"FORMULATION AND CHARACTERIZATION OF TOPICAL GEL CONTAINING BENZOYL PEROXIDE AND CLINDAMYCIN PHOSPHATE "

A Thesis Submitted to

NIRMA UNIVERSITY

in Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL TECHNOLOGY & BIOPHARMACEUTICS

BY

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NAAC ACCREDITED 'A' GRADE

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Month 2016

CERTIFICATE

This is to certify that the dissertation work entitled "Formulation and characterization of Topical gel containing Benzoyl Peroxide and Clindamycin phosphate" submitted by Mr Vora Chirag Chetan with Regn. No. (14MPH102) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Technology and Biopharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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DECLARATION

I hereby declare that the dissertation entitled "Formulation and characterization of topical gel containing Benzoyl peroxide and Clindamycin Phosphate", is based on the original work carried out by me under the guidance of Dr. Renuka Mishra Assistant professor, department of Pharmaceutics, Nirma University and Dr Imran Khan Senior Research scientist, Formulation and Development Department(NDDS) of Cadila Pharmaceuticals Pvt.Ltd Dholka, Ahmedabad. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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"स्थावरं जंगमं व्याप्त यत्कं चि्सिरारम । त्पदं दर्शति येन तस्मै श्रीगुरवे नमः" *means Salutation to the noble Guru, who has made it possible to realise Him, by whom all that is – sentient and insentient, movable and immovable is pervaded. As it is implicated, I owe my first and heartiest indebtedness to my mentors Dr. Renuka Mishra and Dr. Tejal Mehta for their keen interest, valuable and experienced guidance and constant words of encouragement.*

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"May the candle be lightened forever, the joy is not of light alone, but of presence of those, who played the role behind the curtain."

Chirag C. Vora ***NirmaUniversity***
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Dedicated to God, My Family & Friends

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List of ABBREVIATIONS

Sr. No.	Short Form	Abbreviations
1	BPO	Benzoyl peroxide
2	CLP	Clindamycin phosphate
3	GMS	Glyceryl monostearate
4	GMO	Glyceryl monooleate
5	NLC	Nanostructured lipid carriers
6	SLN	Solid lipid nanoparticles
7	EB	Epidermolysis bullosa
8	P.acne	Propionibacterium acne
9	PPARs	Peroxisome proliferator activated receptor
10	TEM	Transmission electron microscopy
11	FTIR	Fourier transform infrared spectroscopy
12	DSC	Digital scanning calorimetry

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Formulation and Characterization of Topical Gel containing Benzoyl Peroxide and Clindamycin Phosphate

ABSTRACT

Acne is a chronic inflammatory disease of pilosebaceous glands. The purpose of this study was to develop topical gel for delivery of Benzoyl peroxide (BPO) and Clindamycin phosphate with an objective to decrease skin irritation and resolve the stability issues. Combination of Benzoyl peroxide and Clindamycin phosphate is more effective than monotherapy as there are less chances of bacterial resistance. Problem of skin irritation is observed in conventional formulations due to conversion of benzoyl peroxide to benzoic acid. Nanostructured lipid carriers (NLC) based formulation of BPO was developed and incorporated into gel in order to obtain controlled release of the drug. Glyceryl monostearate(GMS) was screened as the solid lipid for NLC due to its good solubilizing capacity, Glyceryl monooleate(GMO) as liquid lipid,Tween80 as surfactant and propylene glycol as co-surfactant. Box Benkhen experimental design was used to optimize the amount of lipid (X1), amount of Surfactant (X2) and homogenization time (X3) in the NLC. The design batches were assessed for particle size (nm), Polydispersity index (PDI) and %Encapsulation efficiency (%EE). The NLC consisting of 2.5gm GMS lipid, 3% Tween80 as Surfactant and 10min HSH time was selected as the optimized batch of Benzoyl peroxide loaded NLC. Particle size, PDI and %EE of benzoyl peroxide for optimized NLC were 160.2 nm, 0.582 and 80.3% respectively. Transmission electron microscopy of optimized batch confirmed the size and sphericity of NLC. Further, Carbomer 980was used to convert NLC into gel form for topical application. Thus, it was concluded that developed NLC of Benzoyl peroxide in combination with Clindamycin phosphate could be a promising formulation for effective treatment of acne compared to conventional formulation.

About 95% of the population suffers at some point in their lifetime from acne vulgaris. Acne vulgaris is a multifactorial disease affecting the pilosebaceous follicle and characterised by comedomes, papules, pustules, nodules, and scars. Follicular keratinisation, seborrhoea, and colonisation of the pilosebaceous unit as Propionibacterium acnes are fundamental to the development of lesions. Furthermore, stratum corneum of the skin has remarkable barrier properties which block entry of most topically applied drugs, this posses a significant challenge to administering medications via the skin either for local cutaneous effects or as systemic therapy.

Benzoyl peroxide is a powerful oxidizing agent originally derived from chlorhydroxyquinolin, a byproduct of coal tar. It is composed of white, crystal agglomerates, is soluble in organic solvents and insoluble in water . The compound is highly lipophilic and, as such, is easily able to penetrate the stratum corneum and enter a pilosebaceous follicle. The antibacterial property of BPO is due to the formation of highly reactive oxygen species that oxidize proteins in bacterial cell membranes. BPO is a powerful antimicrobial agent that is toxic to both bacterial organisms and yeasts. Benzoyl peroxide is also believed to be effective in the treatment of acne on account of its anti-inflammatory properties. The released reactive oxygen species kill P. acnes but may also induce injury to the surrounding host cells. Benzoyl peroxide is also an effective comedolytic and keratolytic Agent.

Combination therapy for acne vulgaris uses several arrangements of different agents such as BPO with clindamycin in an effort to increase efficacy while minimizing bacterial resistance and adverse reactions. Resistant strains of P. acnes are found on the skin of individuals worldwide and can result in a poor clinical response to topical antibiotics. P.acne lipase that severs sebaceous triglycerides and therefore release unsaturated fat. These unsaturated fat are both comedogenic and proinflammatory , applying chemotactic impacts. Clindamycin has been appeared to inhibit p.acnes related extracellular lipase generation , while erythromycin did not restrain chemical creation. Therefore combination treatment targeting several of these mechanism appears to be logical approach in treatment of acne. Combination products that contain benzoyl peroxide along with clindamycin phosphate helps to prevent the

antimicrobial resistance in patients who have already developed resistance, this combination product tends to improve acne.

Accordingly treatment focusing on a few of these system seems, by all accounts, to be intelligent methodology in treatment of skin break out. Formulations that contain benzoyl peroxide alongside clindamycin phosphate keeps the antimicrobial resistance in patients who have officially created resistance, this mix item has a tendency to enhance skin inflammation. Joining benzoyl peroxide with clindamycin decreases safe strains and allows longer successful treatment. Not at all like blends benzoyl peroxide/erythromycin, benzoyl peroxide/clindamycin details are steady at room temperature and don't require refrigeration.

As nutshell we can say that by considering factors such as skin type, application and convenience novel combinations such clindamycin phosphate1% / benzoyl peroxide gel proves to be superior to clindamycin or benzoyl peroxide monotherapy in reducing acne lesions counts. Combination therapy should be standard in management of acne in order to decrease p.acne related resistance.

The efficacy of the antiacne topical drugs using novel carrier-based drug delivery system is well established. The local side eefects, however, mainly cutaneous irritation, erythema, dryness, peeling, and scaling, remain major problems. The anti-acne drug-loaded vesicular and particulate delivery systems (polymeric microspheres, and solid lipid nanoparticles, nanostructured lipid carriers) for topical treatment are advantageous compared to conventional available topical delivery system. The encapsulation of antiacne drugs in vesicular and particulate delivery systems represents an innovative and alternative approach for minimizing the side effects and preserving their efficacy.

Hence the aim of the present investigation was to design, develop & characterize the nanostructured based gel for the treatment of acne.

The objective of the present investigation was……..

- ✓ Improve solubility of the drug.

- ✓ Improve the irritation problem occurring due to conversion of benzoyl peroxide to benzoic acid
- ✓ Improve skin permeability of the drug.
- ✓ To reduce systemic toxicity of drug.
- ✓ To deliver the drug to specific site.
- ✓ Further stabilize the NLC, increase viscosity & retention capacity by incorporating into gel.

2. INTRODUCTION

2.1 INTRODUCTION TO SKIN[1]

Skin is one of the most accessible organ in human body for topical administration and is main route of topical drug delivery system. It performs many important functions, that includes protection against external physical, chemical, and biologic factors, as well as prevention of excess water loss from the body and in thermoregulation etc. So an complete understanding of the anatomy and physiology of skin is important. The skin is made up of three layers: the epidermis, the dermis, and subcutaneous tissue. The outer most layer, the epidermis, consists of a specific constellation of cells known as keratinocytes, that synthesize keratin, a long, threadlike protein with a protective role. The middle layer, the dermis, is made up of the fibrillar structural protein known as collagen. The dermis is located on the subcutaneous tissue, which contains small lobes of fat cells known as lipocytes.

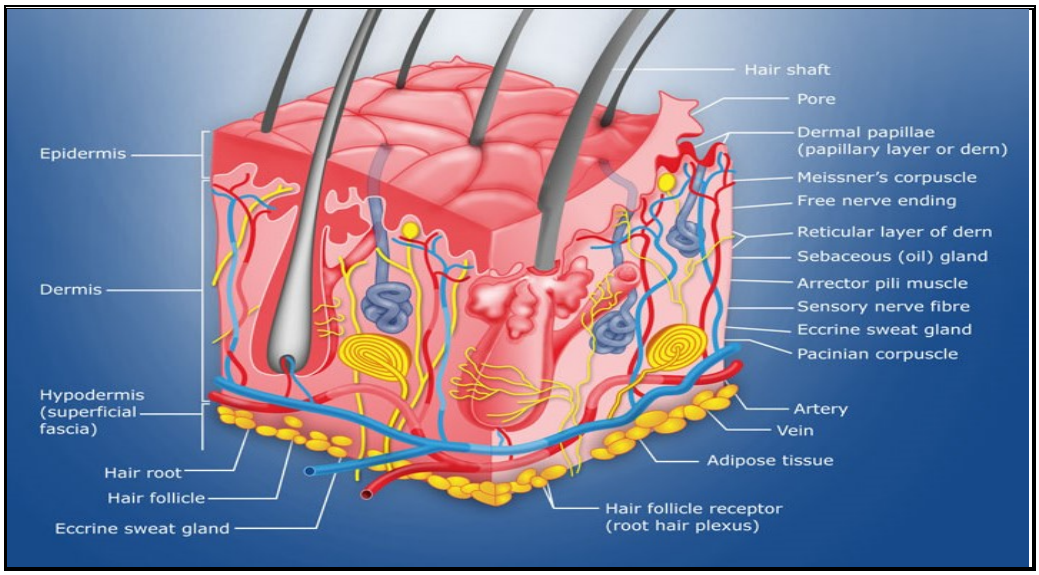

Figure 2.1- Structure of skin

Anatomy of the skin :[2]

The epidermis is the outer layer, which serves as the physical and chemical barrier between the interior body and exterior environment; the dermis is the deeper layer that provides the structural support of the skin, below which is a loose connective tissue layer, the subcutis or hypodermis which is an important depot of fat (table no - 2.2).

Sr No:	Skin layer	Description
1	Epidermis	The external layer mainly composed of layers of keratinocytes but also containing melanocytes, Langerhans cells and Merkel cells.
2	Basement membrane	The multilayered structure forming the dermoepidermal junction.
3	Dermis	The area of supportive connective tissue between the epidermis and the underlying subcutis: contains sweat glands, hair roots, nervous cells and blood and lymph vessels.
4	Subcutis	The layer of loose connective tissue and fat beneath the dermis.

Table 2.1- Layers of the skin[1]

The Epidermis[3]

- Constituted by keratinizing, stratified squamous epithelium. Keratinocytes form 95% of the epidermal cells; the other 5% form the melanocytes, Langerhans cells and Merkel cells .

Epidermis function:

- It acts as a barrier to harmful exogenous substances, chemicals and pathogens. Also it is fundamental as a retainer of tissue proteins along with fluids.

The epidermis can be subdivided into 4 layers of keratinocytes:

- Stratum basale

- Stratum spinosum
- Stratum granulosum
- Stratum corneum

The layers of the epidermis[4]

- **Stratum basale** – It is continuous layer which is 1-3 layers of cuboidal cells that have large nuclei and dense cytoplasm
- **Stratum spinosum** – In this layer, the cells gets augment and procure various desmosomal association plaques that will balance out the system of cells. Prickle cells are rich in tonofilaments, which are constituted by transitional fibers of keratin that connect themselves to the desmosomes. Maladies that influence the desmosomes can prompt breaks in the fabric of the keratinocytes with arrangement of vesicles.
- **Stratum granulosu*m*** – here, the cells turn out to be level and gather thick, basophilic granules in the cytoplasm as keratohyalin granules.These granules contain antecedent of filaggrin, a protein which when enacted is supposed on the grounds that it advances the total of fibers of keratin. By the impact of filaggrin, the keratin fibers adjust into the disulphide cross-connected macrofibres.
- **Stratum corneum** – the outermost layer is the major layer responsible for the barrier function of skin; epidermis is actually devoted to its production. It is composed of 15-20 layers of flattened, non-nucleated keratinized cells; filled with filaments of keratin. The surface cells of the stratum corneum are continuously desquamated .

Glands [5]

- **Sweat glands**

There are 3 types of sweat glands:

- Eccrine
- Apocrine
- Apoeccrine

- *Eccrine glands*

These are found over the entire skin, however are increasingly various on the soles, temple and axillae. Their secretory segment is a convoluted tubule situated at the intersection between the dermis and the subcutaneous fat . There are 3 sorts of cells in this tube: dim cells, clear cells and myoepithelial cells .The discharge of sweat is expert through straight conduit that crosses the dermis, and is proceeded by an intra-epidermal part called the acrosyringium which opens onto the surface of the skin.

Functions:

- Thermoregulation – through dissipation of shallow dampness; eccrine sweat is boring and unscented. The forerunner of sweat is created in the loop as an isotonic arrangement yet experiences reabsorption of sodium chloride in the conduit, delivering a hypotonic sweat. Cholinergic nerves of SNS release acetylcholine, which controls sweat generation. Warm and passionate components are additionally included. Ingested medications can likewise be conveyed to the skin.

Apocrine glands

- These are increasingly various in a few areas, for example, the anogenital skin, axillae and around the umbilicus. Their secretory coild is up to 10x larger than the eccrine organ. The secretory organs are situated in the profound dermis or subcutaneous fat and are lined by vast cells. They shed a portion of their apical cytoplasm into the lumen . They are likewise encompassed by myoepithelial cells .The apocrine pipe does not open onto the surface of the skin; rather it closes in the hair follicle over the sebaceous conduit .

- **Apoeccrine glands**

- These are present in the human axillae . They have a secretory portion indistinguishable from that of apocrine glands, but their duct opens on the surface of the skin. They develop from eccrine glands during puberty, and account for approximately ½ of all axillary sweat glands

Sebaceous glands

- These are holocrine glands where the secretions are produced by the disintegration of cells . They are found on the whole body surface except the palms and soles. Sebaceous glands are lobulated and contain small, germinative basophilic cells at the periphery

Development of the dermis:[6]

The dermis emerges from the mesoderm, which is carried into contact with the inward surface. The mesoderm gives a dermis, as well as key for prompting separation of epidermal structures e.g. the hair follicle. By 12 weeks, the interface is undulated (known as the rete-edge design) and fibrillar parts are clear. By 24 weeks, dermal papillae will be created .

Structure

The dermis is divided into:

- The superficial thin papillary dermis ADVENTITIAL DERMIS– this interdigitates with the ridged underside of the epidermis. Histologically appear pale; consists of abundant ground substance with a highly developed microcirculation but thin irregular collagen fibres, delicate elastic fibres + numerous fibroblasts"
- The larger underlying reticular dermis RETICULAR DERMIS – this blends with the subcutaneous fat

Skin functions[7]

The skin is a complex metabolically active organ, which performs important physiological functions that are summarised in Table

Barrier function and skin desquamation

As the reasonable cells move towards the stratum corneum they start to bunch proteins into granules in the granular layer. The granules are loaded with the protein allagrin, which gets to be complexed with keratin to keep the breakdown of allagrin by proteolytic catalysts. As the declining cells move towards the external layer,

proteins separate the keratin-allagrin complex. Fillagrin frames on the outside of the corneocytes while the water holding keratin stays inside. At the point when the dampness substance of the skin decreases, allagrin is further separated into free amino.

Lipids[8]

The main issue in the upkeep of a wet, adaptable skin limit is the closeness of intercellular lipids. These structure stacked bilayers that envelop the corneocytes and join water into the stratum corneum. The lipids are gotten from lamellar granules, which are released into extracellular spaces of undermining cells in the granular layer; the layers of these cells also release lipids. Lipids join cholesterol, free unsaturated fats and sphingolipids. Ceramide, a kind of sphingolipid, is generally responsible for making the stacked lipid structres that trap water particles in their hydrophilic zone.. After the age of 40 there is a sharp decline in skin lipids thusly extending our lack of protection to dry skin conditions. Shedding (desquamation) of skin cells Shedding the cells of the stratum corneum is a fundamental variable in keeping up skin uprightness and smoothness. Desquamation incorporates the enzymatic method of dissolving the protein interfaces, the desmosomes, between the corneocytes, and the unavoidable shedding of these cells .

UV protection

Melanocytes, situated in the basal layer, and melanin have imperative parts in the skin's hindrance capacity by anticipating harm by UV radiation. In the internal layers of the epidermis, melanin granules frame a defensive shield over the cores of the keratinocytes; in the external layers, they are all the more equitably disseminated. UV radiation prompts keratinocyte multiplication, prompting thickening of the epidermis.

Thermoregulation:

The skin assumes an essential part in keeping up a consistent body temperature through changes in blood stream in the cutaneous vascular framework and dissipation of sweat from the surface.

2.2 INTRODUCTION TO ACNE[1]

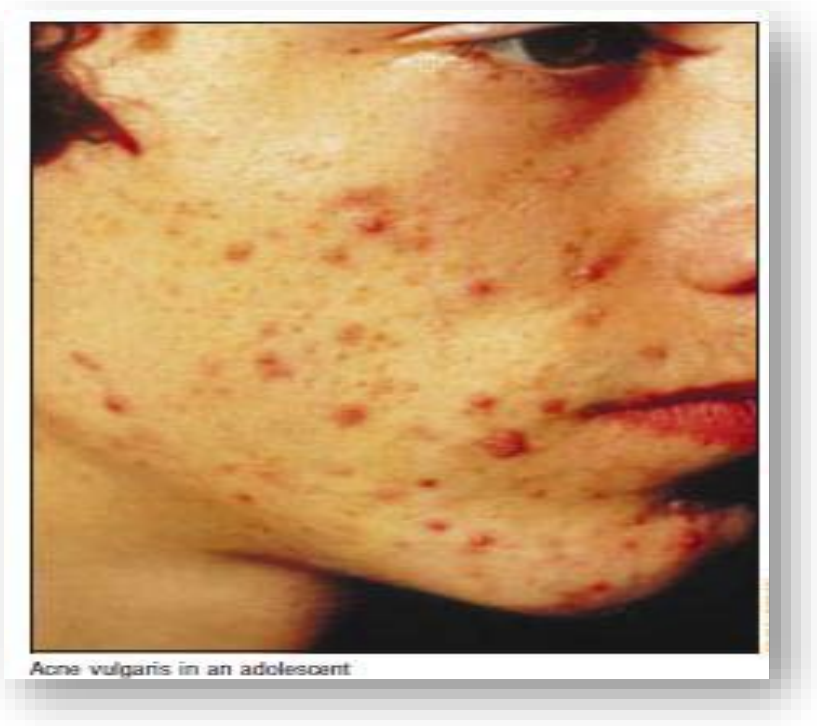

Figure 2.2- Acne Vulgaris in adolescent

2.2.1 INTRODUCTION[9]

Skin inflammation is the term for stopped pores, acne, and significantly more profound protuberances that happen on the face, neck, mid-section, back, shoulders and even the upper arms. Skin break out vulgaris (regularly called skin break out) is a skin condition created by changes in the pilosebaceous units. Skin break out is most normal amid puberty, influencing more than 85% of youngsters, and often proceeds into adulthood. For a great many people, skin break out reduces after some time and has a tendency to vanish. Most of the skin break out sufferers show mellow to direct skin inflammation at first, which advances to the serious structure in certain cases. Regular skin break out sores are comedones, provocative papules, pustules and knobs. A portion of the huge knobs were beforehand called "pimples" and the term nodulocystic has been utilized to depict serious instances of incendiary skin break out.

DEFINITION

A precise definition of acne vulgaris is difficult to frame it can be defined as a chronic, self limiting, inflammatory disease of pilosebaceous unit, manifesting

generally in adolescence with pleomorphic lesions like comedones, papules, nodules and cysts. Extensive scarring can occur.

2.2.2 PATHOPHYSIOLOGY OF ACNE[10]

The pathogenesis of acne is multifactorial. Acne vulgaris can be divided into noninflammatory (open and closed comedones) and inflammatory (papules, pustules and nodules) lesions. The most important factors involved are:

a. Increased sebum production

b. *Propionibacterium acnes* proliferation

c. Altered follicular keratinisation

d. Inflammation

a. Increased Sebum Production

i. Androgen Mediated Sebum Production

Sebum generation is increased,11 - 12, level III either by overstimulation of the organ by abnormal states androgens or by extreme touchiness of typical levels androgens.Androgens, for example, testosterone, dehydroepiandrosterone sulfate (DHEAS) and dihydrotestosterone (DHT), are known not qualities in charge of sebaceous organ development and sebum creation. There is a plausibility of expanded androgen creation inside the pilosebaceous follicle. Testosterone is likewise changed over to the more intense androgen i.e. DHT by the catalyst 5α-reductase. It is not known whether androgens act alone or in combination with development components, for example, fibroblast development element, epidermal development variable or insulin like development element.

ii. Peroxisome Proliferator-Activated Receptors (PPARs)

Sebaceous lipids are at any rate somewhat directed by PPARs and sterol reaction component restricting proteins. PPARs act working together with retinoid X receptors to control epidermal development and separation, and lipid digestion system. Lipid creation is expanded in sebocytes treated with agonists of the PPARs, which are the translation variables required in managing lipogenic qualities**.**

b. *Propionibacterium (P) acnes* Proliferation

Propionibacterium acnes is the essential animal and a run of the mill anaerobic occupant of pilosebaceous unit that colonizes skin break out slanted zones of the skin The extension of these minute living beings is responsible for the begin of irritation. P. acnes releases various chemicals, for instance, proteinases, lipases and hyaluronidases which isolate sebum to free unsaturated fats and peptides. It in like manner releases chemotactic parts which are fundamental to the flammable course. These variables add to the combustible method for skin break out by influencing monocytes to release proinflammatory cytokines,.

d. Inflammation

Cellular products from *P. acnes* stimulate the recruitment of CD4 lymphocytes and subsequently neutrophils. These inflammatory cells penetrate the follicular wall, causing disruption of the follicular barrier. This leads to the release of lipids, shed keratinocytes and *P. acnes* into the surrounding dermis, inciting further recruitment of inflammatory cytokines and neuropeptides including substance P.

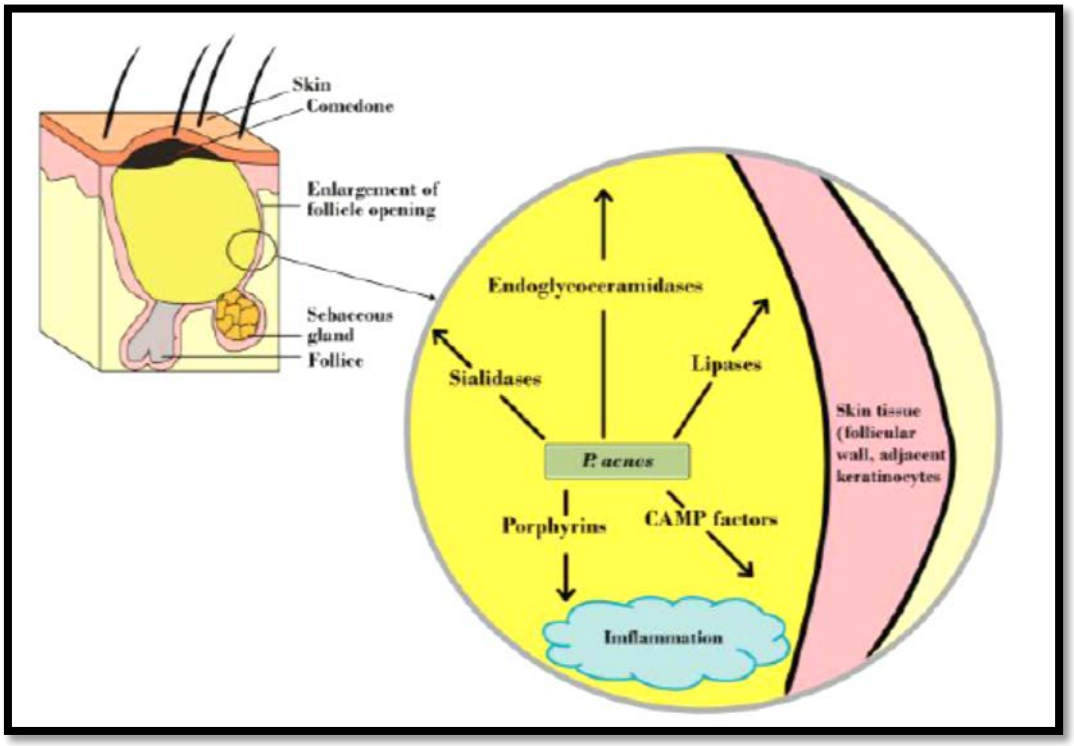

Figure 2.3- Role of P.acne in pathogenesis of acne[11]

2.2.3 DIAGNOSIS AND TREATMENT OF ACNE[3]

2.2.3.1 DIAGNOSIS:

Acne mostly affects the face, especially the T-zone, and trunk. Non inflammatory lesions include open, “black” comedones and closed, “white” comedones. Inflammatory lesions include papules, pustules and nodules are shown in (Figure 2.4).

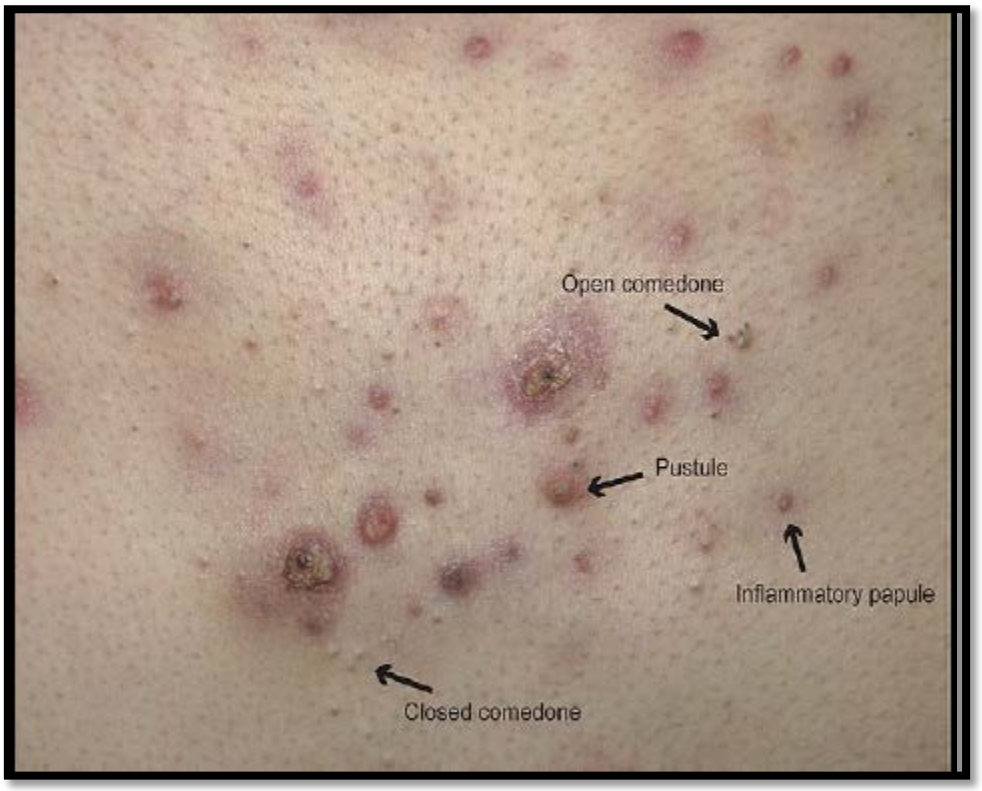

Figure-2.4-Comedones, papules and pustules in mixed comedonal and inflammatory

Secondary bacterial infection may rarely increase inflammation. There are five main types of scars. Ice-pick scars are narrow, tapering deeply into the dermis. Rolling

scars are wide and shallow. Boxcar scars are well demarcated, punched out depressions (Figure 2.5).

Signs of possible hyperandrogenism include, (1) late onset, severe acne, (2) marked seborrhoea, (3) acanthosis nigricans, (4) dysmenorrhoea andinfertility, (5) dyslipidaemia and diabetes and (6) Cushingoid habitus (Cushing's syndrome).

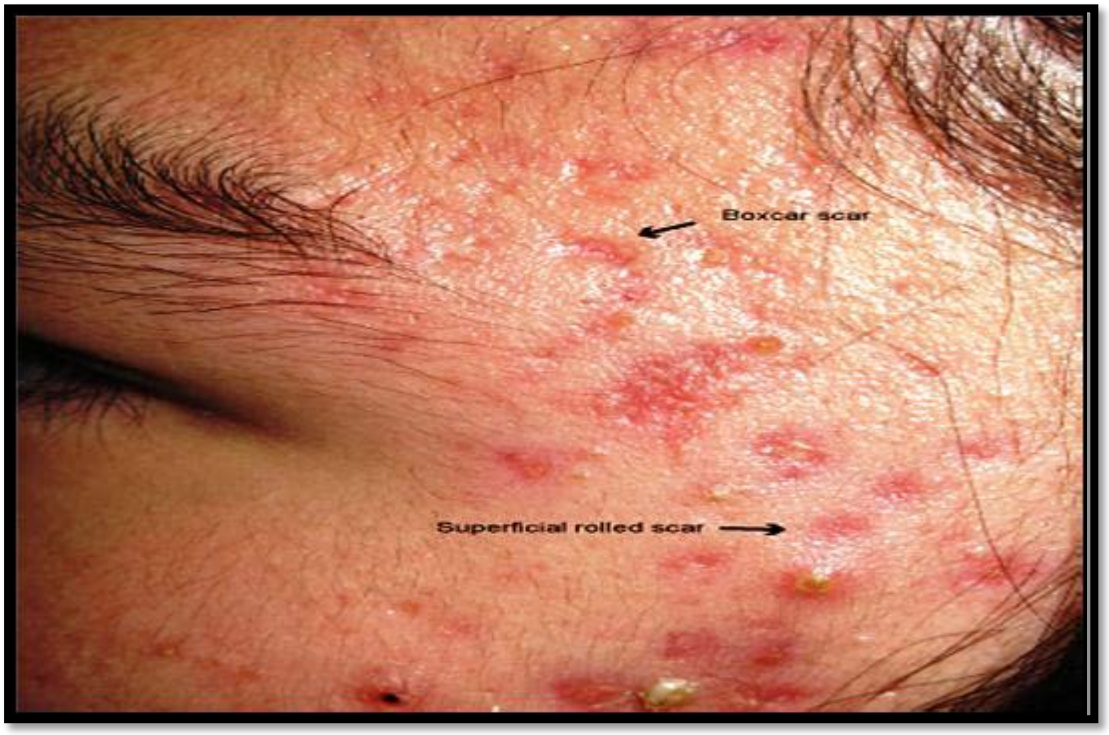

Figure 2.5- Common Acne Scars

- In 1990, the American Academy of Dermatology developed a classification scheme for primary acne vulgaris. This grading scale delineates three levels of acne:

✓ Mild: characterized by the presence of few to several papules and pustules, but no nodules.Moderate: Patients with moderate acne have several to many papules and pustules along with a few to several nodules. Severe: patients have numerous or extensive papules and pustules, as well as many nodules.

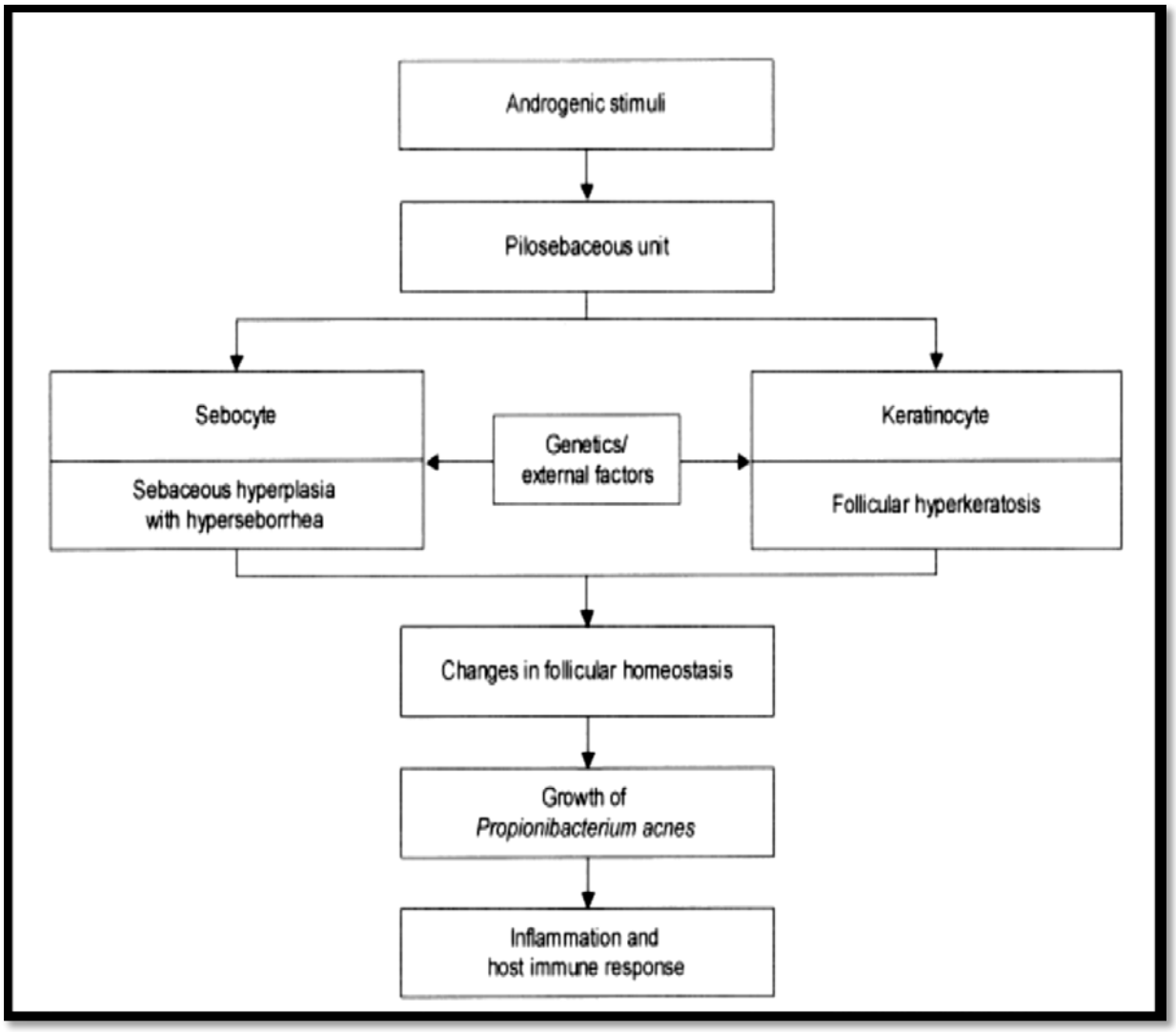

Figure 2.6- Occurrence of Acne[12]

2.2.3.2 Treatment[13]

Successful management of acne needs careful selection of anti-acne agents according to clinical presentation and individual patient needs.

a) Topical Therapy:

Topical therapy is useful in mild and moderate acne, as monotherapy, in combination and also as maintenance therapy.

i. Benzyol Peroxide:

- Benzoyl peroxide is a broad spectrum bactericidal agent which is effective due to its oxidizing activity. The drug has an anti-inflammatory, keratolytic, and comedolytic activities, and is used in mild-to moderate acne. The main

limitation of benzoyl peroxide is concentration dependent that causes irritation or dryness.

ii. Topical Retinoids:

Tretinoin, adapalene, tazarotene, isotretinoin, metretinide, retinaldehyde, and β-retinoyl glucuronide are available topical retinoids.

- Topical retinoids focus on the microcomedo–precursor sore of skin break out. Now a days they are utilized as the primary line treatment, alone or in combination, for mild to-moderate acne causing skin inflammation.
- **iii. Topical Antibiotics:**

Topical antibiotics, for example, erythromycin and clindamycin are the most prominent in the administration through skin. They restrain the development of P. skin break out and lessen irritation. Clindamycin and erythromycin were both powerful against provocative skin break out in topical structure in mixture of 1–4% with or without the use of zinc.

- ✓ Side effects includes erythema, peeling, tingling, dryness,, pseudo membranous colitis which is uncommon, yet has been accounted for use with clindamycin.

iv. Newer Topical agents:

- ➢ **Combination therapy:**
- ➢ Benzoyl peroxide has the advantage to eliminate the growth of *P. acne* resistance. Therefore it is more preferred as combination therapy. Its efficacy and tolerability are enhanced when combined with topical erythromycin or clindamycin, after various study through clinical trials..
- ➢ Benzoyl peroxide can be combined with tretinoin and found superior to monotherapy. Both the combination should not be applied simultaneously as benzoyl peroxide may oxidize tretinoin.
- ➢ A combination of topical retinoid and topical antimicrobial is more effective in reducing both inflammatory and non inflammatory acne lesions.

- ✓ **Salicylic acid:** It has been used for many years in acne as a comedolytic agent,

but is less potent than topical retinoid.

- ***Azelaic acid:*** It is available as 10–20% topical cream which has been shown to be effective in inflammatory and comedonal acne.

- **Dapsone gel 5%:** It is a sulfone with anti-inflammatory and antimicrobial properties. The trials have confirmed that topical dapsone gel 5% is effective and safe as monotherapy and in combination with other topical agents in mild-to moderate acne .

Hormonal Therapy:

- **Oral Contraceptives:**
- Estrogen is commonly combined with progestin to avoid the risk of endometrial cancer. Anti-acne effect of oral contraceptive governed by decreasing level of circulatory androgens through inhibition of luteinizing hormones (LH) and follicle stimulating hormone (FSH).
- **Spironolactone:**
- They works fundamentally as a steroidal androgen receptor blocker. It might bring about hyperkalemia (when higher measurements are recommended or when there is cardiovascular or renal trade off), menstrual abnormalities
- **Flutamide:**

✓ It is useful in acne when given in females with hirsuitism.

- **Oral isotretinoin:**
- It is the principal androgen receptor blocking specialist to be all around examined and found to powerful in skin break out in females. It is likewise consolidated (2 mg) with ethinyl estradiol (35 or 50 μg) as an oral preventative definition to treat skin inflammation.

c) Physical Treatment:

i. Photo therapy:

- **Visible Light:**

✓ They are demonstrated for mellow to-moderate provocative skin break out. In vitro and in vivo presentation of skin inflammation microorganisms to 405–

420 nm of bright free blue light results in the photograph devastation through the impact on the porphyrin delivered actually by P. acne. Use of restricted range wavelength, for example, blue light (top at 415 nm), and blended blue and red light (crest at 415 and 660 nm) have been observed to be viable in lessening skin inflammation sores following 4–12 weeks..

- **Photodynamic therapy:**

➢ This includes pulsed dye laser (585 nm), also effective in acne.

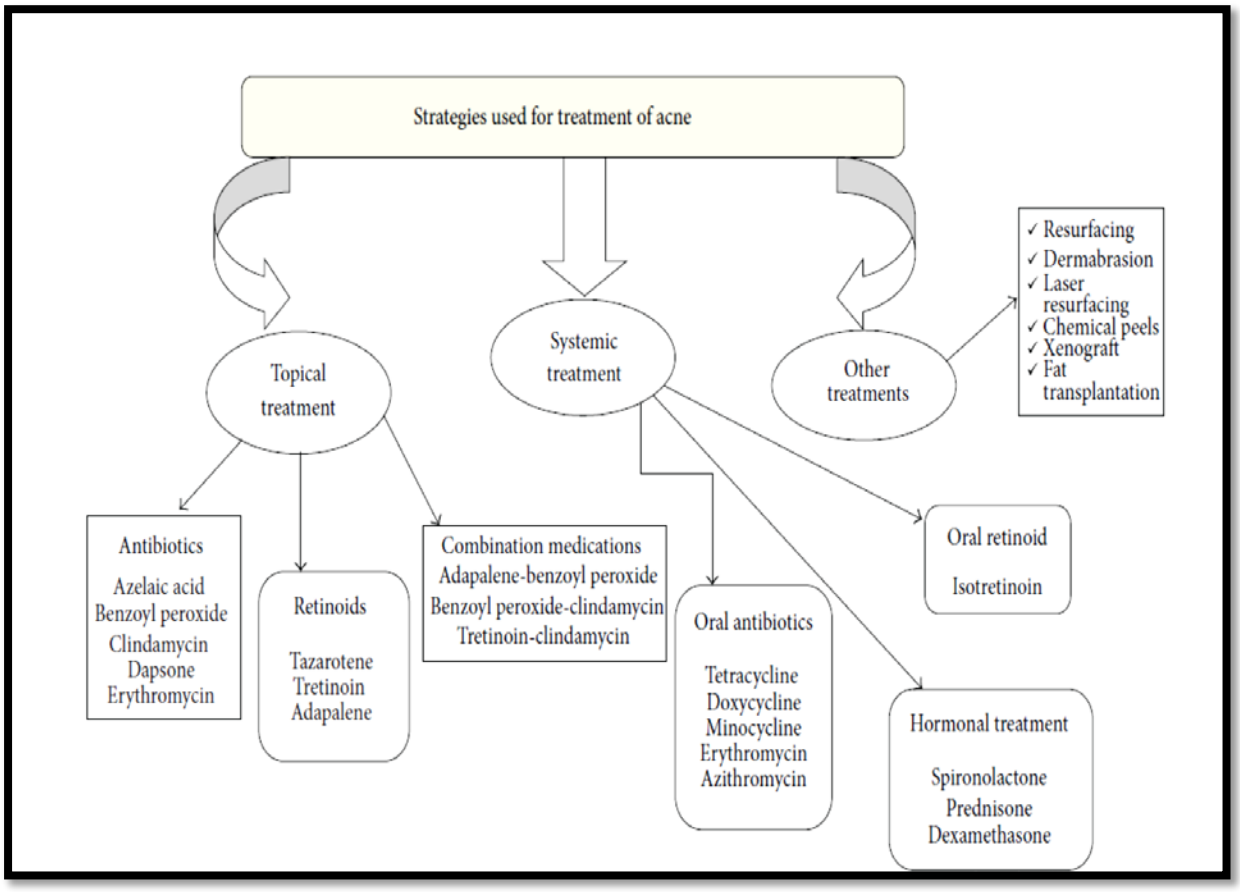

Figure 2.7- Treatment strategies used for acne[14]

2.3 TOPICAL DOSAGE FORM[15]

Topical measurements structures are those which are connected to the skin. These planning are connected to the skin either for their physical impacts, that is for their capacity to go about as skin protectants, oils, emollients, drying specialists, and so forth or for their particular impact ofmedicinal operators present. Arrangements sold over the nation much of the time contain blends of therapeutic substance utilized as a part of the treatment of such condition as minor skin disease, tingling, wound, skin inflammation, psoriasis and dermatitis. Skin application, which require a remedy for the most part contain a solitary restorative specialist expected to counter a particular analyzed condition. Topical measurement shapes have been utilized subsequent to exceptionally old times. The use of therapeutic substance to skin or to different body openings is an idea as old as mankind.

- Various treatments, creams, gels, salves, glues, powders and mortars have been utilized for a long time. The essential topical medication conveyance framework (TDDS) is that they could give controlled steady organization of a medicament by basic application to the skin surface. The topical conveyance has been endeavored and made fruitful utilizing various lipid based frameworks viz., vesicular frameworks, lipid, microsphere, lipid nanoparticles, lipid emulsion and polymeric gels.

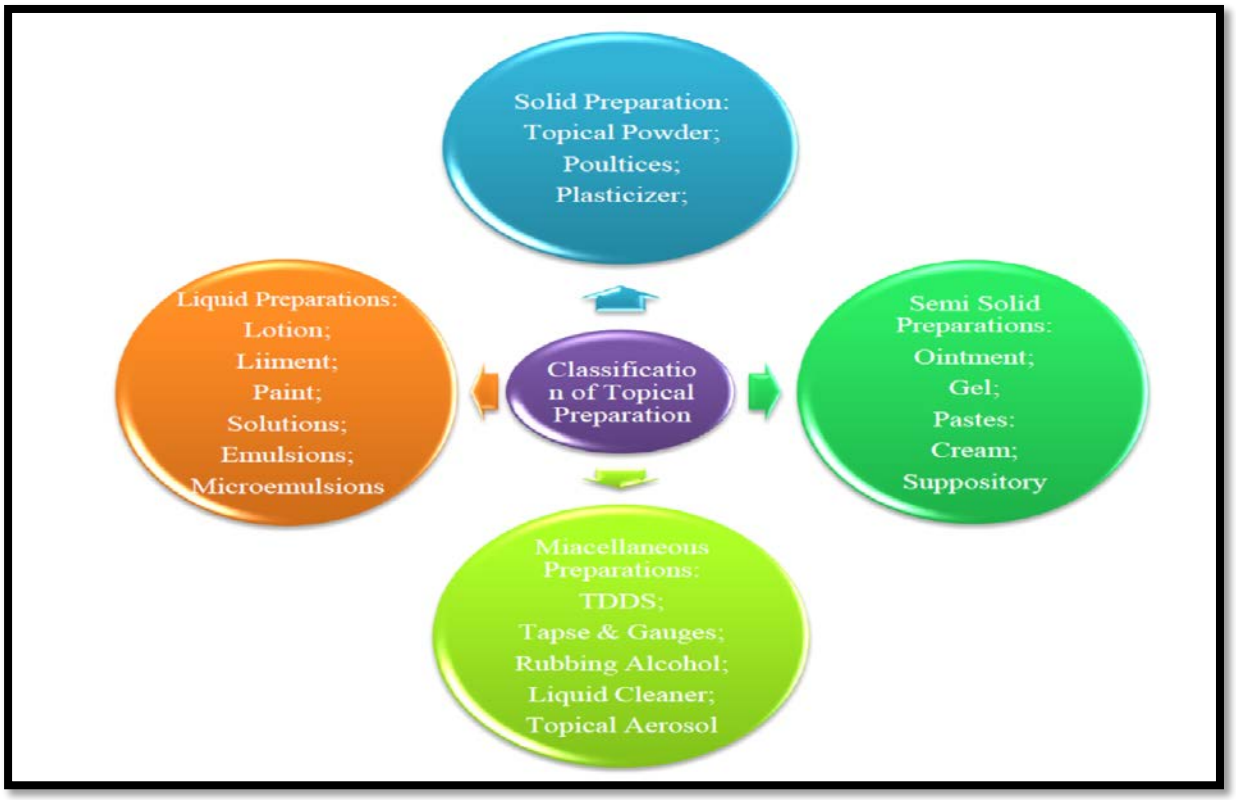

Figure 2.8- Various approaches to Topical Dosage forms[15]

2.3.1 Advantages of topical systems:[16]

They are of slightest restorative intrigue however of pragmatic importance is great patient consistence. The frameworks are anything but difficult to apply and evacuate. It maintains a strategic distance from dangers and disservices connected with intravenous treatment.

- They wipe out the variables, which impacts gastrointestinal retention, for example, nourishment consumption, stomach exhausting, intestinal motility and travel time.
- Produces managed and controlled level of medication in plasma hence lessens the possibility of over or under-dosing.
- Reduces recurrence of medication dosing.

- Topical frameworks are effortlessly retractable along these lines end of medication inaproxenut, if harmful impacts are watched.
- Offers an option course when oral treatment is impractical as if there should arise an occurrence of queasiness and regurgitating.
- Helps in accomplishment of more steady blood levels with lower measurements of medication by constant medication in aproxenut and by-passing hepatic first-pass digestion system and subsequent debasement.
- In certain circumstances, enzymatic change inside epidermis might be utilized to enhance porousness of certain hydrophilic medications when connected to the skin as prodrug.

2.3.2 Limitations of topical systems:

✓ Drugs with low partition coefficient and possessing solubility both in oil and water are most ideal, as drug must diffuse through lipophilic stratum corneum via epidermis to reach the systemic circulation. Only drugs, which are effectively absorbed by the percutaneous routes as such or by using penetration enhancers, can be used.

✓ The route is not suitable for drugs that irritate the skin.

✓ The route is restricted by the surface area of delivery system and the dose that needs to be administered in the chronic state of disease.

2.4 TOPICAL GELS:[5]

- Topical gels are straightforward or translucent semisolid plans containing a high proportion of dissolvable/gelling specialist. Gels are characterized as semirigid framework in which the development of the scattering medium is limited by intertwining threedimensional systems of particles or solvated macromolecules of the scattered stage. A high level of physical or concoction cross-connecting might be included. The U.S.P. characterizes gels as a semisolid framework comprising of scattering made up of either little inorganic molecule or extensive natural atom encasing and interpenetrated by fluid. The inorganic particles shape a three dimensional 'place of cards'

structure. Gel comprise two stage framework in which natural particles are not broke down but rather just scattered

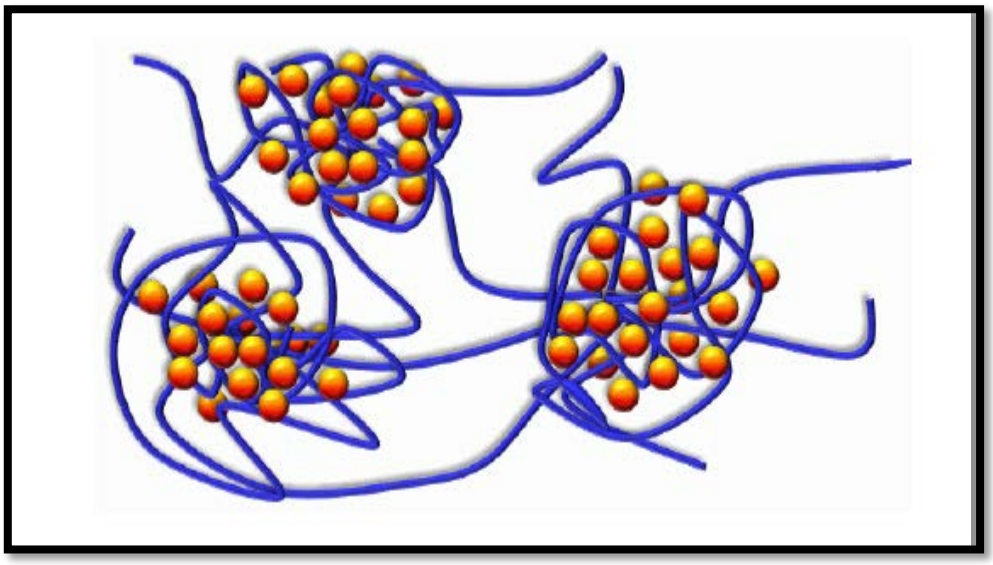

Figure 2.9- Structure of Gel

throughout the continuous phase and large organic particles are dissolved into continuous phase, randomly coiled in the flexible chains. Gels have viscoelastic property. The solid like matrix structure formed during storage breaks easily on a shaking a bottle or squeezing a tube. Thinning under pressure allows it easily applicable on skin and it's solid like matrix makes it adhere onto the skin when application is over.

- Topical drug organization is limited medication conveyance framework anyplace in the body through rectal, ophthalmic, vaginal and skin as topical course. Most topical gels are set up with natural polymers, for example, carbomers, that grant a tastefully satisfying, clear, shimmering appearance to the items and are effectively washed off from the skin with water. Utilization of kind of bases in figuring a topical dermatological item incredibly impacts its viability. Bases containing a lot of oleaginous substances which give an emollient impact to dry aggravated skin. An occlusive hindrance on the skin can frame by non-unpredictable oleaginous substances (e.g. hydrocarbon bases) that keep break of dampness from the skin into the earth.

Advantages of topical gel [17]

- They can avoid gastrointestinal drug absorption difficulties caused by gastrointestinal pH and enzymatic activity and drug interaction with food and drinks. To avoid the first pass effect following gastrointestinal absorption, avoiding the deactivation by digestive and liver enzymes should be done. They are less greasy in nature and can be easily removed from the skin. It is cost effective. Reduction of dose as compare to the oral dosage form. Localized effect with the minimum toxic effects.

Disadvantages-

- It is having Poor permeability of some drugs through the skin
- Possibility of allergenic reactions can be used only for drugs which require very small plasma concentration for delivery of drugs at specific site.
- Enzyme in epidermis may denature the drugs
- Larger particle size drugs may not easily to absorb through the skin.

Classification of gel[18]

Gels can be classified based on the basis of colloidal phases, nature of solvent used, physical nature and rheological properties.

A. Based on colloidal system

- **Two phase system(Inorganic)-** If the particle size of dispersed phase is relatively large and form the three dimensional structure throughout gel such as a system consist of floccules of small particle rather than layer molecule and gel structure in this system is not always stable. E.g.Aluminum Hydroxide Gel USP
- **Single phase system (Organic) -** These consist of large organic molecule existing on the twisted stands dissolved in continuous phase.

B. Based on nature of solvent used

- Hydrogel –Here they contain water as their continuous liquid phase E.g. Gelatin, cellulose derivatives and poloxamer gel.
- Organic gel (with a non-aqueous solvent) - These contain a non –aqueous solvent as continuous phase.

- Xerogels - Xerogels are solid gel with low solvent concentration and produced by evaporation of solvent or freeze drying.E.g. Dry cellulose and polystyrene.

3. Based on rheological properties

Usually gels exhibit non-Newtonian flow properties. They are classified into,

- Plastic gels
- Pseudo plastic gels
- Thixotropic gels

4. Based on physical nature

- Rigid gels
- Elastic gels

Ideal properties of topical gel

- Should be inert, compatible with other additives
- Should be non-toxic
- Should be stable at storage condition
- Should be free from microbial contamination
- Should be economical
- Should be washable with water and free from staining nature
- Should be convenient in handling and its application

Basic components of Topical gel

A) Drug substances-.

Mainly NSAID'S agent, antifungal agent, antibacterial agent etc. used. Judicious choice of the drug plays an important role in the successful development of a topical product. The important drug properties that effect its diffusion through the device as well as through skin are as follows ,

a. Physicochemical properties:

- Less than 500 Daltons molecular weight of drug should be required. Adequate lipophilicity of drug must be required. pH of aqueous solution (saturated) of drug should be required value between 5 and 9.Drugs which

are highly acidic or alkaline in solution are not suitable candidates for topical delivery.

b. Biological properties:

The medication ought not be specifically aggravated to the skin. Drugs,which debase in gastrointestinal tract or are inactivated by hepatic first pass, are appropriate for topical delivery. Resilience to thedrug must not create under the close to zero request release profile of topical delivery. Drugs must be controlled fora whil e or which make unfriendly impacts non-focused on tissue can likewise be figured for topical delivery..

B) Polymers-

Mechanism of drug release depends upon the physicochemical properties of drug and polymer used. Polymers are used to give the structural network, which is essential for preparation of gel. Gel forming polymers are as follow:

Natural Polymers	Semisynthetic Polymers	Semisynthetic Polymers
Gelatin	Carboxy methyl cellulose	Carbopol-940
Tragacanth	HPMC	Poloxamer
Guar gum	Hydroxy ethyl cellulose	Poly vinyl alcohol
Xanthin	Hydroxymethyl cellulose	Polyethylene
Agar	Methyl cellulose	Polyacrylamide

Table 2.2- Examples of Polymers[3]

C)Penetration enhancers

It Promote skin permeability by altering the skin as a barrier to the flux of desired penetrant and are considered as integral part of most topical formulation. Ideally, penetration enhancers should reduce the barrier resistance of the stratum corneum without damaging viable cells. Examples Water, Essential oils, urea and its derivatives.

D)Preservatives- Used to resist microbial attack. Examples- Methyl paraben, Propyl paraben

E)Surfactants- Reduces interfacial tension. Example- sodium lauryl sulphate, sodium glycolate

F)Chelating agent- Bases and medicaments in gels are sensitive to heavy metals, hence added to protect. Example-E.D.T.A., methylated cyclodextrin

Methods of preparation of gel[19]

- **COLD METHOD-**
  - In this method the entire ingredient mixed together to form a homogenous mass, under low temperature at about 50C. In this polymer and penetration enhancer are mixed together to form a solution A, then drug and solvent mixed to form solution B. After that with constant stirring poured solution B into solution A .
- **DISPERSION METHOD-**
  - In this method polymer is dispersed over water for 2 hrs till all the polymer is soaked with water, then addition of remaining ingredients is done with stirring until a homogenous mass is formed.
- **CHEMICAL REACTION-**
  - In this method gel formed by precipretation. Silica gel and aluminium hydroxide gel are the examples. Silica gel is produced by interaction of sodium silicate and acids in aqueous solution.
- **TEMPERATURE EFFECT-**
  - With decreased in temperature, solubility of most lipophilic colloid e.g. gelatin, agar is reduced. So that when cool concentrated hot sol gel are produced.
- **FLOCCULATION–**

- In this method gelatin is produced by addition of sufficient quantity of salt to precipitate out for complete precipretation.

NOVEL APPROACHES FOR GEL FORMULATION:

A) EMULSION
B) Microemulsion
C) Solid Lipid Nanoparticles
D) Nanostructured lipid carriers
E) Liposomes
F) Emulgel
G) Solid dispersion
H) Microsphere
I) Niosomes

2.5 INTRODUCTION TO NANO-STRUCTURED LIPID CARRIERS (NLC):[20]

2.5.1 INTRODUCTION

- Lipid Based Drug Delivery (LBDD) is relatively recent and relates to the developments in the past 10 to 15 years, largely driven by the growing need for novel drug delivery systems to deal with the vast majority of the new chemical entities (NCE) that have poor solubility or permeability, to improve the delivery of existing drugs, and for line extensions.
- Lipidic colloidal formulations (e.g. emulsions and liposomes) already play an important role in pharmaceutical applications. Major concerns of these systems are problems like coalescence and early drug release due to diffusion. These drawbacks can partly be overcome by solid nanoparticles. Extensive research work has been carried out on polymeric nanoparticles containing e.g. polylactic acid/ polyglycolic acid, polycyanoacrylates or proteins.

- These investigations led to the development of first microparticulate products which have already entered the market: Decapeptyl (triptorelin acetate) and Sandostatin (octreotide acetate). Both formulations are based on polylactic acid and polyglycolic acid.
- A major problem with these polymeric nanoparticle dispersions derives from the cytotoxic potency of the formulation. A possible origin for toxicity can be the polymer itself, since nanoparticles can be phagocytized by macrophages due to their small size. Additionally, high quality standards and cost efficient on a large scale manufacturing are rare.
- The use of solid lipids as a matrix material for drug delivery is well-known from lipid pellets for oral drug delivery (e.g. Mucosolvanw retard capsules). Basically, lipids can be used which are well tolerated by the body. Large scale production can be performed in a cost-effective and simple way. The SLN combine the advantages of other innovative carrier system e.g. physical stability, protection of incorporated labile drugs from degradation, controlled release, excellent tolerability. SLN formulation for various application route (parenteral, oral, dermal, ocular, pulmonary and rectal) have been developed and thoroughly characterised in vitro- in vivo .

However, there are also some potential limitations of SLN which might occur:

- Poor drug loading capacity
- Drug expulsion after polymeric transition during storage
- Relatively high water content of the dispersions (70-99.9%)
- The low capacity to load water soluble drugs due to partitioning effects during the production process.
- After preparation by hot homogenization technique, the particle crystallise in higher energy modification α and β. During storage, these modification can transform to the low energy, more ordered β modification. Due to its high degree of order, the number of imperfections in the crystal lattice is reduced thus leading to drug expulsion. (Fig: 2.12) The creation of less ordered solid

lipid matrix is pre requisite for high drug loading. In general, the drug can be located in between the lipid layers and also in imperfections (e. g. amorphous drug clusters. In contrast to SLN being produced from solid lipids, the NLC are produced by controlled mixing of solid lipids with spatially incompatible liquid lipids leading to special nanostructures with improved drug incorporation and release properties. NLC are the identical and second generation of the solid lipid nanoparticles (SLN) .

- The point of preference of the second era innovation is the expanded stacking with actives contrasted with SLN and firmer consideration of the dynamic inside the molecule lattice amid the time span of usability. By setting up the particles from a strong lipid, particularly exceedingly decontaminated strong lipids, the molecule grid tends to frame a moderately consummate precious stone cross section leaving restricted space to oblige the dynamic. This confines the stacking limit and can prompt ejection of dynamic from the lipid framework amid capacity. Conversely, the utilization of a lipid blend with diversely organized (estimated) particles twists the development of an impeccable precious stone. The molecule network contains numerous blemishes giving space to suit the dynamic in sub-atomic structure or as shapeless bunches .One could express that "the flawlessness" of the NLC framework is its "imperfectness" in its crystalline structure.

2.5.2 DRUD INCORPORATION MODELS OF NLC[21]

NLC with special structure for the better drug accommodation in order to increase the payload and prevent drug expulsion during storage.

The three types of NLC can be summarised:

1. The imperfect type (imperfect structured solid matrix):

Especially different lipid are mixed and imperfection in crystal order of lipid nanoparticle. Therefore, the matrix contain imperfection to accommodate the drug in amorphous cluster. Mixing small amount of chemically different liquid lipid (oil) with

the solid lipid in order to achieve the highest incompatibility leads the highest payload.

2. The amorphous type (structure less solid amorphous matrix):

This sort of NLC can be accomplished by mixing strong lipid with liquid lipid eg hydroxyoctacosanyl hydroxystearate, isopropylmyristate or medium chain triglyceride,for example, Miglyol 822. Taking into account this, molecule were created with a high substance of liquid lipids (oils). Among different procedure, the liquid lipid molecule (nanoemulsions) are cooled from the liquid state to room temperature to take shape and from strong molecule. At high oil fixation a miscibility hole of the two lipids (strong lipid and oil) happens among the cooling stage, prompting stage partition that implies precipitation of nanocompartments.

3. The multiple type:

when drug having low solubilty in lipids, addition of higher amount of liquid lipid to lipophilic phase display the advantage of the solid matrix which prevented drug leakage while the region (oily nanocompartment) show comparatively high solubility for lipophilic drug. In type III lipids are mixed in a way that prevent them from crystallization. The lipid matrix is solid, but in an amorphous state. The absence of crystallization avoid drug expulsion by crystallization

2.5.3 Advantages of NLC:[22]

The mostimportant application of NLCs is of a drug nano carrier. NLCs have been developed to deliver the drugs by different routes of administration such as , parenteral injection, topical delivery, oral administration, ocular delivery,
and pulmonary inhalation. Among them, the routes of injection and the skin are the most widely used pathways for NLCs.

1. **Parenteral injection**
2. **Brain targeting**
3. **Tumor targeting**
4. **Antihepatotoxic injection**

5. **Analgesia**
6. **Anti-inflammation**
7. **Topical delivery**
8. **Cosmetic application**
9. **Ocular delivery**
10. **Oral delivery**
11. **Pulmonary delivery**
12. **Gene delivery.**

These are some of the advantages of NLCs by which the drug having poor solubility or permeability or irritation problem and also having poor loading capacity can be resolved by use of NLC and also can be used to target different organs for delivery of the drug.

2.5.4 Components of NLC:[23]

NLC are a nano-particulate carrier system derived from O/W nanoemulsions. The major ingredients of NLC are lipid, surface active agent and water. To obtain NLCs, solid lipids are mixed with liquid lipids (oils), preferably in a ratio of 70:30 up to a ratio of 99.9:0.1. While, the overall solid content of NLC can be up to 95% (w/w).

2.5.4.1 Lipids[8]

Selection of an appropriate lipids blend is crucial for successful production of NLC with suitable physical and chemical characteristics. Usually mono, di- and tri-acylglycerols, fatty acids and waxes are used for preparation. The parameters which are to be undertaken are as follows.

1. The solubility of the active compound in lipid matrices is essential, because it influences onto the drug loading capacity, encapsulation efficiency etc.
2. The molecules of liquid and solid lipids should be spatially incompatible together as much as possible; this means the oil molecules should not effect onto the structure

of solid crystalline matrix and the crystals of solid lipid should not be dissolved in the liquid lipid.

3. The lipids should be biodegradable and is able to produce particles in the nanometric scale. O/W emulsion is produced when the oil phase has a low viscosity.

4. The lipids should have an least toxicological profile and should not lead to production of any toxic residues during NLC formulation.

2.5.4.2 Liquid lipids[8]

Medium chain triglycerides (MCT) and oleic acid are most used liquid lipids for preparing NLC. MCT have been approved as generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA), Oleic acid is a constituent of many natural edible lipids. Natural edible oils such as corn oil, soybean oil and sunflower oil can also be used as liquid lipid for NLC preparation. These liquid lipids may contain natural antioxidants leading to effective protection of drugs from oxidation; some oils have high viscosity and may lead to oxidative instability of NLC (e.g. for soybean oil)

2.5.4.3. Solid lipids:[8]

Glyceryl behenate, glyceryl palmitostearate, glyceryl monostearate/monostearin, cetyl palmitate and stearic acid are mainly used for preparing NLC. Cetyl palmitate, have been accepted as GRAS. Glyceryl behenate for the most part comprises of diacylglycerols of behenic together with variable amounts of mono-and tri-acylglycerol. Glyceryl palmitostearate is utilized for controlled release applications. Glyceryl monostearate is a mixture of mono-and di-acylglycerols. It is utilized as a non-ionic emulsifier, stabilizer, emollient, and plasticizer. Stearic acid is an endogenous in length chain with unsaturated fat and an essential segment of lipids in creature and plant sources which has preferred biocompatibility and lower poisonous quality over the integrated partners.

2.5.4.4 Emulsifiers:[8]

Emulsifiers are kind of surfactants, phospholipids, proteins, and polysaccharides. The most utilized surfactants as a part of preparing NLC are polyoxyethylene sorbitan monooleate (another names: Polysorbate 80, Tween 80), lecithin and Poloxamer 188. At the point when NLC balanced out with non-ionic surfactants, steric aversion is the major colloidal connection among NLC nanoparticles. Soy lecithin is the most generally used surfactant and zwitterionic surfactants, and they can have a net negative, impartial or positive charge. Poloxamer 188 (Lutrol F68; Pluronic F68) is a nonionic surfactantand is widely used. Poloxamers display least toxicities at high temperatures; Sodium dodecyl sulfates (SDS), Polyoxyethylene sorbitan monolaurate (Polysorbate 20/Tween 20), Sodium deoxycholate (SDC) and Polyglyceryl-3-methylglucose distearate have been also used as surfactant as a part of NLC Formulation. Tween 20 is a hydrophilic non-ionic surfactant that is also used widely.

2.5.5. Preparation method of NLC[24]

Preparation techniques of NLC are similar to that of SLN. Many different methods are used for production of lipid nanoparticles. Hot homogenization is the most commonly used method for preparation of both SLN and NLC. This method has many advantages (such as easy scale up, lack of organic solvents and short production time) compared to the other methods.

A. High Pressure Homogenization Technique

In high pressure homogenization technique lipids are passed under high pressure (100-200 bars) through a narrow gap of few micron ranges. So shear stress and cavitationsare the forces which cause the disruption of particle to submicron range. Normally the lipid contents are in the range of 5-10%. In contrast to other preparation technique high pressure homogenization does not show scaling up problem.

Basically, there are two steps for production by high pressure homogenization, hot and cold homogenization techniques. For both the techniques the drug is dissolved or dispersed or solubilized in the lipid being melted at approximately 5-10^{o}c above its melting point.

I. Hot Homogenization Technique

In hot homogenization method the drug and lipid undergoes high shear stress in the fluid surfactant arrangement of same temperature. The pre-emulsion acquired is homogenized by utilizing a cylinder hole homogenizer and the delivered hot o/w nanoemulsion is allowed to cool at room temperature. At room temperature the lipid recrystallizes and leads to development of nanoparticles.

II. Cold Homogenization Technique

Cold homogenization has been developed to overcome the following problems of the hot homogenization technique such as: Temperature mediated accelerated degradation of the drug payload, Partitioning and loss of drug into the aqueous phase during homogenization. In First step is same as cold and hot homogenization but they are differing from next steps. The melted emulsion containing drug is cooled quickly using ice or liquid nitrogen for distribution of drug in the lipid matrix. Cold homogenization minimizes the thermal exposure of the sample.

B. Microemulsion Technique

The lipids are dissolved; drug is fused in liquid lipid. Watery stage containing, surfactant and co-surfactant is warmed to the same temperature as the lipids and added under gentle mixing to the lipid melt. A thermodynamically stable framework is shaped when the mixes are in the right proportions for microemulsion development. This microemulsion is then scattered in a fluid medium under gentle mechanical blending in the proportion of hot microemulsion to water (1:25 - 1:50). This prompts fast recrystallization of the oil beads on scattering in cool watery medium. Along these lines Nanoparticles are shaped because of precipitation .

C. Solvent Emulsification-Evaporation Technique

In solvent emulsification-evaporation method, the lipophilic material and hydrophobic

drug were dissolved in a water immiscible organic solvent (e.g. cyclohexane, dichloromethane, toluene, chloroform) and then that is emulsified in an aqueous phase using high speed homogenizer19. To improve the efficiency of fine emulsification, the coarse emulsion was immediately passed through the micro fluidizer. Thereafter, the organic solvent was evaporated by mechanical stirring at room temperature and reduced pressure (e.g. rotary evaporator) leaving lipid precipitates nanoparticles.

D. Solvent Emulsification-Diffusion Technique

In dissolvable emulsification-dispersion method, dissolvable utilized (e.g. benzyl liquid, butyl lactate, ethyl acetic acid derivation, isopropyl acetic acid derivation) must be half miscible with water and this system can be completed either in aqueous stage or in oil. At first, both the dissolvable and water were commonly soaked keeping in mind the end goal to guarantee the underlying thermodynamic balance of both fluid. At the point when warming is required to solubilize the lipid, the immersion step was performed at that temperature. At that point the lipid and medication were broken up in water soaked dissolvable and this natural stage was emulsified with dissolvable immersed fluid arrangement containing stabilizer utilizing mechanical stirrer. After the arrangement of o/w emulsion, water in run of the mill proportion ranges from 1:5 to 1:10, were added to the framework keeping in mind the end goal to permit dissolvable dissemination into the constant stage, in this way shaping collection of the lipid in the nanoparticles. Here the both the stage were keep up at same temperature and the dissemination step was performed either at room temperature or at the temperature under which the lipid was dissolved. Throughout the procedure consistent mixing was kept up.

E. High Shear Homogenization and/or Ultrasonication Technique

This is a less frequently used method for the preparation of lipid nanoparticles. The particles are prepared by melting the core material, adding phospholipids along with an aqueous medium and dispersing the melted material at increased temperature by mixing techniques, such as mechanical stirring or sonication. This is how nanoparticles are prepared by this method.

F. Double Emulsion Technique

In this method, homogenization is carried out in two steps; in the first step, water soluble drugs are incorporated in the inner aqueous phase and polymer/ lipophilic drugs are added into oil phase then both phases are homogenize by proper mixing to form the primary emulsion. Then, the primary emulsion is emulsified with the outer aqueous phase containing stabilizer to form double emulsion. Formation of double emulsion is followed by evaporation of the organic solvent from the dispersed phase leads to a point of insolubility. The solvent may be evaporated under reduced pressure via rotary evaporator or by simple stirring at ambient temperature depending upon the boiling point of organic solvent. The outer aqueous phase act as dispersion medium and the mixing can be provided either by mechanical stirring or sonication depending upon the nature of drug to be encapsulated and their particle size.

3. REVIEW OF LITERATURE

3.1 BENZOYL PEROXIDE

SR NO:	REFERENCE	WORK DONE
1	**Matt sagransky, brad a yentzer & steven r feldman**[25]	They have done research with antibiotic-resistant bacteria which is becoming more prevalent,and as BPO is a good, effective alternative to antibiotic monotherapy in the treatment of mild to moderate acne vulgaris it has helped them to elucidate the mechanism of action of BPO and provide insight into effective combination therapies. Clindamycin – BPO, adapalene – BPO and butenifine – BPO are all combination therapies that have superior efficacy to monotherapy without a significant difference in adverse effects. Although effective, patients must be fully aware of the limitations of topical BPO. Bleaching of fabric and hair as well as irritant dermatitis are commonly associated with BPO use. However, most of these side effects can be avoided by using BPO washes. A wash, as opposed to a leave-on cream, is used in the shower and thus avoids bleaching clothes. Washes are generally less irritating than leave-on creams. Physicians should choose the appropriate BPO product for a patient based on the severity of their acne and their skin type.
2	**Jelvehgari et al.**[26]	A microspongic delivery system of BPO using an emulsion solvent diffusion technique, by adding an organic internal phase containing benzoyl peroxide, ethyl cellulose, and dichloromethane into a stirred aqueous phase containing polyvinyl alcohol.It was that the presence of emulsifier was essential for microsponge formation and that the drug to polymer ratio, stirring rate

		and volume of dispersed phase influenced the particle size and drug release behavior of the formed microsponges. An increase in the ratio drug to polymer resulted in a reduction in the release rate of BPO from microsponges which was attributed to a decreased internal porosity of the microsponges. Further studies showed that the morphology and particle size of BPO microsponges were affected by drug to polymer ratio, stirring rate and the amount of emulsifier used.
3	**A. R. Gardouh[27], shadeed gad, hassan m. Ghonaim And mamdouh m. Ghorab**	Lipophilic model drugs (Dibenzoyl peroxide, Erythromycin base and Triamcinolone acetonide) which were used to study the feasibility of preparation of solid lipid nanoparticles. The drugs were successfully incorporated into SLNs by high-shear hot homogenization technique. The effects of different formulation parameters like viscosity and surfactant type and concentration on encapsulation efficiency, particle size and physicochemical properties of produced SLNs were investigated. Drug release from arranged SLNs formulae was upgraded contrasted with financially accessible formulae as acquired through in vitro discharge tests. The sort of surfactant furthermore focus close to glycerol as consistency enhancer utilized had an extraordinary force on the physicochemical portrayal of SLNs and the in vitro drug release. Definition F1 containing Tween 80 as a surfactant and the lipid network (10% Glyceryl monostearate and 5% Tween 80 with 1 % lecithin as cosurfactant) demonstrated the best results as indicated by the entanglement effectiveness and in vitro drug release.
4	**Ali nokhodchi, mitra jelveghari,**	Benzoyl peroxide (BPO) is a first-line topical treatment in skin inflammation vulgaris. In this manner, the

	mohammad-reza siahi, siavoosh dastmalchi[28]	motivation behind the present examination was to get appropriate controlled release for BPO. This study inspected whether the sort of topical formulation (cream, gel and foam) can influence the release pattern of BPO from microsponges. Benzoyl peroxide microparticles were readily utilizing an emulsion dissolvable dispersion strategy by including a benzoyl peroxide, ethyl cellulose and dichloromethane into a aqueous stage containing polyvinyl liquor. The stacking limit of the drug content and the mean particle size of microparticles were resolved. BPO microparticles were then fused into different formulations (creams, gels and moisturizers) for release studies. The microparticles are too huge to go through the stratum corneum, subsequently they would be relied upon to stay on the skin surface, thus releasing their substance after some time. This may improve the wellbeing of any topically applied formulation..
5	**James n. Bollingers delma lewis, and victor m. Mendez**[29]	The capacity of benzoyl peroxide within the sight of pharmaceutical gel fixings was researched. At both 30 and 40 °c temperatures, benzoyl peroxide was checked for 1 month in presence of ethanol and acidic chelating agents. The substitution of (CH3)2CO for ethanol, the disposal of chelating agents, and the expansion of sodium hydroxide to gel effect the formulation. By using triethanolamine, bis(2-propanol)amine, sodium hydroxide, carboxypolymethylene, hydroxypropyl methylcellulose, magnesium aluminum silicate, propylene glycol, the polyoxyethylene lauryl ethers, and CH3)2CO, separately it impact onto the capacity of benzoyl peroxide. In this way, the fractional debasement that happened in the CH3)2CO tests containing the acidic chelating agents most likely was because of some

		other liquor or free-radical spreading bunches, since CH3)2CO itself can't be relied upon to respond promptly with benzoyl peroxide. These show that sodium hydroxide prevents the development of "dissolvable" radicals in this manner by lowering benzoyl peroxide decay. As a result, the effects of this examination showed that the dependability of benzoyl peroxide in pharmaceutical gel arrangements is firmly impacted by the concoction cosmetics of the definitions and, by the capacity temperature because of expanded reactivity.
6	**US patent Patent No: 8895070 B2**[30]	As indicated by this patent Benzoyl peroxide is essentially insoluble in water. The disturbance because of use of arrangements containing benzoyl peroxide has been resolved to be brought about by the segment of the benzoyl peroxide that is in suspension, though broke down benzoyl peroxide causes practically no skin aggravation. Comparative clinical hostile to skin break out adequacy acquired by utilization of a watery topical definition containing 5.0% benzoyl peroxide is gotten by giving a fluid topical detailing containing a suspension of a low convergence of benzoyl peroxide in a soaked arrangement of water and a water-miscible natural dissolvable. The definition of the creation lessens skin aggravation because of utilization of the detailing contrasted with use of a comparative 5.0% benzoyl peroxide plan without trading off clinical viability.

3.2 CLINDAMYCIN PHOSPHATE

SR NO:	REFERENCE	WORK DONE
1	**Misra M. Et al**[31]	A steady zinc-clindamycin complex gel was framed by enhancing grouping of clindamycin phosphate and zinc acetic acid derivation get dried out, deciding ideal pH condition (pH 7.5) and balancing out (pH 5-8) the mind boggling utilizing different gelling specialists. Physical parameters like shading, smoothness/dirt, simplicity of use, sleekness/oiliness, skin disturbance, pH and consistency of gel were directed every now and then. Antimicrobial adequacy test were screened against five chose pathogens. A Franz dissemination cells framework was utilized to decide the discharge rate profile of the definition which creates better result practically identical to the advertised item finishes up the topical utilization of gel helpful in skin inflammation vulgaris is fit for capacity amid timeframe of realistic usability for more timeframe without losing its remedial viability and keeping up the consistency
2	**Golmohammadzadeh S et al**[13]	L iposomes encapsulated with clindamycin and tretinoin Liposomes can improve the therapeutic effect of drugs and decrease the adverse effects. Therefore, liposomes containing clindamycin (Lip-CL),liposomes containing tretinoin (Lip-TRT) and liposomes loaded with both tretinoin and clindamycin (Lip-CL-TRT) were prepared and characterized. Lip-TRT were prepared by solvent evaporation method whereas Lip-CL and Lip-CL TRT were prepared by dehydration-rehydration method. The amount of drug which was passed through or retained inside the skin was determined

		by Franz cell diffusion method and compared with the TRT cream.
3	**Hanpramukkun N et al** [32]	The stability of clindamycin phosphate was studied in solution and water-in-oil-in-water (w/o/w) multiple emulsions at 40 oC and 4°C. Clindamycin phosphate in aqueous solution showed degradation by hydrolysis mechanism. The stability of clindamycin phosphate in w/o/w multiple emulsions were better than in solution after 3 months. Increase viscosity of oil middle phases by addition of petrolatum in w/o/w multiple emulsion was found to improve clindamycin phosphate stability compared to w/o/w multiple emulsions without petrolatum. Apparent viscosity of external water phase was unaffected to clindamycin phosphate stability in w/o/w multiple emulsion .
4	**Chaudhary A** [33]	They developed liposomal formulation containing clindamycin were prepared by using lipid film hydration method and the optimum ratios of components were determined. Optimum liposomal formulation has been incorporated in gel base and comparison of that has been made with non liposomal gel both containing 1% CMP. In vitro permeation studies and minimum inhibition concentration were determined .
5	**Parmpreet singh, sunny kalia, rajni bala, naresh singh gill**[14]	Emulgel have emerged as one of the most interesting topical delivery system as it has dual release control system i.e. gel and emulsion. So they have investigated that the major objective behind this formulation is enhancing the topical delivery of hydrophobic drug (Clindamycin phosphate) by

		formulating Clindamycin phosphate into Emulgel using high molecular weight water soluble polymer of Hydroxypropyl methylcellulose (HPMC-5 and HPMC-15), carbopol-934, carbopol-94 that possesses very high viscosity, transparency; film forming properties at low concentration are reported to useful in formation of gel with an objective to increase transparency and spreadability. Oleic acid is used as permeation enhancer. They prepared Emulgel which were evaluated for their physical appearance, pH determination, viscosity, spreadability, extrudability, *in vitro* drug release, anti microbial activity and stability. All the prepared Emulgel showed acceptable physical properties, homogeneity, consistency, spreadability, viscosity and pH value. The best formulation showed better antimicrobial activity .

3.3 NANO-STRUCTURED LIPID CARRIERS (NLC'S)

SR NO:	REFRENCE	WORK DONE
1	**Joshi m, pathak s, sharma s, patravale v**[20]	The goal of the present examination was to investigate the capability of nanostructured lipid transporters (NLC) for the intravenous conveyance of artemether (ARM), an ineffectively water-dissolvable antimalarial operator. The NLC of ARM (Nanoject) were defined by utilizing a microemulsion layout system. The NLC were assessed for molecule size, exemplification

		effectiveness, in vitro drug discharge and in vitro hemolysis. The antimalarial action of the Nanoject and ordinary ARM injectable definition was assessed in Plasmodium berghei tainted mice. In vitro haemolytic studies demonstrated that Nanoject had lower haemolytic potential when contrasted with every one of the segments when concentrated exclusively. Nanoject demonstrated essentially higher (P<0.005) antimalarial action when contrasted with the advertised injectable detailing. The antimalarial movement of Nanoject went on for a more drawn out length (over 20 days) demonstrating that Nanoject might be long-coursing in vivo. Nanoject demonstrated fundamentally higher survival rate (60%) even following 31 days when contrasted with advertised detailing which indicated 0% survival (100% mortality).
2	**Chia-lang fang A, saleh a. Al-suwayeh B,and jia-you fang**[21]	Nanostructured lipid carriers (NLCs) are drug-delivery systems composed of both solid and liquid lipids as a core matrix. It was shown that NLCs shows some advantages for drug therapy over conventional therapy, including increased solubility, the ability to enhance storage stability, improved permeability and bioavailability, reduced adverse effect, prolonged half-life. This review describes recent developments in drug delivery using NLCs strategies. Special attention is paid to parenteral injection and topical delivery since these are the most common routes for investigating NLCs. The related patents of NLCs for drug delivery are also reviewed. Finally, the future development and current obstacles needing to be resolved are elucidated.

3	**Sunil prakash chaturvedi , vimal kumar** [20]	They have studied and review on different production methods developed for lipid nanoparticles and most of the methods use two basic steps; emulsification and size reduction to nano size. Homogenization techniques are most frequently employed using hot and cold homogenization or ultrasonication for the production. On the other hand, few methods based on emulsification are also applied used earlier for polymeric nanoparticle production. Hot high pressure homogenization and ultrasonication are most commonly used method with scale up feasibility but costly equipment is biggest drawback. Other methods used to produce lipid nanoparticles are possible in a laboratory setup with no expensive equipments are needed but scale up is still a problem with such method along with regulatory problems associated with high surfactants concentrations in these formulations. Furthermore, a comparison of the all commonly used methods ha salso been summarized by them.
4	**Wei keat ng, latifah saiful yazan, li hua yap,wan abd ghani wan nor hafiza, chee wun how, rasedee abdullah**[21]	Thymoquinone (TQ) has been shown to exhibit antitumor properties. Thymoquinone-loaded nanostructured lipid carrier (TQ-NLC) was developed to improve the bioavailability and cytotoxicity of TQ. This study was conducted to determine the cytotoxic effects of TQ-NLC on breast cancer and cervical cancer cell lines. TQ-NLC was prepared by applying the hot high pressure homogenization technique. Polysorbate 80 helps to increase the stability of TQ-NLC. The encapsulation efficiency of TQ in TQ-NLC was determined by HPLC analysis. TQ-NLC exhibited antiproliferative activity towards all the cell lines in a dose-dependent manner which was most cytotoxic

		towards MDA-MB-231 cells.. Thus, TQ-NLC has the potential to be developed into a drug for treatment of breast cancer .

3.4 NANOSTRUCTURED LIPID CARRIERS BASED GEL

SR NO:	REFERENCE	WORK DONE
1	**Dilip patel sandipan dasgupta sanjay dey, y. Roja ramani , subhabrata ray, bhaskar mazumder**[34]	NLCs of aceclofenac could be effectively prepared by the melt-emulsification and high speed homogenization techniques. The rapid homogenization strategy produce smaller particles similar to that of melt-emulsification technique. Their study demonstrates that the measure of liquid lipid and lecithin altogether influences the particle size and in addition entrapment efficency. It is additionally found that the release rate, saturation rate, and pharmacodynamic action can be alterd after changing the proportion of solid lipid to liquid lipid ratio. It can be presumed that the enhanced NLC gels gives faster onset and delayed activity when compared with the marketed formulation. Further, in vivo pharmacokinetic studies are important to evaluate the activity of the NLC gel.
2	**Anupam kumar sachan, ankita gupta, mona arora**[35]	Nanostructured lipid carriers (NLC)-based topical gel of Etoricoxib was prepared with the aim for the treatment of inflammation. The composition of NLC consisted of Stearic acid as solid lipid, oleic acid as liquid lipid and tween 80 as a surfactant. NLCs are

		prepared by melt-emulsification and low temperature solidification technique and was characterized by measuring particle size, scanning electron microscopy (SEM), and differential scanning calorimetry (DSC). All of the NLC showed high entrapment efficiency ranging from 69% to 76%. Both the entrapment and release rate are affected by the percentage of oleic acid used. The higher magnitude of zeta potential indicates the stability of formulation. The nanoparticulate dispersion was suitably gelled and measured for Physical appearance, Homogeneity, Spreadability and *in-vitro* permeation study. *In-vitro* drug release pattern of developed NLC dispersion gel showed burst and prolong release. It was concluded that developed NLC gel of Etoricoxib can be used for prolonged release of drug in the skin tissues and can be used for better management of inflammatory conditions.
3	**Bharti gaba, mohammad fazil, saba khan, asgar ali, sanjula baboota, Javed ali**[22]	Their present study was to evaluate Terbinafine HCl (TH)-loaded nanostructured lipid carrier (NLC) for the treatment of fungal infection via topical administration. Fungal infections are tremendously widespread and the treatments are effective but associated toxicities restrict their use. TH-NLC was prepared using high pressure homogenization technique using Glyceryl Monostearate (GMS) as solid lipid, Labrasol as liquid lipid and Pluronic F-127 as surfactant, binary lipid phase was selected in the ratio 6:4 w/w (solid:liquid lipid ratio). Spherical shape and size were confirmed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analysesThe pharmacodynamic studies indicated that TH-NLC gel efficiently reduced

		the fungal burden in shorter duration of time as compared to marketed formulation. Therefore, it can be concluded that the developed NLCs showed a sustained release pattern and reduction of fungal burden in the infected area. Hence, TH-NLC could be a potential alternative for treatment of topical fungal infection after clinical evaluation in near future.
4	**M. Joshi and v. Patravale**[35]	Nanostructured Lipid Carrier (NLC)–based topical gel of Valdecoxib was detailed with the point of speedier onset yet drawn out activity for the treatment of aggravation and united conditions. NLCs arranged by microemulsion format procedure were described by photon relationship spectroscopy for size. Drug embodiment proficiency was resolved utilizing Nanosep® diffusive gadget. The nanoparticulate scattering was appropriately gelled and portrayed as for medication content, pH, spreadibility, rheology, and in-vitro discharge. Wellbeing of the NLC-based gel was surveyed utilizing essential skin bothering studies, and viability was affirmed utilizing pharmacodynamic concentrate, to be specific the Aerosil-instigated Rat Paw edema model. The created NLC-based gel indicated quicker onset and inspired delayed action up to 24 hours.

4. EXPERIMENTAL WORK

4.1 Materials and Equipments

Table 4.1- List of materials used in project work

SR No.	MATERIALS	FUNCTION	COMPANY NAME
1.	Benzoyl peroxide	Antibacterial	P.D Navkar
2.	Clindamycin phosphate	Antiimicrobial	ZHEHANG HISOAR PHARMACEUTICAL CO., LTD.CHINA
3.	Glyceryl monosterate	Solid lipid	Gattefosse
4.	Precirol	Solid lipid	
6.	Compritol 888 ATO	Solid lipid	
7.	Compritol E ATO	Solid lipid	
8.	Stearic acid	Solid lipid	
9.	Imwitor 900	Solid lipid	Gattefosse
10	Glucire 43/01	Solid lipid	
11	PECEOL	Liquid lipid	
12	Cetiol v	Liquid lipid	BASF Corp. Mumbai
13	Miglyol 812	Liquid lipid	
14	MCT	Liquid lipid	
15	TWEEN 80	Surfactant	S D Fine Chemical, Mumbai
16	POLOXAMER 188	Surfactant	SIGMA Aldrich, USA
17	POLOXAMER 407	Surfactant	
18	TWEEN 20	Surfactant	S D Fine Chemical, Mumbai
19	SPAN 20	Surfactant	
20	SPAN 60	Surfactant	

21	CREMAPHORE RH 40	Surfactant	BASF Corp. Mumbai
22	Transcutol	Permeation enhancer	Gattefosse
23	Propylene glycol	Co-surfactant	S D Fine Chemical, Mumbai
24	Carbopol 980	Gelling agent	Lubrizol
25	Potassium hydroxide	Neutralizing agent	Qualigens, Mumbai

Table 4.2- List of equipments used in project work

SR NO:	NAME OF THE EQUIPMENT	NAME OF THE COMPANY
1	UV Spectrophotometer	Shimadzu
2	Magnetic Stirrer	Remi
3	Electrical Balance	Metler toledo
4	Brookfield Viscometer	Anton Paar
5	High speed homogenizer	Kinematica
6	pH meter	Metler Toledo
7	HPLC	Waters
8	Malvern Zeta Sizer	Malvern Z1700
9	Refrigerated Centrifuge	Thermolab
10	Texture Analyser	Brookfield
11	Vortex Shaker Hot	Remi
12	Transmission Electron Microscope	Philips
13	Water bath	Equitron Chennai, India

4.2 Pre-formulation studies

4.2.1 Identification of benzoyl peroxide and clindamycin phosphate

4.2.1.1 Melting point determination:

The melting point of the benzoyl peroxide and clindamycin phosphate was determined by thiel's tube method and it was compared with reported melting point of standard.

Result:

Table 4.3- Melting point determination of Benzoyl Peroxide and Clindamycin Phosphate

SR NO	DRUG	OBSERVED MELTING POINT (°C)	REPORTED MELTING POINT (°C)
1	Benzoyl peroxide	103 ° C	103-106 °C
2	Clindamycin phosphate	115 °C	114-116 °C

Conclusion:

The melting point of the benzoyl peroxide and clindamycin phosphate was found similar to the reported value of melting point, which confirmed identity of procured drug sample.

4.2.2 UV spectrophotometric analysis of benzoyl peroxide:

The standard stock solution of benzoyl peroxide having concentration of 10µg/ml in Methanol/acetone mixture was scanned between 200-400nm in UV-visible Spectrophotometer. The wavelength maxima was found and it was compared with reported wavelength maxima of benzoyl peroxide.

Result:

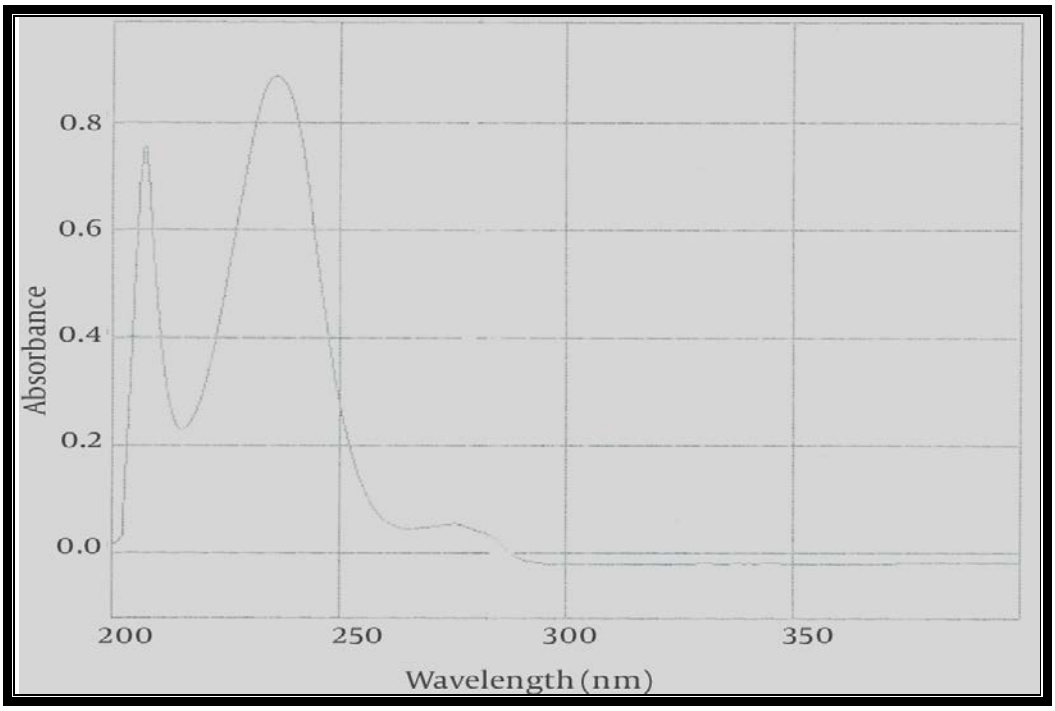

Figure 4.1- UV spectrum of Benzoyl peroxide

Table 4.4 -Wavelength maxima (λmax) of benzoyl peroxide

SR NO:	DRUG	Observe wavelength maxima(λmax)	Reported wavelength maxima(λmax)
1	Benzoyl peroxide	234.8	235

Conclusion:

The UV absorbance of benzoyl peroxide was found at 234.8nm, which was similar to reported value of wavelength maxima (235nm), which again confirmed identity of the procured drug sample.

4.2.3 Development of spectrophotometric method for estimation of benzoyl peroxide

Quantitative data is required on various studies such as purity, evaluation of the drug, compatibility studies, in-vitro diffusion studies. It is essential to develop analytical methods which are precise, specific and accurate. Therefore, following analytical methods were developed and validated for Benzoyl peroxide.

4.2.3.1 Calibration curve of Benzoyl peroxide in methanol:acetone

- **Preparation of stock solution:**

  A standard stock solution("A") of benzoyl peroxide was prepared in 100ml methanol:acetone by dissolving 100 mg benzoyl peroxide in ratio of 50:50 of methanol:acetone mixture.

- **Dilution of stock solution:**

  From the stock solution("A") 10ml was dissolved again in 100ml volumetric flask with methanol to make concentration 100µg/ml (stock solution "B"). Aliquots 0ml, 0.5ml, 1ml, 1.5ml, 2ml ,2.5ml, 3ml, 3.5ml, 4ml, 4.5ml, 5ml of stock solution "B" were pipette out and final volume was made to 100ml with methanol to get concentration in range of 0.5-5µg/ml respectively.

Table 4.5- Calibration curve of Benzoyl peroxide in mixture of Methanol:Acetone

Conc. (µg/ml)	Avg Absorbance.
0.0	0.000
0.5	0.041
1.0	0.084
1.5	0.136
2.0	0.153
2.5	0.195
3.0	0.241
3.5	0.284
4.0	0.344
4.5	0.399
5.0	0.443

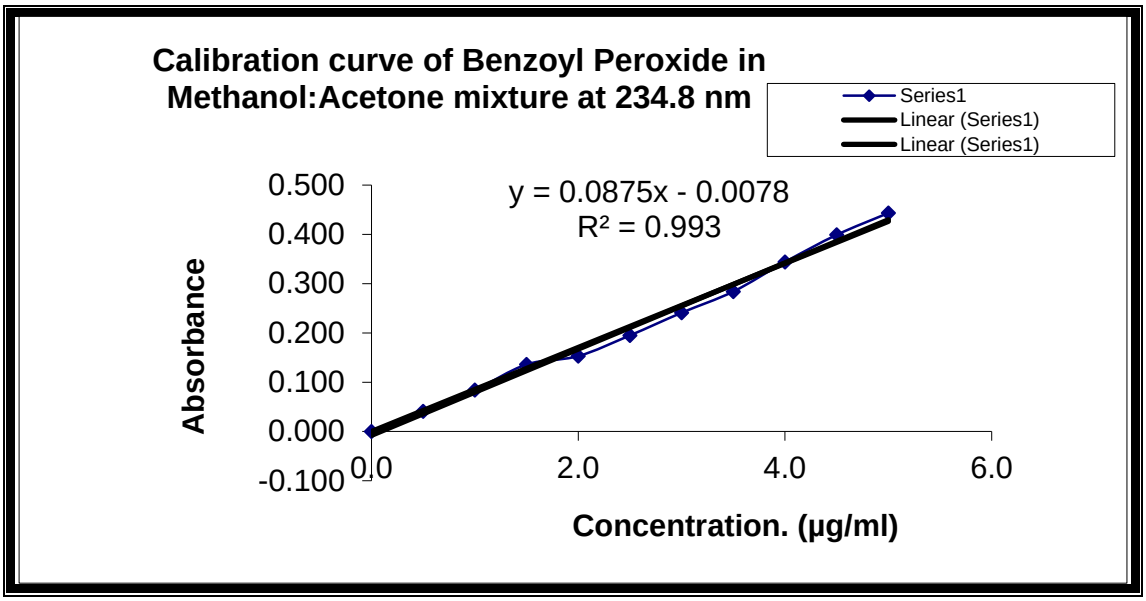

Figure 4.2- Regression analysis of Calibration curve of Benzoyl peroxide in Methanol:acetone mixture

Table 4.6 shows the Regression Analysis of the calibration plot of benzoyl peroxide in methanol:acetone mixture. The correlation coeeficient (r^2) has value 0.993which is near to 1. This shows linearity of the curve.

Table 4.6- Regression Analysis of the calibration plot of benzoyl peroxide in methanol:acetone

Sr. No.	Regression Parameter	Values
1	Correlation Coefficient	0.993
2	Slope	0.0875
3	Intercept	0.0078

4.2.4 DEVELOPMENT AND VALIDATION OF HIGH PERFORMANC LIQUID CHROMATOGRAPHIC METHOD FOR ESTIMATION OF CLINDAMYCIN PHOSPHATE:

4.2.4.1 EXPERIMENTAL:

Apparatus:

- A Waters RP-HPLC instrument equipped with a UV-Visible detector and a photodiode array detector, manual injector with a 20 µL loop, Phenomenex (Torrance, CA) C18 column (250 mm × 4.6 mm id, 5µm particle size) and LC solution software were used.
- Analytical balance
- Ultrasonic cleaner
- Micropipettes

Reagents and Materials

- Clindamycin phosphate was kindly gifted by ZHEHANG HISOAR PHARMACEUTICAL CO., LTD.CHINA

- HPLC grade Methanol (Merck Ltd., Mumbai, India
- HPLC grade Acetonitrile (Finar Chemical Ltd., Mumbai, India)
- RP-HPLC Water (Finar Chemical Ltd., Mumbai, India)
- A nylon 0.45 μm- 47 mm membrane filter (Gelman Laboratory, Mumbai, India)

Chromatographic Condition

The chromatographic estimation was performed using following conditions:

- Stationary phase : phenomenex C18 column (250mm x 4.6 mm).
- Mobile phase : Acetonitrile : Methanol (94 : 6 v/v).
- Flow rate : 1.0 ml/ min.
- Injection volume : 20 Ml.
- Detection : The elution was monitored at 210 nm using UV detector.

All the solvents of mobile phase were filtered through nylon 0.45 um- 47 mm membrane filter, degassed before use and were filled in neat and clean separate bottle. The ratio of mobile phase was set as Acetonitrile: Methanol (94: 6 v/v)

Preparation of CLP Standard stock solutions

Accurately weigh CLP (22mg) was transferred to a separate 100 ml volumetric flask and dissolved and dilute up to mark with water to obtain a standard stock solutions having concentration CLP (220ug /ml).

Determination of analytical wavelength

The standard solutions of CLP was injected under the same chromatographic condition. The elution showed good response at 210 nm.

4.2.4.2 METHOD VALIDATION

Calibration Curve (Linearity)

Calibration curves was constructed by plotting peak areas Vs concentrations of CLP and the regression equations were calculated. The calibration curve was plotted over the concentration range of 22-220 ug/ml for CLP. Accurately measure standard working solutions of CLP (1, 2, 4, 6,8,10 ml) and was transferred to a series of 10 ml of volumetric flasks and diluted to the mark with water. Aliquots (20 uL) of each solution was injected under the operating chromatographic condition.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of CLP by the standard addition method. Known amount of standard solutions of CLP were added at 50,100, 150 % level to prequantified sample solutions of CLP. The amount of CLP was estimated by obtained values to the regression equation of the calibration curve.

Method precision (% Repeatability)

The precision of the instrument was checked by continuously injecting standard solutions of CLP (88 ug/ml) under the same chromatographic condition and measurements of peak area, retention time and tailing factor. Percentage relative standard deviation (RSD) should not be more than 2 %.

Intermediate Precision (Reproducibility)

The intraday and interday precision of the developed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentration of standard solutions of CLP(88 ug/ml). The results was reported in terms of relative standard deviation (RSD) .

Limits of detection (LOD) and limit of Quantification (LPQ)

LOD and LOQ of drug were derived by calculating the signal to noise ratio (S/N, i.e, 3.3 for LOD and 10 for LOQ) using the following equation as per International Conference on Harmonization (ICH) guidelines.

LOD = 3.3 x 6/S

LOQ = 10 x 6/S

Where 6= the standard deviation of the response

S= slop of calibration curve

4.2.4.3 RESULT AND DISCUSSION

Method development

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for CP was obtained with a mobile phase acetonitrile-methanol (94:6 v/v) at a flow rate of 1.0 ml/ min to get better reproducibility and repeatability. Quantification was achieved with LC detection at 210 nm based on peak area. Complete resolution of peaks with clear baseline was obtained in 22 µg/mL (figure-4.2) and 220 µg/mL (figure-4.3). Peak purity of drugs was confirmed by comparing the spectra of different standard solution. The UV-visible spectrum of standard solution shows good correction. System suitability test parameters for CLP for the proposed method are reported in Table-4.5.

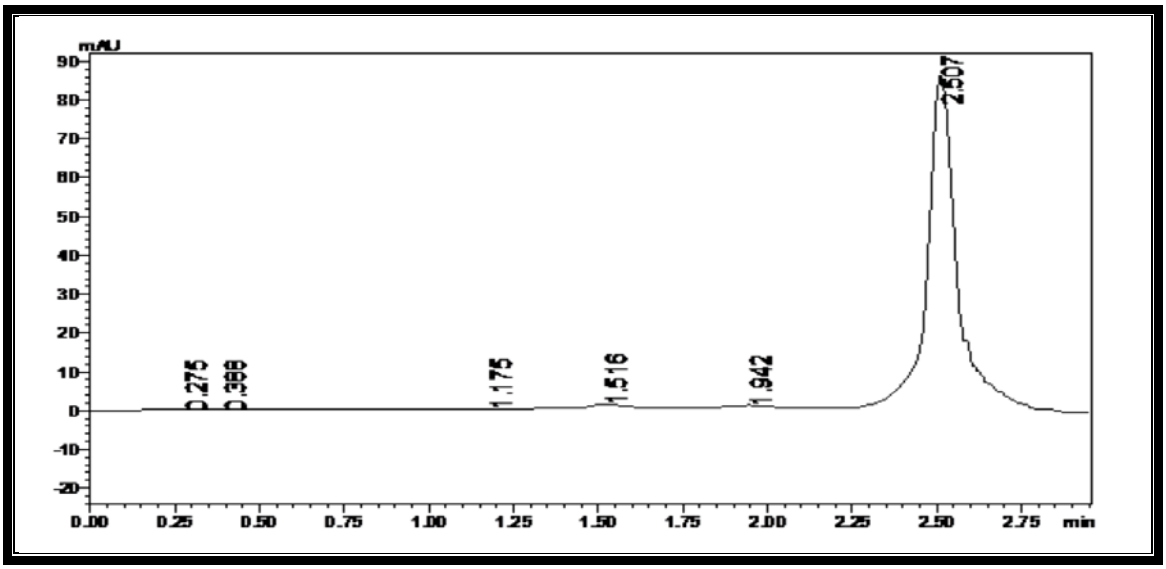

Figure 4.3- Regression Analysis of the calibration plot of benzoyl peroxide in methanol:acetone

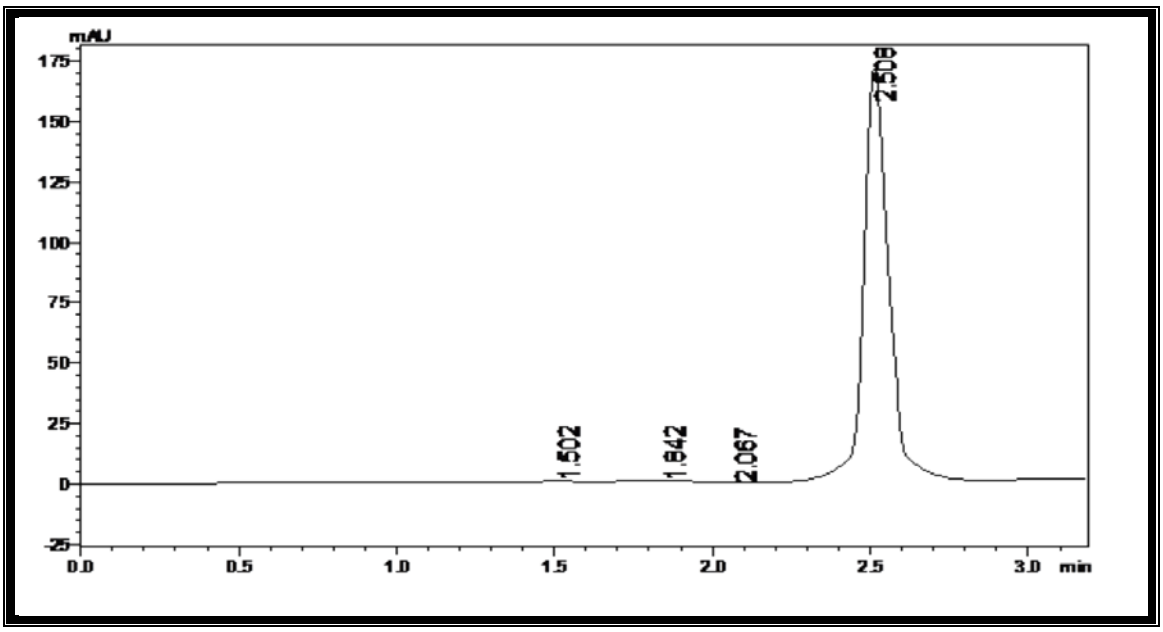

Figure 4.4- Chromatogram of Clindamycin Phosphate 220µg/ml

Table 4.7- System suitability parameters of chromatogram for CLP

Parameters	**CLP (22 µg/mL) ± RSD[a]**	**CLP (220 µg/mL) ± RSD[a]**
Retention time (min)	2.51±0.02	2.50±0.02
Tailing factor	1.10±0.22	1.13±0.25
Theoretical plates	6238.50 ±0.30	6455.89±0.30
Resolution	3.45 ±0.19	3.57±0.11

RSD[a] = Relative Standard Deviation

4.2. 4.4 VALIDATION OF PROPOSED METHOD

Linearity

Linear correlation was obtained between peak area Vs concentrations of CLP in the ranges of 22 -220 µg/mL. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table 4.8) (figure 4.5).

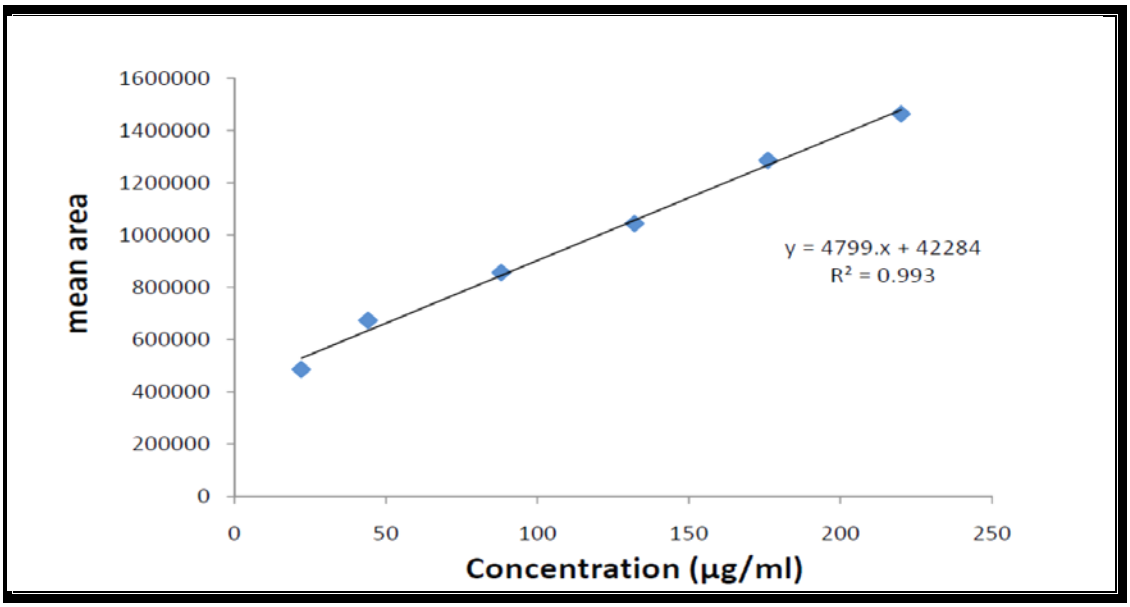

Figure 4.5- Calibration curve of clindamycin phosphate by using HPLC

Table 4.8- Regression analysis data and summary of validation parameter

Parameters	**HPLC Method for CLP**
Detection wavelength (nm)	210 nm
Concentration range	22 -220 (µg/mL)
Intercept	42284
Slope	4799
Correlation coefficient	0.993
LOQ (µg/mL)	6.35 µg/ml
LOD (µg/mL)	2.04 µg/ml
Accuracy S1 S2 S3	 100.6 % ± 0.24% 100.5% ± 0.37% 100.1% ± 0.35%
Repeatability (% RSD)	0.38 %
Precision (% RSD) Intraday Interday	 0.26% - 0.31% 0.31% - 0.36%

LOD = limit of detection

LOQ = limit of quantification

RSD = Relative Standard Deviation

Accuracy

The recovery experiments were performed by the standard addition method. The recoveries obtained were 100.51% ± 1.05% for CLP. The low value of standard deviation indicates that the proposed method is accurate. Result of recovery studies shown in (table4.9)

Table 4.9- Recovery data

DRUG	Level	Amount of Sample Taken (µg/ml)	Amount of Standard Spiked (%)	Mean %Recovery±RSD
CLP	I	70.4	80	100.6± 0.24
	II	88	100	100.5 ± 0.37
	III	105.6	120	100.1± 0.35

Method Precision (% Repeatability)

The RSD values for CLP were found to be 0.38 % (Table 4.8). The RSD values was found to be < 1%, which indicates that the developed method is precise.

Table 4.10- Precision data for CP

CLP (88 µg/mL)	Retention time (min)	Peak area
1	2.505	856297
2	2.507	856184
3	2.495	861104
4	2.508	863412
5	2.504	856995
6	2.492	856897
Mean	2.4990	858486
SD	0.004268750	3031.37
%CV	0.170795535	0.353101

Intermediate precision

The RSD value of interday (0.31% -0.36%) and intraday (0.26% -0.31%) variations for CLP, indicates that the proposed method is precise. (Table 4.10) LOD value for CLP was found to be 2.04 µg/mL and LOQ value was found to be 6.35 µg/mL.

4.2.4.5 Discussion

The described HPLC method provides simple, precise, sensitivity and reproducible quantitative method for routine analysis of clindamycin phosphate. Based on the result, obtained from the analysis of using described method, it can be concluded that the method has linear response in range of 22-44 µg/mL with co-efficient of correlation (r^2) 0.993 for CLP.

4.3- FORMULATION STUDY:

4.3.1 Solubility profiling of benzoyl peroxide in solid lipids, liquid lipids and surfactants:

The solubility of benzoyl peroxide in various solid lipids, liquid lipids and surfactants were determined and the screened excipient in which benzoyl peroxide showed maximum solubility were selected for formulation development.

4.3.1.1 Determination of solubility of benzoyl peroxide in solid lipids:

Fixed amount of each solid lipid (10 mg) was taken in a 5 ml glass vial and is heated above the melting point on a water bath. To the melted lipid, 10 mg of drug was added and vortexed. If drug did not dissolve in this amount of lipid, then the amount of lipid was increased in increments of 10 mg, till entire drug was dissolved. The amount of solid lipid required to dissolve 5 mg of drug was noted.

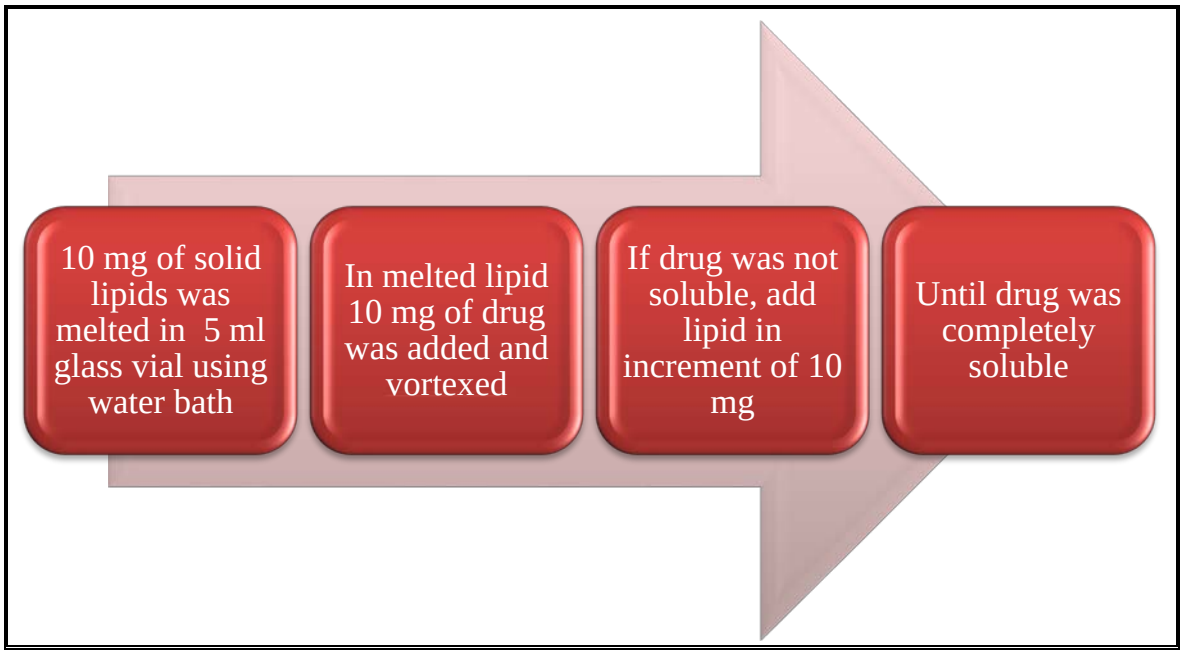

Figure 4.6- Solubility study of BPO in solid lipids

Table 4.12- Screening of lipids

SR NO:	SOLID LIPIDS	Amount of lipids required to dissolve drug (mg)
1	Imwitor 491 (glyceryl monostearate)	150
2	Precirol	470
3	Compritol 888 ATO	500
4	Compritol E ATO	610
5	Stearic acid	190
6	Imwitor 900	420
7	Glucire 43/01	1030

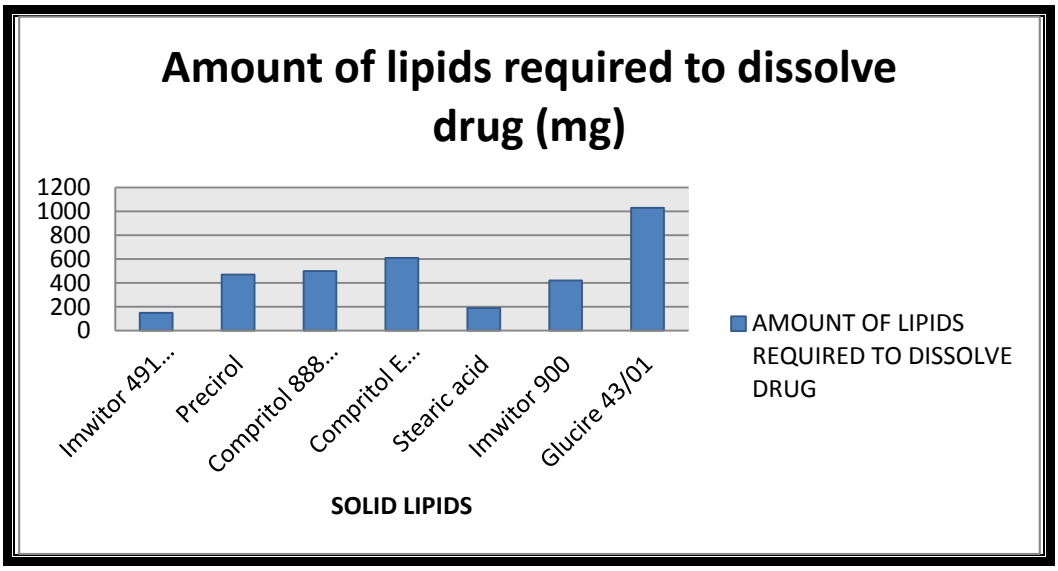

Figure 4.7- Screening of solid lipids

Results and Disussion:

The various solid lipids such as Imwitor491(glyceryl monostearate), Precirol,compritol 888 ATO, compritol E ATO, stearic acid, Imwitor 900, glucire 43/01 were screened for solubility of benzoyl peroxide. The amount of solid lipid required to solubilize 10 mg of Benzoyl peroxide is shown in Figure 4.7 .It was observed that smaller amount of Imwitor 491 (Glyceryl monostearate) was required to solubilize the drug.

4.3.1.2- Determination of solubility of Benzoyl peroxide in liquid lipids:

Fixed amount of each liquid lipid (10 mg) was taken in a 5 ml glass vial and is heated above the melting point on a water bath. To the melted lipid, 10 mg of drug was added and vortexed. If drug did not dissolve in this amount of lipid, then the amount of lipid was increased in the increments of 10 mg, till the entire drug dissolved. The amount of liquid lipid required to dissolve 10 mg of drug was noted.

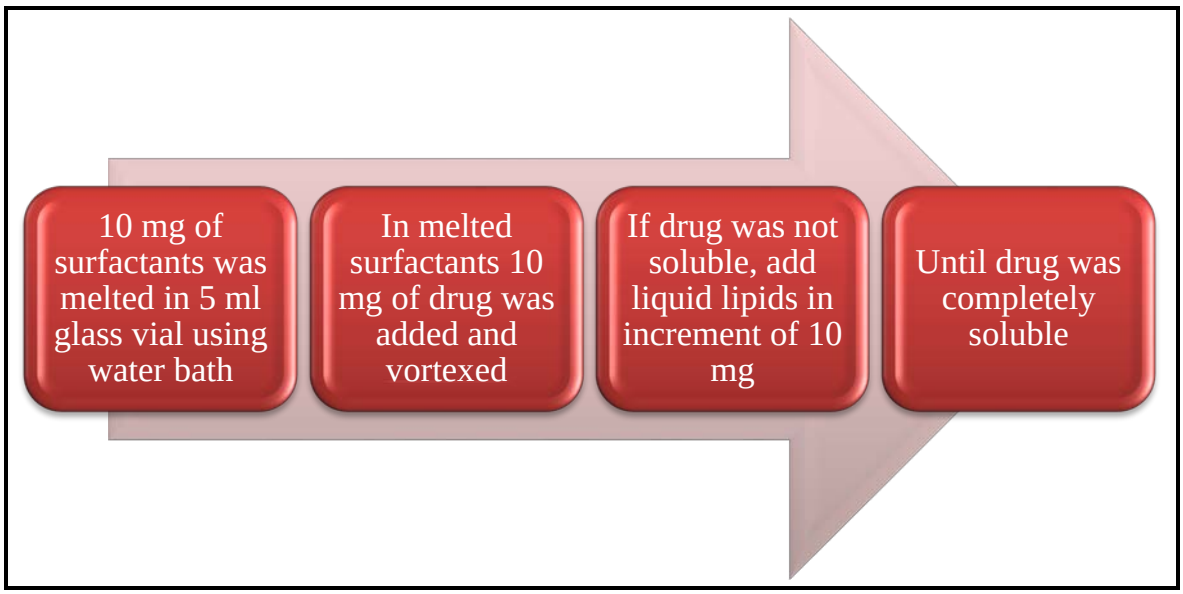

Figure 4.8- Solubility study of BPO in liquid lipids

Table 4.13- Screening of lipids

SR NO:	LIQUID LIPIDS	Amount of lipids required to dissolve drug (mg)
1	Peceol	50
2	Cetiol v	100
3	Miglyol 812	105
4	MCT	80

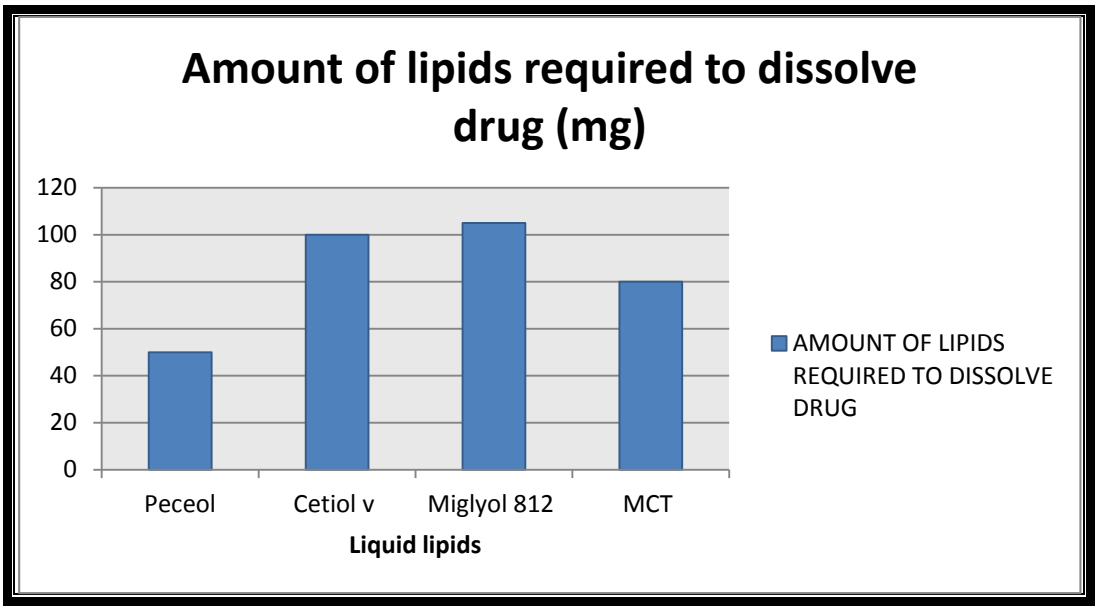

Figure 4.9- Screening of liquid lipids

Results and Discussion:

Solubility of Benzoyl peroxide was studied in a series of liquid lipids/oils. Comparative solubility of benzoyl peroxide in various oils is represented in. As shown in Fig 4.9, among the oils screened, highest solubility of benzoyl peroxide was found in peceol.

4.3.1.3- Determination of solubility of benzoyl peroxide in surfactants:

Fixed amount of each surfactant (10 mg) was taken in a 5 ml glass vial and is heated above its melting point on a water bath. To the melted surfactant, 10 mg of drug was added and vortexed. If drug did not dissolve in this amount of surfactant, then the amount of surfactant was increased in the increments of 10 mg, till the entire drug dissolved. The amount of surfactant required to dissolve 10 mg of drug was noted.

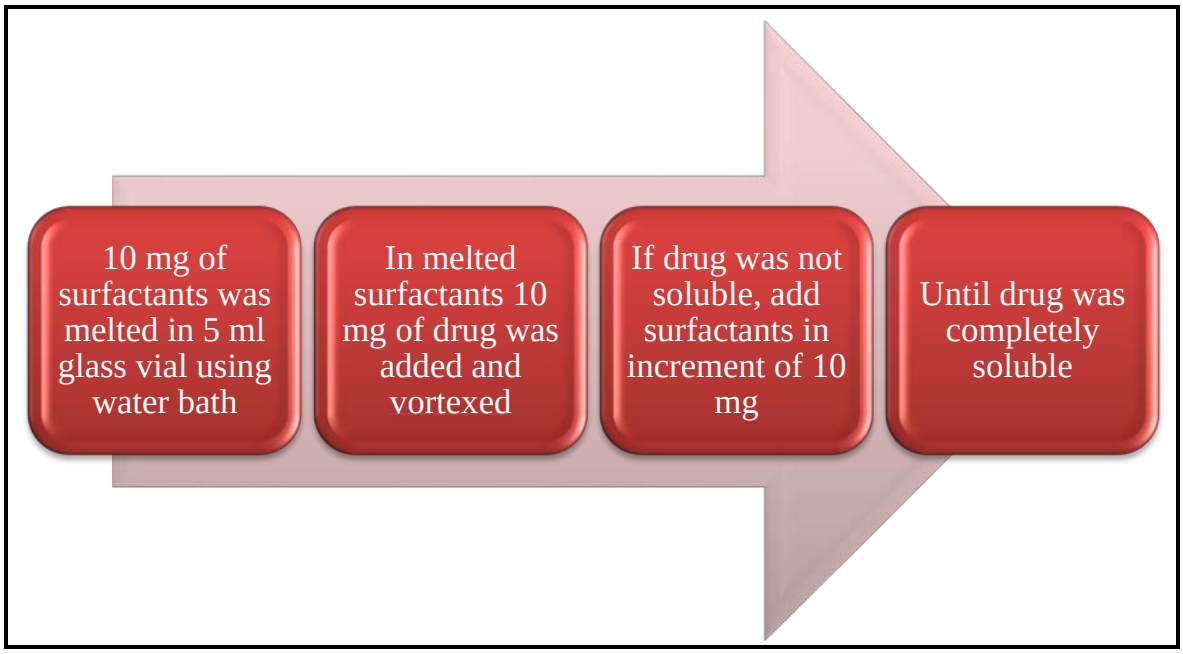

Figure 4.10- Solubility study of BPO in surfactants

Table 4.14- Screening of surfactants

SR NO:	**SURFACTANTS**	**Amount of lipids required to dissolve drug (mg)**
1	Tween 80	125
2	Poloxamer 188	75
3	Poloxamer 407	100
4	Tween 20	80
5	Span 20	105
6	Span 60	45
7	CREMAPHORE RH 40	70

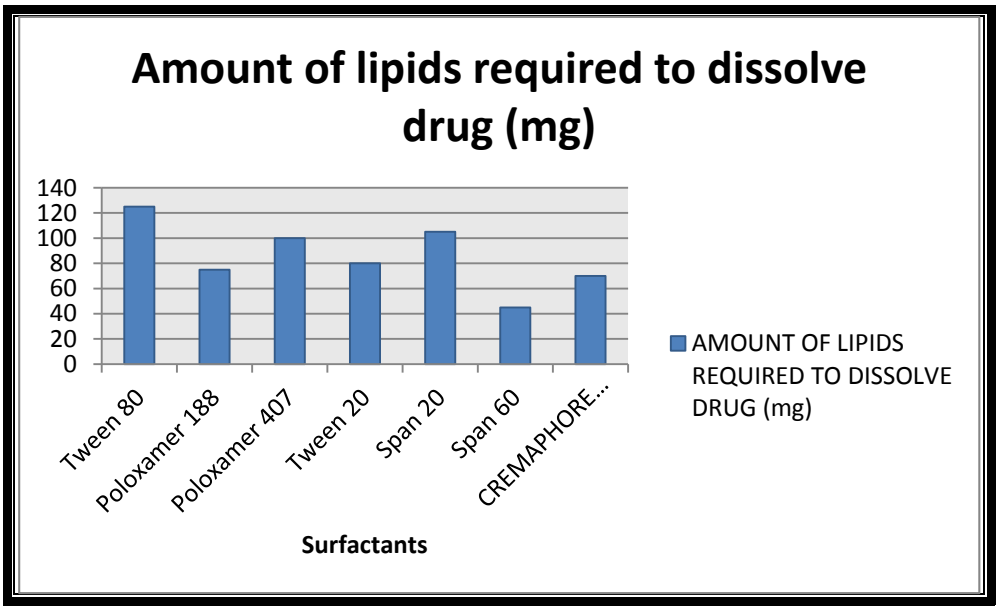

Figure 4.11- Screening of lipids

Results and Discussion:

The various surfactants were screened for solubility of benzoyl peroxide. The amount of surfactant required to solubilize 10 mg of benzoyl peroxide is as shown in figure 4.11. The figure 4.11 shows amount of Tween 80 required to solubilize the drug were very higher as compared to other surfactant.

4.3.2- Drug-Drug and Drug-Excipient compatibility study using FTIR spectroscopy:

Compatibility study of procured drug (benzoyl peroxide and clindamycin phosphate) & different excipients were done by Infrared spectroscopy. KBr disc containing the drug and different excipients mixture was prepared and the spectra was recorded in a range between 4000 & 400 $cm^{-1.}$

Results and discussion:

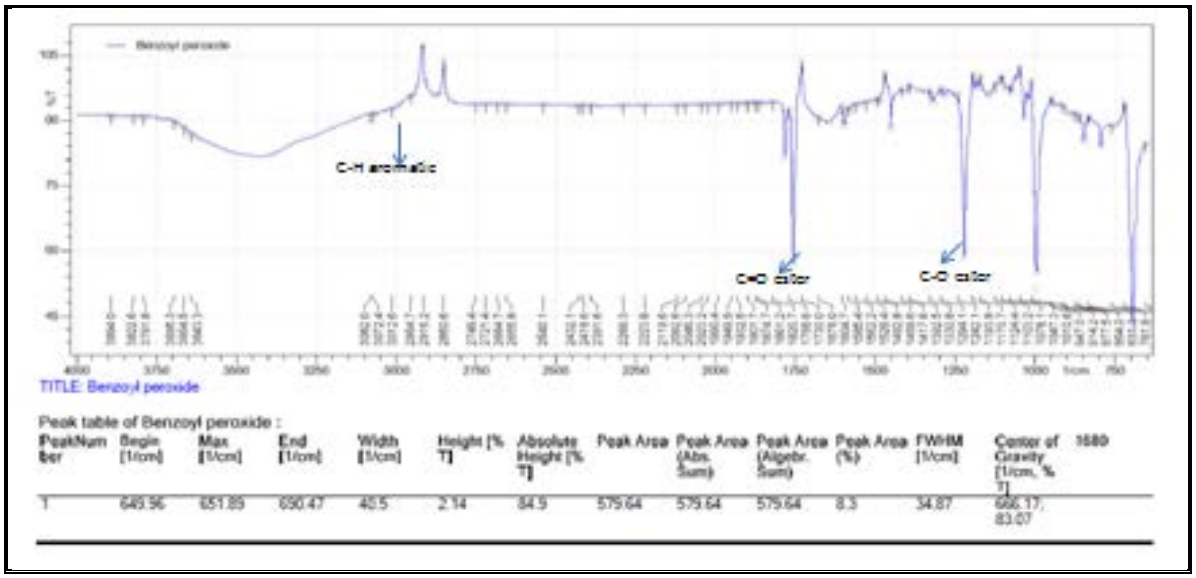

Figure 4.12- FTIR spectra of Benzoyl peroxide

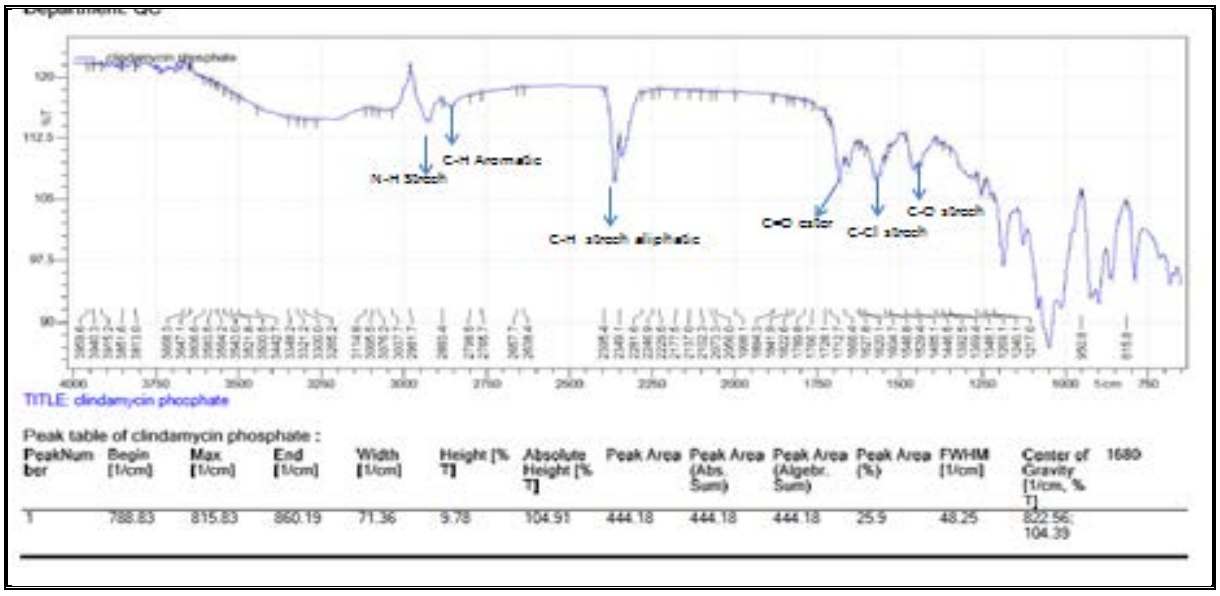

Figure 4.13- FTIR spectra of Clindamycin phosphate

- Here we can conclude that the spectra peak obtained is similar to that of standard spectra of benzoyl peroxide and cindamycin phosphate.

4.3.3- Formulation development and optimization of Nanostructured lipid carriers(NLCs):

4.3.3.1- Development of placebo NLCs-

The placebo NLCs were prepared, using Glyceryl monostearate(GMS) as solid lipids, glyceryl monooleate (GMO) as liquid lipid and Tween 80 (T80) as surfactant and propylene glycol as Co-surfactant. The placebo NLCs were prepared using Hot Homogenization process. Lipids (GMS) and (GMO) along with co-surfactant (PG) were heated to 75-80 ºC on a heating water bath. Aqueous phase containing surfactants (T80) was heated up to the same temperature(75-80 ºC). The aqueous phase was added to melted lipid phase and homogenized by High speed homogenizer (HSH) (Polytron kinematica, Switzerland) to form hot microemulsion. The hot microemulsion was rapidly cooled down at 2-3 ºC on an ice bath to form NLCs. During placebo formulation optimization various process parameters such as homogenization time, amount of lipids and surfactants are summarized in Table 4.15.

Table 4.15- Various process parameters and their range

Variable parameter	**Range**
Homogenization time	5min, 10min, 15min
Amount of solid lipids	1gm, 2gm, 3gm, 4gm, 5gm
Amount of surfactants	2%, 3%, 4%

Preliminary trials for optimizing various process parameters:

Table 4.16- Screening of lipid content

COMPOSITION	15/028/001	15/028/002	15/028/003	15/028/004	15/028/005
GMS (solid lipid)	**1 gm**	**2 gm**	**3 gm**	**4 gm**	**5 gm**
Peceol	500mg	500mg	500mg	500 mg	500mg
Water	40ml	40ml	40ml	40 ml	40 ml
Transcutol	3ml	3ml	3ml	3ml	3ml
Tween 80	4%	4%	4%	4%	4%

Propylene glycol	2%	2%	2%	2%	2%
Process parameters : Hsh time Hsh speed	5min 20,000 rpm	5min 20,000 rpm	5min 20,000 rpm	5min 20,000 rpm	5min 20,000 rpm

Table 4.17- Screening of surfactant contents

COMPOSITION	**15/028/006**	**15/028/007**	**15/028/008**
GMS (SOLID LIPID)	3 gm	3 gm	3 gm
Peceol	500mg	500mg	500mg
Water	40ml	40ml	40ml
Transcutol	3ml	3ml	3ml
Tween 80	**2%**	**3%**	**4%**
Propylene glycol	2%	2%	2%
Process parameters : Hsh time Hsh speed	5min 20,000 rpm	5min 20,000 rpm	5min 20,000 rpm

Table 4.18- Screening of homogenization time

COMPOSITION	**15/028/009**	**15/028/010**	**15/028/011**
Gms (solid lipid)	3 gm	3gm	3 gm
Peceol	500mg	500mg	500mg
Water	40ml	40ml	40ml
Transcutol	3ml	3ml	3ml
Tween 80	3%	3%	3%
Propylene glycol	2%	2%	2%
Process parameters : Hsh time Hsh speed	**5min** 20,000 rpm	**10min** 20,000 rpm	**15min** 20,000 rpm

4.3.3.2 Development of benzoyl peroxide loaded NLCs

The benzoyl peroxide loaded NLCs were prepared, using Glyceryl monostearate(GMS) as solid lipids, glyceryl monooleate (GMO) as liquid lipid and Tween 80 (T80) as surfactant and propylene glycol as Co-surfactant. The benzoyl peroxide NLCs were prepared using Hot Homogenization process. Drug (BPO), Lipids (GMS) and (GMO) and co-surfactant (PG) were heated up to 75-80 ºC on a heating water bath. Aqueous phase containing surfactants (T80) was heated up to the same temperature(75-80 ºC). The aqueous phase was added to melted lipid phase and homogenized by High speed homogenizer (HSH) (Polytron kinematica, Switzerland) to form hot microemulsion. The hot microemulsion was rapidly cooled down at 2-3 ºC on an ice bath to form NLCs.

Table 4.19- Preliminary trial of drug loaded NLC's

COMPOSITION	**15/028/012**	**15/028/013**	**15/028/014**	**15/028/015**
Drug (BPO)	1.63 gm	1.63 gm	1.63 gm	1.63
GMS (SOLID LIPID)	3 gm	3 gm	3 gm	4 gm
Peceol	500mg	500mg	500mg	500mg
Water	40ml	40ml	40ml	40ml
Transcutol	3ml	3ml	3ml	3ml
Tween 80	**3%**	**3%**	**4%**	**4%**
Propylene glycol	**2%**	**2%**	**3%**	**3%**
Process parameters : Hsh time Hsh speed	**5min** 20,000 rpm	**10min** 20,000 rpm	**5min** 20,000 rpm	**10min** 20,000 rpm

4.3.4 Physicochemical characterization of Optimized NLCs

4.3.4.1 Particle size and zeta potential of benzoyl peroxide NLCs

A. Photon correlation spectroscopy

Photon correlation spectroscopy (PCS) is a system utilized to decide the mean molecule size (PCS width) and size determination (poly-dispersity index, PDI). It is a light dissipating test in which the factual power changes in light scattered from the particles are measured. These variances are because of the irregular Brownian movement of the particles. Molecule sizes of the improved NLCs were controlled by light dissipating in view of laser diffraction utilizing Malvern Zetasizer (Nano sizer Z1700, Malvern Instruments, Inc) after appropriate weakening. investigation (n=3) were completed for 60 sec at room temperature at point of identification at 90°.The mechanical assembly comprised of a He-Ne laser (5 mW) and an example holding cell of 5 ml limit.

B. Zeta Potential:

Measurement of zeta potential has become inextricably connected with the study and characterization of colloidal dispersions, as this parameter is highly useful for the measurement of the physical stability of colloidal dispersions. However after the application of an electric field, particles move with velocity related to their zeta potential which is measured using technique called phase analysis light scattering and gets converted to the zeta potential by inbuilt software. For this measurement Malvern zetasizer (Nano sizer Z1700, Malvern Instruments, Inc) was used.

C. Entrapment efficiency (EE) and loading efficiency (LE) of BPO NLCs

The entrapment efficiency of BPO loaded NLCs was determined by centrifugation method. The NLCs dispersion was centrifuged for 45 min at 5,000 rpm (Thermolab). The supernatant was separated and was diluted suitably with methanol:acetone mixture and was determined by UV Visible spectrophotometric analysis at 234.8 nm. The entrapment efficiency and loading efficiency of BPO in NLCs were calculated according to the following equations:

$$\%EE = \frac{\text{total mass of BPO} - \text{mass } of \text{ BPO } in \text{ supernatant}}{\text{total mass of BPO}} \times 100$$

$$\%LE = \frac{\text{total mass of BPO} - \text{mass } of \text{ BPO } in \text{ supernatant}}{\text{total mass of lipid}} \times 100$$

4.3.4.2. *In vitro* drug release study of NLCs based BPO:

The study was completed utilizing a Franz Diffusion cell. Here, a cellophane layer already absorbed phosphate buffer (pH 5.5) was utilized. The acceptor compartment was loaded with 1 g of NLC dispersion. The acceptor compartment was loaded with 20 ml of Phosphate Buffer pH 5.5 and the permeation study was completed for 8 hours. At various time intervals from 0.5 to 8 hrs, 2ml aliquots from acceptor compartment were pulled back and properly weakened. After every withdrawal, the volume of receptor compartment was compensated by 2ml of fresh phosphate buffer pH 5.5. The temperature of asswmbly was kept steady at 370 ± 0.05°C and the volume of collector compartment was continuallystirred utilizing magnetic stirrer at 200 rpm. The centralization of drug in the pulled withdrawn samples was checked with the help of UV-Visiblespectrophotometer at wavelength of 234.8nm.

4.3.4.3 TEM anlaysis:[21]

The Particle Size (PS) was determined for the NLCs using TEM (TECNAI-G2, Philips). A drop of NLC was placed on a paraffin sheet and carbon coated grid was put on sample and left for 1 min to allow NLC to adhere on the carbon substrate. The remaining NLC was removed by adsorbing the drop with the corner of a piece of filter paper. Then the grid was placed on the drop of phosphotungstate (1%) for 10 s. The remaining solution was removed by absorbing the liquid with a piece of filter paper and samples were air dried and examined by TEM.

Results and discussion:

Table 4.20- Evaluation of preliminary batches from 15/028/001 to 15/028/015

Batch NO	PARTICLE SIZE (nm)	PDI	ZETA POTENTIAL (mv)
15/028/001	396 d.nm	0.645	-47.8 mv
15/028/002	354 d.nm	0.485	-55.2 mv
15/028/003	295.7 d.nm	0.589	-45.6 mv

15/028/004	557.8 d.nm	0.41	-18.5 mv
15/028/005	856 d.nm	0.38	-30.8 mv
15/028/006	246 d.nm	0.534	-17.5 mv
15/028/007	178.2 d.nm	0.6	-19.6mv
15/028/008	285.7 d.nm	0.454	-47.8 mv
15/028/009	237.6 d.nm	0.445	-55.2 mv
15/028/010	161.4 d.nm	0.384	-45.6 mv
15/028/011	240 d.nm	0.464	-21.3 mv
15/028/012	168.5 d.nm	0.357	-27.3 mv
15/028/013	397.7 d.nm	0.497	-45.5 mv
15/028/014	194 d.nm	0.632	-43.7 mv
15/02/015	405 d.nm	0.542	-53.7 mv

4.3.5 Optimization using Box-Behnken design:

Box-Behnken design was discovered by George box and Donald Behnken in 1960 for optimization purpose. It is type the response surface methodology design which is rotatable, independent and quadratic. It is mainly used for a spherical domain in which each factor takes 3 levels only. In this design each combination of extreme values of 2 variable is tested and remaining variables consider zero as coded value. Therefore they are subset of three level full factorial design.

Features of Box-Behnken Design:

- It requires fewer experimental runs and less time (3 factors, 3 levels, 15 runs).
- Providing more economical and efficient techniques than the conventional ones.
- Design is used to optimize and evaluate the main effects, interactions, effects and quadratic effects(non linearity effects)
- Design do not consist of axial points and also ensures that all factors are never simultaneously set at their highest or lowest level, so avoiding experiments performed under extreme conditions for which unsatisfactory results may occur.

Table 4.21- Table value of Box Behnken design

Independent variable	Coded value	Actual value
Amount of solid lipid:drug (GMS) X1	-1	2:1
	0	2.5:1
	+1	3:1
Amount of surfactant (Tween80) X2	-1	2%
	0	3%
	+1	4%
Homogenization time X3	-1	5min
	0	10min
	+1	15min

Table 4.22- Composition of batches F01 to F15 using Box-Behnken Design

Batches	X1	X2	X3	Size	Polydispersity index (PDI)	% EE
F01	3	4	10	330.6	0.369	67.6
F02	2	2	10	259.7	0.542	56.6
F03	2	3	15	241.3	0.460	45.3
F04	3	3	15	349.7	0.876	62.1
F05	2.5	3	10	160.2	0.627	80.3
F06	2.5	3	10	161.3	0.542	79.4
F07	2	4	10	207.4	0.465	52.7
F08	2	3	5	398.1	0.315	54.7
F09	2.5	3	10	168.7	0.485	80.2
F10	2.5	2	15	190.4	0.645	49.2
F11	2.5	4	15	262.3	0.512	47.3
F12	2.5	2	5	213.4	0.660	58.7
F13	3	2	10	384.9	0.516	73.2
F14	2.5	4	5	270	0.420	51.3
F15	3	3	5	397.2	0.497	71.9

RESPONSE OF FACTORIAL DESIGN:

1) Particle Size

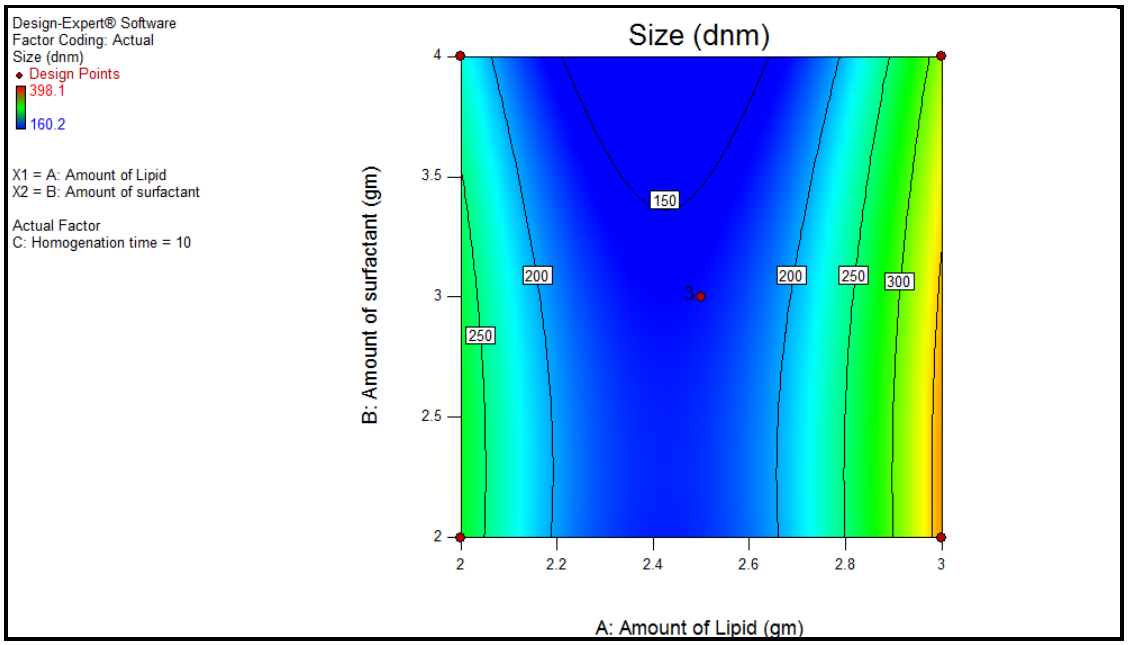

Figure 4.14- Contour plot for particle size:

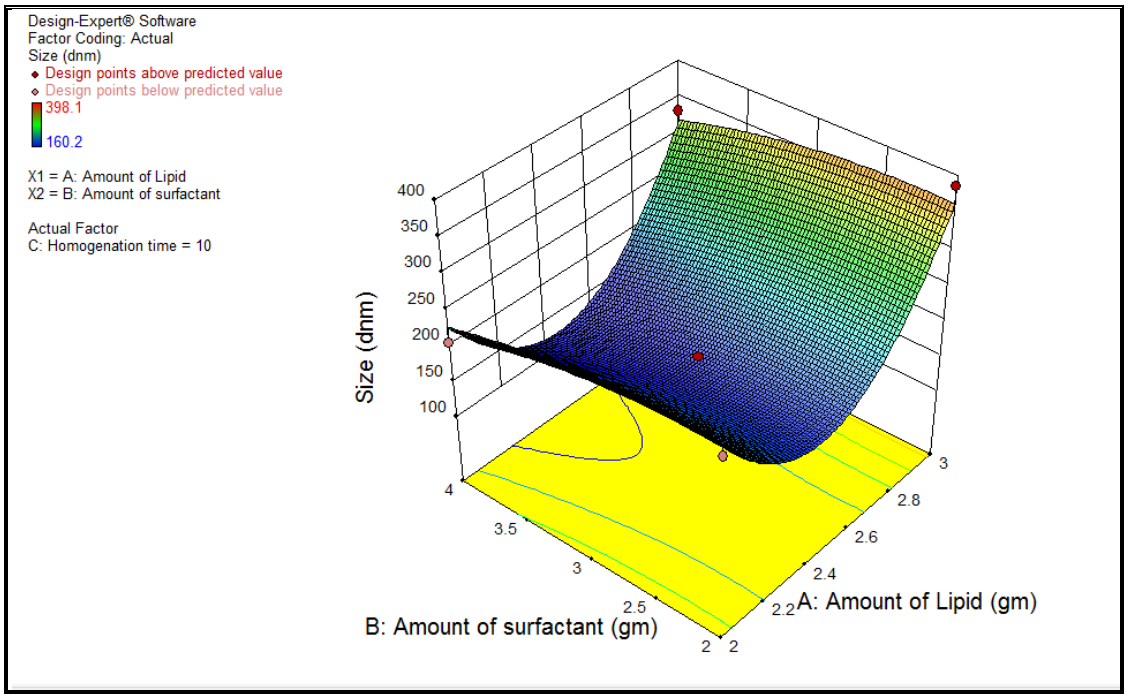

Figure 4.15- 3D Surface plot for particle size

Table 4.23- ANOVA table for particle size using QUADRATIC model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.152E+005	9	12795.91	9.93	0.0105	significant
A-Amount of Lipid	15833.10	1	15833.10	12.28	0.0172	
B-Amount of surfactant	3964.95	1	3964.95	3.08	0.1398	
C-Homogenation time	6903.13	1	6903.13	5.36	0.0686	
AB	1.00	1	1.00	7.757E-004	0.9789	
AC	2986.62	1	2986.62	2.32	0.1885	
BC	58.52	1	58.52	0.045	0.8397	
A^2	80221.88	1	80221.88	62.23	0.0005	
B^2	847.47	1	847.47	0.66	0.4543	
C^2	4725.60	1	4725.60	3.67	0.1137	

Table 4.24- Regression analysis for particle size

Parameter	Result
Std. dev.	35.90
Mean	253.01
% CV	14.19

Final polynomial equation in terms of actual factors: (Equation1)

Particle size = +4046.95000 - 295.32500*X1 + 63.48750*X2 – 64.11500*X3 - 1.00000*X1*X2 - 10.93000*X1*X3 + 0.76500 * X2*X3 + 589.60000*$X1^2$ – 15.15000*$X2^2$ + 1.43100*$X3^2$

From the polynomial equation generated, 3D surface plot and contour plots to study effect of variables on particle size, we can conclude that X1 had a positive effect on particle size (Y1), PDI (Y2), %EE (Y3). It was also observed that as lipid concentration iss increasing, viscosity of the dispersed phase is also increasing which

results in particle size agglomeration with higher size and PDI and decreased efficiency of homogenization. Increase in size and PDI along with higher %EE could be due to presence of higher amount of lipid which provides additional space for drug molecule to embed in thus decreasing its total surface area. This leads to reduction in the diffusion rate of the solute molecules as viscosity of the lipidic phase is high and thus showed higher %EE.

2) PDI:

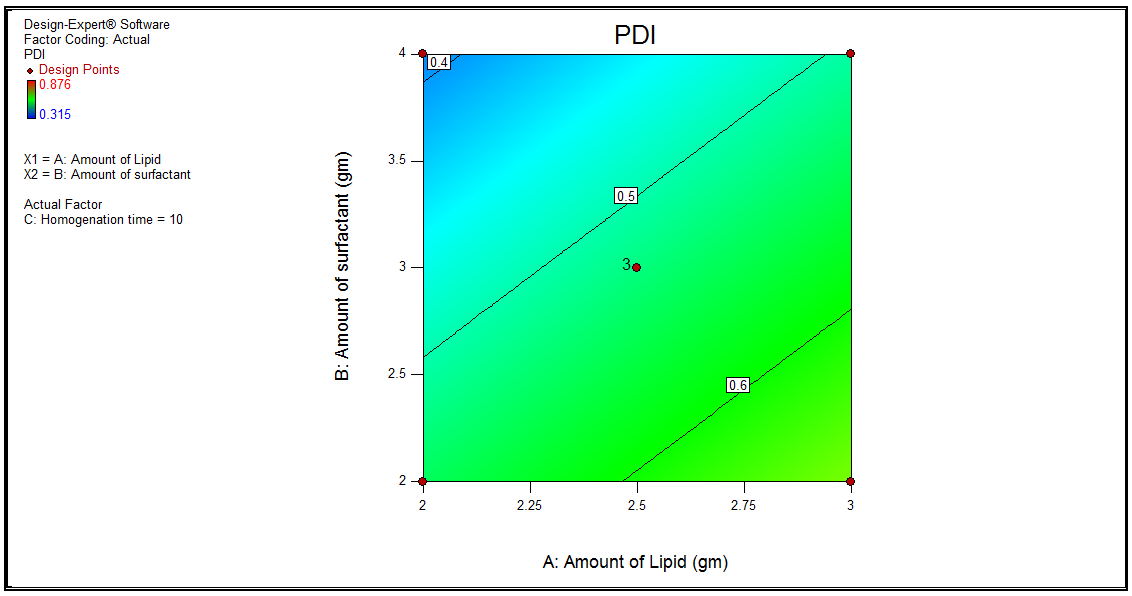

Figure 4.16- Contour plot for PDI:

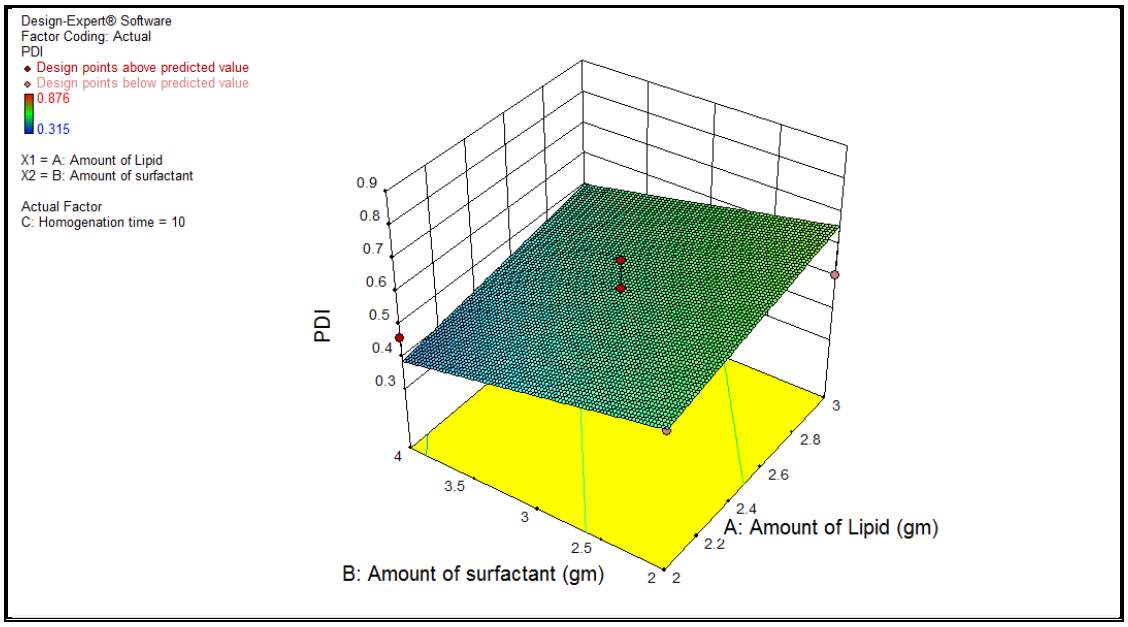

Figure 4.17- 3D Surface plot for PDI

Table 4.25- ANOVA table for PDI using Linear Model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.12	3	0.041	3.22	0.0651	not significant
A-Amount of Lipid	*0.028*	*1*	*0.028*	*2.18*	*0.1680*	
B-Amount of surfactant	*0.049*	*1*	*0.049*	*3.83*	*0.0763*	
C-Homogenation time	*0.046*	*1*	*0.046*	*3.66*	*0.0822*	

Table 4.26- Regression analysis for PDI

Parameter	Result
Std. dev.	0.11
Mean	0.53
% CV	21.39

Final polynomial equation in terms of actual factors: (Equation 2)

PDI = +0.31383 +0.11750*X1 -0.077875*X2 +0.015225*X3

From the polynomial equation generated, 3D surface plot and contour plots to study effect of variables on PDI, we can conclude that as concentration of tween 80 increases from 2% to 3% w/w, it indicates that reduction in interfacial tension of lipid and aqueous phase which may control the aggregation of lipid particle by facilitating the particle partition thus resulting into lower size and PDI. At higher concentration of surfactant lipid matrix will get stabilized by formation of steric barrier on the surface thus avoiding aggregation. Also with increase in the homogenization time from 5 to 10 min size and PDI was gradually decrease while at 15 min both size and PDI increased. Homogenization speed and time for which it is applied was one of the important strategy for applying kinetic energy for longer time that may lead to instability of formed lipidic structures thereby resulting into aggregation and formation of larger particles and PDI. Therefore an optimum homogenization will result into formation of stable particles with uniform size distribution and low PDI.

3) % Entrapment Efficiency:

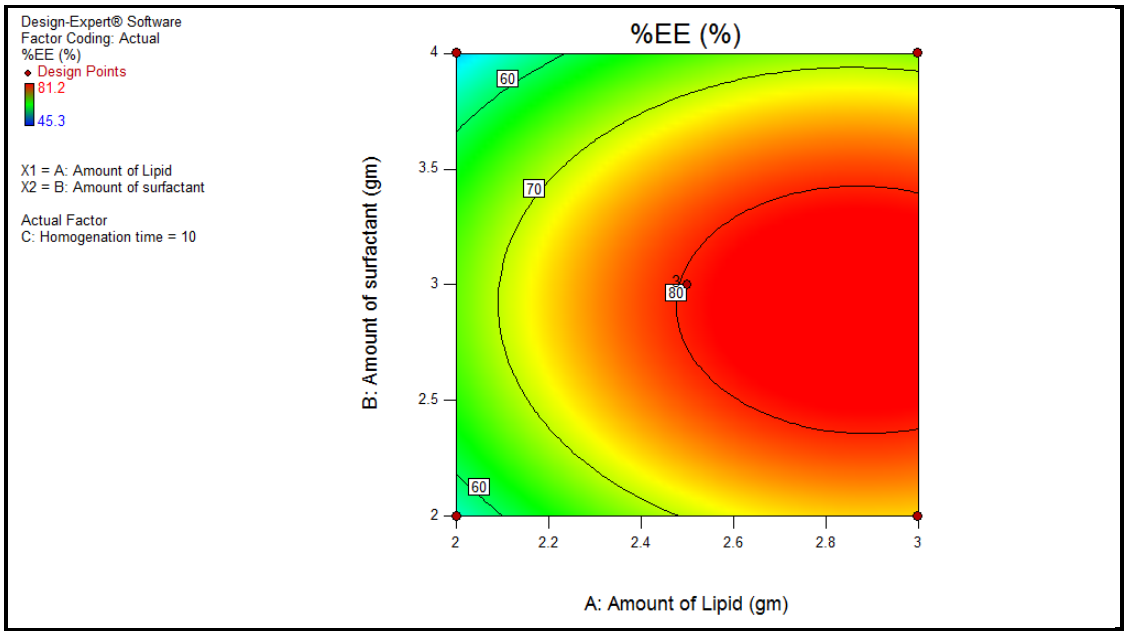

Figure 4.18- Contour plot for %EE

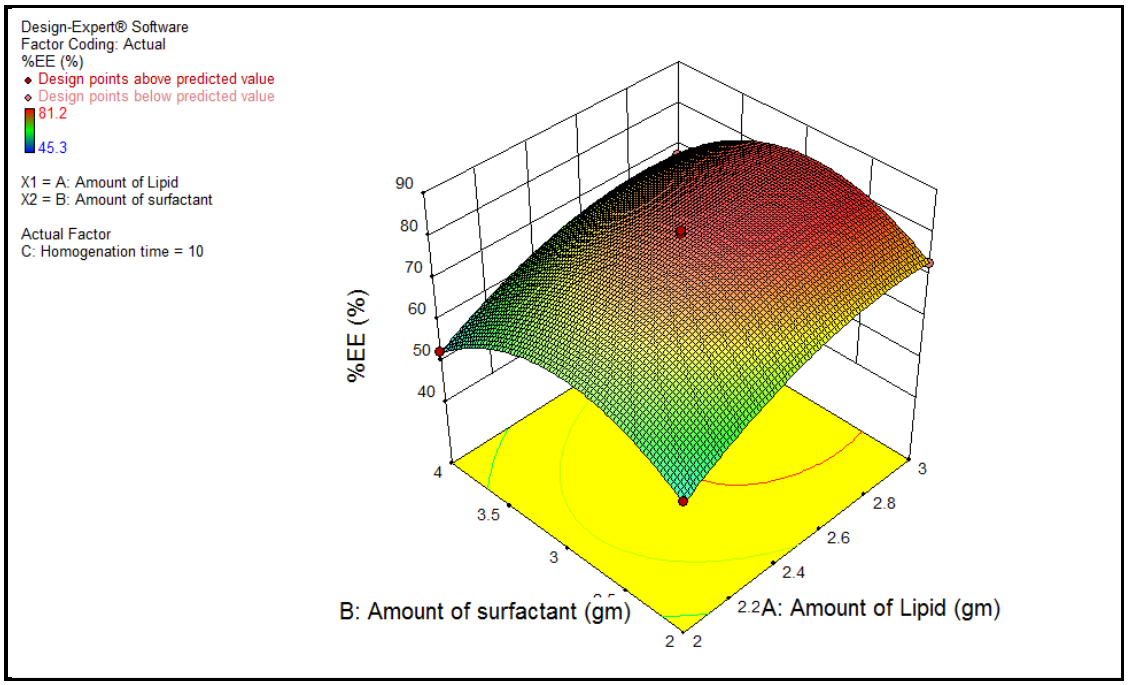

Figure 4.19- 3D surface plot for %EE

Table 4.27- ANNOVA table for %EE using Qudratic Model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2207.63	9	245.29	189.63	< 0.0001	significant
A-Amount of Lipid	536.28	1	536.28	414.60	< 0.0001	
B-Amount of surfactant	44.18	1	44.18	34.16	0.0021	
C-Homogenation time	133.66	1	133.66	103.33	0.0002	
AB	0.72	1	0.72	0.56	0.4885	
AC	0.040	1	0.040	0.031	0.8673	
BC	7.56	1	7.56	5.85	0.0603	
A^2	109.67	1	109.67	84.79	0.0003	
B^2	560.88	1	560.88	433.62	< 0.0001	
C^2	987.04	1	987.04	763.07	< 0.0001	

Table 4.28- Regression analysis for %EE

Parameter	Result
Std. dev.	1.14
Mean	62.10
% CV	1.83

Final polynomial equation in terms of actual factors: (Equation 3)

%EE = -257.11250 +128.32500*X1 +70.97500*X2 +11.53750*X3 -0.85000*X1*X2 -0.010000*X1*X3 +0.27500*X2*X3 -21.80000*$X1^2$ -12.32500*$X2^2$ -0.65400*$X3^2$

From the polynomial equation generated, 3D surface plot and contour plots to study effect of variables on %EE, we can conclude that, decrease in %EE leads to higher solubilisation effect produced by higher concentration of surfactant on benzoyl peroxide. At higher concentration of surfactant, solubility of benzoyl peroxide in external phase may increase due to diffusion of drug from lipid core into aqueous phase leading to reduced %EE. Also %EE was less in 15 min as compared to 10min. This was due to removal of surfactant particle from lipid surface thus causing lipid disruption and escape of entrapped drug into aqueous phase.

Data optimization:

In design expert software, graphical optimization was done by superimposing the critical response contours on contour plot. By doing the overlay plot that consists of two regions i.e yellow region that indicates an area of design space with feasible responses values and grey region indicating an area where response values did not fit the quality product criteria. On the basis of overlay plot the optimized batch was selected.

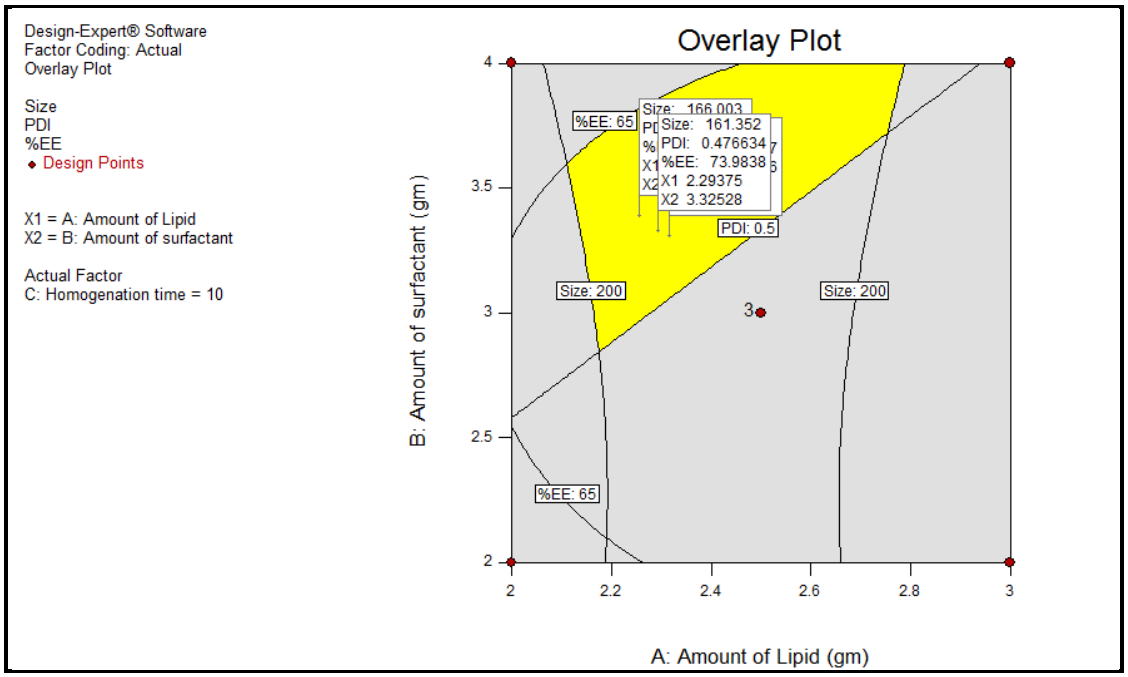

Figure4.20- Overlay plot

CHECK POINT BATCH:

Batch no.		V1	V2	V3
Composition	Solid lipid(GMS)	2.5	2.5	2.5
	Surfactant (Tween 80)	3%	3%	3%
Size	Expected value	161.352	166.3	153.72
	Observed value	165	162.5	160.2
PDI	Expected value	0.476	0.467	0.49
	Observed value	0.480	0.547	0.582
%EE	Expected value	73.98	72.35	74.73
	Observed value	72.80	74.52	70.3

Result and discussion: From design space randomly three points were selected to formulate check point batch. After evaluation it was observed that observed value was almost similar to the expected value which indicate that mathematical model used for optimization is validated.

OPTIMIZED BATCH FORMULATION OF NLC:

Table 4.30- Optimized batch

Ingredients	Quantity
Solid lipid (GMS)	2.5 gm
Liquid lipid (GMO)	500 mg
Drug BPO	1.63 gm
Transcutol	3 ml
Tween 80	3%
Propylene glycol	2%
Water	40ml

Evaluation Parameter:

Table 4.31- Optimized batch evaluation

Parameters	Result
Size	160.2 d.nm
PDI	0.582
Zeta potential	-26.7 Mv
Encapsulation efficiency	80.3%
Loading efficiency	29.83

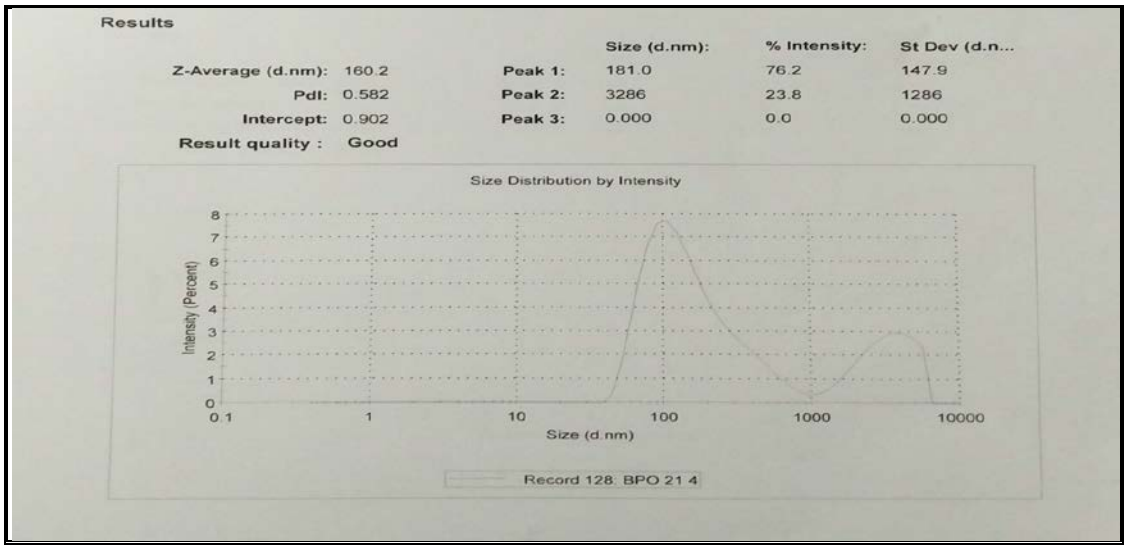

Figure 4.21- Partcle size and PDI of optimized batch

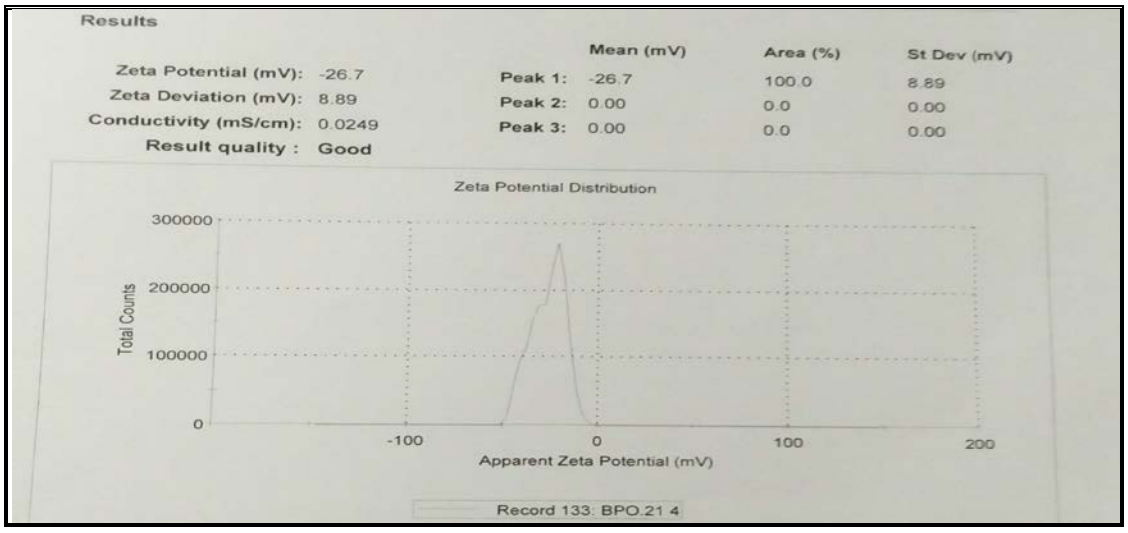

Figure 4.22- Zeta potential of optimized batch

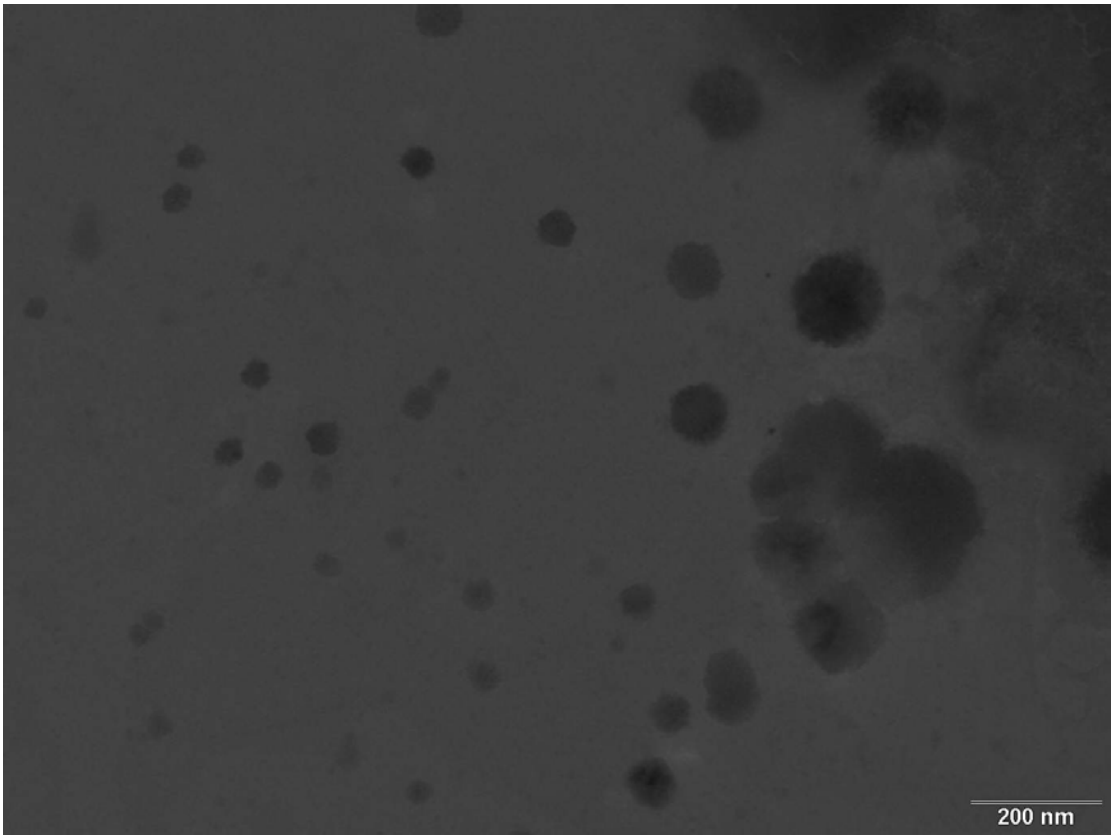

Figure 4.23 TEM image of optimized NLC of BPO

4.3.6- Devlopment and characterization of NLC based topical gel:

4.3.6.1- Formulation of NLC based gel:

Carbopol 980 was selected as a gelling agent due to its widespread use in pharmaceutical formulations and fast dispersion in water. Carbopol 980 was allowed to hydrate in sufficient quantity of water for 24 h at room temperature. Further, clindamycin phosphate was added to the Carbopol 980 dispersion and after that NLC containing benzoyl peroxide was added under magnetic stirring. The dispersion was neutralized with potassium hydroxide to obtain NLC based gel of Benzoyl peroxide and clindamycin phosphate) with 0.25, 0.5, 0.75, 1% concentration. The final NLC containg gel formulations contained 2.5% (w/v) Benzoyl peroxide and 1.2% clindamycin phosphate.

4.3.6.2 Characterization and optimization of NLC based gel of Benzoyl peroxide and clindamycin phosphate:

4.3.6.2.1 pH measurements

The pH is measured for each formulation using a pH meter, which was calibrated with buffered solutions of pH 4 and 7.38 before using it.

4.3.6.2.2 Rheological studies

The viscosity of the optimized formulation was determined at different angular velocities at 30 ±1 °C by using Brookfield Viscometer. A typical run involves by changing the angular velocity from 0.5 to 50 rpm at a controlled ramp speed with 10, 20 30 & 50 rpm. After 6 s at 0.5 rpm, the velocity was relatively increased to 50 rpm, with a similar period at each speed. The angular velocity was then decreased (50-0.5 rpm) for a similar period of 6 s. The average of two readings was used to calculate the viscosity of the formulation.

4.3.6.2.3 Texture analysis

Gel strength was determined using a Brookfield Texture Analyzer (USA) in compression mode. Different formulations were transferred into cylindrical holder (figure 4.24) and care was taken to avoid the introduction of air into the samples. A cylindrical analytical probe (38 mm diameter) is forced down into each sample at a defined rate (20 mm/min) and to a defined depth (10 mm). From the resulting load–time plots, the gel strength (the maximum force required to attain a given deformation i.e. peak load) and adhesive force (the work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe) was calculated.

Table 4.32- Various parameters of texture analyer

Test type	Compression
Trigger point:	5g
Target value:	10mm
Test speed:	20mm/min
Probe:	38 mm

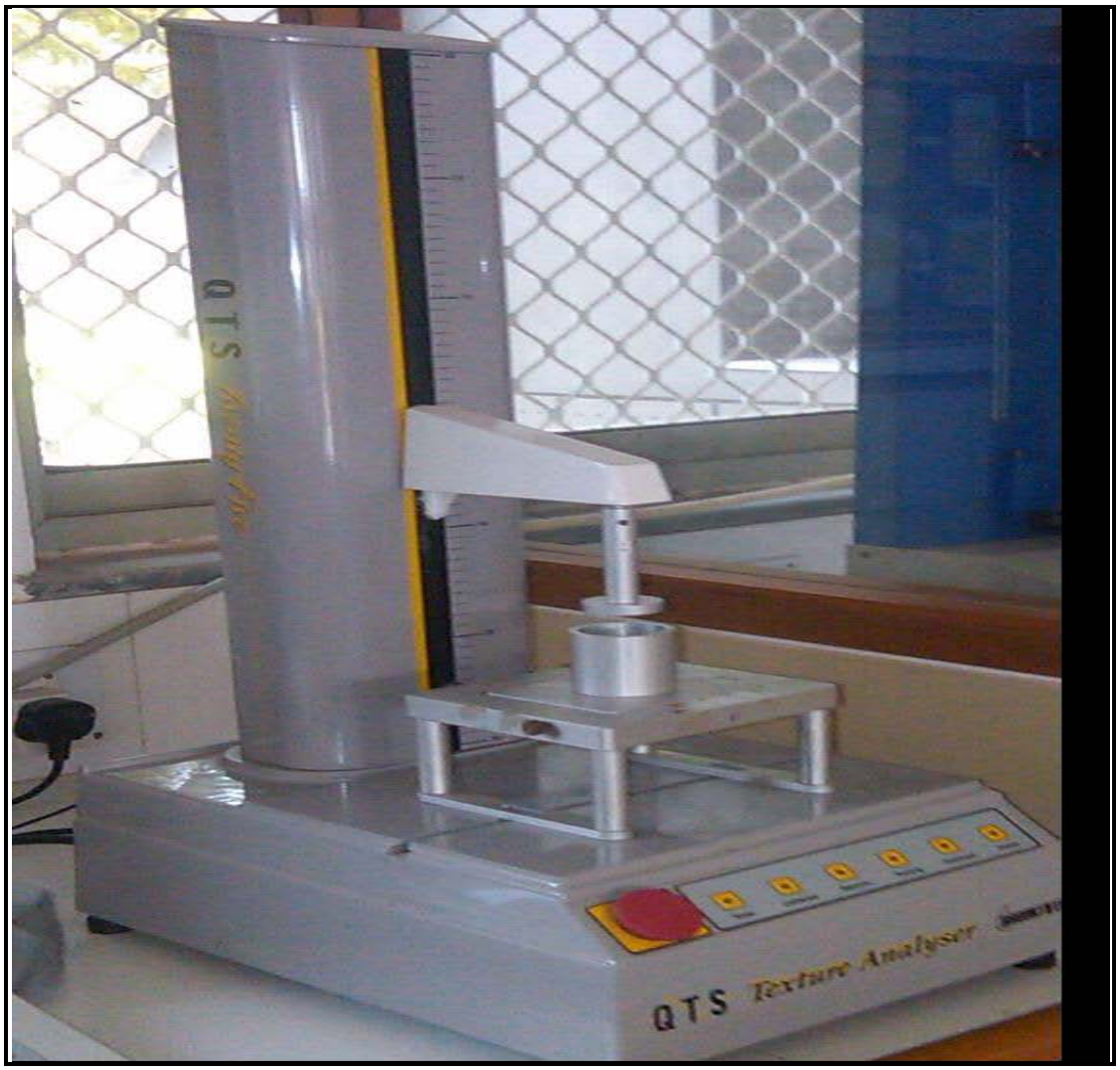

Figure 4.24- Brookfield Texture analyser

4.3.6.2.4- Stability studies:

According to International Conference on Hormonisation(ICH) guidelines, samples of NLC based gel was which was packed in Lami tubes and aluminium tubes were stored at 25°C/60% RH, 40°C/75% RH and at 2-8°C in a stability chamber for a period of 3 months. Assay, pH was performed (initial, 1M, 2M, 3M) after their preparation and comparison was done betwwn lami tubes and collapsible tubes

Results and Discussion:

Table 4.33- Composition of NLC based gel

Sr No:	Components	0.25%	0.5%	0.75%	1%
1	Clindamycin	1.21	1.21	1.21	1.21
2	NLC dispersion	46 ml	46 ml	46 ml	46 ml
3	Carbopol 980	0.25 gm	0.5 gm	0.75 gm	1 gm
4	water	Q.S 100ml	Q.S 100ml	Q.S 100ml	Q.S 100ml
5	KOH	0.5gm	0.5gm	0.5gm	0.5gm

Table 4.34- Evaluation parameters of gel

NLC based gel parameters	0.25%	0.5%	0.75%	1%
pH	5.46	5.52	5.56	5.60
Viscosity	20324	21520	24215	25917
Adhesive force	48	65	227	448
Gel strength	39	73	392	687

Table 4.33- Analytical findings in Lami Tubes

Sr. No.	Parameters	Analytical Findings			
		25°C/60% RH		40°C/75% v RH	2-8°C
		Initial	1M	1M	1M
1	Assay of BPO	100.5	100.2	99.9	100.8
	Assay Of CLP	99.8	99.2	100.2	99.1
2	pH	5.50	5.48	5.49	5.37

Table 4.34- analytical findings in Aluminium tubes

Sr. No.	Parameters	Analytical Findings			
		25°C/60% RH		40°C/75% v RH	2-8°C
		Initial	1M	1M	1M
1	Assay of BPO	100.5	101.2	100.5	101.7
	Assay Of CLP	99.8	98.9	100.8	99.9
2	pH	5.50	5.45	5.47	5.46

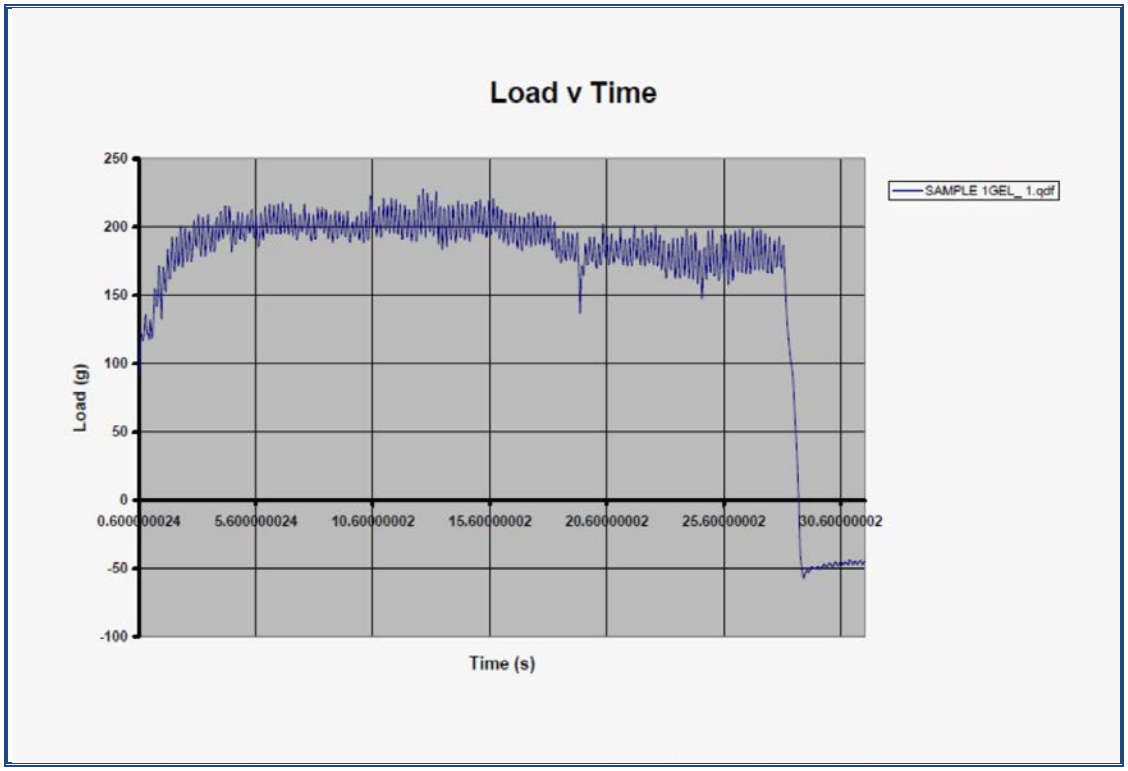

Figure 4.25- Texture graph of optimized NLC based gel

- Acne vulgaris is the most common skin disease, with 80 % of reported occurrence, of adolescents and young adults. Benzoyl peroxide is used to treat acne especially for the patients who are unresponsive to conventional anti-acne agents. Due to low water solubility & side effects associated with oral route, benzoyl peroxide and clindamycin phosphate was a right candidate to formulate it with a NLC based gel.
- Therefore, the objective was to design, develop and characterize the NLC based gel for topical delivery, to limit the side effects of drug improve the solubility & permeability of drug, for the treatment of acne.
- Liquid lipids, solid lipids and surfactant were selected on the basis of solubility of drug. Benzoyl peroxide showed higher solubility in GMS as solid lipids , Peceol as liquid lipids and Tween 80 as surfactant.
- .The influence of different co surfactants (Labrasol, Cremopher EL, Propylene glycol & PEG 400) on the formation of NLC was carried out. Therefore, propylene glycol was selected as co-surfactant.
- The “Box Behnken design” was used to optimize the NLC formulation and to obtain the relationship between the particle size distribution, PDI and %EE in the formulation. A polynomial equation was obtained and contour plot of response was plotted over given design space. An optimum formulation was selected on the basis of size in range of 100-200 nm. Hence the optimized batch of NLC was found with 3% tween80 2.5gm GMS and 2% propylene glycol. For optimized NLC, NLC based gel was developed using carbomer 980 and evaluated for Assay, pH, viscosity, gel strength, adhesive force.
- The pH of all the formulated NLCs based gel was near to skin pH.
- The present study was done with an aim to develop NLC for the treatment of acne in view to overcome the problems associated with conventional topical gels.NLC was found a suitable vehicle due to its excellent permeability in the gel and overcome the irritation problem as it release drug in controlled manner due to its encapsulating property.

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