Asian Journal of Pharmaceutical Education and Research
Vol -8, Issue-3, July-September 2019
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 7.014

# FORMULATION AND EVALUATION OF LIPOSOMAL GEL FOR EFFECTIVE TREATMENT OF ACNE

**Singh Nishantkumar Ramkrishna[1]*, Hemant Kumar Dharma[1], Neelesh Choubey[1], C.K. Tyagi[1], Prabhakar Budholiya[1], Geeta parkhe[2]**

1. College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M. P.).

2. Scan Research Laboratories, Bhopal (M.P.)

*Corresponding Author's E mail: Nks210892@gmail.com

Received 03 June 2019; Revised 08 June 2019; Accepted 16 June 2019, Available online 10 July 2019.

**ABSTRACT**

Approximately 95% of the population suffers at some point in their lifetime from acne vulgaris. Acne is a multifactorial disease of the pilosebaceous unit. This inflammatory skin disorder is most common in adolescents but also affects neonates, prepubescent children and adults. Topical conventional systems are associated with various side effects. Novel drug delivery systems have been used to reduce the side effect of drugs commonly used in the topical treatment of acne. 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. Liposomal formulations have been successfully used in the treatment of a number of dermatological diseases. Various synthetic as well as herbal drugs are incorporated into liposome to improve its efficacy. Clindamycin phosphate is an antibiotic useful for the treatment of a number of infections. It is of the lincosamide class. Clindamycin phosphate is a semi synthetic lincosamide antibiotic that has largely replaced lincomycin due to an improved side effect profile. Clindamycin inhibits bacterial protein synthesis by binding to bacterial 50S ribosomal subunits. Topical Clindamycin phosphate reduces free fatty acid concentrations on the skin and suppresses the growth of Propionibacterium acnes (Corynebacterium acnes), an anaerobe found in sebaceous glands and follicles. Clindamycin loaded liposomes were prepared and characterized for vesicle size, surface charge and vesicle size, entrapment efficiency, pH measurements and stability. Clindamycin liposomal gels were formulated and evaluated comparatively with commercial gel with respect to measurement of viscosity, drug content, extrudability study, spreadability, in vitro drug diffusion study and stability studies. All the prepared liposomal gel showed acceptable physical properties, homogeneity, consistency, spreadability, viscosity and pH value. The best formulation F8 showed better anti-acne activity when compared with all formulation.

**Keywords:** Acne vulgaris, Clindamycin phosphate, Topical treatment, Liposomal.

## INTRODUCTION:

Approximately 95%of the population suffers at some point in their lifetime fromacne vulgaris [1]. Papules, pustules, closed and open comedones, cysts, and scarring may be seen. Having acne can give rise to feelings of embarrassment, loss of self-esteem, and depression, as well as physical symptoms (such as

soreness and pain) associated with individual lesions. Acne is well known to respond to hormones, both endogenous and exogenous. It is the most common dermatologic disorder affecting approximately 85% of the teenagers [2, 3] and a chronic inflammatory follicular disorder of the skin, occurring in specialized pilosebaceous units on the face consisting of the follicular canal with its rudimentary hair, and the group of sebaceous glands that surround and open on to the follicle [4–6]. Acne vulgaris can be defined as the most common skin disease, that results in comedos or severe inflammatory lesions in the face, back, and chest with a large number of sebaceous follicles, and the condition of the disease is associated with the elevated rate of sebum excretion [7]. The pathophysiology of acne includes abnormal proliferation and differentiation of keratinocytes, increased sebum production, hyper proliferation of Propionibacterium acne, and inflammatory response initiated by bacterial antigens and cytokines [8–14]. In the skin with acne due to the excess production of male hormone androgen and oil producing glands in the face comedone occurs on the face [15]. The closed comedone (whitehead) and ripen comedone (blackhead) are the primary two noninflammatory lesions in acne. These lesions may progress to inflammatory papules and pustules when the contents rupture. Larger, more painful lesions, such as cysts and nodules, may also develop [16]. The application of novel delivery systems to the skin distributes the topical agent gradually, reduces the irritancy of some anti-acne drugs and shows good efficacy [17]. Drugs used to treat acne include clindamycin phosphate, azelaic acid, benzoyl peroxide, dapalene, erythromycin, tretinoin and isotretinoin. Limitations of conventional topical formulations include side effects like scaling, erythema, dryness, stinging, irritation, burning, itching, rash, pruritus and sunburn. To reduce side effects, novel carrier-based drug delivery systems are formulated which distribute the topical medicament gradually, reduce the irritancy of drugs and maintain better efficacy. Therefore, penetration is more efficient than non-particulate systems that provide a high local concentration. Vesicles are an example of novel drug delivery systems which act as drug carriers to deliver entrapped drug molecules into or across the skin. They act as enhancers for the penetration of the individual lipid components into the stratum corneum and subsequently altering the inter-cellular lipid lamellae within this skin layer. Vesicles also serve as a depot for sustained release of dermally active compounds and as a rate limiting membrane barrier for the modulation of systemic absorption, therefore, providing a controlled transdermal delivery system [18]. Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of phospholipids with or without some additives [19]. Among a variety of drug lipid carriers, liposomes seem to have the best potential as localizers of topically applied drugs [20]. It was reported that conventional liposomes only enhanced skin deposition, with mostly reduction effect on percutaneous permeation or systemic absorption of drugs with better drug localization at site of action[21]. The aim of this work is to minimize the adverse effects associated with clindamycin application on the

skin. Incorporating clindamycin in suitable liposomal formulation may modify its diffusion parameters in the skin and hence reduce its systemic absorption and consequently its adverse effects. Also, its effectiveness could be improved by maximizing its accumulation into the skin.

## MATERIALS AND METHODS:

### Materials

Clindamycin phosphate was kindly provided as a gift sample from curetech skin formulation Baddi, Himanchal Pradesh, India. Spans 80, Soya PC were purchased from SD Fine Chemicals Mumbai, India. Carbopol 941 was purchased from CDH Laboratories New Delhi, India. Liquid paraffin, propylene glycol and sodium hydroxide extra pure were purchased from Hi-Media laboratories Mumbai, India. Propionibacterium acne was obtained from microbial culture collection, national centre for cell science, Pune, Maharashtra, India. All other chemicals used were of analytical grade and were used without any further chemical modification.

### Determination of λ max of clindamycin

Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.4 pH buffer solution in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 7.4 pH buffer solution prepare suitable dilution to make it to a concentration range of 10-50 µg/ml. 2 ml of standard solution mix with 1 ml methyl orange and extracted with 3 ml of chloroform. Pipette out the chloroform layer and take the absorbance against chloroform as blank. The spectrum of this solution was run in 400-800 nm range in U.V. spectrophotometer (Labindia-3000+).

### Compatibility studies of drug and excipients

Taken 50 mg accurately weigh of clindamycin phosphate dry powder and 50 mg of excipients and mix the blend of drug and excipients and binary/tertiary blends of extract and excipients were prepared and transferred to inert glass vials. The mouths of the vials were covered with rubber closures followed by the aluminum seal caps. Binary/tertiary blends of extract and excipients, clindamycin neat and excipients were stored at 4˚C (refrigerator) as control and at 40˚C/75%RH for accelerated stability studies for 4 weeks. The λmax were recorded for determination of compatibility of clindamycin phosphate with other excipients.

## Method

### Preparation of Clindamycin phosphate loaded liposomes

Liposomes were prepared by rotator evaporation method given by Touitou et al., 2000 [22] with slight modification in which drug was dissolved in methanol to give a concentration of 1.0% w/v of drug solution. The accurately weighed amounts (10% w/v) of phospholipids and surfactant (7:3 ratio) were taken in a clean, dry, round-bottom flask and this lipid mixture was dissolved in minimum quantity of methanol and chloroform mixture in ratio of 2:1. The round bottom flask was rotated at 45º angle using rotator evaporator at 40°C in order to make uniform lipid layer. The organic solvent was removed by rotary evaporation under reduced pressure at the same temperature (40°C). Final traces of solvents were removed under vacuum overnight. The prepared lipid film in the inner wall of round bottom was hydrated with 10% w/v of drug solution in water followed by rotating the flask containing mixture of drug by rotation at speed of 60rev/min for 1 hr. After complete hydration of film, the prepared formulation of liposomes was subjected to sonication at 40C in 3 cycles of 10 minutes with 5 sec rest between the cycles. The prepared formulation was stored at 4°C in closed container till further use for analysis.

### Preparation of Gel Base

Carbopol 934 (1-3%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10ml of propylene glycol was added to this solution. The obtained slightly acidic solution was neutralized by drop wise addition of 0.05 N sodium hydroxide solutions, and again mixing was continued until gel becomes transparent. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.5. Gel was also prepared with plain drug by adding 10 mg of drug and dispersed properly by following same procedure given above. Liposomes preparation corresponding to 1% w/w (10mg of drug in 100 mg of gel) of drug was incorporated into the gel base to get the desired concentration of drug in gel base.

In the liposomal formulations (F1 to F10), the lipid: surfactant ratio was optimized by taking their different ratio such as 5:5, 6:4, 7:3 and 8:2 ratio, drug concentration was optimized by taking different concentration of drug and sonication time was optimized by sonicating the formulation for different time i.e 30, 60 and 90 sec at 40C in 3 cycles of 10 minutes with 5 sec rest between the cycles. all other parameters were kept remain constant. The prepared formulations were optimized on the basis of average particle size and % entrapment efficiency. The composition of different formulation was given in table 1

**Table 1 Different formulas of clindamycin liposomal**

| F. code | Soya PC: Span 80 (% w/v) | Drug (% w/v) | Sonication time (Sec) |
|---|---|---|---|
| F-1 | 5:5 | 1.0 | 60 |
| F-2 | 6:4 | 1.0 | 60 |
| F-3 | 7:3 | 1.0 | 60 |
| F-4 | 8:2 | 1.0 | 60 |
| F-5 | 8:2 | 1.0 | 60 |
| F-6 | 8:2 | 1.5 | 60 |
| F-7 | 8:2 | 2.0 | 60 |
| F-8 | 8:2 | 1.5 | 30 |
| F-9 | 8:2 | 1.5 | 60 |
| F-10 | 8:2 | 1.5 | 90 |

**Table 2 Optimized formulation of liposomes**

| Formulation code F-8 | |
|---|---|
| Phospholipid: Surfactant (10 % w/v) | 8:2 |
| Drug (% w/v) | 1.5 |
| Sonication time (sec) | 30 |

## Characterization of Liposomes

### Vesicle size

Microscopic analysis was performed to determine the average size of prepared liposomes. Formulation was diluted with distilled water and one drop was taken on a glass slide and covered with cover slip. The prepared slide was examined under trinocular microscopic at 400 X. The diameters of more than 150 vehicles were randomly measured using calibrated ocular and stage micrometer. The average diameter was calculated using the flowing formula.

$$Average\ Diameter = \frac{\Sigma n.d}{\Sigma\ n}$$

Where n = number of vesicles; d = diameter of the vesicles

### Surface charge and vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the liposomes was based on the zeta potential that was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field

strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9 % NaCl adjusted to a conductivity of 50 lS/cm.

**Entrapment efficiency**

Entrapment efficiency of Clindamycin phosphate liposomal formulation was determined using the Sephadex G-50 column. The weighed amount of Sephadex G-50 was properly mixed with sufficient amount of distilled water in a beaker and kept for 24 h for complete swelling. After complete swelling, Sephadex dispersion was placed in a 1 mL capacity of PVC syringe (Dispovan) packed with glass wool and a small piece of Whatman filter paper at the bottom end to provide stability for the Sephadex column at 3,000 rpm. The entrapment efficiency of Clindamycin phosphate in liposomes vesicle was determined by ultracentrifugation, 10mL of liposomes formulation were passed from the column. The amount of drug not entrapped in the liposomes was determined by passing the formulation from the Sephadex column, centrifuging at 3,000 rpm, and collecting the elution using the equation given below. After removing the un-entrapped drugs, the liposomes were collected and lysed using 1 % Triton X100; and then centrifuged. The supernatant layer was separated, diluted with water suitably and drug concentration was determined using UV spectrophotometer.

**pH measurements**

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted.

Characterization of Liposomes Containing Gel

**Measurement of viscosity**

The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer. The viscosity was measured using spindle no. 63 at 10 rpm and 250C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25 ±/10C) before the measurements.

**Extrudability study**

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube. Extrudability of gel required 155 grams of weight to extrude a 0.6cm ribbon of gel in 6 seconds.

**Spreadibility**

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. It was determined by method reported by Multimer et al., 1956 [23]. An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadibility, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 6cm upon adding 50g of weight was noted. Good spreadibility show lesser time to spread.

**In Vitro drug diffusion study**

The in-vitro diffusion study is carried by using Franz Diffusion Cell. Egg membrane is taken as semi permeable membrane for diffusion. The Franz diffusion cell has receptor compartment with an effective volume approximately 60 ml and effective surface area of permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32 ± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of 486nm.

**Stability Studies**

Stability study was carried out for drug loaded liposomes at two different temperatures i.e. refrigeration temperature (4.0 ±0. 2°C) and at room temperature (25-28±2°C) for 3 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any physical changes and drug content.

**RESULTS AND DISCUSSION**

λ max of clindamycin was found to be 486 nm by using U.V. spectrophotometer (Labindia-3000+) in linearity range 10-50µg/ml. Clindamycin phosphate was freely soluble in ethanol, methanol, soluble in phosphate buffer pH 7.4 and slightly soluble in 0.1 N HCl, water. Identification of clindamycin was done by FTIR spectroscopy with respect to marker compound. It was identified from the result of IR spectrum as per specification. Loss on drying value, moisture content and melting point of clindamycin phosphate was found to be 0.72, 0.565 and 140-142oC respectively. In the compatibility testing program, blends of drug and excipients are prepared by triturating the drug with individual excipients. The λmax were recorded for determination of compatibility of clindamycin phosphate with other excipients. Vesicle size and zeta potential of the ethosomes were measured by photon correlation spectroscopy using a Malvern

Zetasizer and entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium by UV spectrophotometer the results shown in table 3. Average vesicle size, % entrapment efficiency and zeta potential of optimized formulation (F-8) was found to be 225.65±4.23, 75.65±1.52 and -32.25±0.25 respectively. Results of evaluation of liposomal gel formulation (LG-1) of optimized formulation (F8) were incorporated into carbapol gel concentration 1 % w/w Table 4. Release of drug from clindamycin liposomes was significantly slower, which confirmed that slight prolonged drug release rate. Incorporation of carbomer affected the release rate of the drug. By increasing the amount of carbomer, the release rate of the drug decreased, which could be related to the increased rigidity of the formulation, followed by its decreased permeability for the drug. Optimized formulation F8 shows significantly improved in drug release rate as compare to marketed preparation. It was concluded that developed formulations deliver the drug for the treatment of acne vulgaris table 5. Stability study was carried out for drug loaded liposomes at two different temperatures i.e. refrigeration temperature (4.0 ±0. 2°C) and at room temperature (25-28±2°C) for 3 weeks. The formulations were analyzed for any physical changes and drug content (Table 6).

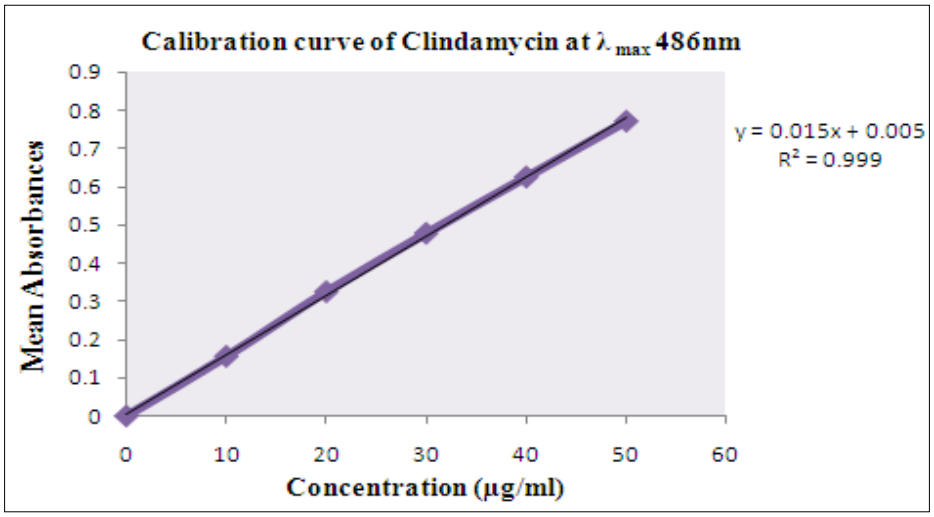


**Figure 1 Calibration curve of clindamycin phosphate in buffer pH 7.4 at 486nm**

**Table 3 Result for vesicle size and entrapment efficiency of drug loaded liposomes**

| Formulation code | Average vesicle size (nm) | % entrapment efficiency |
|---|---|---|
| F-1 | 365.56±0.45 | 48.85±0.32 |
| F-2 | 325.45±0.85 | 55.56±0.41 |
| F-3 | 295.65±0.65 | 69.98±0.52 |
| F-4 | 253.32±0.25 | 78.98±0.32 |
| F-5 | 265.56±0.65 | 70.23±0.32 |
| F-6 | 242.25±0.23 | 76.65±0.54 |
| F-7 | 285.65±0.11 | 69.98±0.41 |
| F-8 | 225.65±0.65 | 75.65±0.36 |
| F-9 | 195.56±0.32 | 70.23±0.54 |
| F-10 | 145.56±0.41 | 62.23±0.85 |

**Table 4 Characterization of gel based formulation of liposomes**

| Characterization | Viscosity (cps) | Release after 12hr | Extrudability (g) | Spreadibility (g.cm/sec) |
|---|---|---|---|---|
| LG-1 | 2550±25 | 95.56±0.52 | 155±4 | 11.23±1.65 |

**Table 5 *In vitro* drug release study of prepared gel formulation**

| S. No. | Time (hr) | % Cumulative Drug Release |
|---|---|---|
| 1 | 0.5 | 12.25 |
| 2 | 1 | 24.56 |
| 3 | 2 | 39.98 |
| 4 | 4 | 46.63 |
| 5 | 6 | 59.98 |
| 6 | 8 | 68.85 |
| 7 | 10 | 79.98 |
| 8 | 12 | 95.56 |

**Table 6 Stability study of prepared gel formulation**

| Characteristic | Time (Month) 1 Month | | 2 Month | | 3 Month | |
|---|---|---|---|---|---|---|
| **Temp.** | 4.0 ±0. 2°C | 25-28±2°C | 4.0 ±0. 2°C | 25-28±2°C | 4.0 ±0. 2°C | 25-28±2°C |
| **Average particle size (nm)** | 230.23±0.45 | 285.65±1.23 | 235.62±2.65 | 330.25±2.12 | 220.32±1.74 | 415.65±3.45 |
| **% EE** | 75.20±1.45 | 65.56±2.32 | 73.23±1.74 | 55.23±1.35 | 70.24±1.65 | 42.23±2.45 |
| **Physical Appearance** | Normal | High turbid | Normal | High turbid and agglomeration | Normal | High turbid and agglomeration |

## Conclusion

Overall results obtained during this work have shown that liposomes may be an interesting carrier for clindamycin phosphate in skin disease treatment, where greater drug retention and reduced percutanous absorption were observed leading to less irritancy and better drug tolerance compared to commercial gel. Liposomes formulae should be stored at refrigerated temperature to minimize the drug leakage from the vesicles and avoid any physical change in the liposomes as sedimentation or color change. The release of clindamycin phosphate from liposomes dispersions was mainly dependant on lipid composition where the vesicle size showed no influence.

## References

1. Ho V, Schacter D and Miller R. Acne management for the 90s: current treatment guidelines. The Canadian Journal of Diagnosis. 1995; 12: 1–25.
2. Arora MK, Yadav A and Saini V. Role of hormones in acne vulgaris. Clinical Biochemistry. 2011; 44(13): 1035–1040.
3. Falcocchio S, Ruiz C, Pastor FIJ, Saso L and Diaz P. Propionibacterium acnes GehA lipase, an enzyme involved in acne development, can be successfully inhibited by defined natural substances. Journal of Molecular Catalysis B. 2006; 40 (3-4): 132–137.
4. Bloom DF. Is acne really a disease? A theory of acne as an evolutionarily significant, high-order psychoneuroimmune interaction timed to cortical development with a crucial role in mate choice. Medical Hypotheses. 2004; 62(3): 462–469.
5. Lucky AW, Cullen SI, Funicella T, Jarratt MT, Jones T and Reddick ME. Double-blind, vehicle-controlled, multicenter comparison of two 0.025% tretinoin creams in patients with acne vulgaris. Journal of the American Academy of Dermatology. 1998; 38(4): S24–S30.
6. Jain A and Basal E. Inhibition of Propionibacterium acnes induced mediators of inflammation by Indian herbs. Phytomedicine. 2003; 10(1): 34–38.
7. Leyden J and Kligman A. Acne vulgaris: new concepts in pathogenesis and treatment. Drugs, 1976; 12(4): 292–300.
8. Palombo-Kinne E, Schellschmidt I, Schumacher U and Gr¨aser T. Efficacy of a combined oral contraceptive containing 0.030mg ethinylestradiol/2mg dienogest for the treatment of papulopustular acne in comparison with placebo and 0.035mg ethinylestradiol/2mg cyproterone acetate. Contraception. 2009; 79(4): 282–289.
9. Yamaguchi N, Satoh-Yamaguchi K and Ono M. In vitro evaluation of antibacterial, anticollagenase, and antioxidant activities of hop components (Humulus lupulus) addressing acne vulgaris," Phytomedicine. 2009; 16(4): 369–376.

10. Leyden J, Shalita A, Hordinsky M, Swinyer L, Stanczyk FZ and Weber ME. Efficacy of a low-dose oral contraceptive containing 20 $\mu$g of ethinyl estradiol and 100 $\mu$g of levonorgestrel for the treatment of moderate acne: a randomized, placebo controlled trial. Journal of the American Academy of Dermatology. 2002; 47(3): 399–409.
11. Kawashima M, Harada S, Loesche C and Miyachi Y. Adapalene gel 0.1% is effective and safe for Japanese patients with acne vulgaris: a randomized, multicenter, investigator-blinded, controlled study. Journal of Dermatological Science. 2008; 49(3): 241–248.
12. Huber J and Walch K. Treating acne with oral contraceptives: use of lower doses. Contraception. 2006; 73(1): 23–29.
13. Thiboutot DM, Weiss J and Bucko A. Adapalene-benzoyl peroxide, a fixed-dose combination for the treatment of acne vulgaris: results of a multicenter, randomized double-blind, controlled study. Journal of the American Academy of Dermatology. 2007; 57 (5): 791–799.
14. Yang D, Pornpattananangkul D and Nakatsuji T. The antimicrobial activity of liposomal lauric acids against Propionibacterium acnes. Biomaterials. 2009; 30(30): 6035-6040.
15. Mitchell T and Dudley A. Acne: The at Your Fingertips Guide, Class Publishing, 2002.
16. Brieva J, McCracken GA and Diamond B. Update and treatment of acne vulgaris, Medical Update for Psychiatrists. 1997; 2(6): 161–163.
17. Leyden JJ. Topical treatment of acne vulgaris: retinoids and cutaneous irritation. Journal of the American Academy of Dermatology. 1998; 38 (4): S1–S4.
18. Prasanthi D and Lakshmi PK. Vesicles-Mechanism of Transdermal Permeation: A Review, Asian J. Pharm. Clin Res. 2012; 5(1):18-25.
19. El-Sayed MA, Abdallah OY, Naggar VF and Khalafallah NM. Lipid vesicles for skin delivery of drugs: Reviewing three decades of research. Int. J. Pharm. 2007; 332 (1-2): 1-16.
20. El-Maghraby GM, Williams AC and Barry BW. Liposomes and skin: From drug delivery to model membranes. Eur. J. Pharm. Sci. 2008*;* 34 (4-5): 203-222.
21. El-Maghraby GM, Williams AC and Barry BW. Can drug-bearing liposomes penetrate intact skin? J. Pharm. Pharmacol.2006; 58 (4): 415-429.
22. Touitou E, Dayan N, Bergelson L, Godin B and Eliaz M. Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release. 2000; 65: 403-418.
23. Multimer MN, Riffskin C, Hill JA, Marry E, Cyr NG and Glickman GJ. Synthesis of methyl silyl derivatives of procaine and their diffusion. J Am Pharm Assoc. Sci, 1956; 45: 212.