ORIGINAL ARTICLE

Topical Combination Delivery of Benzoyl Peroxide and Adapalene Niosomal Gel for Acne Treatment

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Abstract

Aim and Objective: Adapalene (ADP) is very effective in 0.01% strength, but it causes skin erythema in the applied area. ADP and benzoyl peroxide (BPO) are the most commonly used drugs in treatment of acne. ADP acts as keratolytic agent and BPO acts as potent antibacterial agent, both the drugs are used individually in a cyclic manner. The combination of both drugs if used judiciously can be used to cure mild to moderate acne effectively by acting on pathogenetic site. The successful treatment of acne depends on the maintenance of effective drug concentration levels at the affected site. The main benefit over liposome is that the lipids are replaced by non-ionic vesicles and hence the preparation is totally non-antigenic. **Materials and Methods:** The non-ionic surfactants like SPANs are obtained from synthetic sources, and hence the quality is maintained same all the time. The ADP was incorporated into niosomes using SPAN 60 and cholesterol was used as a stabilizer. Various ratios of SPAN 60, cholesterol, Stearic acid, BPO, and ADP were tried and optimized. Various process parameters were also optimized for the rotary flask evaporation method. The present study investigates the effect of niosomal coadministration of BPO and ADP in term of *in vitro* skin retention study and *in vivo* antiacne effects. These vesicular carriers, because of their improved percutaneous delivery and better skin retention, have proved to be very useful in enhancing therapeutic index of drugs used for tropical diseases. The niosomal dispersion was incorporated into Carbopol gel. The gel was kept for 3 months accelerated stability studies. **Results and Discussion:** The niosomal dispersion was evaluated for various parameters such as vesicle size, shape, and morphology by transmission electron microscopy. *In vitro* and *in vivo* studies were carried out. The drug release pattern from gel was evaluated on the basis of *in vitro* studies and skin irritation studies on rabbit skin. **Conclusion:** The *in vitro* study shows sustained release gel effects whereas the *in vivo* study shows no signs of irritation on the applied skin area.

Key words: Adapalene, benzoyl peroxide, niosomes, SPAN 60

INTRODUCTION

Skin and topical drug delivery[1]

The skin is the largest and most readily accessible organ of the human body, accounting for approximately 16% of total body mass of an adult and spanning an average surface area of 2 m^2. This large area of skin offers many convenient sites for administration of topical as well as systemic drugs.

Transdermal drug delivery presents many advantages over other routes of administration. Active pharmaceutical ingredients (APIs) applied topically avoid hepatic first-pass metabolism. Better patient compliance exists due to noninvasive and painless aspects of the formula which can be applied repeatedly without the complications that exist with oral or parental daily dosing. The major pharmacokinetic advantages include elimination of dose dumping, sustained API delivery, steady plasma levels, and reduction in dosage frequency, and systemic toxicity. Although a number of advantages exist, formulators are still challenged to overcome the natural barrier function of the skin for optimal transdermal drug delivery.

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The skin forms a unique interface between the human host and the external environment, where its most important function is to act as a barrier and protect our bodies from the outside world. This protection refers to the chemical, physical, immune, pathogen, and ultraviolet (UV) radiation defenses. Other functions of the skin include sensory reactions to temperature, pain, pressure, and touch, thermoregulatory mechanisms such as insulation, sweating, and control of blood flow and it also performs endocrine functions such as Vitamin D synthesis and peripheral conversion of prohormones.

Transdermal drug delivery system is one among of the various modes of drug delivery which facilitates passage of therapeutic quantities of drug substances through the skin and for systemic (or) local effects. Discovering a new medicine is a very expensive and time-consuming work. However, redesigning the modules and means to transport medicine into the body is a less demanding and more lucrative task. In the normal drug release, if the medication may not be absorbed means it will release too slowly (or) if it delivered too fastly means, the patient may suffer untoward effects. To rectify the above drawback, one of the solutions developed was transdermal drug delivery systems. Human skin is a uniquely engineered organ that permits terrestrial life by regulating heat and water loss from the body while preventing the ingress of noxious chemicals or microorganisms. It is also the largest organ of the human body, providing around 10% of the mass of an average person, and it covers an average area of 1.7 m^2. While such a large and easily accessible organ apparently offers ideal and systemic actions; human skin is a highly efficient self-repairing barrier designed to keep the insides in and the outside out. Skin membranes can be examined at various levels of complexity; the membranes can be regarded as a simple physical barrier more complexity can be introduced by viewing skin as various barriers in series. We can then introduce barriers in parallel by considering drug transport through pores in the tissue.

Factors affecting drug delivery[2]

The transdermal route should have the capability to deliver the drug, regardless of size (or) structure at a predetermined rate. However, there are some factors which influence the rate of drug delivery,

A. Biological factors
   - Skin condition
   - Skin age
   - Amount of blood flow
   - Regional skin sites
   - Skin metabolism
   - Species differences.

B. Physiochemical factors
   - Skin hydration
   - Temperature and pH
   - Diffusion coefficient
   - Drug applying surface area
   - Drug concentration
   - Partition coefficient
   - Molecular size and shape.

Types of treatment achieved by topical drug delivery[3]

- Camouflage
- Protection effects
- Antimicrobial
- Antifungal
- Depilatory
- Anti-inflammation
- Anti-purity
- Local anesthetic
- Polyunsaturated fatty acids and photodynamic therapy
- Antihistamine
- Anti-angina
- Anti-ischemic.

Various type of dosage form used in topical drug delivery

- Liquid preparations
- Gels (jellies)
- Powders
- Ointments
- Creams
- Paste
- Aerosols
- Poultice
- Transdermal patch.

Role of niosomes in transdermal drug delivery system[4]

Niosomes can be used to deliver both hydrophobic and hydrophilic drugs through transdermal route. Although niosomes were tried for various routes, it is used in the market for transdermal route (Novasome Products Such as 30% Petrolatum Novasomes and 10% Salicylic Acid Novasomes). Studies showed that an enhanced delivery of drugs when encapsulated in niosomes. Niosomes increase skin penetration of drugs and it can act as local depot for sustained release of dermal active compounds. When non-ionic surfactants are incorporated into niosomes they are much better tolerated by the skin than when they are used in emulsion.[4]

Acne

Acne vulgaris is a common chronic inflammatory disease of the skin. It is found in about 80% of young adults and adolescents. It is a disease that affects the pilosebaceous units of the skin and may result in inflammatory or non-inflammatory lesions. Acne is a chronic inflammatory

dermatitis which consists of open comedones (blackheads), closed comedones (whiteheads), and inflammatory lesions such as nodules, pustules, and papules. Acne should be recognized as a chronic disease which may also affect the patient psychologically.

In recent years, acne has been observed in younger patients due to the earlier onset of puberty. It is more common in girls in the age range of 12 years and younger, but it presents more in boys in the age range of 15 years or older. In most cases, acne disappears within the patient's early twenties; however, acne may persist into adulthood which usually occurs more often in females. Acne has many negative effects on young adolescents. It causes discomfort, emotional stress, disfigurement, and even permanent scarring to the skin. Some of these factors include genetics, male sex, youth, stress, and smoking as well as comedogenic medications such as androgens, halogens, corticosteroids, and pore-clogging cosmetics. Past research suggests that genetic influence combined with comedogenic hormones (especially androgens) produce abnormal volumes of sebum which contribute to acne lesions.

Pathogenesis of acne[5]

Acne affects the pilosebaceous units of the skin, which presents with a variety of lesions at various inflammatory stages, including acne scars and hyperpigmentation. Acne lesions are most commonly present on the face, chest, upper back, and upper arms which are known to have a high density of sebaceous glands. The four main pathological factors involved in the development of acne are the increased sebum production, irregular follicular desquamation, Propionibacterium acnes proliferation, and inflammation of area.

Excess sebum production[6,7]

Androgen hormones (especially testosterone) stimulate increased production and secretion of sebum. Increased sebum production directly correlates with the severity and occurrence of acne lesions, and for this reason, it is an important factor that should be taken into consideration when dealing with patients suffering from acne vulgaris.

MATERIALS AND METHODS

Standard curve for adapalene (ADP)[8]

Preparation of calibration medium

The ideal property of a solvent should be that the drug should be completely soluble in the solvent used. In the present study, the solvents, namely, tetrahydrofuran (THF) and methanol were used.

Determination of absorption maximum λmax) by UV spectrum

UV spectrum is obtained for 10 µg/ml concentration of ADP using THF solution.

Calibration curve for ADP[9]

ADP (10 mg) was dissolved (by sonication) in 1 ml of THF and 30 ml of methanol and diluted to 100 ml with methanol so as to obtain a concentration of 100 µg mL^{-1}. It was further diluted to obtain ADP solution of 10 µg ml^{-1}. The absorption spectrum of this solution was recorded in 237 nm.

Calibration curve for benzoyl peroxide (BPO)

The standard stock solution was prepared by dissolving BPO in methanol to make final concentration of 10 µg/ml. Different aliquots were taken from stock solution and diluted with methanol to prepare series of concentration from 1 to 9 µg/ml. The λ_{max} was found to be 234.8 nm in methanol. The λ_{max} was found to be 234.8 nm. Absorbance was measured at 234.8 nm using ethanol as blank, and calibration curve was prepared.

Differential scanning calorimetry[10]

Thermal analysis was conducted using thermal analyzer (PHOENIX DSC-204 F1, Nietzsche-Gerätebau GmbH, and Germany). Temperature axis and cell constant were calibrated using indium (In). Accurately weighed samples (2 mg) were transferred to aluminum pans and sealed. Samples were heated over a temperature range of 30–200°C, under dry nitrogen purging (50 ml/min) in pin-holed aluminum pans.

Preparation of ADP loaded BPO niosomes[11]

Multilamellar niosomes were prepared by thin-film hydration method. Accurately weighed quantity of surfactant and cholesterol was dissolved in chloroform: methanol (2:1) in round bottom flask. The organic solvent was evaporated at 45°C under reduced pressure at 160 rpm using rotavapor film evaporator (Rotary Vacuum Digital Bath, Popular, India). After complete evaporation of organic solvent, the flask was kept under vacuum overnight to remove the residual solvent. Hydration of thin film was carried out using saline (0.9%) and water (214). The empty niosomes also prepared by the same method without the drug for further evaluation Table 1.

Evaluation of niosomal formulation[11,12]

Drug content analysis

The amount of drug in the formulation is determined after lysing the niosomes using 50% n-propanol. Niosomes preparation equivalent to 200 µg of ADP (1 ml) is pipette out in 100 ml standard flask. To this sufficient quantity of 50%, n-propanol is added and shaken well for the complete

Table 1: List of materials

Name of compound	Purchased from
Drug-adapalene	Systopic labs, Ahmadabad
Benzoyl peroxide	Micro lab, Hosur India
Polyacrylic acid (Carbopol 940)	Dr. Reddys Lab, Hyderabad
Cholesterol	S. D. Fine Chem, Mumbai
Stearic acid	S. D. Fine Chem, Mumbai
Span-40	HiMedia Lab, Mumbai
Span-60	HiMedia Lab, Mumbai
Span-85	Reachem, Chennai
Chloroform	HPLC, Mumbai
Methanol	Rankem, New Delhi
Isopropanol	S. D. Fine Chem, Mumbai
Sodium chloride	Central Drug House
Potassium dihydrogen orthophosphate	HPLC, Mumbai
Disodium hydrogen orthophosphate	HPLC, Mumbai
Dialysis membranes 50 - LA 387	HiMedia Lab, Mumbai
Tetra hydro furan	S. D. Fine Chem, Mumbai
Triethanolamine	S. D. Fine Chem, Mumbai

lysis of the vesicles. The volume is made up to 100 ml with the buffer phosphate-buffered saline pH 7.4. The absorbance is measured at 237 nm in the UV-visible spectrophotometer (Shimadzu UV-1700 Pharma spec Japan) using empty niosomes as blank. The drug content is calculated from the standard curve, using the following formula,

Drug Content = Sample absorbance/Standard absorbance × 100.

Estimation of entrapment efficiency[13]

Drug loaded noisome preparations (1 ml) are centrifuged at 14,000 rpm for 120 min at 4°C using a refrigerated centrifuge (Eppendorf, 5417R, Germany) to separate niosomes from un entrapped drug (215). The free drug concentration in the supernatant layer after centrifugation is determined at 237 nm using UV-visible spectrophotometer (Shimadzu UV-1700 Pharma spec Japan). The percentage of drug entrapment in niosomes is calculated using the following formula,

% Drug entrapment = (Total drug−Unentrapped drug)/Total drug × 100.

In vitro drug release studies[14,15]

In vitro release pattern of niosomes suspension is carried out by dialysis bag (HiMedia M. W. 12000). The niosomal preparation of ADP is placed in a dialysis bag with an effective length of 5 cm which acts as a donor compartment. Dialysis bag is placed in a beaker containing 250 ml of buffer phosphate-buffered saline pH 7.4, which acts as receptor compartment. The temperature of receptor medium maintained at 37 ± 1°C and the medium is agitated at 50 rpm speed using magnetic stirrer. Aliquots of 5 ml samples are collected at predetermined time and replenished immediately with the same volume of fresh phosphate-buffered saline pH 7.4. The sink condition is maintained throughout the experiment. The collected samples are analyzed spectrophotometrically at 375 nm using UV-visible spectrophotometer (Shimadzu UV-1700 Pharma spec Japan). Each study is performed in triplicate (126, 128). The *in vitro* release studies are also carried out for the pure drug by the same method.

Characterization of niosomes[16,17]

Morphological analysis by transmission electron microscopy (TEM)

A drop of diluted noisome dispersion was applied to a carbon-coated 300 mesh copper grid and left for 1 min to allow some of the niosomes to adhere to the carbon substrate and stained with 1% phosphotungustic acid. The remaining dispersion was removed by absorbing the drop with the corner of a piece of filter paper. Then, samples were examined and photographed with Hitachi, TEM at 100 KV.

Particles size determination[18]

Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful techniques for routine measurements of particle size. The Coulter method is rarely used to measure niosomes particle size because of difficulties in the assessment of small nanoparticle and the need of electrolytes which may destabilize colloidal dispersions. PCS (also known dynamic light scattering [DLS]) measures the fluctuation of the intensity of the scattered light which is caused by the particle movement. This method covers a size range from a few nanometers to about 3 µ. This means that PCS is a good tool to characterize nanoparticles, but it is not able to detect larger microparticles. They can be visualized by means of LD measurements. This method is based on the dependence of the diffraction angle on the particle radius (Fraunhofer spectra). Smaller particles cause more intense scattering at high angles compared to the larger ones. A clear advantage of LD is the coverage of a broad size range from the nanometer to the lower millimeter range. The development of polarization intensity differential scattering technology greatly enhanced the sensitivity of LD to smaller particles. However, despite this progress, it is highly recommended to use PCS and LD simultaneously.

It should be kept in mind that both methods do not "measure" particle size. Rather, they detect light scattering effects which are used to calculate particle size.

For example, uncertainties may result from non-spherical particle shapes. Platelet structures commonly occur during lipid crystallization and have also been suggested in the solid lipid nanoparticles. Further, difficulties may arise both in PCS and LD measurements for samples which contain several populations of different size. Therefore, additional techniques might be useful. For example, light microscopy is recommended, although it is not sensitive to the nanometer size range. It gives a fast indication of the presence and character of microparticles (microparticles of unit form or microparticles consisting of aggregates of smaller particles). Electron microscopy provides, in contrast to PCS and LD, direct information on the particle shape. However, the investigator should pay special attention to possible artifacts which may be caused by the sample preparation. For example, solvent removal may cause modifications which will influence the particle shape.[19]

DLS, also known as PCS or quasi-elastic light scattering, records the variation in the intensity of scattered light on the microsecond time scale. This variation results from interference of light scattered by individual particles under the influence of Brownian motion and is quantified by compilation of an autocorrelation function. This function is fit to an exponential, or some combination or modification thereof, with the corresponding decay constant(s) being related to the diffusion coefficient(s). Using standard assumptions of spherical size, low concentration, and known viscosity of the suspending medium, particle size is calculated from this coefficient. The advantages of the method are the speed of analysis, lack of required calibration, and sensitivity to submicrometer particles.

Particle size was determined using a LD particle size analyzer, Malvern Mastersizer. The prepared niosomes were suspended in the chamber of the particle size analyzer containing distilled water, and the particle size was determined using the software provided by the manufacturer.

Surface charge of the particles determination by zetasizer[20]

Zeta potential is an important product characteristic of niosomes since its high value is expected to lead to deaggregation of particles in the absence of other complicating factors such as steric stabilizers or hydrophilic surface appendages. It is usually measured by zeta meter.

A diluted suspension (1 µg/ml) of the niosomes was prepared. Size and surface charge of the particle was determined by Zetasizer Nano ZS (Malvern Instruments, UK) (216).

Encapsulation efficiency

Niosomes entrapped BPO and ADP could be separated from the free drug by dialysis method. The prepared niosomes were filled into dialysis bags and the free drugs dialyzed for 24 h into 100 ml of phosphate buffer saline (PBS, pH7.4). After 24 h dialysis, niosomal suspension contains only entrapped drug. From this niosomal suspension, 0.5 ml was taken and added isopropanol up to 5 ml then volume made up to 10 ml with respective solvent in which the drug is freely soluble then absorbance of resulting solution was measured at 234.8 nm and 237 nm for BPO and ADP, respectively.

Stability studies of niosomal formulation[21,22]

The ability of vesicles to retain the drug was assessed by keeping the niosomal gel at three different temperature conditions, i.e., refrigeration temperature (4–8°C), room temperature (25 ± 2°C), and oven (45 ± 2°C). Throughout the study, niosomal gel formulations were stored in aluminum foil-sealed glass vials. The samples were withdrawn at different time intervals over a period of 1 month, and drug leakage from the formulations was analyzed for drugs content using UV spectrophotometer.

Stability studies

To determine the shelf life of a developed formulation stability studies was carried out. Stability testing gives idea regarding temperature, humidity other environmental condition impact on product quality. Drugs and their formulations are exposed to variable storage conditions throughout their shelf life, during storage, shipment, and handling. In addition to this, diversity of conditions with respect to temperature and humidity, in various countries, also propel us to investigate the stability of drugs and their formulation under influence of various storage conditions.

"Stability testing of new drug substances and products was carried out using International Conference on Harmonization (ICH) Q1A (R2)" (ICH, 2003). The guideline recommends the following time period and condition was used during stability evaluation.

Preparation of niosomal gel formulations[23]

Preparation of ADP niosomal gel

Carbopol 940 gel bases are prepared by homogenizing 1% (w/w) Carbopol dispersion in sufficient water using a magnetic stirrer for 30 min and leaving it to equilibrate for 24 h. After that, pH is adjusted to 5–7 with Triethanolamine (48). The ADP loaded niosomes is added to the prepared plain gel base during the stirring process, and the step is completed as mentioned for Carbopol plain gel bases.

Evaluation of niosomal gel formulations[15]

Drug content analysis

An accurately weighed quantity of each ADP niosomal gel (100 mg) is dissolved in 50 ml of phosphate buffer

(pH 7.4). These solutions are quantitatively transferred to a volumetric flask, and appropriate dilutions are made with the same buffer solution (59). The resulting solutions are then filtered through membrane filters (pore size 0.45 mm) before subjecting the solution to spectrophotometric analysis for ADP at 237 nm (Shimadzu UV-visible spectrophotometer).

PH measurements

The pH is measured in each niosomal gel using a pH meter. This is calibrated before each use with buffered solutions at pH 4, 7, and 10. 1 g of gel is taken and diluted with 100 ml of distilled water and stored for 2 h. The electrode of the pH meter is immersed in the prepared base solution for pH determination. The pH determination is carried out in triplicate, and the average reading is recorded (217).

Rheological studies

The viscosity of gel formulation is carried out on Brook-field viscometer using spindle number S-06, and the determinations are carried out in triplicate, and the average of three reading is recorded.

TEM[19]

The morphology of the ADP niosomal gel dispersions is determined by TEM. A drop of niosomal gel dispersion is applied to a carbon-coated 300 mesh copper grid and left to adhere on the carbon substrate for about 1 min. The remaining gel dispersion was removed by a piece of filter paper. A drop of 2% aqueous solution of uranyl acetate was applied for 35 s and again the solution in excess was removed by the tip of filter paper. The sample was air dried and observed under the TEM at 90 kV.

Table 2: Rheological studies of niosomal gel

RPM	Viscosity in CPS		
	F8	F6	F2
0.1	22000	19000	16500
0.5	6900	5200	4750
1.0	4300	2300	1600
5.0	2180	1580	1120
10.0	940	620	505
20.0	610	300	195
50.0	320	185	110
100.0	190	90	65

Table 3: Drug content in niosomal gel

Formulation	Drug content (%)
F2	92
F6	94.6
F8	97.2

Particle size analysis

The vesicle size distribution is determined using a laser technique on a master sizer (X Ver. 2.15; Malvern instruments Ltd. Malvern, UK). The measurements were performed at 25°C using a 45 mm focus lens and a beam length 2.4 mm.

In vitro release studies

In vitro release study is carried out by taking 1 g of gel formulations into dialysis bag and placed beaker containing 100 ml PBS pH 7.4 at 37 ± 10°C. The beakers placed over a magnetic stirrer and stirred at 50 rpm. Aliquots of samples are withdrawn at specified time intervals and analyzed at 237 nm using a UV spectrophotometer to determine the percentage

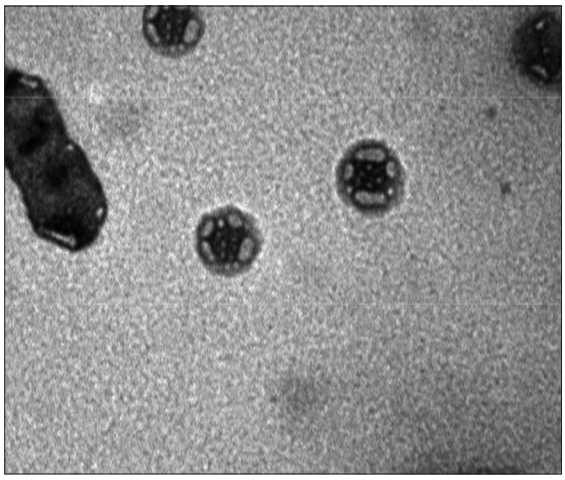

Figure 1: Transmission electron microscopy image of niosomal gel-F8

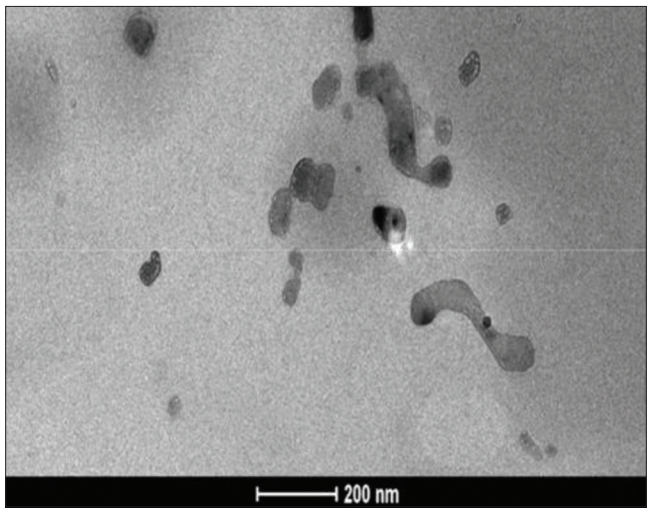

Figure 2: Transmission electron microscopy image of niosomal gel–F2

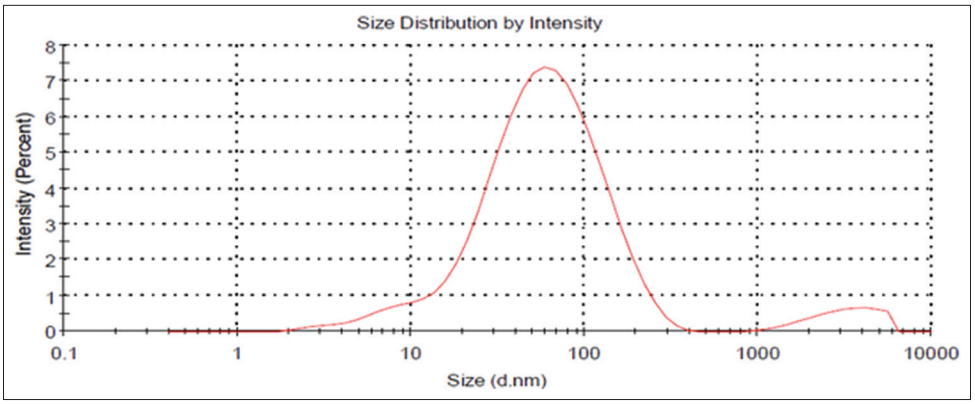

Figure 3: Particle size distribution of niosomal gel-F8

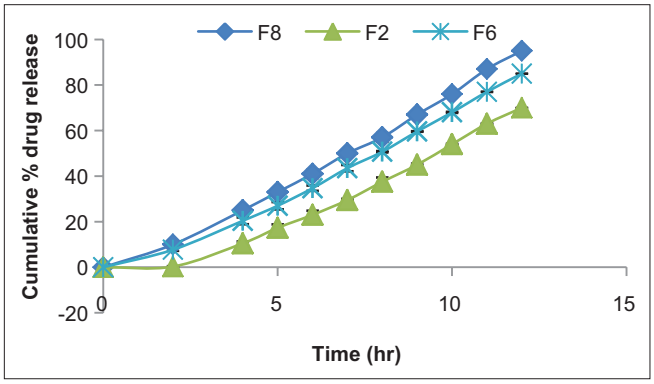

Figure 4: Cumulative % drug release of niosomal gel

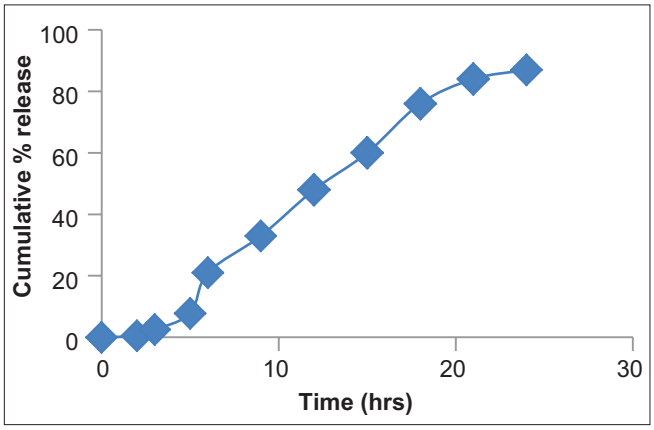

Figure 5: Zero order

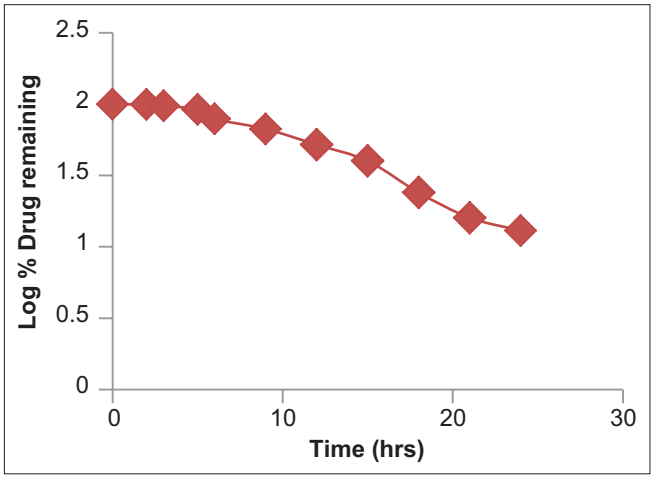

Figure 6: First order

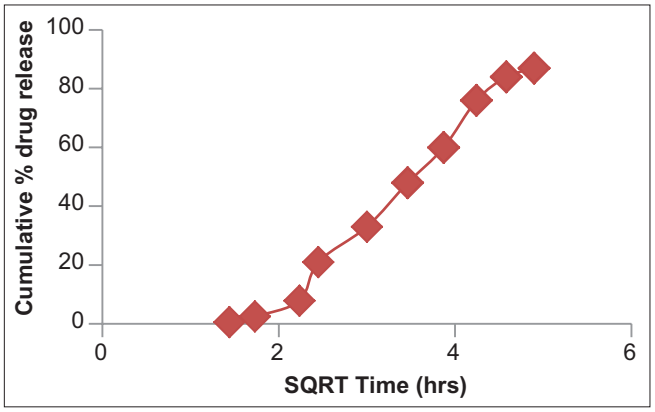

Figure 7: Higuchi plot

drug released and replaced with equal volume of fresh PBS pH7.4.

Kinetics of drug release[16]

To understand the pharmacokinetics and mechanism of drug release, the result of *in vitro* drug release study of niosomes gel is fit with various pharmacokinetic equations such as zero-order (cumulative % release vs. time), first-order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time), and the Korsmeyer–Peppas (log cumulative % drug release vs. log time), and Hixson-Crowell models (cubic root of drug remaining vs. time). The r^2 and k values were calculated for the linear curve obtained by regression analysis.

Stability studies

"Stability testing of new drug substances and products was carried out using ICH Q1A (R2)" (ICH, 2003). There were

no changes in the viscosity of the final niosomal formulation containing niosomal loaded BPO and ADP. On the basis of stability study, we can conclude that niosomal gel formulation was stable.

In vitro permeation study

Permeation study of prepared antiacne niosomal gel[14]

In vitro, skin permeation studies were performed using vertical Franz diffusion cells with an effective diffusion area of 2.54 cm^2. The study was conducted using waster rat skin. The skin was mounted on the receptor compartment with the stratum corneum side facing upward into the donor compartment. The donor compartment was filled with weighed amount 200 mg of antiacne niosomal gel containing 0.1% ADP and 0.600% BPO. A 25 ml aliquot of 1:1 (ethanol/methanol: Saline) v/v was used as receptor medium to maintain a sink condition. The receptor compartment was maintained at 37°C and stirred by a magnetic bar at 600 rpm. At appropriate time interval, 3 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution up to 24 h.

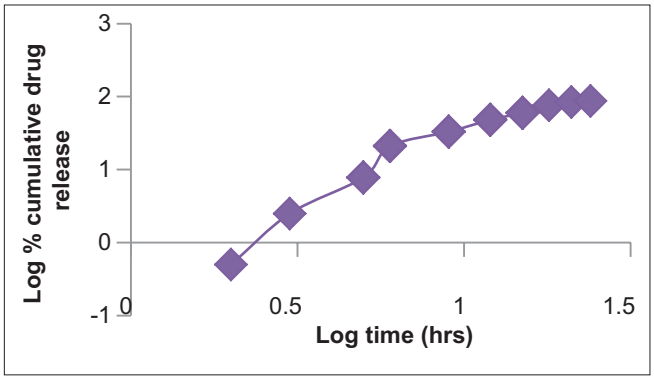

Figure 8: Korsmeyer-Peppas plot

The samples were analyzed by UV spectrophotometer at 234 nm for BPO and 237 nm for ADP. The flux was calculated for each component from niosomal gel formulation using waster rat skin.

In vitro skin retention study

The ability of vesicles to help retain the drug within the skin milieu (i.e., depot-effect) was investigated by determining the amount of drug retained in the skin samples employed in permeation studies. After completion of the permeation experiment, skin mounted on the diffusion cell was removed. The skin was cleaned with cotton dipped in saline solution and blotted with tissue paper to remove

Table 4: Cumulative percentage of drug release of niosomal gel

Time (min)	Cumulative % drug release of gels±SD*		
	F8	F2	F6
0.25	−4.03±1.92	−9.1±2.5	−7.4±2.62
0.50	−1.00±1.95	−11.13±2.01	−4.84±2.37
1.0	4.48±1.58	−6.24±2.83	1.49±1.52
2.0	10.01±1.75	0.31±2.41	7.69±1.85
3.0	16.90±2.13	5.34±1.95	13.16±2.99
4.0	25.23±2.80	10.71±2.34	20.39±3.83
5.0	33.81±2.20	17.36±2.05	27.32±3.06
6.0	41.32±2.30	22.83±3.37	34.72±3.43
7.0	50.10±3.60	29.53±3.72	43.46±2.18
8.0	57.74±3.28	37.57±3.75	50.70±2.87
9.0	67.03±3.03	45.26±2.10	59.60±2.54
10.0	76.39±3.28	53.82±2.73	68.12±2.85
11.0	87.04±2.75	63.29±2.43	77.05±2.17
12.0	95.68±3.53	69.92±2.53	85.57±2.77

SD: Standard deviation

Table 5: Release kinetics of niosomal gel

Formulation code	R^2 values of mathematical models of dissolution studies				
	Zero-order	First-order	Higuchi model	Peppas model	
				R^2	N
F2	0.980	0.849	0.992	0.985	0.920
F6	0.981	0.871	0.993	0.964	0.728
F8	0.986	0.875	0.994	0.952	0.781

Table 6: Stability studies of niosomal gel

Formulation	Condition	Percent drug retained (Time in weeks)					
		Initial	1	2	3	4	6
Niosomal gel	2–8°C	101.44	100.64	98.69	97.52	96.13	93.12
	25°C/60% RH	101.44	99.16	98.14	96.29	94.13	91.94
	40°C/75% RH	101.44	95.44	89.56	82.76	75.39	63.54

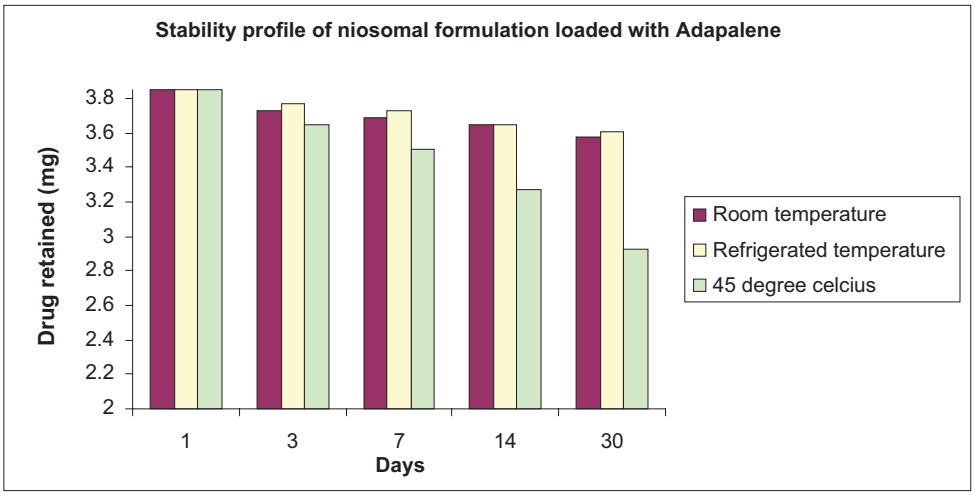

Figure 9: Adapalene retained in niosomes at various temperatures for 30 days

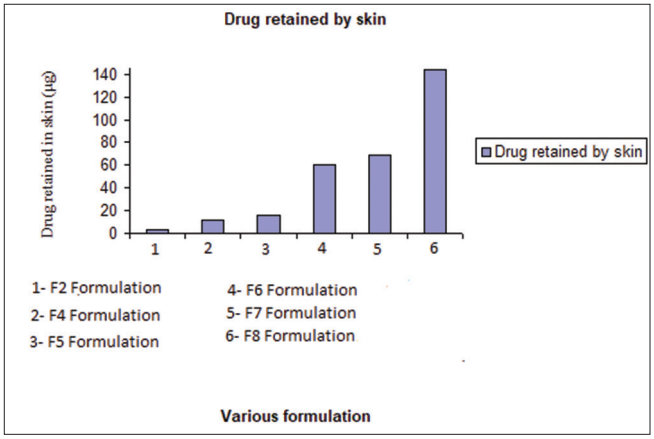

Figure 10: Comparison of *in vitro* retention study of niosomal gel

any adhering formulation. Subsequently, the skin sample was homogenized with 20 ml of chloroform: methanol/ethanol mixture (2:1, v/v), for the extraction of homogenate suspension thus obtained was filtered using filter paper and quantified for the drugs content using UV spectrophotometer at respective absorption maxima for BPO and ADP.

In vivo study[22]

The rabbit ear model was used to study comedo formation to assess the comedo genicity of cosmetics, toiletries, and dermatological preparation and to evaluate the potential of anti-acne drugs. This comedo induction takes place after about 2 weeks of repeated topical application of a chemical comedo gen such as 50% oleic acid. One rabbit was treated as control which receives no treatment, remaining two rabbits receive treatment of 50% oleic acid and dimethyl sulfoxide up to 28 days on the ventral aspect of the pinnas once a day. Rabbits were treated for 3, 7, and 28 days and skin biopsies performed from the treated pinnas at the end of treatment Table 2.

RESULTS AND DISCUSSION

Evaluation of niosomal gel formulations

Drug content analysis

An accurately weighed quantity of each ADP niosomal gel (100 mg) is dissolved in 50 ml of phosphate buffer (pH 7.4). These solutions are quantitatively transferred to a volumetric flask, and appropriate dilutions are made with the same buffer solution (Abd-Allahetal, 2010). There resulting solutions are then filtered through membrane filters (poresize 0.45 mm) before subjecting the solution to spectrophotometric analysis for ADP at 237 nm. The percentage drug content of all the formulations was found to be 81.65%–95.54% ensured the uniformity of drug content in all formulations.

Rheological studies

A viscometer (Brookfield + IILVviscometer) was used to measure the viscosities (in cps) of the gels. The spindle (TF64) was rotated at 0.5 rpm up to 100 rpm. Samples of the gels were to settle over 30 min at the assay temperature (28 ± 1°C) before the measurements were taken. The viscosity of F8 gel was found to be 22,000 cps in 0.5 rpm. The viscosity of F2 gel was found to be 16,500 cps in 0.5 rpm.

TEM

Results of TEM study of niosomal gel prepared from FG2 and FG3 formulations were shown Figure 1. Most of the vesicles were well identified, spherical and discrete with sharp boundaries having large internal aqueous space (Mansroietal, 2008) Figure 2.

Particle size analysis

The vesicle size distribution is determined using a laser technique on a master sizer (X Ver. 2.15; Malvern instruments Ltd Figure 3 and Table 3. Malvern, UK). The measurements

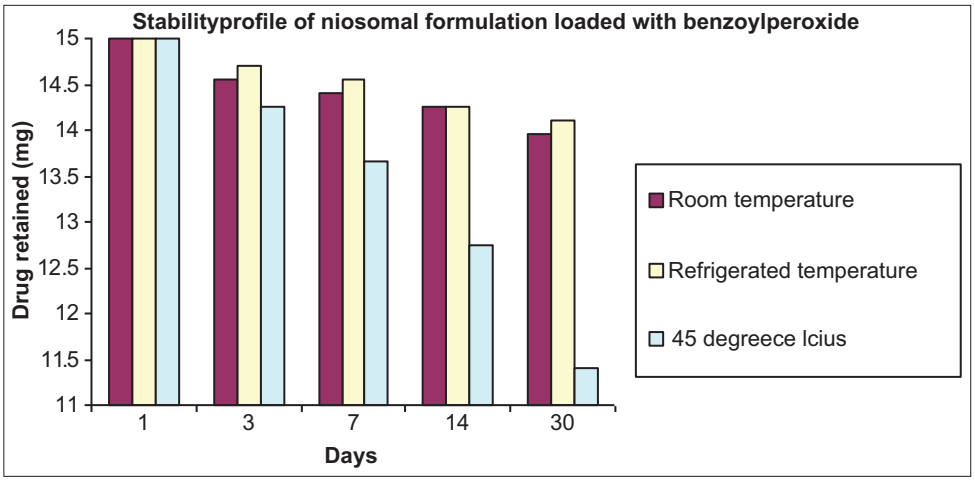

Figure 11: Benzoyl peroxide retained in niosomes at various temperatures for 30 days

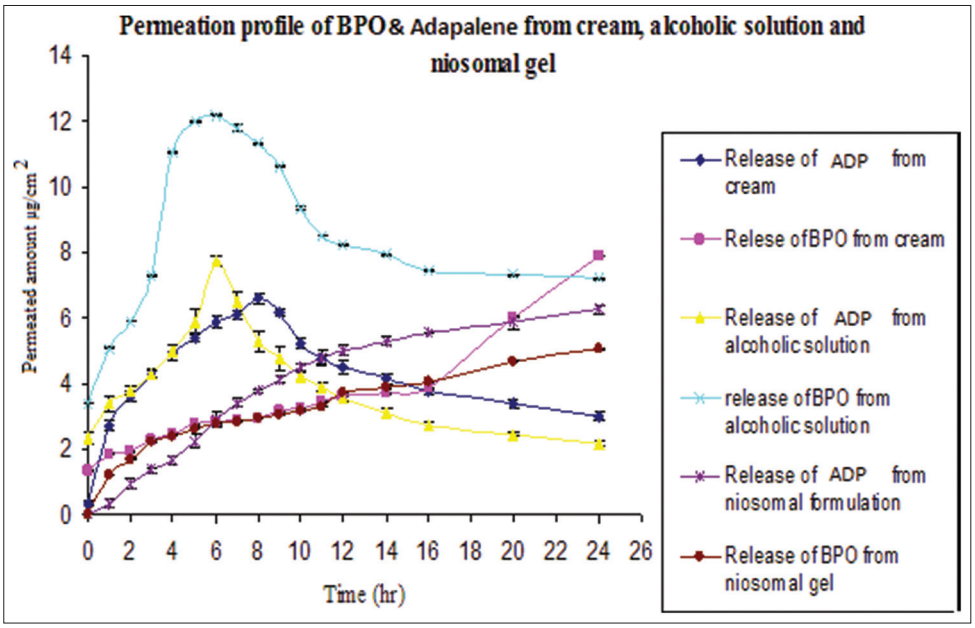

Figure 12: Permeation profile of benzoyl peroxide and adapalene from niosomal gel

Table 7: Percent drug retained

Formulation	Condition	Percent drug retained (Time in weeks)					
		Initial	1	2	3	4	6
Niosomal gel	2–8°C	101.44	100.64	98.69	97.52	96.13	93.12
	25°C/60% RH	101.44	99.16	98.14	96.29	94.13	91.94
	40°C/75% RH	101.44	95.44	89.56	82.76	75.39	63.54

were performed at 25°C using a 45 mm focus lens and a beam length of 2.4 mm (Nasr *et al*., 2009) Figure 4.

In vitro release studies

In vitro release studies were performed for the niosomal gel by dialysis bag method. The cumulative percentage drug release of the formulation containing (F8) ADP plain gel showed 95.68% at 12 h. The cumulative percentage drug release of formulation containing (F2) Span 60 niosomal gels showed 69.92% at 12 h. The cumulative percentage drug release of formulation containing (F6) niosomal gel showed 85.57% at 12 h. The results showed prolonged drug release in the order F8> F6> F2 (Ruckmani *et al*., 2010) Table 4.

Kinetics of drug release

Linear regression analysis for the release was performed to determine the proper order of drug release Figure 5 and Table 5. All the formulations follow zero-order kinetics

Table 8: Observations and calculations of *in vivo* studies

Symptoms	Plain drug gel			Niosomal gel		
	A	B	C	A	B	C
Scaling	0	1	1	0	0	0
Lesions	1	2	3	0	0	0
Erythema	1	2	3	0	0	1
Total score	4	4	7	0	0	1

Where, A: Observation after 12 h, B: Observation after 24 h, C: Observation after 48 h

Figure 6. Calculation of Higuchi correlation coefficient confirms that the drug release was proportional to the square root of time indicating that ADP release from niosomal gel was diffusion controlled Figure 7. The formulations F8 (0.995), F2 (0.992), and F6 (0.993) were followed the Higuchi diffusion-controlled model Figure 8.

Stability studies

The results showed that niosomal gel formulation was quite stable at refrigeration and room temperatures as not much leakage of the drug was found at these temperatures. Drug retained at 45°C might have decreased due to the melting of the surfactant and lipid present in the formulation. Therefore, the niosomal gel formulations can be stored at either refrigeration or room temperature Table 6.

In vitro permeation study

The mean amount of ADP and BPO permeated per unit of surface area was determined during 24 h and experiments Figure 9. Figure 10 shows permeation profiles of niosomal gel (cumulative amounts of ADP and BPO permeated vs Figure 11. time). The 6.25 ± 0.14 μg/cm^2 was permeated amount of ADP from niosomal gel in 24 h and 5.04 ± 0.014 μg/cm^2 was permeated amount of BPO from niosomal gel in 24 h.

In vitro skin retention study

The drug content retained in the layers of skin from cream was 2.68 μg from F2 formulation, 11.54 μg from F4 formulation, 15.54 μg from F5 formulation Table 7, 59.98 μg from F6 formulation, 68.85 μg from F7 formulation, and 143.78 μg from F8 formulation from niosomal gel Figure 12 and Table 8.

In vivo studies

From the result, the beneficial role of niosomal formulations over the plain drug formulations was indicated with regard to higher skin permeation and retention. Hence, it was thought worthwhile to investigate the effect of ADP niosomal formulations. The rabbits treated with plain gel are showing more irritation characteristics. The erythematic signs are continuously increasing at regular time intervals (initially it was marked as one but after 48 h it was three). The scaling was absent with plain gel initially, but after 12 h it appeared on applied skin portion. The lesions are also increasing as the therapy time increased.

The rabbits treated with niosomal gel are showing comparatively less irritation characteristics. The erythema signs were absent initially, but after 48 h it showed slight erythema signs. The scaling was completely absent with niosomal gel initially as well as even after 48 h. The lesions were also completely absent initially and even after 48 h. Hence, from the above comparisons of data it can be concluded that niosomal gel is more effective than plain gel.

There was no change in the niosomal formulation containing loaded BPO and ADP. On the basis of stability study, we can conclude that niosomal gel formulation was stable.

Summary

It is evident from the aforementioned study that niosomes showed better therapeutic activity than conventional dosage forms using formulations through the same route of administration. The greatest challenge with topical drug delivery is the barrier nature of skin, which restricts the entry of most drugs. Here, the present data proved that niosomes acted as the best vesicles in dermal drug delivery due to its nanometer size and their elastic nature. They acted as a drug carrier to deliver entrapped drug molecules into or across the skin and due to the individual lipid components, enhanced penetration into the stratum corneum and subsequently, the alteration of the intercellular lipid lamellae within this skin layer. *In vivo* experiments demonstrated an interesting correlation between the better permeation capabilities of niosomes in comparison to other conventional dosage forms in terms of a better therapeutic efficacy at the affected site at lower doses of drugs present in the niosomal gel formulation.

CONCLUSION

- ADP niosomes were prepared using different ratios of non-ionic surfactants with cholesterol and the same concentration of drug by thin-film hydration method.
- The niosomal formulations were evaluated for drug content, entrapment efficiency, *in vitro* release studies, and kinetic drug release.
- The percentage drug content of all the formulations was found to be in the range of 84–97.86%.
- The entrapment efficiency was found to be higher for niosomal formulation F8 when compared to other niosomal formulations due to the length of alkyl chain of Carbopol with cholesterol.
- The *in vitro* release kinetics also evaluated that all niosomal formulations obey the Higuchi diffusion model.
- From the above study, it is concluded that the niosomes

can be considered as promising drug delivery vehicles which increase the duration of the drugs. Thus, niosomes help in formulating dosage forms having more prolonged action.

- The niosomal delivery of ADP in Carbopol gel base acts as a suitable topical drug delivery system which may be given safely to all patients suffering from rheumatoid arthritis and osteoarthritis. The stability of niosomes improved after incorporation into gel base may be due to prevention of fusion of niosomes.

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