



Formulation Development and Evaluation of Niosomal anti-acne gel of Clindamycin phosphate

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ABSTRACT

Acne is a cutaneous pleomorphic disorder of the pilosebaceous unit involving abnormalities in sebum production and is characterized by both inflammatory (papules, pustules and nodules) and noninflammatory (comedones, open and closed) lesions. Propionibacterium acnes and Staphylococcus epidermidis are common pus-forming microbes responsible for the development of various forms of, acne vulgaris. Commonly used topical treatments include benzoyl peroxide, antibiotics, sulfur and sodium sulfacetamide, azelaic acid and retinoids. Topical treatment of acne with active pharmaceutical ingredients (API) makes direct contact with the target site before entering the systemic circulation which reduces the systemic side effect of the parenteral or oral administration of drug. Novel drug delivery systems display a strong rationale for topical treatment of acne in order to enhance the therapeutic performance of the topical antiacne agents with improved patient compliance. Clindamycin Phosphate is an antibiotic widely used for the treatment of acne. The pseudomonas colitis occurs with oral dosage form while in topical dosage forms it has side effects like irritation, skin rash, itching etc. its topical bioavailability is also less. An attempt has been made to overcome these limitations for the preparation of niosomal gel of clindamycin phosphate.

Keywords Clindamycin Phosphate, Acne, Niosomal Gel, anti- acne gel.

1 Introduction

Acne

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous units. It is a pleomorphic disorder with multifactorial pathogenesis. Acne vulgaris is a distressing condition related to the pilosebaceous follicle and which is considered as an 'adolescent' disorder. It is characterized by spontaneous resolution in the late teens or early twenties in the majority of cases-

Previous years have shown that among the girls 61% had acne lesions at 12 years and 83% at 16 years with a maximum between 15 and 17 years. Among the boys, the prevalence of acne was only 40% at 12 years but increased to 95% at 16 years with a maximum of frequency between 17 and 19 years (Rademaker M., et al., 1989).

The prevalence of acne in adolescents and adults varies among countries and ethnic groups (Kilkenny M. et al., 1998; Freyre EA et al., 1998). In Australia, acne was observed in 27.7% of student's aged 10-12 and in 93.3% of adolescents aged 16-18. A study in Peru reported a prevalence of 16.33% and 71.23%, in students aged 12 and 17, respectively. In countries including Belgium and China, prevalence in adolescents is high, about 90%, while in England it is estimated at 50%. It is generally assumed that acne occurs in 70-80% of adolescents (Dreno B., 2010; Ghodsi SZ et al., 2009).

Types of Acne

1. Mild acne: this includes whiteheads (closed clogged pores) and blackheads (clogged pores that are open at the skin surface and more easily noticeable).
2. Moderate or severe inflammatory acne: includes whiteheads and blackheads plus papules (reddened areas that are elevated above the skin surface) and areas of pustules (pimples—small bumps on the skin that contain visible fluid)
3. Nodulocystic acne: nodules are deeply embedded solid, often painful lesions. These may develop additional infections and may eventually lead to scarring if not treated. Nodules can be greater than 5 mm in diameter (Kaisar R, et al., 2012).

Table 1: Types of acne lesions (Kaisar R, et al, 2012)

| S.No | Type | Indication |
|------|-------------------------------------|--|
| 1. | Open Comedo (Non-inflammatory) | Blackhead in which pigment is invariably visible and consist of dilated orifice. |
| 2. | Closed Comedo (Non-inflammatory) | Absence of orifice leads to inflammatory lesion in the dermis. |
| 3. | Pustule (Inflammatory) | A small inflammatory lesion emerging from the microcomedo& results in cap of pus. |
| 4. | Papule & Nodule (Inflammatory) | A rigid and deeper lesion than a pustule. Its results in more severe form of acne. A larger and still deeper inflammatory lesion that invariably produces a deep scar |

Pharmacology of Clindamycin

a) Mechanism of action- 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. It does so by binding to the 50S rRNA of the large bacterial ribosome subunit, overlapping with the binding sites of the oxazolidinone, pleuromutilin, and macrolide antibiotics, among others. The binding is reversible. Clindamycin is more effective than lincomycin. The X-ray crystal structures of clindamycin bound to ribosomes (or ribosomal subunits) derived from *Escherichia coli*, *Deinococcus radiodurans*, and *Haloarcuramarismortui* have been determined; the structure of the closely related antibiotic lincomycin bound to the 50S ribosomal subunit of *Staphylococcus aureus* has also been reported.

b) Pharmacodynamics- Clindamycin exerts its bacteriostatic effect via inhibition of microbial protein synthesis. Clindamycin has a relatively short T_{max} and half-life necessitating administration every six hours to ensure adequate antibiotic concentrations.

Clindamycin is active against a number of gram-positive aerobic bacteria, as well as both gram-positive and gram-negative anaerobes. Resistance to clindamycin may develop, and is generally the result of base modification within the 23S ribosomal RNA. Cross-resistance between clindamycin and lincomycin is complete, and may also occur between clindamycin and macrolide antibiotics (e.g. erythromycin) due to similarities in their binding sites.

c) Pharmacokinetics

- **Absorption-** After topical application of 1% Clindamycin Phosphate, an average of 4% to 5% of clindamycin appears to be absorbed systemically.
- **Volume of distribution-** Clindamycin is widely distributed in the body, including into bone, but does not distribute into cerebrospinal fluid. The volume of distribution has been variably estimated between 43-74 L.
- **Metabolism-** Clindamycin undergoes hepatic metabolism mediated primarily by CYP3A4 and, to a lesser extent, CYP3A5. Two inactive metabolites have been identified - an oxidative metabolite, clindamycin sulfoxide, and an N-demethylated metabolite, N-desmethylclindamycin.
- **Route of elimination-** Approximately 10% of clindamycin bioactivity is excreted in the urine and 3.6% in the feces, with the remainder excreted as inactive metabolites.
- **Half life-** The elimination half-life of clindamycin is about 3 hours in adults and 2.5 hours in children. Half-life is increased to approximately 4 hours in the elderly.

d) Indication- In oral and parenteral formulations, clindamycin is indicated for the treatment of serious infections caused by susceptible anaerobic bacteria, as well as susceptible staphylococci, streptococci, and pneumococci.

e) Dose- Gel should be applied once a day to affected areas after washing in the evening before retiring. A thin film of the gel should be applied, avoiding eyes, lips, and mucous membranes.

During the early weeks of therapy, an apparent exacerbation of acne may occur. This is due to the action of the medication on previously unseen lesions and should not be considered a reason to discontinue therapy. Therapeutic results should be noticed after eight to twelve weeks of treatment.

f) Adverse Effects- Some adverse effects such as erythema, scaling, dryness, pruritus, and burning will occur in 10-40% of patients.

The following additional adverse experiences were reported in approximately 1% or less of patients:

skin irritation, burning/stinging, erythema, sunburn, and acne flares.

2 Materials and Methods

Preformulation Studies

The drug Clindamycin phosphate was obtained as a gift sample from M/S Glen-mark pharmaceuticals Pvt. Ltd., Baddi, India for the present work. The drug was authenticated by characterizing certain properties and comparing the same with the official standards or those described in the literature. The experimental details are described in the following paragraphs.

Physical Properties

The physical examination of the drug was performed in day-light and drug was observed for its appearance, color, and odor (Chandira R.M et al., 2010).

Melting Point

Melting point was determined in a melting point apparatus (Top Tech Lab Equipment Pvt. Ltd., Mumbai, India). Drug was filled in capillary tube, previously heat sealed from one end. The drug filled capillary was placed in the apparatus and the temperature was raised gradually and temperature was recorded.

Partition Coefficient

Partitioning of clindamycin was determined in Octanol and water system, Isopropyl myristate and buffer system (pH 5.5) by calculating log P values in respective systems (Surabhi K et al., 2010; Prasanna K et al., 2015).

The partition coefficient is defined as the ratio of unionized drug distributed between the organic and aqueous phase at equilibrium.

$$P_{o/w} = [C_{org} / C_{aq}]_{equilibrium}$$

Solubility Studies

Solubility is defined in quantitative terms as the concentration of solute in a saturated solution at a certain temperature and in qualitative terms it may be defined as the spontaneous interaction of two or more substances to form a v/v homogeneous molecular dispersion., analysed spectrophotometrically (Chandira R.M et al., 2010).

Drug compatibility studies

Any chemical or physical interaction between drugs and excipients can affect stability and bioavailability of drug. A small mixtures of drugs and excipients (1:1) ratio was prepared. The mixtures were placed above in vials. The rubber closures were placed on the vials and sealed it hermetically. The sample was assayed by FTIR (Bruker, ECO-ATR) (Ankit K et al., 2016).

Method for Quantitative Estimation

A spectrophotometric method based on UV-visible absorption provided convenient, precise and accurate mode to estimate the drug concentration in the microgram range. Estimations were carried out in PBS of pH 6.8.

Standard Curve of Clindamycin phosphate in water

CLP (10 mg) was dissolved in phosphate buffer pH 6.8 and volume was made up to 100 ml in 100 ml volumetric flask. This solution (100 µg/ml) was further diluted with phosphate buffer to obtain solution of 2 to 14 µg/ml. Peak area of each solution was measured at 210 nm using HPLC Shimadzu LC- 2010. The standard curve was generated at 210nm using Shimadzu Corp.81195, UV -1800Visible spectrophotometer against PBS pH 6.8 as blank. The standard curve was prepared between absorbance and concentration which was linearly regressed. The standard curve procedure was repeated three times and observations were recorded (Roshan R et al., 2015).

3 Results

Preformulation Studies

Table 2: Physical Properties of Clindamycin Phosphate

| Properties | Experimental Observation |
|-----------------------|--------------------------|
| Physical status | White Crystal Powder |
| Melting point | 112-116°C |
| Partition coefficient | 2.16 |

Table 3: Solubility of Clindamycin phosphate in Various Solvents

| Solvent | Solubility |
|------------------|------------|
| Distilled Water | +++ |
| Ethanol | + |
| PBS(6.8) | ++ |
| Methanol | ++ |
| Propylene glycol | +++ |
| DMSO | +++ |
| THF | ++ |
| Tetrahydrofuran | ++ |

--- Insoluble, +sparingly soluble, +++ very soluble ++ soluble

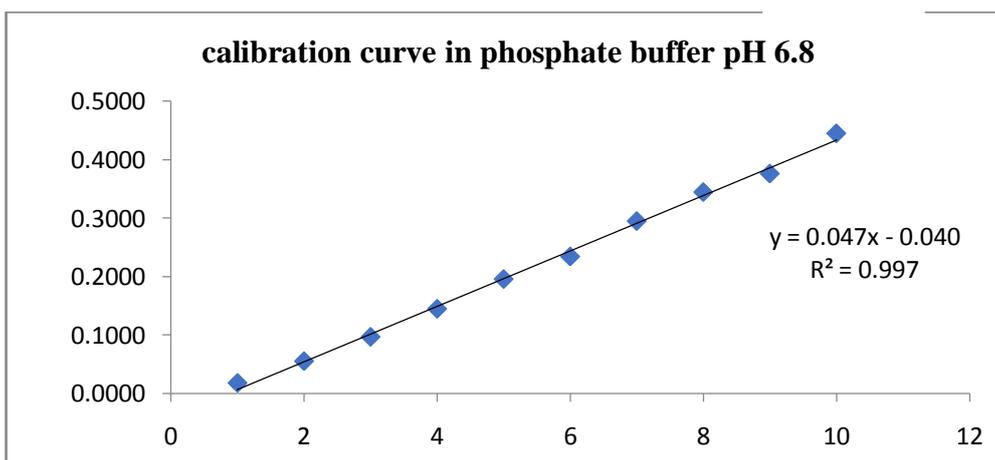


Fig 1: Standard Curve of Clindamycin phosphate in PBS (pH6.8)

Preparation & Characterization of NIOSOMES aspenetration enhancervesicles (PEVs)

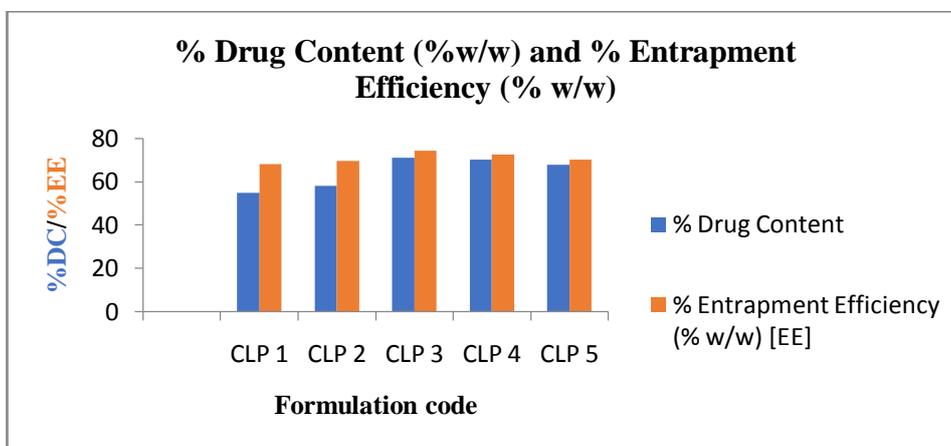
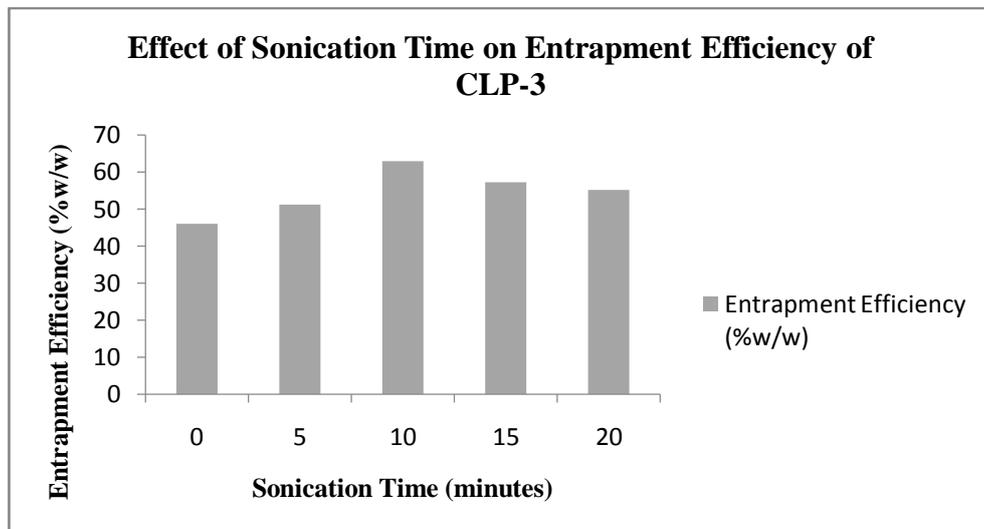
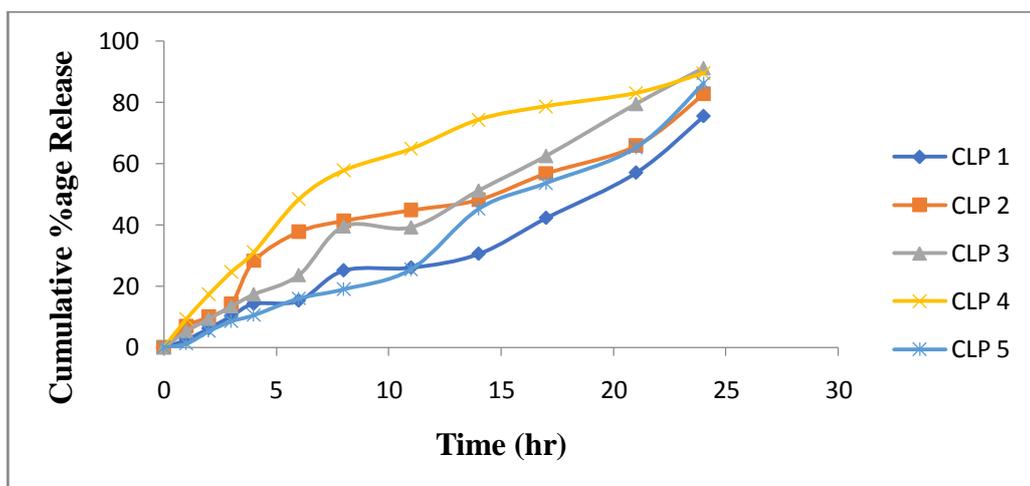


Fig 2: % Drug Content and % Entrapment Efficiency of Clindamycin phosphate Niosomes containing Span-60

Table 4: Effect of Hydration Time on Entrapment Efficiency of CLP-3

| S. No. | Hydration Time (minutes) | Entrapment Efficiency (%w/w) |
|--------|--------------------------|------------------------------|
| 1 | 30 | 31.06±2.52 |
| 2 | 45 | 39.5±1.15 |
| 3 | 60 | 65.2±0.16 |
| 4 | 75 | 63.53±1.55 |
| 5 | 90 | 61.27±1.11 |

**Fig 3: Effect of Sonication Time on Entrapment Efficiency of CLP-3****Fig 4: *in-vitro* release profile of clindamycin Niosomes**

Characterization of NIOSOMAL GEL

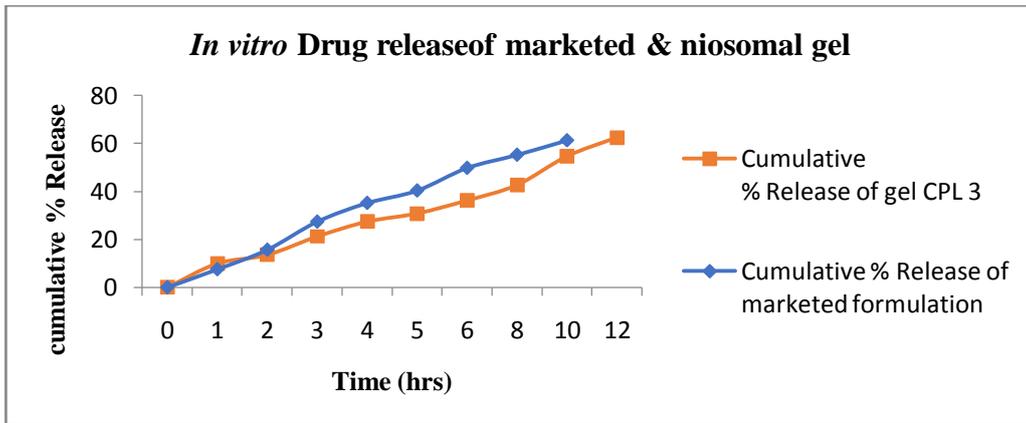


Fig 5: In vitro Drug Release of Niosomal gel & marketed formulation

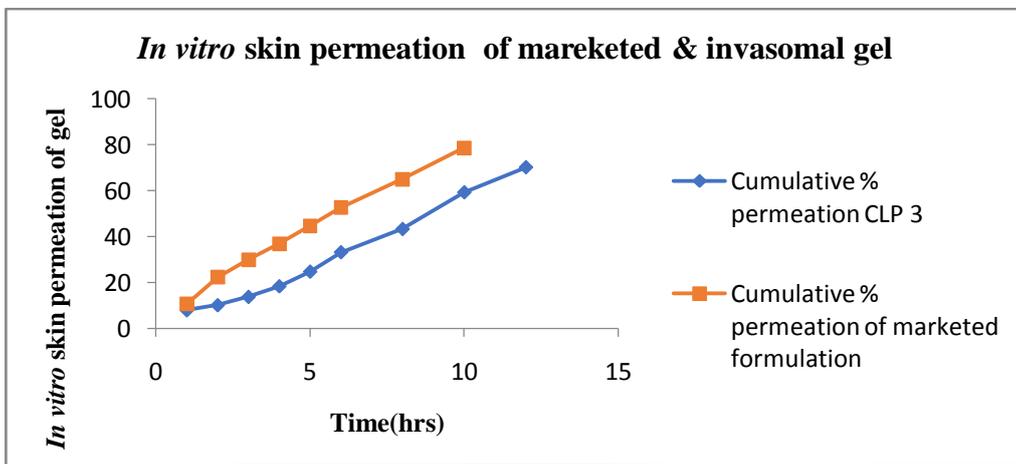


Fig 6: In vitro skin permeation of both Niosomal gel & marketed formulation

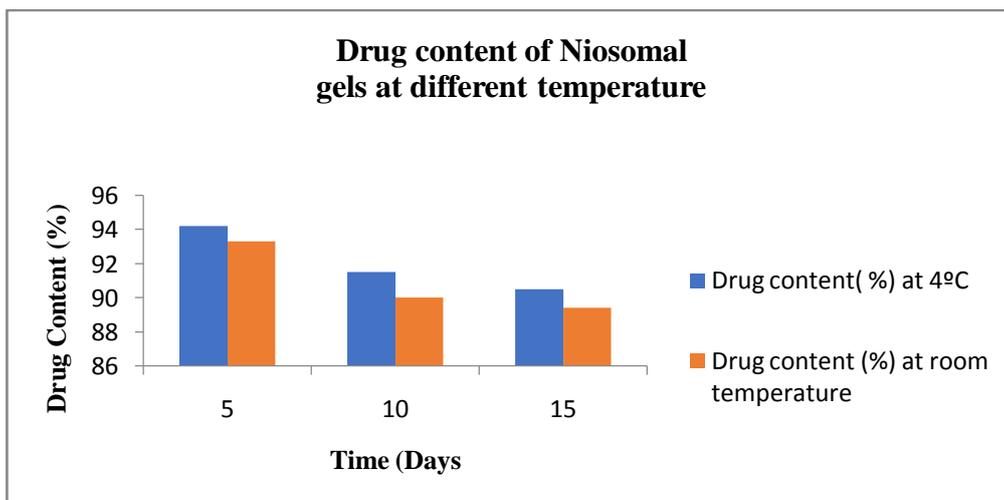


Fig 7: Drug content of Niosomal gel at different temperature

4 Conclusion

Clindamycin phosphate Niosomal gel was formulated which reduced the side effects produced by oral conventional doses. Characterization of the selected CLP3 formulation showed stability.

The entrapment efficiency of drug in vesicles was found to be dependent on drug to Cholesterol ratio. By optimizing the concentration of different variables Niosomes were characterized by determining the morphology, particle size, zeta potential. The TEM photograph revealed that the carrier systems are spherical in shape. It was reported an increase in concentration of cholesterol in vesicles resulted in increase in negative surface charge upto certain level then start decreasing. Wherein Niosomes containing 1M Moles (10mg) of cholesterol possessed a surface charge of -31.2 mV and Niosomes containing 3 M Moles (30mg) of cholesterol possessed -48.25 mV. It has been reported that negatively charged vesicles promoted permeation of drug. Negative charge may be imparted due to the presence of ethanol. The entrapment efficiency of Clindamycin phosphate loaded niosomes ranged from 65%-75%.

In vitro skin permeation studies were performed using male rat abdominal skin selected clindamycin phosphate gel formulations with HPMC E-15 (CLP 3 gel) & marketed gel formulation. Permeation was determined in terms of the mean cumulative amount diffused at each sampling point for a period of 12 hrs. Cumulative drug permeated was found to be $70.11 \pm 2.48\%$ for CLP 3 gel which was less than the marketed formulation $78.63 \pm 1.69\%$. Mean cumulative amount Drug diffused after 24 hrs was found to be $79.56 \pm 1.14\%$ for CLP 3 gel, on other and permeation of marketed formulation diminish after such interval. This is due to better penetration of niosome as well as release of medicament for longer duration.

Optimized niosomal gel was sealed in 10 ml glass vial and stored at refrigeration temperature 4°C and at room temperature for 4 weeks physical appearance, pH and drug content was determined at regular intervals. The drug content after a period of 4 weeks at 4°C and room temperature were found to be between the range of 90-95% respectively. The results indicate that more than 90% of the drug was retained in the Niosomal formulation for a period of 4 week at 4°C. The pH of all the formulation ranged from 6.21 -6.25 appropriate for the skin. There was no change in the physical appearance of niosomal gel.

In conclusion, results of present study showed the potential use of novel niosomal vesicular carrier, niosomes for enhancing permeation of Clindamycin phosphate across skin. It anticipates numerous advantages like counting higher bioavailability; less long term effects due to smaller amounts of drug administered and most importantly increased patient-compliance.

Conflict of Interest

The authors declare that there is no conflict of interest.

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