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PREPARATION AND EVALUATION OF NIOSOMAL GEL LOADED WITH COMBINATION OF ISOTRETINION AND CLINDAMYCIN

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Abstract

The objective of the present study was to formulate topical niosomal gel loaded with combination of Isotretinion and Clindamycin for the beneficial of acne patients, to provide sustained release effects, to prevent their side effects. First generation topicalsretinoids (Isotretinion) and antibacterials (Clindamycin) target comedones, *Propionibacterium acnes* or inflammation. The combination of Isotretinion and Clindamycin is reported to treat almost all type of acne and have potential to increase both efficacies, patient adherence as compared to single agent treatment. Purpose of this study was to develop a stable formulation that allows progressive follicle penetration and increased efficacy. Preformulation studies such as melting point, FTIR spectroscopy, UV spectroscopy, solubility, partition coefficient (log P) were performed for both drugs. The niosomal combination of both drugs was incorporated into the carbopol gel. The pH and viscosity of gel was performed.

Keywords: Niosomes; Isotretinion; Clindamycin; Carbopol gel; Acne; Ether injection method.

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1. Introduction

Using nano-vesicular systems for topical application has gained growing interest over the years. They increase drug solubility and prolong its release and accordingly enable dose reduction with subsequent decrease of drug side effects [1, 2]. Niosomes are promising drug carriers for the delivery of pharmaceuticals and cosmetics. This type of delivery system improves the stability and enhances skin penetration of drugs [3, 4]. They are very popular in topical drug delivery due to their outstanding characteristics like enhancing the penetration of drug, providing a sustained release pattern of drug release, improves therapeutic index of drug by restricting its action to target cells and ability to carry both hydrophilic as well as lipophilic drugs [5, 6]. Also, niosomes have the ability to improve the drug residence time in the epidermis and stratum corneum and reduce the systemic absorption of the drug at the same time [7]. Budhiraja & Dhingra (2015) proved that gel containing antimicrobial-loaded niosomes is effective for acne vulgaris treatment by enhancing the time and amount of drug retention within the skin and improve the therapeutic efficacy of the drug [8].

ITR is one of the best treatment approaches for acne [9]. ITR reduces sebum production, number of acne causing bacteria (Propionibacterium acnes) on the skin's surface along with alleviation of inflammation. However, the drug administered by oral route is coupled with several adverse effects viz. teratogenicity, cheilitis dermatitis, conjunctivitis, blepharitis, skin fragility and xerosis. In recent times, topical ITR has been found to be favorable for acne management due to its anti-pathogenic effect, safety profile and being devoid of systemic side effects in comparison to oral ITR. Acne recommends combination of retinoids and antimicrobials for acne treatment owing to its superior efficacy than monotherapy. The main hindrance in combination therapy is the inconvenience of double topical application. Topical antibacterials like clindamycin phosphate (CLIN) and erythromycin act as bacteriostatic agents for P. acnes [10]. These agents also exhibit anti-inflammatory activity by inhibition of lipase production by P. Acnes [11]. The combination of both the agents reduces comedogenesis and contributes in the healing of acne lesions [12].

2. Meterials and Methods

2.1. Materials

Isotretinionwas received from Allwell Pharmaceutical company, India, whereas Clindamycin HCl purchased from Jakson Lab, India. Surfactants (Span 60, Span 80, Tween 60, Tween 80) were purchased from SD Fine chem. Ltd., India. Cellophane membrane was received from CDH Analytical Reagents, New Delhi, India while Triethanolamine, Sodium chloride, Disodium hydrogen orthophosphate, Potassium dihydrogen orthophosphate purchased from local store.

2.2. Methods

2.2.1. Preparation of Isotretinion and Clindamycin loaded Niosomes

Isotretinion as well as clindamycin loaded carrier system was prepared using ether injection method. In this method lipid was first dissolved in an organic solution which was then brought into contact with aqueous phase containing materials to be entrapped within the vesicles. In brief, surfactant i.e. (span 60, span 80, tween 60, tween 80) and cholesterol in different ratio were dissolved in 10 ml diethyl ether. Drug solution was prepared by adding drug into 10 ml phosphate buffer pH 7.4. Then dissolved surfactant/lipid were injected slowly at the rate of 0.25 ml/min through 23-gauge needle into 10 ml drug solution which is magnetically stirred continuously and maintained at 60 ◦C for 1 hr to ensure complete evaporation of solvent and to get uniform suspension of niosomes. Concentration of niosomal ingredients and process variables were optimized on the basis of size, shape, zeta potential and entrapment efficiency [13].

2.2.2.Preparation of gel

As a vehicle for incorporation of niosomes for skin delivery, carbopol gel was made. Carbopol 934 (450 mg) was dispersed in distilled water (60 ml) and allowed to swell overnight. Swelled carbopol was stirred at 800 rpm for 60 min. Mixture was neutralized by dropwise addition of triethanolamine. Mixing was continued until a transparent gel appeared, while the amount of base was adjusted to achieve a gel with pH 5.5 [14, 15].

2.2.3. Incorporation of niosomes of isotretinoin and clindamycin into the carbopol gel

Carbopol 934 (450 mg) was dispersed in distilled water (60 ml) and allowed to swell overnight. The swelled carbopol was stirred at 800 rpm for 60 min. The mixture was neutralized by dropwise addition of triethanolamine. Mixing was continued until a transparent gel appeared, while the amount of base was adjusted to achieve a gel with pH 5.5. Niosomes of isotretinion and clindamycin was dispersed in the carbopol gel with slow agitation.

2.3. Characterization and evaluation of fabricated formulation

Fabricated formulations are characterized based on their size, morphological characteristics, and surface charge. The size distribution, average particle diameter, and surface charge influence the physical stability, redispersibility, and in vivo performance of formulation.

2.3.1. Particle size and Zeta potential

The particle size measurements were taken by using Beckman coulter counter size analyzer at a temperature of 20◦C under a fixed angle of 90◦. Dispersions were diluted suitably with distilled water. Zeta potential was measured by using flow through cell cuvette, working on the principle electrophoretic light scattering (ELS), which determines electrophoretic movement of charged particles under an applied electric field from Doppler shift of scattered light [16].

2.3.2.Percentage entrapment efficiency

Percentage of isotretinion and clindamycin hydrochloride entrapped in the niosomes was determined by centrifugation of formulation at 25000 rpm for half an hour at controlled temperature of 4 ◦C. Supernatant was withdrawn and measured by UV spectrophotometer at 352 nm and 202 nm respectively [17,18]. Entrapment efficieny was calculated by using following formula [19]:

$$\% \text{ Entrapment efficiency} = \frac{\text{entrapped drug}}{\text{total drug}} \text{x} 100$$

2.3.3.Morphology Observation

With direct observation of the formulation, the electron microscopy-based approach evaluates their size, shape, and surface morphology. The solution was first converted into a dry powder, which was then deposited on a sample holder before being sputter-coated with a conducting metal (such as gold). The entire sample was scanned with a focused fine beam of electrons for analysis, and surface characteristics were determined [20].

2.3.3.1. Fourier-Transform Infrared Spectroscopy

FT-IR spectroscopy was used to confirm the purity of drug. FT-IR studies were performed on pure drug (Figures 5 and 6). Drug samples were scanned in the wavelength range of 400- 4000 cm−1 in an FT-IR spectrophotometer, and the spectra were recorded.

2.3.3.2. Transmission electron microscopy (TEM)

Niosomes preparations were characterized for their shape as well as surface morphology using transmission electron microscopy. For TEM imaging, copper grids having a thin layer of carbon were loaded with T-MNLC dispersion. Sample was allowed to dry under IR lamp and images were captured [21].

2.3.4. In-vitro drug release study

In-vitro release kinetics of isotretinion and clindamycin was performed using dialysis method. Incubator shaker was kept at constant temperature 37 ◦C with 100 rpm.Semi permeable cellophane membrane (previously immersed in phosphate buffer pH 7.4 for 24 hrs) was firmly stretched over the lower open end of a glass tube made watertight by rubber band (donor compartment). The tube was then immersed in a beaker containing 200 ml of phosphate buffer pH 7.4 (receptor compartment). Samples were analyzed spectrophotometrically at respective λmax [22].

2.3.5. In vitro skin permeation study

It is the diffusion of drug across the cellophane membrane into the receptor domain. *In vitro* skin permeation was

conducted on modified Franz diffusion cell. Cumulative amount of drug was assessed by plotting the % cumulative drug permeated against time. Study was conducted for 8 hrs duration. Sampling time was 0, 1, 2, 4, 6 and 8 hrs [23].

3. Result and Discussion

3.1. Particle size and Zeta potential

Particle size of the optimized formulations of Isotretinoin and Clindamycin hydrochloride was found to be 506.8 nm and 511.8 nmwithpolydispersity index of 0.824 as well as 0.610.Zeta potential of the optimized formulations was found to be -6.26 mV and -11.8 mV. Particle size of the particulate system generally affects its penetrationinto skin.

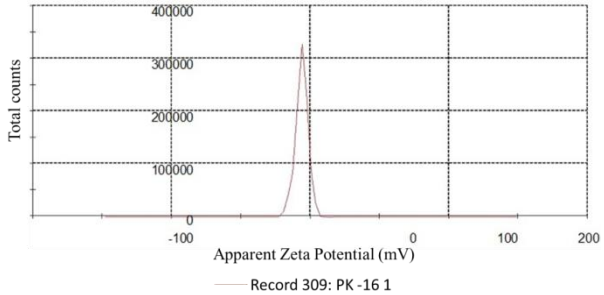

Fig. 1. Zeta potential distribution curve for Isotretinoin

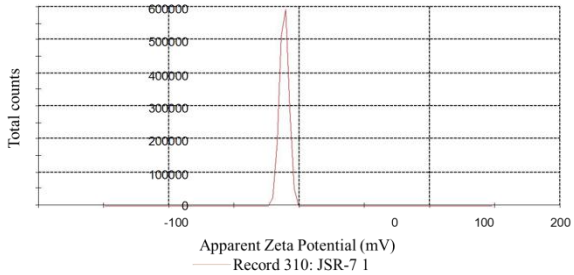

Fig. 2. Zeta potential distribution curve for Clindamycin hydrochloride

3.2. Entrapment Efficacy

The prepared formulations were evaluated to get an optimized surfactant: cholesterol ratio. Three ratios 1:0.5:1, 1:1:1, 1:2:1 was chosen for the further study. Best ratio was selected on the basis of percentage entrapment efficiency EE (%).

Table.1. Entrapment efficiency of different niosomal formulations of Isotretinoin

Formulation	Surfactant	Drug: Surf: Chol	Entrapment efficiency (EE %)
N1	Span 60	1:0.5:1	47.81%
N2	Span 60	1:1:1	52.64%
N3	Span 60	1:2:1	66.26%
N4	Span 80	1:0.5:1	30.79%
N5	Span 80	1:1:1	35.25%
N6	Span 80	1:2:1	45.64%
N7	Tween 60	1:0.5:1	25.37%
N8	Tween 60	1:1:1	30.79%
N9	Tween 60	1:2:1	48.38%
N10	Tween 80	1:0.5:1	32.33%
N11	Tween 80	1:1:1	43.68%
N12	Tween 80	1:2:1	54.21%

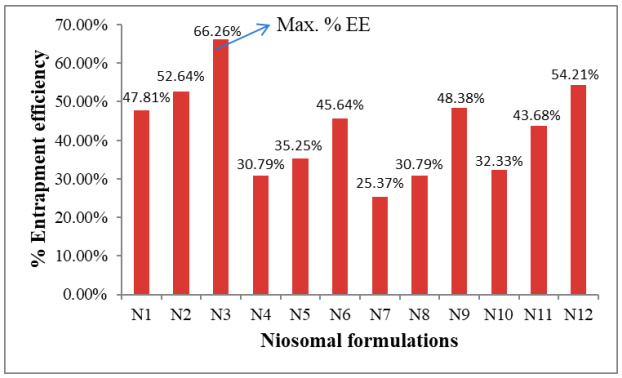

Fig. 3. Graph showing Comparison of various Niosomal formulations of Isotretinoin in term of % Entrapment efficiency

Among all the surfactants, Span 60 demonstrated maximum entrapment efficiency. It was selected as it is solid at room temperature and has highest phase transition temperature (52 °C). Span 60 is a good surfactant as it has a CPP of 0.5-1 and hence forms spherical vesicles.

Table.2. Entrapment efficiency of different niosomal formulations of Clindamycin hydrochloride

Formulation	Surfactant	Drug: Surf: Chol	Entrapment efficiency (EE %)
C1	Span 60	1:0.5:1	21.32%
C2	Span 60	1:1:1	32.37%
C3	Span 60	1:2:1	48.38%
C4	Span 80	1:0.5:1	25.37%
C5	Span 80	1:1:1	32.23%
C6	Span 80	1:2:1	52.64%
C7	Tween 60	1:0.5:1	43.68%
C8	Tween 60	1:1:1	55.23%
C9	Tween 60	1:2:1	71.45%
C10	Tween 80	1:0.5:1	33.32%
C11	Tween 80	1:1:1	47.81%
C12	Tween 80	1:2:1	62.21%

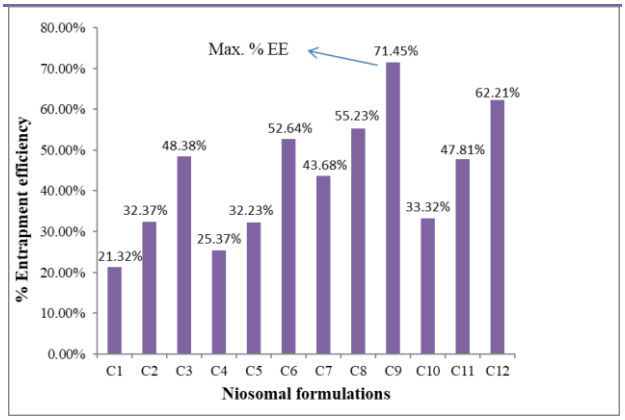

Fig. 4.Graph showing Comparison of various Niosomal formulations of Clindamycin hydrochloride in term of % Entrapment efficiency

Niosomal formulations prepared using Tween 60 showed higher entrapment efficiency. Among all the surfactants, entrapment efficiency for niosomes prepared using Tweens was superior to those prepared using Spans. This can be explained by the fact that large hydrophilic head Tweens is capable of solubilizing greater amount of Clindamycin hydrochloride which is extremely hydrophilic. Furthermore, with increase in concentration of surfactants (Tweens, Spans), entrapment efficiency was found to be increased.

3.3. Morphology Studies

3.3.1. Fourier Transform Infra-Red Spectroscopy (FTIR Analysis)

Fig. 5 illustrates FTIR spectra of Isotretinoin. Table 3 showed the frequency of observed bands and its interpretation confirming the purity of sample.

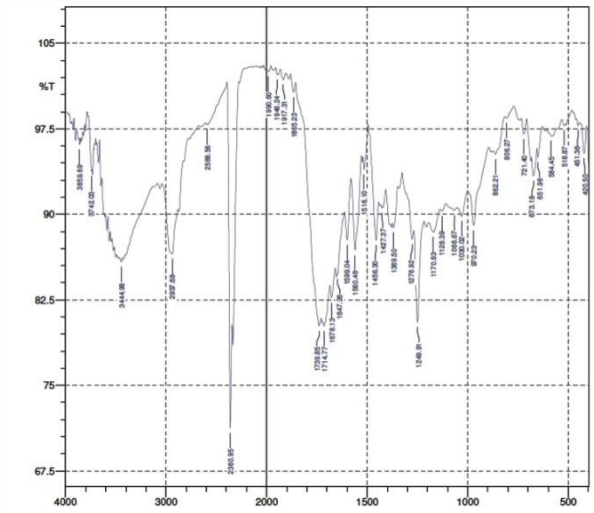

Fig.5. FTIR spectra of Isotretinoin

Table 3 FTIR Interpretation data of Isotretinoin

Observed peak (cm-1)	Standard peak (cm-1)	Interpretation
3444.98	3570-3450	-OH (stretch)
2937.68	2960-2850	-CH (stretch)
1647.26	1680-1640	-C=C (stretch)
1714.77	1750-1680	-C=O (stretch)

Fig. 6 illustrates FTIR spectra of Clindamycin hydrochloride. Table 4showed the frequency of observed bands and its interpretation confirming the purity of sample.

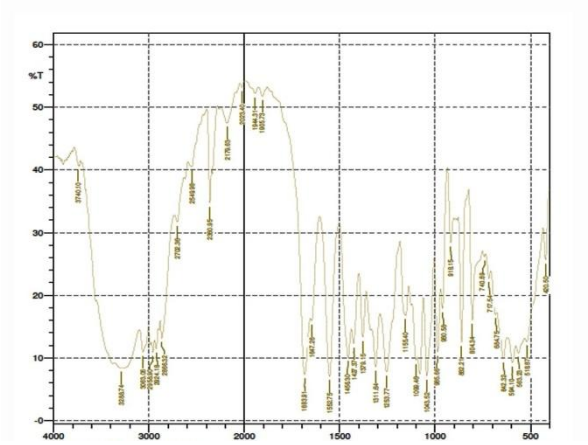

Fig. 6. FTIR spectra of Clindamycin hydrochloride

Table 4 FTIR Interpretation data of Clindamycin hydrochloride

Observed peak (cm-1)	Standard peak (cm-1)	Interpretation
3288.74	3570-3450	-OH (stretch)
2866.32	2960-2850	-CH
3740.10	3500-3350	-NH
1683.91	1750-1680	-C=O
684.75	800-600	-C-Cl
		-S-CH3
1155.40	1470-1070	-C-N

3.3.2. Transmission Electron Microscopy (TEM) images of niosomes

TEM images showed that diameter was found to be in size range of 100 nm. This size is suitable for dermal delivery of niosomes. Noisome of size 100-300 nm can easily permeate through skin.

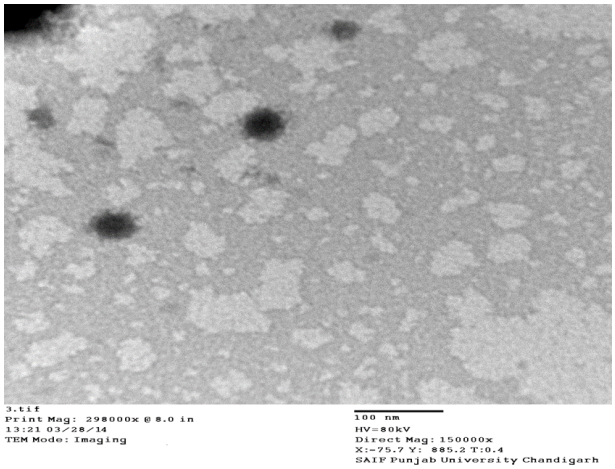

Fig.7. Transmission electron micrograph of Niosomal dispersion (N3)

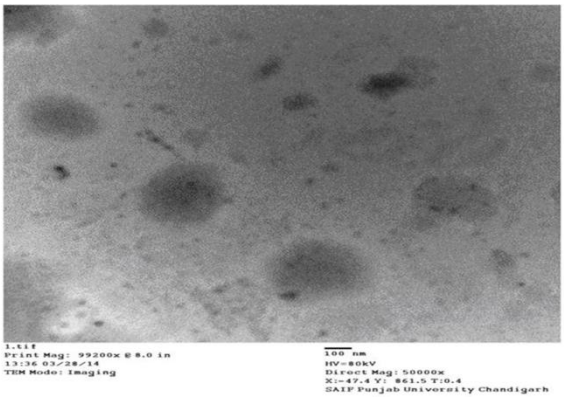

Fig. 8. Transmission electron micrograph of Niosomal dispersion (C9)

3.4. Solubility Studies

The solubility of isotretinion and clindamycin were tested in various solvents such as distilled water, ethanol, methanol, chloroform and PBS pH 7.4. The quantitative solubility of Isotretinoin and Clindamycin hydrochloride was determined in various solvents at room temperature. Data of solubility in various solvent has been explained in table 14 and 15. These data suggest that Isotretinoin had good solubility in methanol, ethanol and less soluble in water which confirmed its lipophilic nature. In the other hand Clindamycin hydrochloride had good solubility in water and very slightly soluble in ethanol and acetone which confirmed its hydrophilic nature.

Table.5. Solubility profile of Isotretinoin

S.No.	Solvent	Standard	Observed	Interpretation
1	Methanol	+++++	+++++	Freely soluble
2	Ethanol	+++++	+++++	Freely soluble
3	Water	++	++	Practically insoluble/ very sparingly soluble
4	PBS	+++	+++	Sparingly soluble
5	Chloroform	+++++	+++++	Freely soluble

Table.6. Solubility profile of Clindamycin hydrochloride

S.No.	Solvent	Standard	Observed	Interpretation
1	Water	+++++	+++++	Freely soluble
2	Ethanol	++	++	Slightly soluble
3	Acetone	++	++	Slightly soluble

3.5. In vitro drug release study

Comparative % age cumulative drug release profile of drug solution and niosomal solution is shown in Table 7-8. Drug release of the niosomes loaded with Isotretinoin as well as Clindamycin hydrochloride was carried out in phosphate buffer at pH 7.4 for 8 hrs. As it is clear from the Fig. 9-10, the release rate of niosomal formulation was slower than observed with drug solution.

Table.7. In vitro comparison of drug release for niosomal solution and plain drug solution (Isotretinoin)

Time (hrs)	Cumulative % drug release	
	Niosomalsolution	Plaindrug solution
0	0.00	0.00
1	22.06±2.19	26.02±1.33
2	28.35±1.15	36.91±0.91
4	36.73±2.39	43.34±0.34
6	42.16±2.73	48.62±0.59
8	47.34±2.48	53.74±0.51

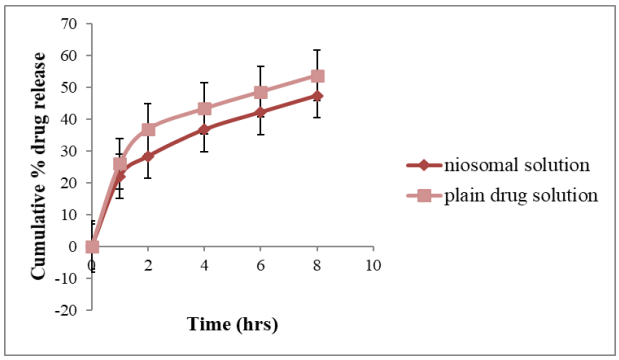

Fig. 9. Comparative *invitro* drug release profile of niosomal solution and plain drug solution (Isotretinoin)

Table.8. In vitro comparison of drug release for niosomal solution and plain drug solution (Clindamycin hydrochloride)

Time (hrs)	Cumulative%drugrelease	
	Niosomalsolution	Plaindrug solution
0	0.00	0.00
1	29.16±0.19	30.98± 0.82
2	35.37±1.15	37.91±1.91
4	40.12±2.29	42.34±0.31
6	50.46±2.71	49.62±0.61
8	58.34±2.21	60.74±1.71

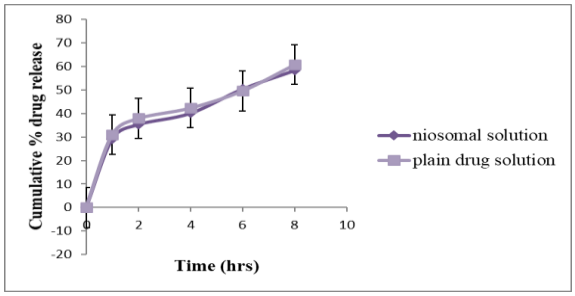

Fig. 10. Comparative *invitro* drug release profile of niosomal solution and plain drug solution (Clindamycin hydrochloride)

3.5.Permeation study

Cumulative amount of drug loaded in niosomal gel was assessed by plotting the %cumulative drug permeated against time. It showed better skin permeation in 8 hrs.

Table.9. In vitro permeation studies

Sr.No.	Time (hrs)	Niosomal gel (% Cumulative release)
1	0	0
2	1	26.78±0.41
3	2	28.73±0.23
4	4	30.42±1.24
5	6	35.76±1.51
6	8	39.61±1.37

4. Conclusion

The present studies were designed to develop niosomal gel loaded with combination of isotretinion and clindamycin for the treatment of acne vulgaris. The noisomes were prepared by ether injection method. The study design focuses on development and characterization of niosomes for delivery of isotretinoin as well as clindamycin.

In preformulation study, FTIR spectrum reveals that drugs were in pure state. Both drugs were compatible with various surfactants (Span 60, Span 80, Tween 60 and Tween 80).The constituents of the carrier system were optimized using different ratio of excipients with maximum entrapment efficiency 66.26% and 71.45% respectively. Nanovesicles were evaluated for entrapment efficiency, microscopy, particle size analysis, zeta potential and *in vitro* release study. Morphological evaluation by optical microscopy and TEM revealed the presence of discrete uniform spherical vesicles. Particle size of niosomes of isotretinoin and clindamycin was found to be 506.8 nm and 511.8 nm respectively. Zeta potential values of both drugs were -6.26 mV and -11.8 mV which indicate niosomal dispersions were stable. Niosomal formulation with best encapsulation efficiency (N3) and (C9) was formulated into gel form using carbopol as gelling agent. Niosomal gel was evaluated for pH, Viscosity and *invitro*Skin permeation.

In vitro skin permeation studies of niosomal gel were carried out in modified Franz diffusion cell using cellophane membrane which inferred that % cumulative release was 39.61% of the initial dose in 8 h. It was confirmed that niosomes showed sustained drug release as compare to plain drug solution. Combination of isotretinion and clindamycin reveal better therapeutic efficacy and patient compliance as compared to individual agent. Niosomes play vital role in improving photostability of drug.

From the research findings, it can be concluded that Isotretinoin as well as Clindamycin hydrochloride was successfully integrated into niosomal gel by ether injection method for topical application in the treatment of acne.

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Conflicts of Interests

There are no conflicts of interest.

Funding

Nil

Authors Contributions

All the authors have contributed equally.

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