Available online on 15.03.2024 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

Formulation and Evaluation of Niosomal gel using Tretinoin and Clindamycin combination

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Article Info:

Article History:

Received 09 Jan 2024
Reviewed 04 Feb 2024
Accepted 25 Feb 2024
Published 15 March 2024

Cite this article as:

Palak, Rani R, Kumar R, Singh AP, Singh AP, Formulation and Evaluation of Niosomal gel using Tretinoin and Clindamycin combination, Journal of Drug Delivery and Therapeutics. 2024; 14(3):106-114

DOI: http://dx.doi.org/10.22270/jddt.v14i3.6477

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Abstract

The objective of the present study was to formulate topical Niosomal gel loaded with combination of Tretinoin, also known as all-*trans* retinoic acid (ATRA), and Clindamycin for the beneficial of acne patients, to provide sustained release effects, to prevent their side effects. Purpose of this study was to develop a stable formulation that allows progressive follicle penetration and increased efficacy. Niosomes was prepared by ether injection method. Preformulation studies such as melting point, FTIR spectroscopy, UV spectroscopy, solubility, partition coefficient (log P) were performed for both drugs. In preformulation study, FTIR spectrum reveals that drugs were in pure state. Combination of Tretinoin and clindamycin reveal better therapeutic efficacy and patient compliance as compared to individual agent. From the research findings, it can be concluded that Tretinoin as well as Clindamycin hydrochloride was successfully integrated into niosomal gel by ether injection method for topical application in the treatment of acne.

Keywords: Niosomes; Tretinoin (ALL TRANS RETINOIC ACID); Clindamycin; Carbopol gel; Acne; Ether injection method.

INTRODUCTION

Niosomes are promising drug carriers for the delivery of pharmaceuticals and cosmetics. These are the microscopic, biodegradable in nature, less toxic, lamellar structures composed of hydrated mixtures of non-ionic surfactant (alkyl ethers/alkyl esters) and cholesterol. This type of delivery system improves the stability and enhances skin penetration of drugs.[1] Improve therapeutic effectiveness of drugs by delayed clearance. Acne vulgaris is a chronic inflammatory dermatosis which is notable for open and/or closed comedones (blackheads and whiteheads), and inflammatory lesions including papules, pustules, or nodules and is very common and normally happens to everyone once in their lifetime.[2]

Tretinoin, also known as all-*trans* retinoic acid (ATRA), is a naturally occurring derivative of vitamin A (retinol) that act by binding to two nuclear receptor families within keratinocytes: the retinoic acid receptors (RAR) and the retinoid X receptors (RXR). It is a medication used for the treatment of acne and acute promyelocytic leukemia. Tretinoin (along with other retinoids) are vitamin A It is in the retinoid family of medications. It is on the World Health Organization's List of Essential Medicines. Tretinoin is available as a generic medication. In 2019, it was the 244th most commonly prescribed medication in the United States, with more than 1 million prescriptions.[3]

Clindamycin is an antibiotic medication used for the treatment of a number of bacterial infections, including osteomyelitis (bone) or joint infections, pelvic inflammatory disease, strep throat, pneumonia, acute otitis media (middle ear infections), and endocarditis. It is of the lincosamide class and works by blocking bacteria from making protein. Clindamycin was first made in 1966 from lincomycin. Clindamycin has a primarily bacteriostatic effect. At higher concentrations, it may be bactericidal. It is a bacterial protein synthesis inhibitor by inhibiting ribosomal translocation, in a similar way to macrolides. Clindamycin hydrochloride is an antibiotic derivative of lincomycin, topically reduces free fatty acid concentrations on the skin and suppresses growth of *Propionibacterium acnes,* an anaerobe found in sebaceous glands as well as follicles. It possesses antibiotic and anti-inflammatory activity.[4]

Combination products with advanced vehicle formulations offer simplified treatment regimen and target multifactorial acne pathophysiologies, for example combination of Clindamycin + Tretinion or Adalpane/Clindamycin + Benzoyl peroxide (BP).[5]

MATERIAL AND METHODS

Materials

Tretinion was 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.

Methods

Ether injection method was used for preparation. In this method, organic solvent containing surfactant and lipids added slowly into the aqueous solution of drug with the help of needle. Vaporization of ether results in the formation of single layered vesicles.[6]

Preformulation studies

It is an important tool for determination of physical and chemical properties of drug before incorporating in formulation development. This is the first step in rational development of dosage forms of a drug substance which gives information needed to define the nature of drug substance and provide framework for the drug combination with pharmaceutical excipients. The nature of the drug highly affects the processing parameters like method of preparation, entrapment efficiency, compatibility and pharmacokinetic response of the formulation. These are indispensable protocol for the development of safe, effective as well as stable dosage form. Thus, in order to ensure optimum condition for clinically beneficial delivery system, preformulation studies were carried out.

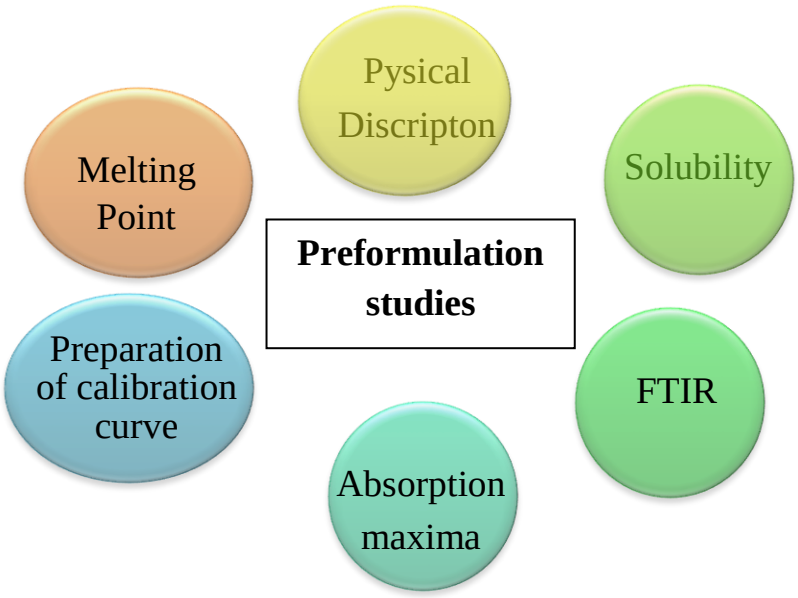

Figure 1. Various Preformulation parameters[7,8,9]

Physical Description

The drugs were inspected visually.

Melting point

It is a criterion for purity as well as for identification. Capillary melting point apparatus was used to determine melting point of Retinoic acid and clindamycin. Small amount of Retinoic acid was filled in capillary and temperature at which drug melted was noted down. Same procedure was repeated for clindamycin.[7]

Fourier Transform Infrared spectroscopy

FTIR studies investigate any physicochemical interactions between components in the formulation and can therefore be applied to the selection of suitable chemically compatible excipients. FTIR spectroscopy of Retinoic acid and clindamycin were performed by using FTIR 8400S Shimaadzu.[8]

Solubility

The solubility of Retinoic acid and clindamycin were tested in various solvents such as distilled water, ethanol, methanol, chloroform and PBS pH 7.4.[9]

Determination of (λmax) absorption maxima (IP 2007)

A 10 ug/ml solution of Retinoic acid as well as clindamycin were prepared in methanol and distilled water respectively. This solution was further scanned in the range of 200-400 nm using UV-visible spectrophotometer.

Preparation of calibration curve

Accurately weighed 100 mg of Retinoic acid was dissolved in 100 ml of methanol to get stock solution of 1000 ug/ml, from this stock solution 10 ml was withdrawn and further diluted to 100 ml with methanol to obtain 2nd stock solution having of 100 µg/ml. 10 ml solution was taken from later stock solution and diluted to 100 ml with methanol. Aliquots of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 ml were taken in volumetric flask and volume was made upto 10 ml. Absorbances of all solutions were measured at 352 nm. Similarly weighed 100 mg of clindamycin dissolved in 100 ml of water to get stock solution of 1000 µg/ml and same procedure was repeated as above at 210 nm.

Partition coefficient

It provides thermodynamic measure of hydrophilicity-lipophilicity balance of a chemical compound which was determined in n-octanol: water system.[10] The n-octanol: Aqueous mixture (1:1 v/v) was kept on shaker for shaking after adding the drug in it. Next day kept it on plain surface for 24 hrs

till it attains equilibrium. Then both phase get separately and the concentration of drug in each phase gets measured.

$$\log P = \log\left[\frac{\text{concentration of solute(Octanol)}}{\text{concentration of solute(Aqueous)}}\right]$$

Formulation of niosomes

Retinoic acid 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. Following steps were used for both drugs.[10]

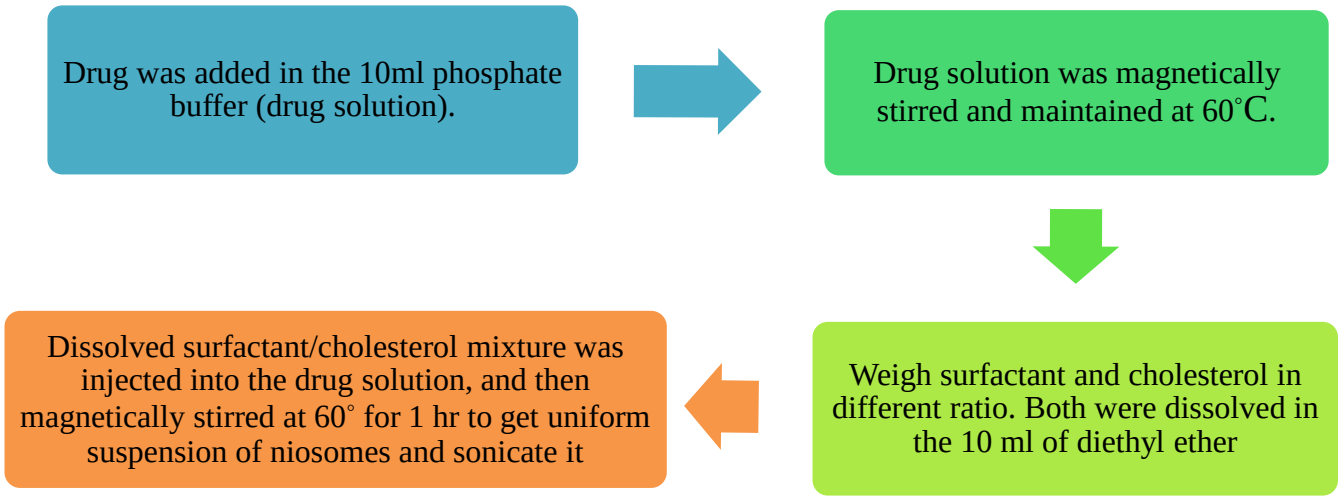

Figure 2: Formulation of Niosomes[10]

Evaluation parameters of niosomes

Particle size and polydispersity index (PI) measurements

The 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

It is a scientific term for electro-kinetic potential in vesicle systems. This is potential difference between dispersion medium and stationary layer of fluid attached to the dispersed particle. 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.[11]

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.[12]

Percentage entrapment efficiency

Percentage of Retinoic acid 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.[13,14] Entrapment efficieny was calculated by using following formula[15]:

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

In- vitro drug release study of niosomes loaded with Retinoic acid and Clindamycin

In-vitro release kinetics of Retinoic acid 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.[16]

Preparation of cellophane membrane sac

A 10 cm long portion of the cellophane membrane was made in the form of sac by folding and tying up one end of the membrane with thread, taking care to ensure that there would be no leakage of the contents from the sac. It was soaked overnight in the buffer medium.

Cellophane membrane set up

The wet sac was gently opened and was washed with phosphate buffer pH 7.4. It was filled with 3 ml of formulation and suspended in a beaker containing 200 ml of phosphate buffer pH 7.4. Temperature at about in the shaking incubator was maintained at temperature 37 °C with 100 rpm. Beaker

was closed with the aluminium foil to prevent any loss during the experimental run.

Sampling

At predetermined time intervals, 5 ml aliquots were withdrawn from the receptors compartment and were equally replenished with phosphate buffer pH 7.4 and subjected to analysis.[16] Spectroscopical analysis was carried out immediately after withdrawal of samples with the help of UV – spectrophotometer. The duration of release study was 8 hrs.

Formulation 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.[17,18]

Incorporation of niosomes of Retinoic acid 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 Tretinoin and clindamycin was dispersed in the carbopol gel with slow agitation. [17,18]

Characterization of gel formulation

Physical examination

The prepared gel formulations were inspected visually for their colour, homogeneity and consistency.

pH of gel

pH is a measure of the concentration of hydrogen ions in a solution. Numerically it is the negative logarithm of that concentration expressed in moles per liter. pH of the prepared gel was measured by a pH meter.

Viscosity of gel

Viscosity measurements were carried out at room temperature (25-27 °C) using a Brookfield viscometer.[18] Sample volume used was 100 ml. Suitable spindle was employed for each treatment, while shear rate was set up at 10 rpm.

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.[19]

RESULT

Physical description

Table 1: Tretinoin

Parameters	Observations
Physical state	Solid (crystalline powder)
Colour	Yellowish orange
Odour	Characteristic

Table 2: Clindamycin hydrochloride

Parameters	Observations
Physical state	Solid(powder)
Colour	White
Odour	Odourless

Melting point

Table 3: Melting point of Tretinoin

Tretinoin	Observed M.P. (°C)
Sample 1	179-182
Sample 2	178-181
Sample 3	180-183

Table 4: Melting point of Clindamycin hydrochloride

Clindamycin hydrochloride	Observed M.P.(°C)
Sample 1	140 -142
Sample 2	139-141
Sample 3	139-141

Fourier Transform Infra Red Spectroscopy (FTIR Analysis)

Table 5: FTIR Interpretation data of Tretinoin

Observed peak (cm^{-1})	Standard peak (cm^{-1})	Interpretation
3448.98	3570-3450	-OH (stretch)
2938.68	2960-2850	-CH (stretch)
1648.24	1680-1640	-C=C (stretch)
1716.78	1750-1680	-C=O (stretch)

Table 6: FTIR Interpretation data of Clindamycin hydrochloride

Observed peak (cm^{-1})	Standard peak (cm^{-1})	Interpretation
3286.74	3570-3450	-OH (stretch)
2864.32	2960-2850	-CH
3748.10	3500-3350	-NH
1684.91	1750-1680	-C=O
682.75	800-600	-C-Cl
		$-S-CH_3$
1158.40	1470-1070	-C-N

Solubility determination

Table 7: Solubility profile of Tretinoin

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 8: Solubility profile of Clindamycin hydrochloride

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

Determination of λmax

The λmax value for the Tretinoin as well as Clindamycin hydrochloride was found to be 352 nm and 202 nm respectively

Calibration curve of Tretinoin in phosphate buffer (pH 7.4)

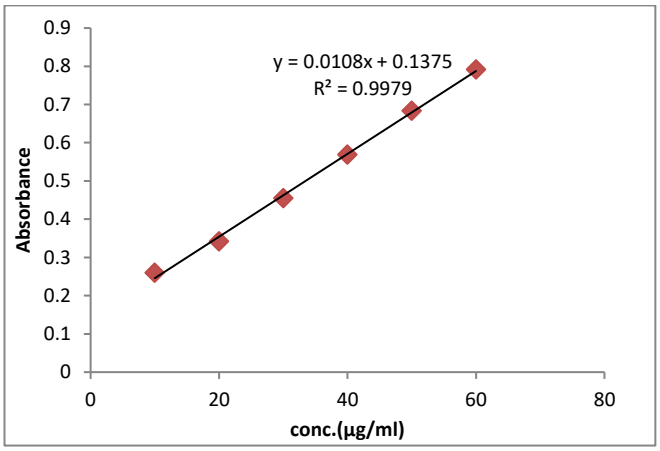

Figure 3: Standard plot of Tretinoin in PBS (pH 7.4) at 352 nm

Calibration curve of Clindamycin hydrochloride in phosphate buffer (pH 7.4)

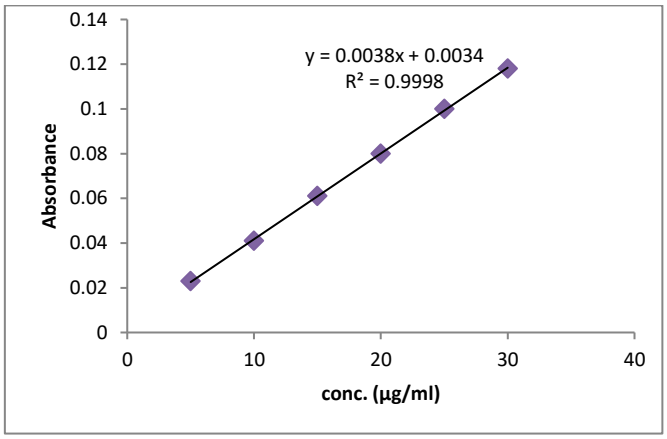

Figure 4: Standard plot of Clindamycin hydrochloride in phosphate buffer (pH 7.4) at 202 nm

Partition coefficient

Table 9: Partition coefficient of Tretinoin

Compound	Observed Log P
Tretinoin	5.62

Table 10: Partition coefficient of Clindamycin hydrochloride

Compound	Observed Log P
Clindamycin hydrochloride	1.75

Compatibility studies

Thus, both drugs were fairly compatible with the excipients physically as well as chemically.

Figure 5: Chemical characterization of Tretinoin - excipients mixtures at different conditions (a) 5 ° ±3 ° C (b) 25 ° ±2 ° C (c) 40 ° ±2 ° C

Table 11: Physical characterization of Tretinoin - excipients mixtures at different storage conditions on 0th, 7th, 15th, 30th day

		0th day			7th day			15th day			30th day		
Drug+ excipient	**Ratio**	**5°C ±3°C**	**25°C ±2°C**	**40°C±2 °C**	**5°C±3 °C**	**25°C±2 °C**	**40°C±2 °C**	**5°C±3 °C**	**25°C±2 °C**	**40°C±2 °C**	**5°C±3 °C**	**25°C±2 °C**	**40°C± 2°C**
Drug + Chol + PBS	1:1	√	√	√	√	√	√	√	√	√	√	√	√
Drug + Tween 60 + PBS	1:1	√	√	√	√	√	√	√	√	√	√	√	√
Drug + Tween 80 + PBS	1:1	√	√	√	√	√	√	√	√	√	√	√	√
Drug + Span 60 +PBS	1:1	√	√	√	√	√	√	√	√	√	√	√	√
Drug + Span 80 + PBS	1:1	√	√	√	√	√	√	√	√	√	√	√	√
Drug	1:1	√	√	√	√	√	√	√	√	√	√	√	√

Chol = Cholesterol, PBS = Phosphate Buffer Solution, √ = No physical change

Figuren 6: Chemical characterization of Clindamycin hydrochloride - excipients mixture at different storage conditions (a) 5 ° ±3 ° C (b) 25 ° ±2 ° C (c) 40 ° ±2 ° C

Table 12: Physical characterization of Clindamycin hydrochloride - excipients mixtures at different storage conditions on 0th, 7th, 15th and 30th day

		0th day			7th day			15th day			30th day		
Drug+excipients	**Ratio**	**5°C± 3°C**	**25°C±2 °C**	**40°C±2 °C**	**5°C±2 °C**	**25°C±2 °C**	**40°C±2 °C**	**5°C±3 °C**	**25°C±2 °C**	**40°C±2 °C**	**5°C±3 °C**	**25°C±2 °C**	**40°C±2 °C**
Drug+ Chol+ PBS	1:1	√	√	√	√	√	√	√	√	√	√	√	√
Drug + Tween 60 +PBS	1:1	√	√	√	√	√	√	√	√	√	√	√	√
Drug + Tween 80 +PBS	1:1	√	√	√	√	√	√	√	√	√	√	√	√
Drug + Span 60 +PBS	1:1	√	√	√	√	√	√	√	√	√	√	√	√
Drug + Span 80 + PBS	1:1	√	√	√	√	√	√	√	√	√	√	√	√
Drug	1:1	√	√	√	√	√	√	√	√	√	√	√	√

Chol = Cholesterol, PBS = Phosphate Buffer Solution, √ = No physical change

Optimization of surfactant: cholesterol ratio for Tretinoin

Table 13: Tretinoin

Formulation	Surfactant	Drug:Surf:chol	Entrapment efficiency (EE %)
N1	Span 60	1:0.5:1	47.82%
N2	Span 60	1:1:1	51.64%
N3	Span 60	1:2:1	66.28%
N4	Span 80	1:0.5:1	31.79%
N5	Span 80	1:1:1	35.45%
N6	Span 80	1:2:1	45.66%
N7	Tween 60	1:0.5:1	26.37%
N8	Tween 60	1:1:1	30.78%
N9	Tween 60	1:2:1	48.36%
N10	Tween 80	1:0.5:1	32.34%
N11	Tween 80	1:1:1	44.68%
N12	Tween 80	1:2:1	54.22%

Table 14: Clindamycin hydrochloride

Formulation	Surfactant	Drug:Surf:chol	Entrapment efficiency (EE %)
C1	Span 60	1:0.5:1	21.34%
C2	Span 60	1:1:1	33.37%
C3	Span 60	1:2:1	48.48%
C4	Span 80	1:0.5:1	26.37%
C5	Span 80	1:1:1	33.23%
C6	Span 80	1:2:1	52.66%
C7	Tween 60	1:0.5:1	43.88%
C8	Tween 60	1:1:1	55.24%
C9	Tween 60	1:2:1	71.46%
C10	Tween 80	1:0.5:1	33.34%
C11	Tween 80	1:1:1	47.82%
C12	Tween 80	1:2:1	62.24%

Niosomes Particle size measurement and zeta potential

Particle size of the optimized formulations of Tretinoin and Clindamycin hydrochloride was found to be 506.8 nm and 511.8 nm with polydispersity 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.

Niosomes Optical microscopy

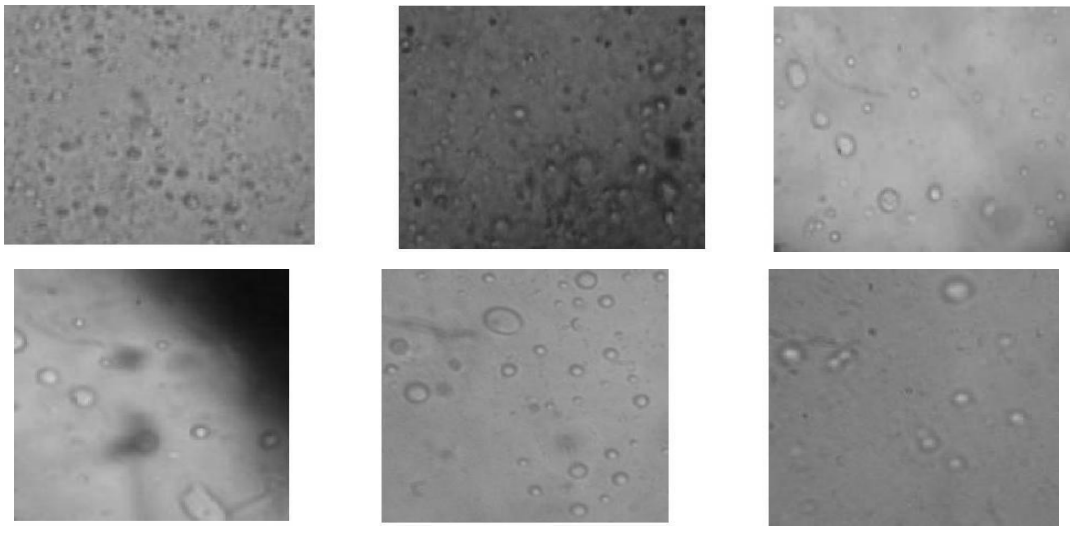

Figure 7: Optical photomicrographs of some niosomal formulations (Tretinoin)

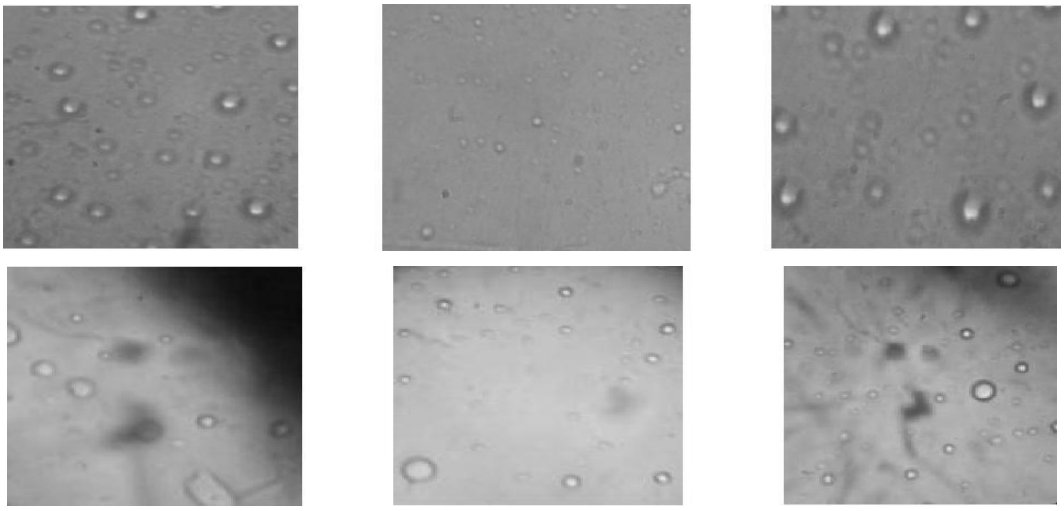

Figure 8: Optical photomicrographs of some niosomal formulations (Clindamycin hydrochloride)

Niosomal In vitro drug release study

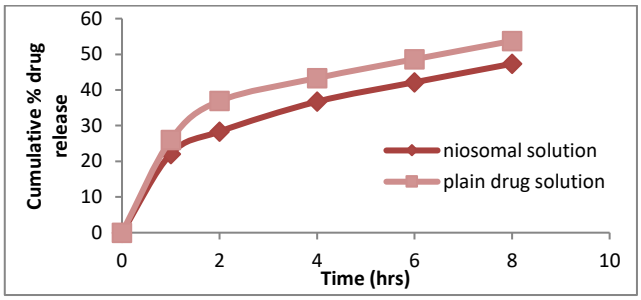

Figure 9: Comparative *in vitro* drug release profile of niosomal solution and plain drug solution (Tretinoin)

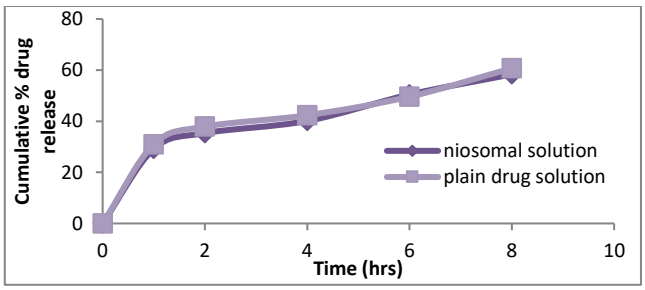

Figure 10: Comparative *in vitro* drug release profile of niosomal solution and plain drug solution (Clindamycin hydrochloride)

Gel formulation

The prepared gel formulation was observed visually and was found to be transparent white having Ph 6 and viscosity 0.5283 (Pa/s) determined with the help of Brookfield DV-1 viscometer.

Permeation study

Table 15: In vitro permeation studies

Sr.No.	Time (hrs)	Niosomal gel (% Cumulative release)
1	0	0
2	1	27.78±0.41
3	2	28.73±0.23
4	4	32.42±1.24
5	6	35.76±1.51
6	8	40.61±1.37

DISCUSSION

The study was conducted to formulate topical Niosomal gel loaded with combination of Tretinoin, also known as all-*trans* retinoic acid (ATRA), and Clindamycin for the beneficial of acne patients, to provide sustained release effects, to prevent their side effects. Niosomes of Retinoic acid and clindamycin were Incorporated into the carbopol gel. Preformulation studies were performed for Tretinoin as well as Clindamycin hydrochloride. Various preformulation parameters such as melting point, IR analysis, solubility, determination of λmax, calibration curve and partition coefficient of drug were determined. A capillary melting point apparatus was used to determine melting point of Tretinoin as well as Clindamycin hydrochloride. The observed melting point was 178-183 ℃ and 139-142 ℃. Spectral analysis was also done that shows the frequency of observed bands and its interpretation confirming the purity of sample. The quantitative solubility of Tretinoin and Clindamycin hydrochloride was determined and data suggested that Tretinoin 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. Absorbance values of Tretinoin were taken at 352 nm and plotted with concentrations. Lambert Beer law has obeyed in the concentration range of 10-60 µg/ml with correlation co-efficient (R^2) = 0.997. The standard regression equation was found to be y = 0.010x + 0.137. Absorbance values of Clindamycin hydrochloride were taken at 202 nm and plotted with concentrations. Lambert Beer law obeyed in the concentration range of 5-30 µg/ml with correlation co-efficient (R^2) = 0.999. The standard regression equation found to be y = 0.003x + 0.003. Calibration data and calibration curves are shown in table 17 and fig. 17 respectively.

Concentration of Tretinoin and Clindamycin in both phases was estimated and partition coefficient was calculated using the formula, partition coefficient = conc. in organic phase (n-octanol)/ conc. in aqueous phase (water). Data obtained suggested that Tretinoin is lipophilic in nature and Clindamycin hydrochloride is hydrophilic.

Compatibility studies were done to check out any physical and chemical interaction between drug and excipients. both drugs were fairly compatible with the excipients physically as well as chemically.

The prepared formulations were evaluated to get an optimized surfactant: cholestrol ratio. Three ratios 1:0.5:1, 1:1:1, 1:2:1

was choosen for the further study. Best ratio was selected on the basis of percentage entrapment efficiency EE (%). 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. Photomicrographs of niosomes of Tretinoin and Clindamycin hydrochloride were obtained by optical microscope. The results revealed the presence of uniform, spherical single layered vesicles (Unilamellar).

Comparative % age cumulative drug release profile of drug solution and niosomal solution is shown in Tables. Drug release of the niosomes loaded with Tretinoin as well as Clindamycin hydrochloride was carried out in phosphate buffer at pH 7.4 for 8 hrs. The release rate of niosomal formulation was slower than observed with drug solution.

CONCLUSION

In preformulation study, FTIR spectrum reveals that drugs Tretinoin as well as Clindamycin hydrochloride were in pure state. Both drugs were compatible with various surfactants (Span 60, Span 80, Tween 60 and Tween 80). Among all the surfactants, Span 60 demonstrated maximum entrapment efficiency for Tretinoin and tween 60 for clindamycin hydrochloride. 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 Tretinoin 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 *in vitro* 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 Tretinoin 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 Tretinoin as well as Clindamycin hydrochloride was successfully integrated into niosomal gel by ether injection method for topical application in the treatment of acne.

Acknowledgment: It's our privilege to express the profound sense of gratitude and cordial thanks to our respected chairman Mr. Anil Chopra and Vice Chairperson Ms. Sangeeta Chopra, St. Soldier Educational Society, Jalandhar for providing the necessary facilities to complete this review/research work.

Conflicts of Interests: There are no conflicts of interest.

Funding: Nil

Authors Contributions: All the authors have contributed equally.

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