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Research Article

Preparation and Characterization of Ethosomes for Topical Delivery of Clindamycin

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Abstract

Topical medication administration is the simplest and most convenient method of delivering localised drugs to any part of the body via ophthalmic, rectal, vaginal, and cutaneous channels. These are used in a wide range of cosmetic and dermatological preparations on both healthy and sick skin. Drugs are applied topically to have an effect at the application site or to have systemic effects. The goal of this study was to develop and characterise clindamycin ethosomes that could carry the medicine to the target site more efficiently while also avoiding the complications associated with oral drug delivery. The formulations were tested for vesicle size, shape, and surface morphology, entrapment efficiency, and in vitro drug penetration using different amounts of ethanol (10-50 percent), HPMC (1-4 percent), and PVC (5-20 percent). Transmission electron microscopy and surface electron microscopy revealed ethosomes with an average size of 1.112m and a spherical shape with a smooth surface. The maximum percentage of ethosomes entrapped was 91.060.79%. The total amount of medication that pierced the biological membrane was found to be between 0.250.014 and 0.480.032 mg/cm2. The stability profile of the constructed system was evaluated for 45 days, and the results revealed that very little drug degradation occurred during storage.

Keywords: Clindamycin, Ethosomes, Topical delivery, HPMC, Acne, Vesicle size

INTRODUCTION

Transdermal drug delivery systems are intended to provide a therapeutically appropriate amount of medicine over a lengthy period of time over a patient's skin[1]. Around 40% of medication candidates are now undergoing clinical trials for transdermal administration. Avoidance of first pass metabolism, predictable and extended duration of activity, minimising undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding drug level fluctuations, inter and intra patient variations, and most importantly, patient confidence are all potential advantages of the transdermal route over traditional routes. However, a key issue with the transdermal route is the drug's limited penetration rate through the skin's outermost layer[2]. For transdermal drug administration of various medicinal compounds, non-invasive techniques such as permeation enhancers, vasicular approach, ablation, microneedle array, ultrasound, iontophoresis, electroporation, and so on are used[3]. The vesicular drug delivery technique is one of them. The use of the vesicular system in medication transport has altered the definitions of diagnosis and treatment in various areas of biomedicine. Liposomes, niosomes, ethosomes, sphinosomes, transferosomes, and pharmacosomes are examples of vesicular systems that are utilised to improve the therapeutic index of both existing and new pharmacological molecules by encapsulating the drug inside a vesicular structure[2]. Ethosomes are novel nanovesicles that hold the drug in a lipid, ethanol, and water matrix. The ethosomes are soft, and a highly flexible vesicle penetrates the skin efficiently, increasing drug delivery of drug molecules. Ethosomes are Phospholipid-based elastic vesicles that contain 20-45 percent ethanol. Ethanol also improves permeation by dissolving the lipids in the skin. The elastic vesicles can squeeze themselves between the skin pores because ethosomes are exceedingly flexible. Topically applied ethosomes increase the drug molecule's residence duration in distinct layers of the skin, such as the stratum corneum and epidermis, and inhibit systemic absorption. Ethosomes bind to skin lipids and release the medication into the skin's deeper layers[4-7]. Acne vulgaris is a prevalent dermatological condition that affects up to 85 percent of the population in different regions[8]. It has a considerable effect on the patients' quality of life[9, 10], psychosocial development, and self-esteem[11]. It primarily affects areas of the skin with the largest concentration of sebaceous follicles, such as the face, upper chest, and back[12]. Antibiotic treatment for P. acnes is one of the most important aspects of acne vulgaris treatment. Despite the fact that systemic antibiotics have been used to lower the population of P. acnes for some years, topical antibiotics are more acceptable due to their fewer side effects and interactions. Clindamycin, among the commonly used topical antibiotics for acne vulgaris, has shown to be more effective

over time[13]. Clindamycin also contains anti-inflammatory characteristics, which are expected to play a key role in its anti-acne therapeutic effects[14]. The efficacy of topical antimicrobial medicines is affected by the concentration of the medication in the pilosebaceous ducts. Furthermore, the formation of resistance is linked to low and fluctuating drug concentrations in the pilosebaceous ducts[15, 16]. As a result, reliable drug delivery systems that allow for improved drug penetration can improve efficacy while also preventing the development of resistance. The goal of this study was to statistically optimise the ethosomes for improved clindamycin skin delivery, which was a successful option for acne treatment.

MATERIALS AND METHODS

Material

Clindamycin was given to me as a gift from Alembic Ltd. in Vadodara, India. Himedia Laboratory, Mumbai, sold HPMC and PVC. CDH Chemical Pvt. Ltd. provided the ethanol. Ltd. New Delhi is the capital of India. Himedia Laboratory in Mumbai provided a Mol Wt cutoff 1200 dialysis membrane. Freshly produced double distilled water was utilized whenever necessary. The rest of the components and compounds were of analytical quality

Preformulation studies

Physical characteristics

Physical characteristics such as colour, texture, and scent were used to identify the drug via a visual examination[17].

Solubility

The drug's solubility was evaluated by placing a little amount of the drug (approximately 1-2 mg) in a test tube and adding 5 ml of the solvent (water, ethanol, methanol, 0.1N HCL, 0.1N NaOH, chloroform, and 7.4 pH buffers) and shaking vigorously for several minutes. Take note of the drug's solubility in various solvents (at room temperature).

Melting point

In a fusion tube, a small amount of powder was inserted. The castor oil-filled tube was inserted in the melting point determining equipment (Chemline). The temperature of the castor oil was gradually raised, and the temperature at which the powder began to melt was recorded, as well as the temperature at which the powder was completely melted.

FTIR spectroscopy

Clindamycin was identified via FTIR spectroscopy in relation to a marker chemical. Clindamycin is a crystalline powder that is white to off white in colour. It was detected based on the IR spectrum's result, which met the requirements.

Determination of λ_{max} of clindamycin

In a 10 ml volumetric flask, 10 mg of medication was accurately weighed and dissolved in 10 ml of phosphate buffer pH 7.4 solution. The resulting solution has a density of 1000g/ml, so take 1 ml of it and pipette it into a 10 ml volumetric flask, and then top it up with phosphate buffer pH 7.4 solutions. Prepare an appropriate dilution to bring the concentration down to 10-50g/ml. In ultraviolet light, the spectrum of this solution was run in the 400-800 nm regions.

Methods

Preparation of clindamycin loaded ethosomes

Touitou et al[7] reported that ethosomes were stimulated. In brief, HPMC (1-4 percent w/v) was used in a small RBF and was solubilized with ethanol (10-50 percent v/v) containing medication while being stirred with a magnetic stirrer. To prevent ethanol evaporation, the spherical bottom flask was covered. To make the ethosomal colloidal suspensions, distilled water was gently added with constant stirring. The finished ethosome suspension was held at room temperature for 30 minutes with constant stirring. Vesicle size, vesicular shape, surface morphology, entrapment efficiency, in vitro drug permeation study, and stability study were all performed on the formulations while they were kept in the refrigerator.

Table 1: Ethosomal formulations with different concentration

Formulation code	HPMC Concentration (%)	PVC Concentration (%)	Ethanol Concentration (%)
ETE1	3	-	10
ETE2	3	-	20
ETE3	3	-	30
ETE4	3	-	40
ETE5	3	-	50
ETL1	1	-	30
ETL2	2	-	30
ETL3	3	-	30
ETL4	4	-	30
ETP1	2	5	30
ETP2	2	10	30
ETP3	2	15	30
ETP4	2	20	30

Characterization of ethosomes

Drug entrapment efficiency

A cooling ultracentrifuge (Remi) was used to centrifuge aliquots of ethosomal dispersion at 12000 rpm. The clear supernatant was carefully syphoned off to remove the unentrapped Clindamycin, and the absorbance was measured using a UV/Vis spectrophotometer at max 210nm (Shimadzu UV 1700). To lyse the vesicles, 1 ml of 0.1 percent Triton X 100 was added to the sediment, which was then diluted to 100 ml with methanol and the absorbance measured at 210nm. The total amount of clindamycin in a 1ml dispersion was calculated by adding the amounts of clindamycin in the supernatant and sediment. The formula was used to compute the % entrapment.

% entrapment= amount of aceclofenac in sediment/amount of aceclofenac added×100

Vesicle size and surface morphology

Using a computerized inspection system, dynamic light scattering (DLS) was used to estimate size and size distribution (Malvern Zetamaster ZEM 5002, Malvern, UK). Surface morphology was assessed using TEM, which involved placing a drop of the material on a carbon-coated copper grid and negatively staining it with a 1 percent aqueous solution of phosphotungstic acid after 15 minutes. The grid was fully dried by air, and samples were examined using a transmission electron microscope (TEM, FEI-Philips Tecnai 10). A drop of ethosomal system was mounted on a clear glass stub; air dried, and coated with Polaron E 5100 Sputter coater (Polaron, UK) and observed under a scanning electron microscope to describe the surface morphology of the ethosomal vesicles.

In vitro drug permeation study

The in vitro permeation study was conducted using a modified Franz diffusion cell with an egg membrane. In the experiment, phosphate buffered saline was used (pH 7.4). In the donor co mpartment, the formulation was applied to the upper surface of the skin (equal to 2.5 mg of medication). A consistent tempe rature of 37±2°C was maintained throughout the construction.

The sampling tube was used to take samples from the receptor media every hour, and the same amount of fresh receptor me dia was supplied to generate a sink condition. The clindamycin constant in withdrew samples was determined using a UV/VI S spectrophotometer.

Stability study

For the stability research, optimized ethosomal formulations were chosen. The formulas were kept at 4°C, 8°C and at room temperature. At various time intervals, the percentage of drug entrapment was determined (1, 15, 30 and 45 d).

RESULTS AND DISCUSSIONS

Clindamycin was discovered to be a white to off white powder with no odour or flavour. Clindamycin (pure drug) melting point was discovered to be 140-142°C. Clindamycin was easily soluble in ethanol, methanol, 0.1 N HCl, distilled water, and phosphate buffer pH 7.4; it was mildly soluble in 0.1 N HCl, distilled water and soluble in phosphate buffer pH 7.4. Clindamycin was identified via FTIR spectroscopy in relation to a marker chemical. The result of the IR spectrum was used to identify it, as shown in fig. 1. Clindamycin calibration curve at 210nm was found to be linear in the concentration range of 10-50µg/ml. In this study, an ethosomal formulation was created and tested to improve clindamycin transdermal penetration. The reported method was used to make colloidal ethosome suspensions. The ethosomal system was discovered to be simple to make and was mostly consisted of HPMC and ethanol, both of which are often present in medicinal preparations. Malvern Zetamaster determined that the average vesicle size of improved formulations was 1.112±0.053µm. TEM images revealed the ethosomes' surface shape as well as the presence of a unilamellar vesicular structure (fig. 2). SEM revealed the flat surface of the vesicles (fig. 3). For all formulations, the ethosome entrapment efficiency was determined. The % drug entrapment of ethosomes was shown to be affected by ethanol concentration. The greatest entrapment efficiency for formulation ETE3 was found to be 91.06±0.79%, while the minimum entrapment efficiency for formulation ETE5 was determined to be 52.36±0.82%. With an increase in ethanol concentration, there was an increase in percent drug entrapment, however when ethanol concentration above 30%, there was a drop in percent drug entrapment. Higher concentrations of ethanol improved clindamycin aqueous solubility, which could be attributed to the co-solvent action of ethanol. When a result, more drugs could be accommodated in the aqueous core of the vesicles, but as the concentration of ethanol went over 30%, drug leakage from the fluidized bilayer of the vesicles occurred. The entrapment efficiency rose as the concentration of HPMC increased, however there was no significant rise in percent entrapment after 3 percent HPMC concentration. Formulation ETL1 had the lowest percent entrapment at 76.03±0.80 percent, while Formulation ETL4 had the highest at 90.21±0.80 percent. This could be owing to the drug's lipophilic nature, which could cause it to become more encapsulated in the formulation's lipid bilayer. PVC content rises, which reduces drug entrapment in formulations. ETP1 and ETP4 formulations were found to have 89.11±0.1 percent and 67.31±0.90 percent entrapment, respectively. The results showed that as the permeation enhancer concentration grew, the fluidity of the bilayer increased as well, leading the medication to leach out of the vesicles. Table No. 2 Table 2 shows the effects of ethanol, HPMC, and PVC on the drug permeation rate of clindamycin. Drug penetration was shown to rise as the quantity of ethanol was increased from 10% to 30%, but as the ethanol concentration was increased further, drug permeation was found to decrease, which could be related to the rupture of bilayer vesicles at higher ethanol concentrations. At 30 percent ethanol concentration, the maximum penetration was reported to be 0.40±0.080 mg/cm2. HPMC plays a vital role in formulation because it is the major component of the ethosomal system. The effect of HPMC concentration on drug permeability was also investigated in this study in order to improve the formulation for system-wide sustained release features. As the concentration of HPMC in the produced ethosomal system went above 2%, drug permeability reduced from 0.41±0.022 mg/cm2 to 0.41±0.022 mg/cm2 due to vesicle bilayer deformability. The amount of drug penetrated was similarly affected by variations in PVC content. The greatest amount of medication penetrated was 0.48±0.032 mg/cm2 (formulation ETP2), and the minimum system played an essential part in formulation. The effect of HPMC concentration on drug permeability was also investigated in this study in order to improve the formulation for system-wide sustained release features. As the concentration of HPMC in the produced ethosomal system went above 2%, drug permeability reduced from 0.41±0.022 mg/ cm2 to 0.41±0.022 mg/ cm2 due to vesicle bilayer deformability. The improved formulation was chosen for the research of vesicle stability at various temperatures. All of the formulations were kept at varying temperatures in an amber glass container. Table 3 shows the drug content after treatment with triton X100, as well as the percent clindamycin residue. Only 2±1% deterioration was seen at 37±2°C, and only 3% degradation was observed at ambient temperature; however, all formulations were almost

stable between 8±2°C and 4±2°C, with only 1.57±0.2 percent clindamycin degradation, indicating that the created system is stable at low temperatures.

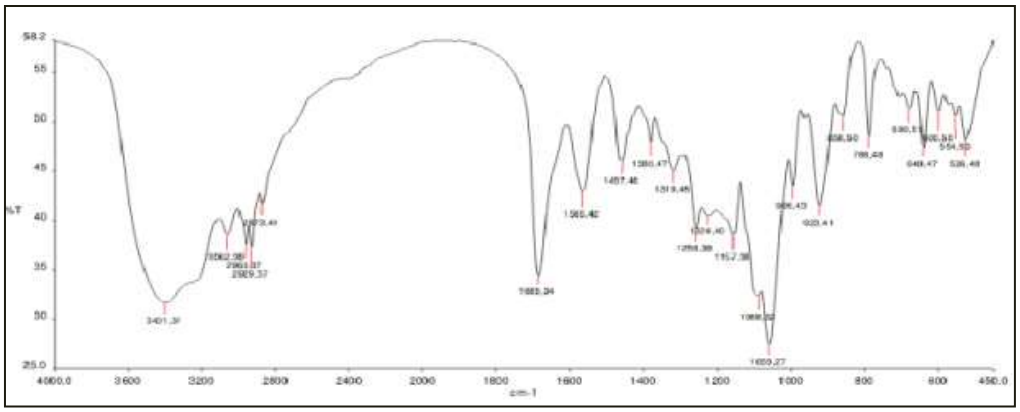

Figure 1: FT-IR spectrum of pure drug (Clindamycin)

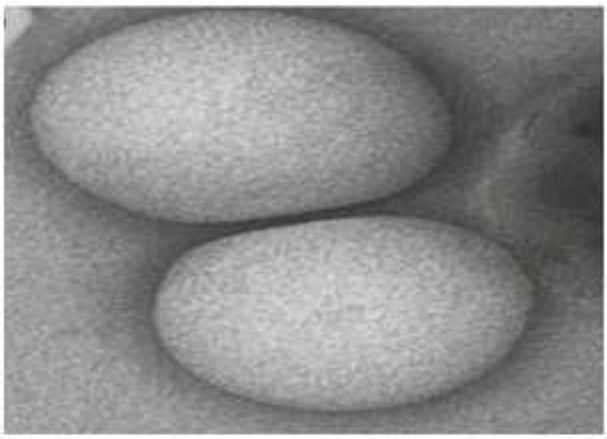

Figure 2: Transmission electron microphotograph Visualization of ethosomes by transmission electron microscopy (˜8400)

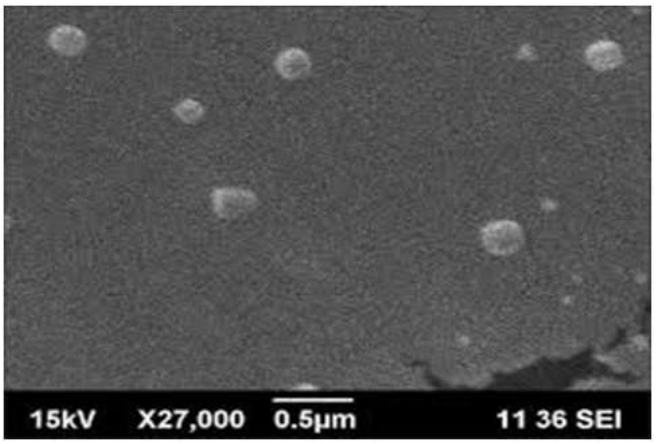

Figure 3: Scanning electron microphotograph Visualization of ethosomes by scanning electron microscopy (bar 2 µm)

Table 2: Entrapment efficiency and cumulative amount of drug permeated after 24hr

S. No	Formulation code	Entrapment efficiency	Cumulative amount of drug permeated (mg/cm²)
1	ETE1	83.59±0.86	0.29±0.010
2	ETE2	86.45±0.81	0.37±0.012
3	ETE3	91.06±0.79	0.40±0.080
4	ETE4	61.31±0.84	0.34±0.022
5	ETE5	52.36±0.82	0.25±0.014
6	ETL1	76.03±0.80	0.31±0.067
7	ETL2	89.01±0.92	0.41±0.022
8	ETL3	90.06±0.88	0.40±0.014
9	ETL4	90.21±0.79	0.32±0.036
10	ETP1	89.11±0.10	0.44±0.054
11	ETP2	88.86±0.85	0.48±0.032
12	ETP3	82.65±0.93	0.31±0.073
13	ETP4	67.31±0.90	0.26±0.080

Table 3: stability of vesicles on storage

Time (days)	Entrapment (%)			
	4±2 °C	8±2°C	37±2°C	Room temp.
1	89.86±0.85	89.86±0.85	89.86±0.85	89.86±0.85
15	89.82±0.76	89.73±0.73	88.88±0.71	88.78±0.75
30	89.53±0.92	88.58±0.90	87.07±0.85	87.67±0.86
45	88.97±0.83	88.29±0.82	87.89±0.89	86.89±0.89

CONCLUSION

These flexible vesicles could be a promising carrier for clindamycin transdermal distribution. It aids in the reduction of medication doses given topically. The facts given here show that systemic treatment of acne vulgaris could be replaced. As a result, gastrointestinal-related adverse effects would be reduced, potentially boosting patient compliance. Preclinical investigations of the optimized formulation will be conducted in future study.

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