concentration of total drugs

Cf is the unentrapped drug

Preparation of Gel base:

The gel matrix was formulated by dispersing 1 gram of carbopol 934 into 80 milliliters of double distilled water. The solution underwent agitation at a speed of 800 revolutions per minute for a period of 60 minutes. A quantity of 10 milliliters of propylene glycol was added to the previously mentioned solution. The neutralization of the mixture was achieved by slowly adding a 10% NaOH solution, while ensuring constant agitation, until a transparent gel was produced. Subsequently, the ultimate volume was modified to 100 milliliters. Pavelic et al. (2005) reported that the pH of the gel base was adjusted to 6.5[15].

INCORPORATION OF NIOSOMES INTO GEL:

The current investigation encompassed the incorporation of niosomes within a gel matrix. In order to achieve the desired drug concentration in the resulting gel formulation, a niosomal formulation was prepared with a drug content of 6.0% w/w (6.0 mg in 100 mg of gel) and then blended with the gel base.

The assessment of the physical stability of a niosomal gel intended for topical application. A comprehensive examination of the physical stability of the prepared topical niosomes gel was performed. The aim of the study was to investigate the release of the drug from the niosomes at different temperature settings, specifically at a refrigeration temperature of 4±5˚C and at room temperature of 25±31˚C. The drug content within the niosomes was observed.

Table 2. Observation table of physical stability of Topical Niosomes based gel with respect to vesicle size and entrapment efficiency.

SF2	T_1		T_2	
	1 month	2 month	1 month	2 month
Vesicle size	1.9±0.3	2.1±0.1	1.5 ±0.4	2.4±0.5
% entrapment efficiency	69.10±4.5	65.20±1.7	68.56±2.34	67.68±0.18

Where, T_1 =Refrigerator condition, T_2 = Room temperature

Viscosity: The viscosity of a topical niosome-based gel was determined using a Brookfield viscometer with spindle no. 63 and a speed of 10rpm. The resulting viscosity value was 3776cps.

Drug content: A precisely quantified quantity of 100 mg of topical niosomal gel was introduced into a beaker and subsequently combined with 20 ml of hydrochloric acid solution with a concentration of 0.01N. The solution was subjected to extensive mixing and subsequently filtered using Whatman filter paper no.1. Following this, a 1.0 mL portion of the filtered solution was transferred into a 10 mL volumetric flask and the volume was then made up to 10 mL using a 0.01 N hydrochloric acid (HCl) solution. The solution in question was subjected to analysis using UV-Spectroscopy, specifically at a wavelength of maximum absorption of 251 nm.

Table.3 Drug content of Niosomal gel

S.no	Optimized niosomes	Drug content
1	SF_2	6.7%

Drug release: The study focused on evaluating the release of niosomal gels that were formulated for drug delivery. The experiment was carried out utilizing a Franz diffusion cell comprising of phosphate-buffered saline (PBS) with a pH value of 7.4. The experiment conducted by Ning et al. (2005)[16] involved the application of a 1mL dosage of topical niosomal gel onto a cellophane membrane measuring 2.5 cm in diameter and possessing a molecular weight of 1200 Daltons. The membrane was subsequently examined at different time intervals. Mathematical approach Employed a range of mathematical models, including zero order, first order, Hixsons Crowell, Higuchi, and Krosmeyer Peppas equations, in order to examine the in-vitro release data. The study additionally presented the correlation coefficients derived from the analysis.

Table 4. The cumulative percentage release of optimized formulations

Time(hr)	% Release of topical niosomal gel (SF_2)
0.25	3.21±1.12
0.5	4.23±2.54
1	13.25±0.45
2	18.75±4.40
4	25.73±1.56
6	29.9±1.77
8	34.56±2.60

10	38.45±4.20
12	41±2.44
24	52.65±5.30

Fig.2 In- vitro release study of Topical Niosomal base Gel of Loxapine Succinate

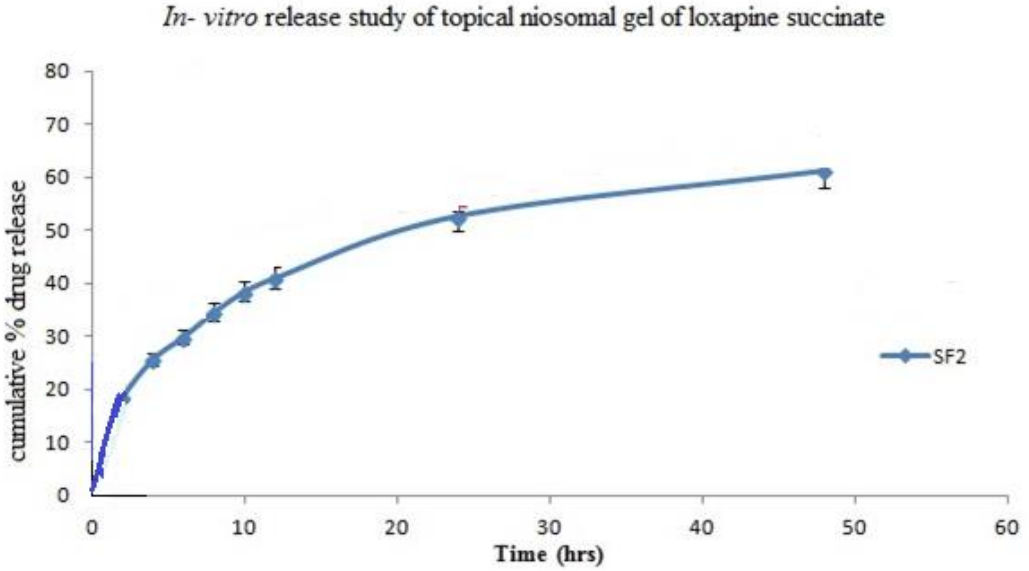

Table 5 Observation table for release order of optimized formulation of SF_2

S.no	Release order	Equation	r^2 value
1.	Zero order	y=2.307x+ 19.988	0.7333
2.	First order	y=-0.0104x+1.9063	0.8573
3.	Hixson crowell	y=0.2286x+4.5943	0.9709
4.	Higuchi	y= 10.395x+6.7999	0.9434
5.	Krosmeyer peppas	y=0.4722x+1.1535	0.9148
6.	Higuchi	y-0.10.385x+6.68787	0.9321

In-vivo Evaluation

Table 6 .Tail suspension test : Immobility Time (sec.) –

S. No.	TREATMENT	IMMOBILITY TIME (sec.)
1	Control	222.33±7.711
2	Pure Drug (Loxapine succinate)	127.50±5.577*
3	Formulation (SF_2)	156.33±7.607*

Values are expressed as MEAN±SD at n=6, One-wayAnova followed by Bonferroni test, *P<0.001 compared to the control group

Table 7. Haloperidol Induced Catatonia: Catalepsy (sec.) –

S No	TREATMENT	Catalepsy (sec.)				
		0 min	30 min	60 min	90 min	120 min
1	Control	1.17±0.408	64.83±8.329	85.50±6.565	113.50±7.662	98.67±5.922
2	Pure Drug (Loxapine succinate)	$1.33±0.516^{NS}$	23.50±4.416*	34.83±6.646*	26.83±5.115*	15.83±3.061*
3	Formulation(SF2)	$1.50±0.548^{NS}$	29.33±3.077*	48.0±5.762*	61.67±7.421*	34.33±4.179*

Values are expressed as MEAN±SD at n=6, One-way Anova followed by Bonferroni test, *P<0.001 compared to the control group

Conclusion

The present study involved the formulation of topical niosomal gels using the hand shaking technique, which was chosen as the preferred method due to its higher drug entrapment efficacy and smaller vesicle dimensions. The choice of spans has been determined with the objective of facilitating niosome formation. The assessment was conducted to determine the entrapment efficiency of Span 60 and Span 80, which were used in different molar ratios. The optimized formulation, particularly SF2 (Span 60), demonstrated enhanced drug entrapment with an efficiency of 67%. The results obtained from the in vivo evaluation indicate that SF2 exhibits heightened cataleptic effects when compared to both the control group and the unmodified form of the drug (Loxapine succinate). According to available reports, it has been observed that SF 2 exhibits a calming effect on animals for a period of 120 minutes. This effect is purportedly linked to the gradual release properties of the drug. The primary objective of the present investigation was to tackle the obstacles associated with oral drug administration, with the ultimate aim of improving patient compliance by facilitating sustained release of the medication. The solubility and chemical composition of a pharmaceutical compound are crucial considerations in the context of transdermal drug delivery. The investigation into the physical stability of niosomes demonstrated that there was a higher level of drug leaching at room temperature. However, it was observed that the niosomes remained stable when stored under refrigerated conditions. The addition of a gel base to niosomes has been shown to improve their stability by effectively inhibiting the fusion of niosomes[17].

The results of the study indicate that the application of a topical niosomal formulation has the potential to result in a reliable and prolonged release of Loxapine Succinate. The prolonged activity of the encapsulated medication may lead to a decrease in the adverse effects typically observed with frequent ingestion of the medication.

The viability of topical administration of Loxapine Succinate can be inferred. The results indicate that the application of a topical niosomal gel as a method of drug delivery shows promise as a vehicle for the delivery of Loxapine Succinate.

Conflict of Interest:

None

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