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IN-VITRO CHARATERIZATION AND DEVELOPEMENT OF ACETRIN LOADEDNANO STRUCTURED LIPID CARRIERS FOR ENHANCED SKIN RETAINED TOPICAL DELIVERY FOR TREATMENT OF PSORIASIS.

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CHAPTER 1

INTRODUCTION

1.1PSORIASIS

The research and development efforts that shape today's drug delivery systems are mostly focused on developing novel medicine formulations and novel drug administration techniques. Determining the mechanisms by which drugs are delivered to their intended targets within the body has recently seen an increase in development. As a result, more patients are following their treatment regimens. The main objective of the current study, which attempts to create an improvised drug delivery system for Act, is to increase the local bioavailability of the anti-psoriatic pharmaceutical acitretin (Act).

Acitretin, also known as 13 cis-trans retinoic acid, is a psoriasis therapy drug licensed by the US Food and Drug Administration (June,1997) (Wiegand andChou, 1998).

The control of epithelial cell proliferation and differentiation, sebum production, and collagen synthesis are just a few of its favorable physiological effects that have garnered a lot of attention. Currently, only oral capsules are manufactured and sold.

The two main objectives of research into novel drug delivery systems are improved therapeutic benefit from already existing medications and the safe and efficient distribution of new therapeutics to meet the body's spatial and temporal requirements.

We refer to a colloidal drug delivery system (CDDS) as particle or vesicular dosing with a size range of 1 nm

to 0.5 m. It facilitates medicine targeting, which increases bioavailability and lowers drug loss and breakdown.

The percentage of drug accumulation at the therapeutic site increases, systemic side effects are greatly decreased, and harmful toxic effects are completely eliminated with this highly selective technique.

Acitretin, a medicine that effectively treats both psoriasis and acne, has lately showed promise when used topically rather than orally (orally, in the digestive tract).

Through this method, potentially fatal systemic adverse effects may be mitigated.

The safe and effective distribution of new treatments to satisfy the body's spatial and temporal requirements is one of the two main goals of research into novel drug delivery systems. The other is to increase the therapeutic benefit from currently available drugs.

With a size range of 1 nm to 0.5 m, we refer to a colloidal drug delivery system (CDDS) as particle or vesicular dosing. It makes medication targeting easier, which raises bioavailability and reduces loss and breakdown of the drug.

With this highly selective approach, the percentage of drug accumulation at the therapeutic site increases, systemic side effects are significantly reduced, and detrimental toxic effects are entirely eliminated.

Recently, topical use of the medication acitretin, which effectively treats both psoriasis and acne, has shown promise in comparison to oral (orally, via the digestive system) use.

Common psoriasis triggers include infections (such as streptococcus throat or skin infections), skin damage, stress, smoking, heavy drinking, a lack of vitamin D, and some medicines. Acne frequently appears on the shoulders, back, neck, chest, and upper arms.

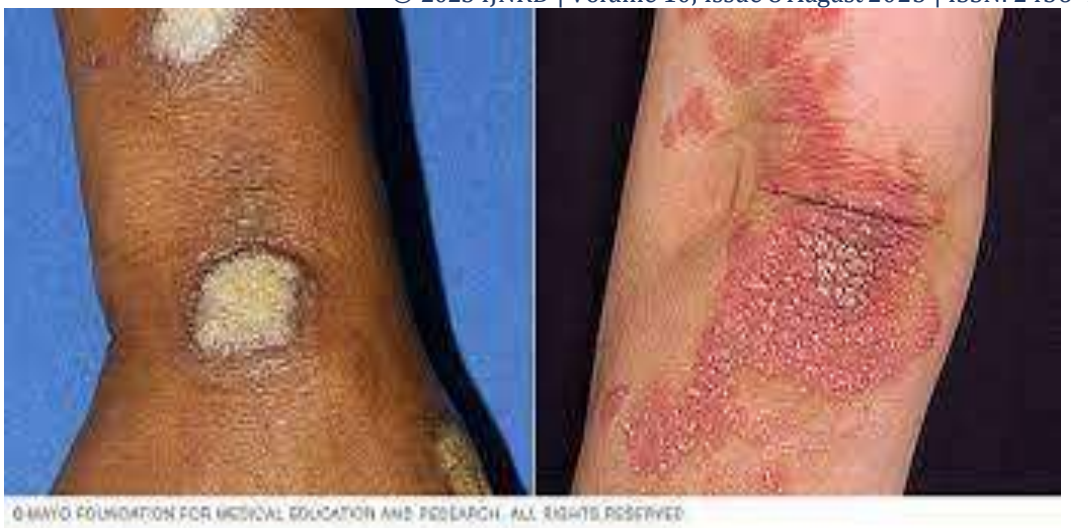
Acne arises when oil, dead skin cells, or bacteria clog skin pores. Acne outbreaks may be exacerbated by several medications, including testosterone and lithium, greasy cosmetics, hormonal changes, stress, and menstruation.

Going the topical route has enormous advantages, including:

1) Stay away from the body's first metabolic process the medicine can be applied and discontinued whenever necessary; patient compliance is increased.

2) Distributed without parenteral administration's risks.

3) Drug administration to a speciic site while preventing systemic absorption



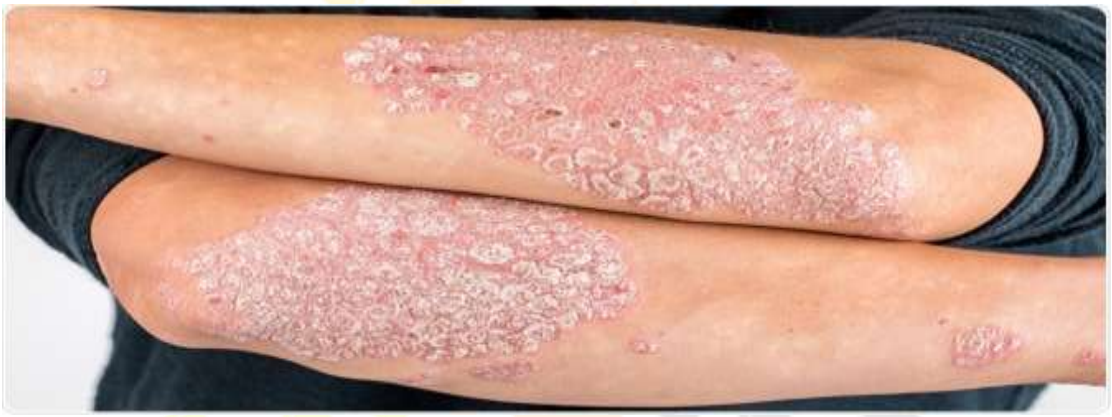


Figure 1: Different Images showing Psoriasis.

1.2 RESTRICTIONS OF PRESENT MARKET FORMULAS

The FDA in the USA has issued a black box warning for acitretin (Katz et al., 1999). The black box warning, which is the very worst thing a medicine can contain for your health, is that. The medication comes with the following warnings (Katz et al., 1999):

Teratogenicity: The teratogenic effects of retinoids are a defining feature of hypervitaminosis A. Hip deformity, meningomyelocele, meningoencephalocele, multiple synostoses, and craniofacial dysmorphisms including a high palate and anophthalmia are a few of the teratogenic consequences.

Hepatotoxicity: Acitretin increases levels of lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate transaminase (AST), and other liver serum enzymes.

Ophtalmologic repercussions are adverse effects on the eyes, such as dryness and irritation. Some people might not be able to wear contact lenses while undergoing treatment due to these side effects.

In patients with pancreatitis, triglyceride levels rise during acitretin therapy. Hypertriglyceridemia is more likely to occur in patients with diabetes mellitus, obesity, heavy alcohol consumption, or a family history of these conditions.

Rarely, systemic retinoids like acitretin have been related to a condition known as pseudotumor cerebri, also known as benign intracranial hypertension, which can be controlled by dietary adjustments along with a reduced dose of acitretin. If papilledema is suspected, it is important to check for it as a way.

1.3 DIFFERENT OTHER SIDE EFFECTS CAN INCLUDE:

- Dry, cracked lips; peeling skin on the palms, soles, and fingers
- Scratchy, flaky skin all over the body
- Thin nails
- Skin that is either very sticky or extremely delicate
- Signs include: a stuffy or dry nose, or nosebleeds
- Hair loss, itchy eyes, and dry lips
- Stiffness, soreness, and aching joints and muscle
- Cholesterol Problems

1.4 CLINICAL EXPRESSIONS OF PSORIASIS

Plaque-type psoriasis

Plaque-type psoriasis, or psoriasis vulgaris, is the most common form, occurring in 75% to 80% of all psoriasis patients. When fully developed, the lesion is a well-demarcated, red-violet, round or oval plaque 1 cm or larger in diameter surmounted by white silvery scales, overlying bony prominences (FIGURE 1). In darkly pigmented patients, lesions are hyperpigmented with various shades of brown or black, especially if the patient scratches or rubs them. The most commonly involved areas are the elbows, knees, scalp, sacrum, umbilicus, intergluteal cleft, and genitalia, and all of these areas should be examined: any one of them may be the solitary site.

Guttate psoriasis

Guttate psoriasis, named for its droplet-shaped lesions (FIGURE 2), accounts for about 18% of all cases, more commonly among children and young adults.5 Guttate lesions range in diame ter from 0.1 to 1.0 cm and are not as indurat ed or scaly as the lesions of plaque-type psori asis. They predominate on the trunk and prox imal areas of the extremities and are likely to involve the face. Guttate psoriasis may be the initial mani festation of psoriasis, or it may represent an acute flare of preexisting chronic plaque-type psoriasis. Patients frequently have a history of upper respiratory tract infection, laryngitis, or tonsillitis.

Streptococci and guttate psoriasis.

Some cases of acute guttate flares are believed to have been precipitated by infection with groups A, C, and G streptococci.6 Leung et al7 showed that acute guttate psoriasis follow ing streptococcal throat infection in 10 patients was the result of streptococcal exotoxin C, which acts as a superantigen and activates CD4+ and CD8+ T cells in the lesions and the areas around them. Researchers hypothesize that these T cells persist in the skin of patients who go on to develop chronic plaque-type psoriasis, because the T cells mistakenly recognize skin autoantigens such as keratins and carbohy drates as bacterial antigens.

Pustular psoriasis

Pustular psoriasis accounts for perhaps 1.7% of cases.4 It is characterized by sterile pus tules either localized to the palms and soles or generalized. The average age at onset is 50 years. Localized pustular psoriasis. The erup tion is chronic and recurring and is recogniz able by yellowish pustules on a background of redness and scaling on the palm (thenar and hypothenar eminences) or on the instep of the sole and side of the heel (FIGURE 3), or on both areas.

Lesions are observed in all stages of development, including vesicles, vesico pustules, frank pustules, and dried brown maculopapules. There is a female predomi nance.

Generalized (von Zumbusch) pustular psoriasis

It may develop de novo or from preex isting plaque-type psoriasis and is character ized by fiery-red, irregular patches with round, arcuate, serpiginous borders, over which are seen tens of thousands of 1-mm to 2-mm superficial pustules (FIGURE 4). These tend to occur in flexural or skin-fold areas (armpits, groin, under the breasts) but may occur any where. The pustules coalesce into lakes of pus, desquamate, and form new pustules as the bor der moves in waves every 24 to 72 hours. Most patients have fever, leukocytosis, hypocal cemia, and hypoalbuminemia.

The incidence is equal in men and women. Ryan and Baker8 reported that 37 (24%) of 155 patients had their first attack of generalized pustular psoriasis within 1 month of either starting or stopping systemic corticosteroids, and they concluded that the steroids provoked the attacks. Other precipitating factors includ ed infection and other drugs. Methotrexate was less effective in patients who previously received systemic corticosteroids. Ohkawara et al9 recently reviewed 208 cases of recurrent generalized pustular psoria sis and found that cases in patients with pre ceding plaque-type psoriasis were more likely to have been triggered by corticosteroids, whereas cases in patients

without a history of psoriasis were more likely to have been trig gered by infection.

1.5 PSORIASIS THERAPY: AN OVERVIEW

Treatments for psoriasis are divided into five levels: The choice of treatment depends on the severity and response in the individual patient.

Level 1:

Topical treatments Emollients (bland lubricants) should be tried first, followed by keratolytic lotions. Topical corticosteroids and calcipotriene ointment or cream can be used by internists for psoriasis involving less than 20% of the body surface area. Most non-dermatologists would probably not use anthralin, crude coal tar, or tazarotene, as these are inelegant (messy, inconvenient to use, and with a bad odor) and have a high irritation potential; coal tar gels, often available over the counter, are more elegant though less efficacious.

Level 2:

Phototherapy All forms of phototherapy are highly effective (80% to 100%) at clearing skin, but some maintenance is necessary. Disadvantages are the requirement for specialty care, the need for office visits two or three times a week, expense, maintenance therapy, theoretical short-term risks of sunburn, and long-term risk of skin cancer.

Beyond natural sunlight and tanning beds, phototherapy is given by dermatologists in the office or clinic and is reserved for patients with widespread lesions involving 20% or more of the body surface area. Specialized equipment and training is neces sary for delivery of phototherapy.

Level 3:

Systemic treatments Systemic treatments are more effective than level 2 treatments but are often more expensive and have greater potential for toxicity. (In general, topical therapies are less toxic than phototherapies, which are less toxic than systemic therapies.) Systemic treatments are generally prescribed only by a dermatologist. Levels 4 and 5: Experimental treatments Treatments in level 4 and level 5 are not approved by the US Food and Drug Administration. Level 4 treatments are weakly to moderately effective but are less toxic than level 5 treatments, which are reserved for the most severe and recalcitrant cases, including arthropathy.

1.6 Management of severe psoriasis

The management of patients with psoriatic erythroderma or pustular psoriasis includes admission to the hospital for evaluation, sup portive care, and conservative treatment until the patient is stable enough to receive aggres sive, conventional antipsoriatic therapy. Severe psoriasis can usually be controlled eventually with the standard regimen of ultra violet B light plus coal tar, or with psoralen ultraviolet A light therapy, acitretin, metho trexate, or cyclosporine. The disease reverts back to its previous state (either plaque-type psoriasis or clear). Systemic and potent topi cal corticosteroids are to be avoided. A minor ity of patients remain unstable and have repeated episodes of

erythroderma or pustula tion during their lifetime.

1.7 EXPERIMENTAL TREATMENTS FOR PSORIASIS

Vitamin D3 and analogs

Vitamin D3 (calcitriol) and the synthetic analogs calcipotriene and tacalcitol, all in top ical form, are effective for plaque-type psoria sis and are free of any serious toxicity. 24 In double-blind studies, patients applied cal cipotriene to one side of their body and corti costeroids to the other side. Calcipotriene was equivalent to or better than medium and potent corticosteroid ointments without the risk of skin atrophy or rebound flare-ups upon discontinuation of therapy. In open trials,25 patients taking oral calcitriol showed dramat ic improvement but experienced significant hypercalcemia, hypercalciuria, and nephro toxicity. No placebo-controlled study of oral calcitriol alone has been performed. However, oral calcitriol had no additive effect compared with placebo when combined with 21 erythe mogenic ultraviolet B treatments.

Hydroxyurea in recalcitrant psoriasis

The antimetabolite hydroxyurea plays a minor role in the treatment of recalcitrant psoriasis. In one trial,27 60% percent of patients achieved "complete to near complete clear ing" with a starting dose of 1.5 g daily and a maintenance dose ranging from 0.5 to 1.5 g daily. Adverse reactions are common, notably cytopenia and macrocytosis. Hydroxyurea is not effective for pustular or erythrodermic psoriasis or psoriatic arthritis.

Thiopurine antimetabolites

Thiopurine antimetabolites—ie, azathioprine, mercaptopurine (6-mercaptopurine, 6-MP), and thioguanine (6-thioguanine, 6-TG)—are used as immunosuppressive and steroid-spar ing agents for many diseases. All inhibit DNA synthesis. Azathioprine is metabolized to 6 MP, which is then converted to 6-MP ribotide. In a study of azathioprine in 29 patients with severe plaque-type psoriasis as well as ery throderma and pustular variants, 19 (66%) of 29 benefited with 50% to 100% improvement on doses of 75 to 200 mg/day.28 The main side effects are gastrointestinal and hematologic. In a study of 6-TG in patients with recal citrant plaque-type psoriasis, marked improve ment occurred in 50% to 70% of patients treated with a pulse-dosing schedule of 120 mg twice weekly to 160 mg thrice weekly.29 Palmoplantar pustulosis and generalized pus tular psoriasis may also respond very well to 6 TG.30 The response is usually evident within 12 weeks of starting therapy. The chief toxici ty of 6-TG is myelosuppression.

Mycophenolic acid

Mycophenolic acid inhibits de novo synthesis of guanosine nucleotide, thereby selectively suppressing proliferation of T and B lympho cytes.31 Double-blind placebo-controlled trials confirmed its efficacy in psoriasis (68% decrease in mean severity scores) after 12 weeks, and patients were subsequently treated with 1.6 to 4-8 g/day for 2 years.32 The main side effects are gastrointestinal and genitouri nary. The incidence of herpes zoster was increased. The

sponsor discontinued the national clinical trial in 1977.

Sulfasalazine steroid-sparing agent

Sulfasalazine is inflammatory bowel disease and is also a second-line therapy for rheumatoid arthritis and ankylosing spondylitis. It may have anti -inflammatory activity in these diseases and psoriasis by inhibiting 5-lipoxygenase. In a double-blind study,33 17 patients with psoriasis received sulfasalazine 3 to 4 g/day. Of these, 7 had a marked response, 7 had a mod erate response, and 3 had a minimal response. Side effects (anorexia, nausea, vomiting, fatigue, headaches, cutaneous eruptions) are common and, while usually not severe, frequently lead to discontinuation of treatment before any therapeutic benefit can occur. The incidence of drug-induced rash may be as high as 18%.

1.8 GENERAL HALLMARKS OF PSORIASIS

Psoriasis is a single disease with several morphologic expressions and a full range of sever ity. The form that psoriasis takes in an individual patient likely depends on genetic influences, environmental factors (eg, trau ma and climate), associated diseases (espe cially infections), medications, and immuno logic status. The mean age at onset is 30 years, but the range is the full spectrum of human life. Men and women are affected equally, but women may be affected earlier than men.

The severity ranges from a single fingernail pit to skin lesions covering the entire body surface and disabling arthritis. The most common pattern is symmetric, inflammatory, and papulosquamous. The classic skin lesions—thick, erythematous, itchy patches covered with silvery scales— usually confirm the diagnosis, but examina tion of scales using potassium hydroxide to look for hyphal elements (ie, fungal infec tion), serologic testing to rule out syphilis, and skin biopsy to rule out other inflammato ry skin diseases such as eczema, pityriasis

1.9 Pathogenesis

Psoriasis is a hyperproliferative skin disease with increased rate of epidermal turnover. The pathogenesis of psoriasis is linked to various cellular mechanism and the role of T cells, antigen presenting cells (APCs), keratinocytes, Langerhans cell, macrophages, natural killer cells, an array of Th1-type cytokines, as well as certain growth factors like vascular endothelial growth factor (VEGF), keratinocytes growth factor (KGF), etc., have been suggested to play a key in the pathogenesis of psoriasis. Psoriasis is an immunologically mediated disease, the activation of T lymphocytes leads to the inflammation in the dermal component and secondary to the inflammatory events there is also that epidermal hyperproliferation.

Various mechanisms are hypothesized to be involved in the pathogenesis of psoriasis:

- T cell function
- Role of dendritic cell
- Hyperproliferation of keratinocytes
- Angiogenesis

- Cytokine mediators
- Reduced apoptosis
- Genetic factors
- Role of oxidants and antioxidants in psoriasis

1. T cell function

T lymphocytes consist of a functionally distinct population of helper T cells and cytolytic T cells. The principal function of T cells is to recognize the processed peptide antigens that are attached to proteins encoded by the MHC class II genes. Therefore, for activation, T cells need APCs to process and present peptide fragments on the APC cell surface. T cells secrete various lymphokines. T cells may also inhibit immune responses; in this role, these are known as suppressor T cells. Distinct cell membrane proteins are expressed by different populations of T cells. CD4 positivity is shown by most of the helper T cells while cytolytic and suppressor cells are CD8 positive. Activation of T cells requires three steps:[12] a. Binding b. Antigen-specific activation (signal 1) c. Non-antigen-specific cell-cell interaction (signal 2)

2. Role of dendritic cells

Dendritic cells serve as a major class of antigen presenting cells found in increased abundance in psoriatic skin lesions.Langerhans cells are a type of immature dendritic cell (iDC) found in normal epidermis and can also be found in psoriasis lesions. iDCs are derived from blood monocytes or other myeloid precursors and have a immunostimulatory role. These iDCs are further stimulated to become mature DCs (mDCs). Psoriasis lesions show a marked increase in dermal DCs. XIIIa and CD11c are expressed by myeloid DCs or iDCs, and CD83 and DC-LAMP proteins are positive for mDC.

3. Hyperproliferation of keratinocytes

The skin provides a protective mechanism through its multilayered structure. The epidermis consists of five layers, stratum basale, stratum spinosum, stratum granulosum, stratum lucidium, and stratum corneum. Mainly the keratinocytes are formed in the stratum basale and further they migrate towards the stratum corneum. As cells move toward the surface, their organelles disappear and are filled with keratin. The topmost layer of keratin provides a protective feature. In normal conditions the epidermal cell cycle is completed in about four weeks. But in psoriatic skin, the epidermal cell cycle is accelerated. Cell division in the basal layer occurs every 1.5 days, and the migration of keratinocytes to the stratum corneum occurs within approximately 4 days. This results in hyperproliferation of keratinocytes.

4. Angiogenesis

Keratinocytes produce proangiogenic cytokines (VEGF, IL-8), but the precise mechanism for angiogenesis in psoriasis is still unknown. In psoriasis the endothelial cells swell and become activated these activated endothelial cells migrate, sprout, and lay down a basement membrane with pericytes for structural support to form novel vessel

networks.[15] This results in widening of the intercellular spaces, and hence, dermal blood vessels dilate thus making it easier for leukocytes to migrate into the skin.[16]

5. Cytokine mediators

In psoriasis, the production of cytokines result in epidermal hyperproliferation, vascular dilatation, and dermal inflammation. The cytokines involved in the development of psoriasis include granulocyte–macrophage colony stimulating factor (GMCSF), epithelial growth factor (EGF), IL-8, IL-12, IL-1, IL-6**, IFN-γ, and TNF-α.**These cytokines result in keratinocyte proliferation, neutrophil migration, potentiation of Th1 type of responses, angiogenesis, upregulation of adhesion molecules, and epidermal hyperplasia.

7. Reduced apoptosis

In order to maintain a constant thickness of the epidermis proliferation of keratinocytes in normal epidermis is regulated by apoptotic cell death. The epidermal hyperplasia characteristic of psoriasis is suggested to be due to P53 overexpression and these proliferating cells typically express Bcl-2 that protects them against apoptotic stimuli, while terminally differentiated cells lose Bcl-2 expression.

Psoriasis and the Eye

Psoriasis and the eye for patients with psoriasis, uveitis had been commonly thought to occur only in conjunction with psoriatic arthritis, however, there have been many case reports of psoriatic uveitis presenting independent of joint disease. Furthermore, the temporal relationship of these two entities has been disputed. Some recent studies suggest that inflammatory joint manifestations precede uveitis. Nevertheless, some cases of uveitis have been reported to occur even before psoriatic skin disease, and uveitis has been reported as the first presenting sign of psoriatic arthritis in 0% to 11.4% of cases. The severity of ocular inflammation does not necessarily correlate with extent of joint findings but may correlate with skin disease.

Since the latency period for development of symptomatic ocular abnormalities may be longer than 5 years, continued surveillance and continued use of appropriate ocular protection by all patients treated with PUVA is indicated.

1.10 Histopathology

Histopathologic findings in psoriasis vulgaris vary with the age and dynamics of the lesions. The earliest changes are nonspecific and consist of spare superficial perivascular T-lymphocyte infiltration with neutrophil exocytosis, followed by dermal edema and dilation of blood vessels within dermal papillae. The early stages of plaque formation further show epidermal hyperplasia, parakeratosis aggregates, and infiltration with lymphocytes, histiocytes, and neutrophils in the dermis.

Mature plaques show important epidermal hyperplasia with characteristic features such as uniform elongation of the epidermal rete ridges and enlargement of their tips; dilated tortuous capillaries and fibrillary collagen (reciprocal elongation of dermal papillae); thinning of the suprapapillary plate; pallor of the superficial epidermis and minimal

spongiosis, marked hyperkeratosis, parakeratosis, and subjacent hypogranulosis; and collections of neutrophils within the parakeratosis, specifically Munro's micro abscesses and less frequently within the spinous layer—spongiform pustules of Kogoj .

Histopathological and immunohistochemistry analysis may be a useful tool for decoding physiopathological mechanisms in psoriasis, such as the recruitment of circulating lymphocytes to inflammatory sites and their Lymphocyte Function-Associated Antigen 1 (LFA-1) dependent adhesion stimulated by endocan . The cytotoxicity of CD8+ T cells and Natural Killer (NK) cells has also been found to be mediated by the perforin/granzyme pathway, explaining why perforin expression is upregulated in activated CD8+ T cells, which are abundantly expressed in the epidermis of psoriasis plaques. Further, Simonetti et al. identified key factors in the link between inflammation and angiogenesis, specifically Vascular Endothelial Growth Factor (VEGF).

They found that Hypoxia-Inducible Factor 1-alpha (HIF-1α) and Matrix Metallopeptidase 2 (MMP-2), which control the rate of *VEGF* gene transcription and play an important role in the remodeling of endothelial cell membranes, were both positively correlated with VEGF.Moreover, different immunohistochemical studies found a correlation between psoriasis severity and Smadand Plexin-B2 expression. Smad 7 and Plexin-B2 are both activators of keratinocyte proliferation that act through distinct mechanisms.

Firstly, the CD-100-Plexin-B2 complex activates NF-κB and NLRP3 inflammasome in keratinocytes , while Smad7 inhibits the TGF-β1 signaling pathway, which normally directly inhibits keratinocyte growth (inhibitor of the inhibitor). Lastly, immunohistochemistry reveals new targets for the development of therapeutic agents. It was found that the Fib3 antibody blocked the Fib3 function of promoting VEGF production, keratinocyte proliferation, and migration .

1.11 REASONS WHY SOLID LIPID NANOPARTICLES SHOULD BE USED

Since acitretin is a BCS class IV drug, its solubility profile is poor. Topical administration also causes decreased absorption through the skin. A diffusion barrier for the majority of substances is also provided by the stratum corneum, which is composed of corneocytes embedded in a lipid matrix. To enable skin penetration and the desired therapeutic impact, acitretin must be enclosed inside a lipid nanoparticle system.

Because of their improved drug delivery and stability, solid lipid nanoparticles (SLNs) have attracted a lot of attention as a novel type of nanoparticulate carrier system. SLNs are made of biocompatible lipids and are covered in an amphiphilic surfactant (Muller et al., 2007). They function better than liposomes, polymeric nanoparticles, and fat emulsions (Jain et al., 2014). According to Sala et al. (2018), SLN has advantages in terms of biocompatibility, good stability, release of drug control, availability of scalability, and sterilising method.

By offering higher protection against chemical drug degradation, they circumvent the limitations of liposomes

and other carriers (Soppimath et al., 2001; Garg et al., 2012; Jain et al., 2010). Other advantages of SLNs are their biocompatibility and possibility for scaling and sterilisation. These systems might also be suitable for manufacturing because they require no or very little organic solvents (Mühlen et al., 1998). The most desired characteristic of a topical formulation is possessed by SLNs since they do not cross the transdermal barrier and remain in the epidermis and dermis. (Jenning et al., 2000(b); Maia et al., 2002) provide evidence for this.

In order to administer medication subcutaneously, lipid nanoparticles (SLNs) are used as the delivery system. The SLN-loaded gels are an appealing method of administering medication since they are straightforward to use and widely accepted. These gels are incredibly dependable and simple to use. Such systems may easily include pharmaceuticals, both hydrophilic and hydrophobic, increasing skin permeability and drug deposition.

Gels like this one increase the stability of formulations by strengthening a rigid network. These gels also prevent the skin from drying out and peeling. These gels' thixotropy, grease lessness, ease of spreading, emollience, absence of discoloration, extended half-life, stability, and aesthetically pleasing appearance make them superior to others. The transparent gels of Carbopol 934P are what make it the best for gelling solid lipid.

1.12 TOPICAL DRUG DELIVERY SYSTEM

Topical drug delivery devices are used to give medications directly to the skin. The drug is intended to permeate numerous skin layers. The largest organ in the body, the skin serves as a barrier to protect the body from the outside environment. The epidermis and dermis, the two outermost layers of skin, can both be reached by a medication when it is applied directly to the skin. Acitretin is a superb option for drug delivery through topical application because to its brief biological half-life, restricted oral bioavailability, and different dose-dependent adverse effects. In the usual formulations, only oral capsules are offered, and they have serious systemic side effects. Therefore, several drug delivery techniques are used to get around Topical drug delivery devices are used to give medications directly to the skin.

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Therefore, several drug delivery techniques are used to get around limitations of traditional dosage forms.Nanoparticles (Shah et al., 2007), chitin nanogel systems (Divya et al., 2016),liposomes (Singh et al., 2009), niosomes (Hashim et al., 2018), ethosomes (Jain etal., 2007), nanoemulsions (Sarkar, 2005), nanostructured lipid carriers (Agarwal etal (Benson, H.A, 2005). In this age of regulated and targeted medication delivery, acarrier based approach for topical drug administration is rapidly evolving. Giventhat they avoid direct contact with the skin, these carrier-based devices have a greatdeal of promise as a targeting tool.

All of these methods have the potential toimprove the efficiency of the medicine by increasing its skin penetration in avariety of species that have been carefully characterized.

Since acitretin is extremely lipophilic, it may be easily encapsulated in a lipid- based delivery system. The outcome will be increased systemic bioavailability and fewer adverse effects.

The following are some of the benefits that may be gained by using topical drug delivery:

Since topical administration enables self-application of the medication, it improves patient compliance and prevents the active ingredient from being metabolised in the body's first metabolic step.

The patient has the right to cease taking medication at any time.

It improves the therapeutic effectiveness of the medication by preventing phenomena such as first-pass metabolism, gastrointestinal (GI) irritation, gastrointestinal (GI) breakdown, and insufficient absorption.

1.13 JUSTIFICATION OF TOPICAL DRUG ADMINISTRATION

In order to solve the biopharmaceutical issues, we may take one of two main steps.

Helping or altering the skin's barrier function is the initial step. Topical antibiotics aid in repairing a compromised barrier and sun blocks strengthen the horny layer's ability to shield vital tissues from damaging UV rays.

The second method bypasses the need for oral, systemic, and other medicines by penetrating the horny layer at the molecular level to deliver pharmaceuticals to healthy epidermal and dermal tissues.

The epidermis might be difficult to reach when treating skin infections with a systemic approach. Moreover, the need for relatively large doses means that it might induce negative effects.

However, the medicine is able to permeate into the living cutaneous tissue at therapeutic quantities when administered through the topical route, sparing the patient from any adverse pharmacological effects.

Therefore, the skin serves as a necessary barrier, keeping harmful external compounds out while keeping vital endogenous ones in.

1.14 SOLID LIPID NANOPARTICLES AS A TREATMENT FOR PSORIASIS

To fill the gap between lipid (emulsion and liposome) and polymeric nanoparticle (NP) delivery technologies, SLNs were first introduced in 1991 (Sala et al., 2018). SLNs are highly sought-after as skin medicine carriers because of their lipid makeup. In fact, the lipid interactions between skin and SLNs may enhance the skin penetration of the encapsulated medications (Zhai and Zhai, 2014). The interfacial area increases with decreasing typical particle size of SLNs, which is between 40 to 1000 nm (Pardeike et al., 2009), increasing the possibility of drug penetration into deeper skin layers. Additionally, it has been asserted that SLNs are the only ones that can target the epidermis and hence improve the benefit to risk ratio of topical treatment (Porter et al., 2016). A solid lipid or a combination of

solid lipids dispersed in water at a rate of 0.1% (w/w) to 30% (w/w) constitutes the lipid matrix.

According to Pardeike et al. (2009), it is customary to utilise a surfactant at a concentration of 0.5% (w/w) to 5% (w/w) to keep the system stable. Solid lipid nanoparticles are the ideal carriers for topical applications, according to a 2012 study by Mehnert and Mäder.

Controlled drug release and drug targeting may be possible;

- Drug stability may be improved; drug payload may be increased;
- Both lipophilic and hydrophilic medicines may be included without compromising the carrier's safety;
- There need be no biotoxicity to the drug;
- Organic solvents may be avoided.
- No issues with large-scale manufacturing or sterilizing

1.15 METHODS OF PREPARATION OF SOLID LIPID NANOPARTICLES

- Solvent injection technique
- Cold homogenization technique
- Ultrasonication or High-Speed Homogenization
- Solvent emulsification-evaporation technique
- Solvent emulsification-diffusion technique
- Micro emulsion-based method
- Hot homogenization technique

1.16 THE IMPORTANCE or NEED OF ONGOING RESEARCHES

The US Food and Drug Administration has approved the systemic medication acitretin for the treatment of severe, drug-resistant psoriasis. Psoriasis is a T-cell mediated skin disorder characterised by constrained, red, thicker plaques with an overlying silver-white scales and recurrent flares of inflammation and hyperkeratosis. The underlying cause of psoriasis is obscure. Present-day treatments for the condition include topical therapy, phototherapy, and systemic therapy, albeit none of these approaches has been demonstrated to be effective in promoting recovery. Acne frequently appears on the shoulders, back, neck, chest, and upper arms. According to Ravisankar et al. (2015), acne forms when bacteria, dead skin cells, or oil block the skin's pores.

Acne is currently thought to be caused by a variety of factors, including increased sebum production, altered sebum lipid quality, androgen activity, interaction with NPs, pro- and anti-inflammatory properties, hyperkeratinization of the hair follicle, and the growth of Propionibacterium acnes within the follicle (Georgel et al., 2005 and Zouboulis CC., 2004). Topical treatments are frequently used to treat both diseases first. Dermatitis, inflammation, and modifications to skin tone or colour are examples of side effects. Phototherapy is frequently suggested as an option if topical therapies are ineffective.

However, phototherapy has a number of adverse effects, including skin cancer, hypertension, renal toxicity, hepatotoxicity, and hyperlipidemia (Pradhan et al., 2013).

Patients are less likely to take their medication as prescribed when it causes nausea and vomiting and has to be taken many times daily (often as many as four times).

One of the main reasons for inadequate treatment is the lack of a perfect anti-psoriatic and anti-acne drug carrier. Researchers are looking at the use of colloidal carriers such liposomes, ethosomes, transferosomes, nanostructured lipid carriers, microspheres, micelles, dendrimers, etc. to enhance therapeutic regimens.

A topical carrier-based method that makes it easier for the medicine to enter the skin and stay there, preventing organ damage, will boost the effectiveness and appeal of acitretin as a treatment for psoriasis. There are several psoriasis carrier-based formulations on the market, such as Lipotar S Gel (coal tar liposomes).

Due to the drug's physicochemical characteristics, such as its extremely low water solubility (0.0729 mg/ml), instability when exposed to light, air, and heat, and skin irritation episodes characterised by erythema, burning, and peeling of the treated area, it is challenging to develop a topical formulation of acitretin (Hashim et al., 2018). Act must therefore be used topically right away in order to facilitate a more secure treatment plan while also raising local bioavailability of the drug at the action site and lowering the danger of systemic exposure.

The stratum corneum serves as the main obstruction to topical therapy due to its barrier properties. The significantly thickened and inflamed stratum corneum, in particular for psoriasis and acne, may restrict the efficacy and anti-psoriatic activity of the medications given topically as standard dose forms (Hashim et al., 2018).

Lipid carrier systems, such as solid lipid nanoparticles, are responsible for transporting the medicine deeper into the dermis.

1.17 DRUG PROFILE

ACITRETIN

IUPAC NAME

(all-E)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7- dimethylnona2,4,6,8-tetraenoic acid (Porwel et al., 2014).

The FDA-approved systemic retinoid is (13-cis-trans retinoic acid). It is the first line of defence against psoriasis. Although its exact method of action is unknown, it works by preventing the increased cell proliferation and keratinization that characterise psoriasis. As a result, it lessens scaling, plaque formation, and skin thickness. Additionally, nodulocystic acne appears to benefit from it (Andrew J. Scheman, 2002).

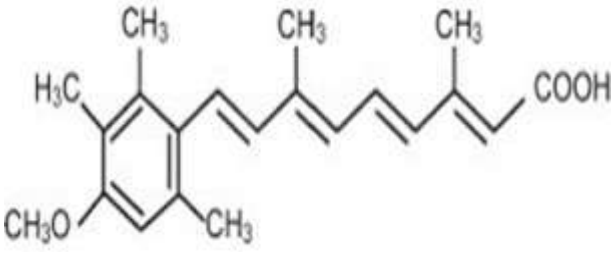

Figure 2: Structure of acitretin

Table 1: Drug parameter details

Parameter	Drug Specifications
Brand name	Soriatane; Neotigason
Chemical Formula	$C_{21}H_{26}O_3$
Molecular Weight	326.429 g/mol
Solubility	0.0729mg/L
Metabolism	Hepatic
Melting point	230˚C
Bioavailability	60%
Protein Binding	> 99.9%
Elimination half life	49 days
BCS Classification	II class (low solubility and high permeability)

Drug interactions: The drugs which can interact with acitretin thus affecting itsaction are:

Table 2: Drug Interaction

S.No.	Drug	Interaction
1.	Tetracycline	Increased photosensitivity, Pseudotumor cerebri
2.	Minocycline	Pseudotumor cerebri
3.	Alcohol	Increased conversion to etretinate, hepatotoxicity
4.	Other Retinoids or Vitamin A	Hypervitaminosis
5.	Corticosteroids	Hyperlipidemia
6.	Methotrexate	Increased methotrexate level, hepatotoxicity.
7.	Minipill	Interfere with the contraceptive effect

Table 3: Commercially available formulations of Acitretin

Name	Strength/Capsule	Pharmaceutical company	Route	Dosage
Aceret 25	25mg	Actavis Specialty Pharmaceuticals Co	Oral	Capsule
Soriatane	25mg	Teva Pharmaceuticals USA, Inc.	Oral	Capsule
Acitretin	17.5mg	Impax Generics	Oral	Capsule
Acrotec 25	25mg	Sigma Pharm Laboratories, Llc	Oral	Capsule
Acetec 10	10mg	Mylan Pharmaceuticals Inc.	Oral	Capsule

CHAPTER 2

LITERATURE REVIEW

Table 4: Literature review table

S.NO	AUTHORS	JOURNAL NAME	YEAR
1.	Yadav SK, Mishra MK.et.al;	Int J Curr Pharm Research	1998
2.	Mousaviasl, S.; Saleh, T.; Shojaosadati, et.al;	Int J Biol Macromol	1999
3.	Rote, A. R.; Kumbhoje, P. A.; Bhambar, et.al;	Pharm Methods	2000
4.	Porwal, P. K.; et.al;	Acta Pharm Sin B	2000
5.	Divya, G.; Panonnummal, R.; et.al	Eur J Pharm Biopharm	2001
6.	Sanzhakov, M. A.; Ignatov, D. V; et.al	Biomed Khim	2001
7.	Adi, A. C.; Christanto, C.; Rachmawati, H	Pharm nanotechnol	2002
8.	Panonnummal, R.; Jayakumar, R.; et.al	Eur J Pharm Sci	2003
9.	. Sinha, P.; Srivastava, S.; Mishra, N.; et.al	Drug Dev Ind Pharm	2004
10.	Avasatthi, V.; Pawar, H.; Dora, C. P.; et.al	Pharm Dev Technol	2004
11.	M. Pradhan, D. Singh, et.al	J. Control	2004
12.	Chalakanti et al	Pharm. Res.	2005
13.	L. Malpezzi, G.A. Magnone, et.al	J. Pharm.Sci	2005
14.	G.M.G. Chiara, et.al	Biol. Macromol.	2006
15.	P. Gisondi, G. Malara, M. Ardigo, et.al	Eur. Rev. Med. Pharmacol. Sci.	2007
16.	Gardouh A, et.al	International Journal of Pharmaceutical Sciences and Research	2008
17.	B.P. Ravi, S. et.al	J. Control. Release	2009
18.	N. Sanoj Rejinold, et.al	J. Colloid Interface Sci	
19.	Gupta PC, Kapoor , et.al	European Journal of Pharmaceutical and Medical	2010

		Research	
20.	Natarajan J, Karri VSR. et.al	Global Journal of Nanomedicine	2011
21.	B.P. Ravi, S. Luis, W. Hanping, et.al	J. Control. Release	2011
22.	. Khurana, N.K. et.al	European Journal of Pharmaceutical and Medical Research	2012
23.	Jain, P.M. Bedi, et.al	International Journal of Pharmaceutical Sciences and Research	2013
24.	A. Sharma, T. Garg, A. Aman, et.al	Global Journal of Nanomedicine	2013
25.	K. Paschal, R. Sharma,et.al:	European Journal of Pharmaceutical and Medical Research	2014
26.	Sanzhakov, M. A.; et;al.	European Journal of Pharmaceutical and Medical Research	2015
27.	Ignatov, D. V. ;et;al.	International Journal of Pharmaceutical Sciences & Research	2016
28.	Ipatova, O. M., et;al.	International Journal of Pharmaceutical Sciences & Research	2016
29.	Prozorovskyi, V. N. ;et;al.	International Journal of Pharmaceutical Sciences & Research	2017
30.	Medvedeva, N. V. ;et;al.	European Journal of Pharmaceutical and Medical Research	2018
31.	Druzhilovskaya, O. S. ;et;al.	International Journal of Pharmaceutical Sciences & Research	2019
32.	Kostryukova, L. V. ;et;al.	European Journal of Pharmaceutical and Medical Research	2020
33.	Connors KA, ;et;al.	Scientia Pharmaceutica	2021
34.	Kennon L. ;et;al.	Scientia Pharmaceutica	2022
35.	Amidon GL; ;et;al.	European Journal of Pharmaceutical and Medical Research	2022
36.	Shinde U; et;al.	Pharm nanotechnol	2023
37.	Modani S. ;et;al.	Scientia Pharmaceutica	2023
38.	Pokharkar S, ;et;al.	Pharm nanotechnol	2024
39.	Kaur I, ;et;al.	J. Control	2024
40.	Bansal G;et;al.	Pharm. Res.	2024

41.	Bansal Y, ;et;al.	J. Pharm.Sci	2025
42.	Kaur J, ;et;al.	European Journal of Pharmaceutical and Medical Research	2025
43.	Suthar N, ;et;al.	International Journal of Pharmaceutical Sciences & Research	2025
44.	Allen LV, ;et;al.	International Journal of Pharmaceutical Sciences & Research	2025
45.	Ansel HC and Ans; et;al.	International Journal of Pharmaceutical Sciences & Research	2025
46.	Popovich NG;et;al	European Journal of Pharmaceutical and Medical Research	2025

CHAPTER 3

3.1 Aim of work

They were created with the intention of improving skin permeability. The inter-corneocyte gaps enlarge and the corneocytes cluster closer together when water loss from the skin is prevented. As a result, drugs have a better chance of penetrating the skin Due to higher occlusion and skin contact surface, smaller particle size for SLNs has been associated with improved skin penetration. Normally, particles have to be smaller than 260 nm

The study's findings suggest that the action is localised and does not penetrate the skin when there is an increase in SLN levels in the upper dermis. NLCs with a lower liquid lipid content permeate the skin more thoroughly, according to research on skin permeation. This supports the notion that SLNs have greater skin permeability than NLCs

The current study's primary objective was to create a safe and effective targeted drug delivery system for the topical route of administration in order to circumvent the aforementioned drawbacks of the standard dose form. Thus, an effort was undertaken in the current research to create a topical acitretin formulation based on a carrier.

OBJECTIVE

a. Pre-formulation studies

b. Preparation of standard curve of drug

c. Drug-excipients compatibility study

d. Preparation and optimization of the formulation

e. Characterization of formulation.

f. *In-vitro* drug release study

RESEARCH ENVISAGED

Psoriasis is thought to be an immune system problem. Triggers include infections, stress and cold.
The most common symptom is a rash on the skin, but sometimes the rash involves the nails or joints.

Treatment aims to remove scales and stop skin cells from growing so quickly. Topical ointments, light therapy and medication can offer relief.

CHAPTER 4

4.1 PREFORMULATION STUDIES

A. Pre-formulation studies

Identification of drug

Physical appearance

Melting point

I.R. spectroscopy

UV spectroscopy

Solubility studies

Preparation of standard curve of drug

B. Preparation and optimization of the formulation

C. Characterization of formulation.

Appearance

pH

D. In-vitro drug release study

E. Results and Discussion

4.2 PREFORMULATION STUDIES OF ACITERTIN

Preformulation studies must be carried out to characterize the physicochemical, natural features of the drug that may affect the development of an effective dosage form prior to the synthesis and characterization of pharmaceutical dosage form containing therapeutic component. The physicochemical characteristics of the new compound that may have an impact on therapeutic performance and the creation of an effective dosage form should be the main focus of preformulation investigations. For the design of a delivery system to attain stability and maximal bioavailability qualities, preformulation data must be produced.

4.3 PREFORMULATION STUDIES

4.3.1 Drug Identification Tests

4.3.2 Physical appearance

4.3.3 Light absorption test for Acitretin

4.3.4 Melting point

4.3.5 IR spectroscopic analysis

4.3.6 DSC of ACITERTIN

4.4 SOLUBILITY PROFILE

4.5 UV SPECTROSCOPIC ANALYSIS

4.5.1Determination of absorbance maxima

4.5.2Preparation of standard curve of ACITERTIN in phosphate buffer pH 7.4

4.6 PARTITION COEFFICIENT STUDIES

4.7 DRUG IDENTIFICATION TESTS

A) PHYSICAL APPEARANCE

Green-yellow crystalline powder was discovered to be present in the medication ACITERTIN. photographic characteristics According to Makin et al. (1989), acitretin is a yellow to greenish-yellow powder with a maximal absorption at 352 nm (methanol) in the UV—visible spectrum. Acitretin can easily undergo photoisomerization processes when exposed to light, especially in solution, because of its conjugated tetraene structure.

B) SOLUBILITY

Soluble in most organic solvents, fats and oils; low solubility in water probably similar to that of alltrans-retinoic acid, i.e. 0.21 jimol/L .

C) LIGHT ABSORPTION TEST FOR Acitretin

UV and visible spectrum: = 352 nm in Phosphate Buffer 7.4 (Makin et al., 1989)

D) **MELTING POINT**

Table 5: MP of Acitretin determined through the MP apparatus was found to be 228-230 °C.

Properties	Observations	Standard (I.P.,2010)
Color	Yellow	Yellow
Odor	Slightly ethanolic	Slightly ethanolic
Physical Appearance	Crystalline powder	Crystalline powder
Melting Point	228-230°C	224C

Determination of UV Absorbance Maxima of Acitretin

The standard solution of Acitretin (10µg/ml) was scanned in the range of 200-400 nm to record the spectra against pH 1.2 HCl buffer as a blank using UV Spectrophotometer. The wavelength maxima of acitretin were determined from the spectra recorded. The λ max of Acitretin was found to be 353 nm as shown in Figure:

Preparation of standard calibration curve in pH 1.2 HCl buffer Preparation of stock solution

Accurately weighted 10 mg of Acitretin is transferred to 100 ml volumetric flask. Then volume was made up to 100 ml with methanol to get the concentration of drug 100 µg/ml.

Making a standard curve

To generate acitretin concentrations of 2, 4, 6, 8, 10, 12, and 16 g/ml, respectively, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 ml from the stock solution were transferred to a 10 ml volumetric flask and diluted with pH 1.2 HCl buffer up to the mark. Using a UV visible spectrophotometer, the absorbance of each solution was determined at 353 nm. In Microsoft Excel, the absorbance v/s concentration (g/ml) graph was drawn, and the data were then submitted to a linear regression analysis.

Table 6: Standard curve data of ACITRETIN in phosphate buffer 7.4

S.NO	CONCENTERATION(µG/ML)	ABSORBANCE AT 352NM
1.	2	0.09
2.	4	0.241
3.	6	0.345
4.	8	0.401
5.	10	0.521
6.	12	0.621
7.	14	0.776
8.	16	0.816

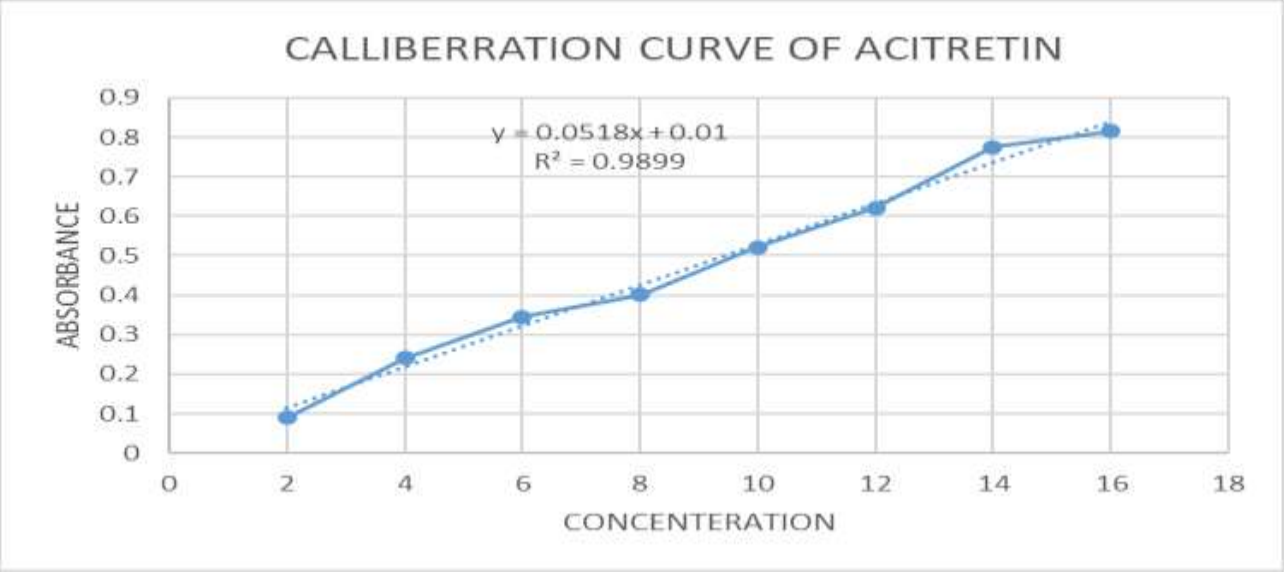

Figure 3: Calibration curve of ACITRETIN in Phosphate buffer 7.4.

IR SPECTRA

Both C=O and C-O vibrations Ketones are expected to have a C=O stretch in the range of 1740–1660 cm-1, and carboxylic acids have a C=O stretch that is identical to this [20]. Due to its great intensity, the band is rather simple to identify.

In the current investigation, the band identified as the C=O vibration is seen at 1782 cm-1 in the FT-IR and 1767 cm-1 in the FT-Raman, and it agrees with the calculated value of 1791 cm -1 with an 80% PED contribution. Due to the high polarity of this multiple bonded group, it exhibits a strong infrared absorption band. The absorption of the carbonyl group is sensitive to both the carbon and oxygen atoms in the C-O band. The C-O vibration has been impacted, according to what we can see.However, these bands overlap with otherbands that are due to aromatic vibrations causing that their undisputed assignment is often difficult.

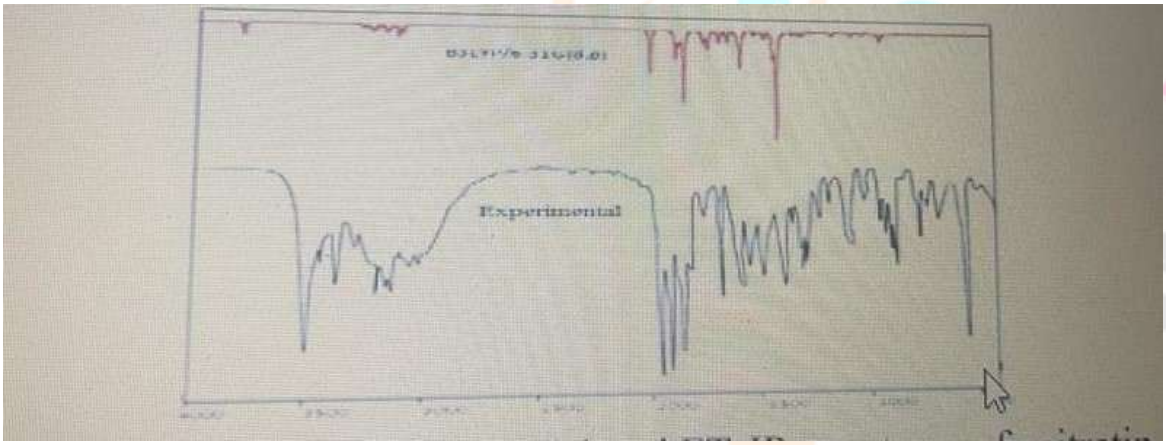

Figure 4: IR of Acitretin

DIFFERNTIAL SCANNING COLORIMETRY

DSC reveals all of the sample's physical characteristics, including its crystalline and amorphous nature, and illustrates potential interactions between drugs and polymers. Acitretin's DSC curve displayed a single endothermic peak at 225 oC. A low intensity peak at roughly 62 oC and an increase in the curve at 220 oC are visible in the optimise formulation's DSC profile. The decrease in crystallinity in a formulation is so established. Figure 5 depicts the physical mixing of the drug and polymers, as well as the thermal behaviour of Acitretin.

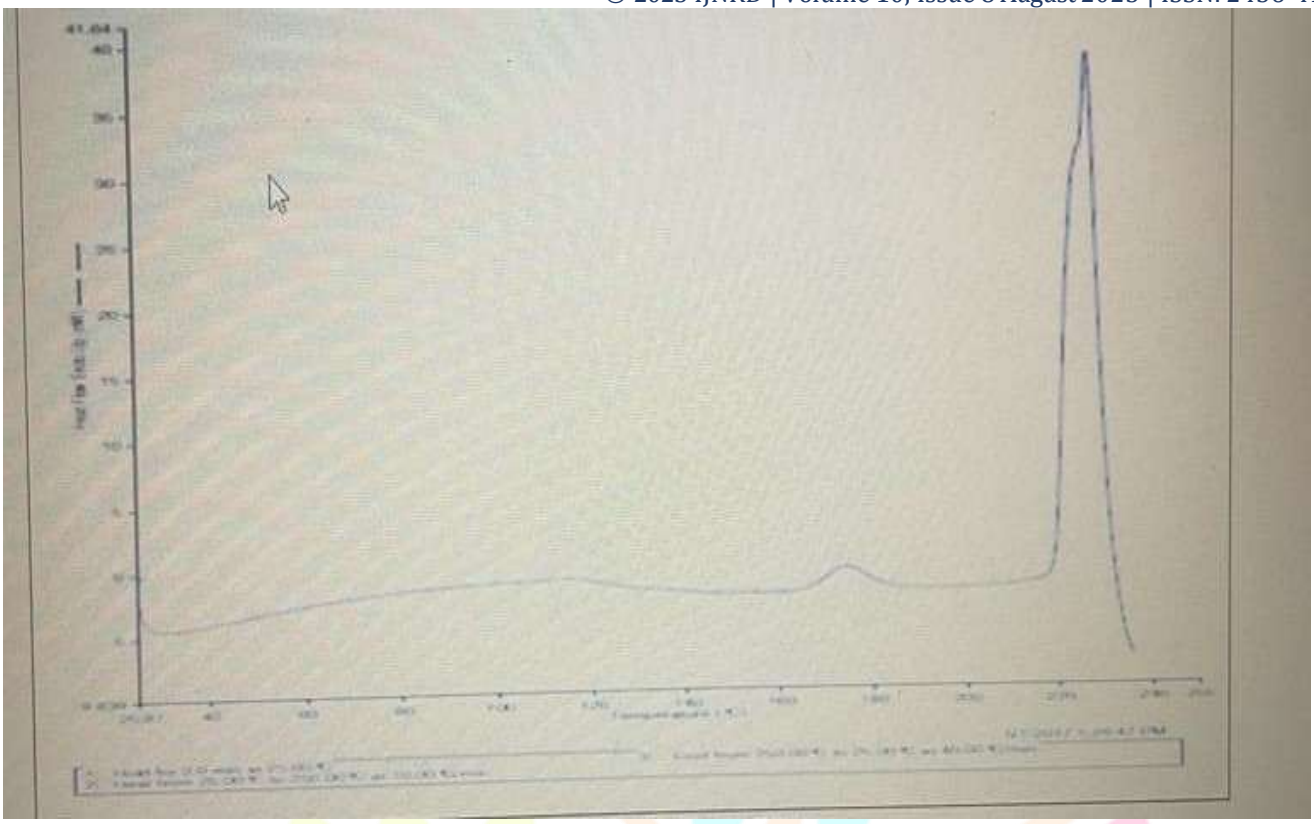

Figure 5: DSC of Acitretin

SOLUBILTY STUDIES

Acitretin is sparingly soluble in aqueous buffers. For maximum solubility in aqueous buffers, acitretin need to be dissolved in DMF and then diluted with aqueous buffers.

Acitretin has solubility of 0.2 mg/ml in 1:4 solution of DMF: PBS (PH7.4).

Table 7: Solubility of ACITRETIN

SOLVENTS	SOLUBILITY
DMF: PBS (pH 7.4)	++++ (VERY SOLUBLE)

PARTITION COEFFICIENT

Partition coefficient (P) or distribution coefficient (D) is the ratio of concentrations of a compound in a mixture of two immiscible solvents at equilibrium

The log P value is a measure of lipophilicity or hydrophobicity

Partition of drugs from oil phase n- octanol and PBS.

In n-octanol:PBS (pH 7.4), the drug's partition behaviour was investigated.It was discovered by placing medication (10) mg in two separate funnels, one of which contained 10 ml sections of n-octanol and the other 10 ml of PBS (pH 7.4). For equilibration, the separating funnel was shaken for 24 hours in a wrist motion shaker. Drug concentration in the aqueous phase was examined at 352 NM following separation of the two phases.it was determined using the following formula:

P O/W = [C ORGANIC / C AQUEOUS], equilibrium

Table 8: Partition Coefficient of ACITRETIN.

Medium	**Partition coefficient**
n-octanol: PBS	**0.64**

CHAPTETR 5

METHODOLOGY

5.1 Method for Preparation of Nanostructured Lipid Carrier (NLC)

Preparation of Acitretin-loaded NLCs by Hot Homogenization Method: The drug Acitretin was dissolved in ethanol and mixed with an acetone solution containing a blend of lipid phase stearic acid and oleic acid. The mixture was then added slowly dropwise to a combination of tween 80 and sodium lauryl sulfate were used to stabilize NLC. The mixture was sonicated at 85 ºC for 30 min at 1200 rpm using magnetic stirrer 8 . This primary emulsion was converted to the NLC system using hot homogenizer at 15000 PSI. The emulsion obtained was subsequently cooled down to room temperature with continuous stirring, and the lipid was recrystallized to form a nanostructured lipid carrier (NLC) . The obtained NLC dispersions were lyophilized. The formulations of different ingredients with composition were tabulated in the Table 9

Table 9: Various Formulations of ACITRETIN

Sample	Drug(%w/v)	Ratio of oleic acid and stearic acid	Tween 80(%v/v)	SLS(%w/v)
F1	12 mg	8:2	2	2
F2	12 mg	8:2	2	3

F3	12 mg	8:2	2	4
F4	12 mg	8:2	3	2
F5	12 mg	8:2	3	3
F6	12 mg	8:2	3	4
F7	12 mg	8:2	4	2
F8	12 mg	8:2	4	3
F9	12 mg	8:2	4	4

5.2 Preparation of Acitretin Loaded NLCs Gel

NLC formulation is determined by the characterizations of the NLC dispersion (particle size, trapping effectiveness, and in-vitro release profile) described above. The best physicochemical properties of International Journal of Pharmaceutical Sciences and Research 3383 were chosen 15. Continuous stirring was used to integrate the NLC dispersion into the HPMC gel. A homogenizer was then used to homogenise the dispersion. To release trapped air, the gel was probe-sonicated for at least one hour before being left to stand overnight.

Table 10: Formulation chart of methyl cellulose & Triethanolamine.

Sample code	Methyl Cellulose(%w/v)	Neutralizing agent(%v/v) **Triethanolamine**.	pH
G1	1.5	6	5.2
G2	2.5	4	6.6
G3	3.5	2	8.5

"Neutralizing agent" is added to increase the pH and cause the dispersion to thicken and gel. Some neutralizing agents are sodium hydroxide, potassium hydroxide, and triethanolamine."

5.3 Evaluation of NLC Gel: Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container.

They were tested for their appearance and presence of any aggregates.

5.4 Spreadability Study of NLC Gel

One gramm of gel was placed between two horizontal plates (20 20 cm2), and its diameter was measured after one minute to test the spreadability of the gel formulations 24 hours after production. The upper plate's standardised weight was 220gm. The spreadability was determined using the formula below. S = M × L/T Where S stands for spreadability, M for weight secured to the upper slide, L for glass slide length, and t for processing time.

5.5 Viscosity measurement

DVE Digital Viscometer's direct torque% display, spindle, and speed torque measurement precision, 1% whole range repeatability, and 0.2% full range compatibility with all Brookfield accessories traceable viscosity standards make it affordable and simple to use. 18 speed for a wider speed range (0.3 to 100rpm).

Table11: Evaluation of Plain Methyl Cellulose gel

S.no	PERCENTAGE OF GEL (%)	Spreadibility (g×cm/sec)	Viscosity(cgs)
1	1.5	3.23	520
2	2.5	4.9	2341
3	3.5	10.2	8765

5.6 Determination of Entrapment Efficiency & Drug loading

A volume of 2.0 ml of drug-loaded sample was centrifuged at 2500 rpm for 90 min to separate the lipids and aqueous phase. The supernatant solution was separated and analyzed by using UV spectrophotometer at 352 nm using phosphate buffer pH 7.4 as blank. The drug entrapment efficiency of NLC was calculated as

% Entrapment efficiency (EE)=(WA-WS) *100/WA

Drug loading (DL)=WA-WS/WA-WS+W1*100

Where, EE is entrapment efficiency, DL is Drug loading, Wa stands for the mass of Acitretin added to the formulation, Ws is the analyzed weight of the drug in the supernatant, and Wl is the weight of lipid added.

Table 12: Drug loading & Entrapment Efficiency of Acitretin gel

S.NO	FORMULATIONS	DRUG LOADING	ENTRAPMENT EFFICIENCY
1.	F1	24	19
2.	F2	53	35
3.	F3	14	27
4.	F4	32	30
5.	F5	61	52
6.	F6	45	49
7.	F7	54	52
8.	F8	65	60
9.	F9	85	88

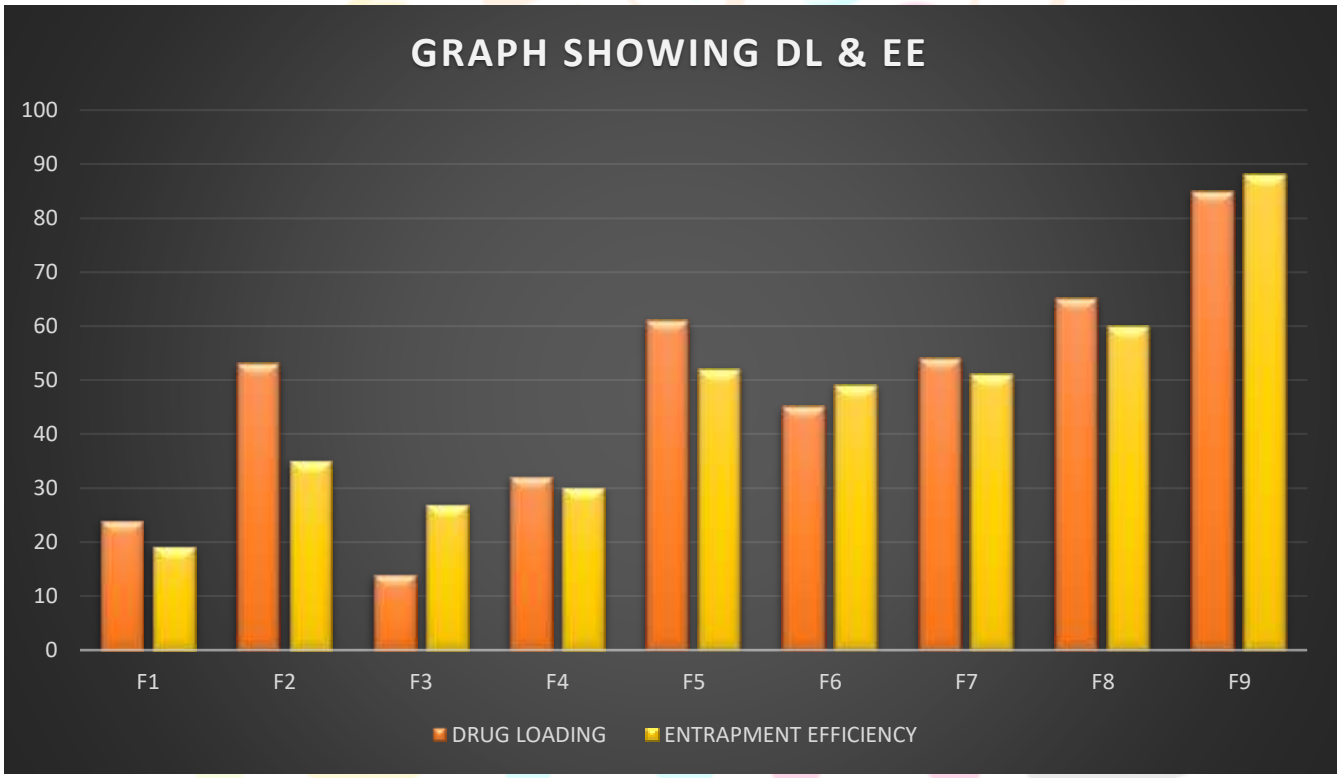

Figure 6: Graphical representation of Drug loading & Entrapment Efficiency

5.7 In-vitro Release Study of Nanostructured Lipid Carrier (NLC) of Gel

Utilising a modified Franz diffusion cell, in-vitro drug release studies were carried out to assess the Acitretin release profile from each formulation. A synthetic cellophane membrane was put on the Franz diffusion cell.

To avoid different concentrations within the acceptor medium and to reduce stagnant layers, the receptor medium was roughly 45 ml of phosphate buffer with a pH of 7.4 and was swirled by the magnetic bar at 700 rpm. In the donor compartment, NLC gel dispersion (1 mg of medication) was made. The solution on the receptor side was kept at 37

0.5 oC throughout the trials.

Three millilitres of the sample medium were removed from the receiver compartment through the side tube after a predetermined amount of time, and the same amounts of freshly prepared receptor media were then added. At 352 nm, a UV-Visible spectrophotometer was used to examine the materials.

Table 13: Formulations F9 showing % DR & % CDR

S.NO	TIME	ABSORBANCE	CONCENTERATION	% DRUG RELEASE	%CUMULATIVE DRUG RELEASE
1.	8	0.002	0.15	0.015	0.015
2.	16	0.004	0.24	2.98	2.995
3.	24	0.005	0.42	8.56	11.55
4.	32	0.007	0.61	11.98	23.53
5.	40	0.008	0.83	15.04	38.57
6.	48	0.01	1.78	17.12	55.69
7.	56	0.04	3.76	18.15	73.84
8.	64	0.05	4.25	19.71	93.55

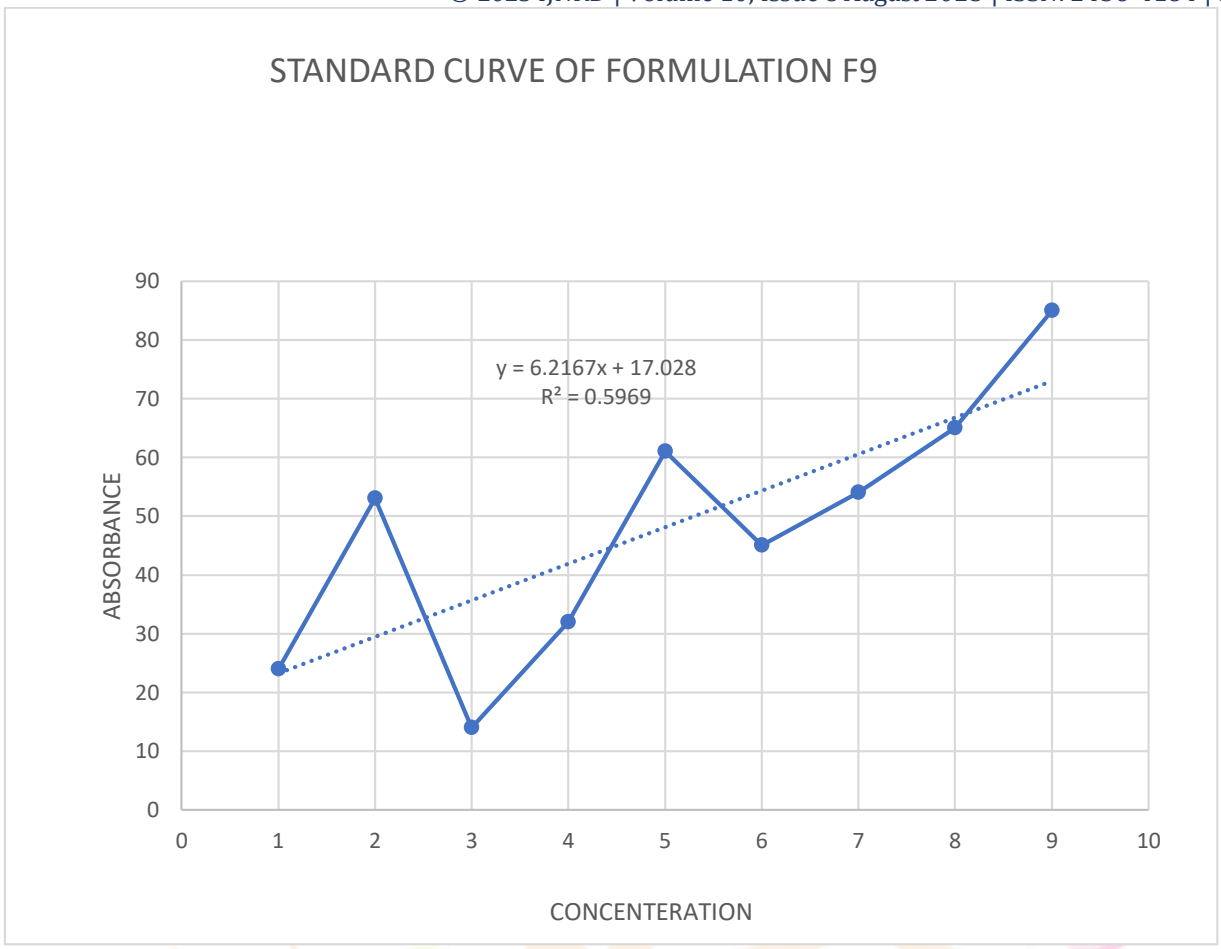

Figure 7: Standard Curve of Formulations F9

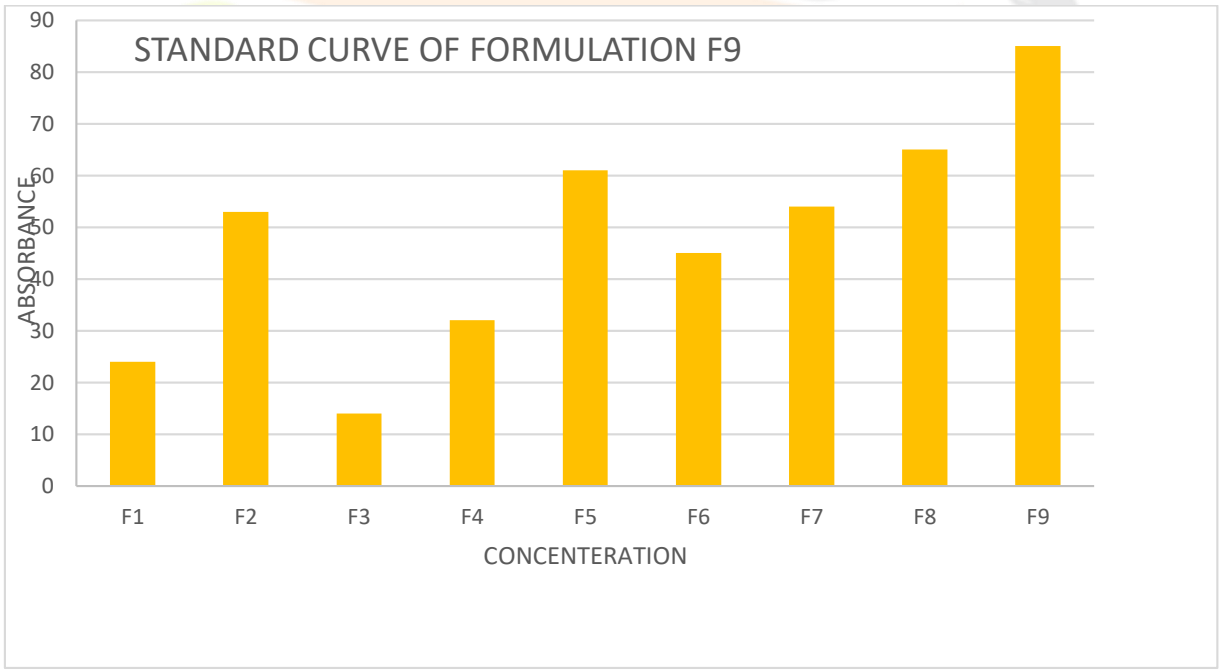

Figure 8: Graphical representation of F9

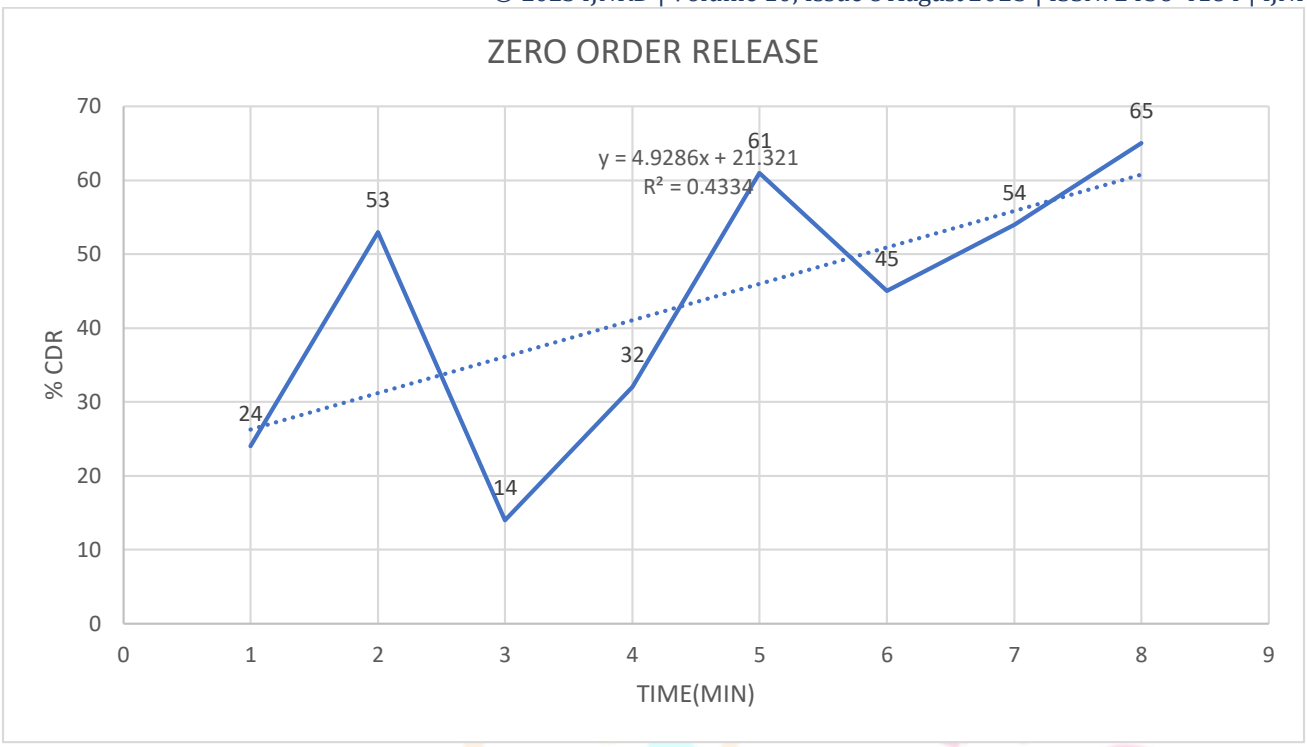

Figure 9: Standard curve of F9 showing zero order release

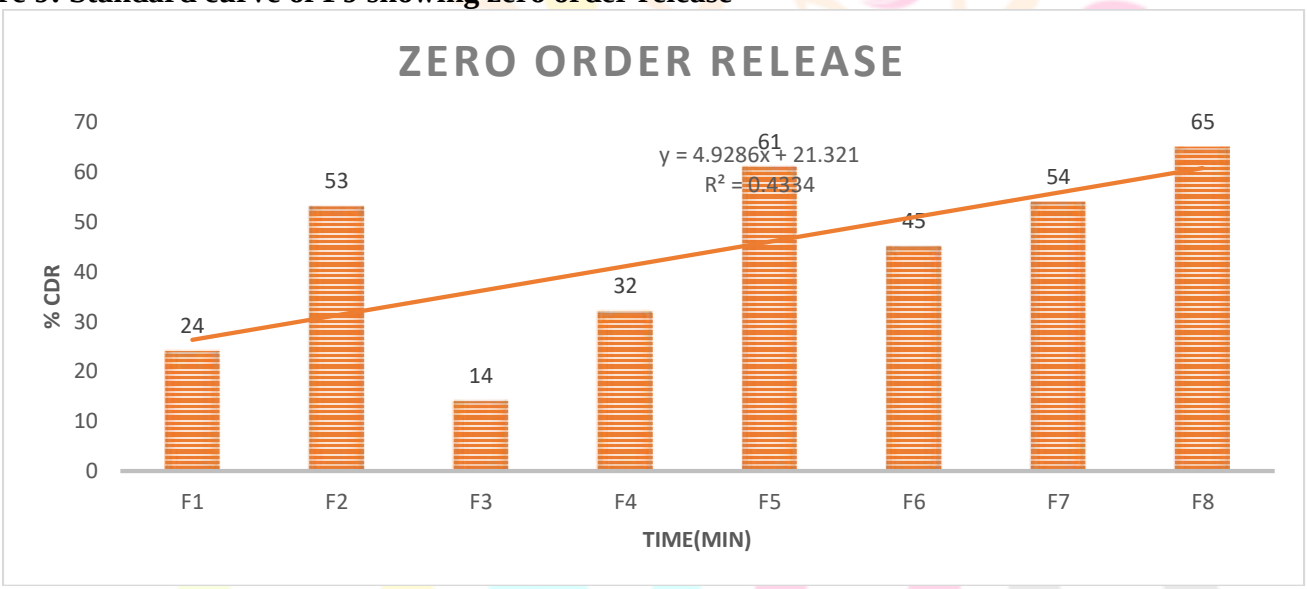

Figure 10: Graphical representation of F9 showing zero order release

5.8 Determination of TEM

The NLC gel micrograph showed spherical droplets in the nanometer range. Using 500ml of phosphate buffer pH 7.4, selected nanostructure lipid carrier formulations (1gm) were diluted. A drop of the emulsion was applied on a film that had a copper grid coating, and any extra droplets were quickly removed with filter paper. The grid was then loaded into the microscope after drying in the air at a specific ambient temperature. The material that had been dried overnight was put onto a copper grid within the TEM's vacuum chamber to produce the TEM images. The findings demonstrated that the particles were spherical, and there were no apparent drug crystals in the particles in Fig. 11. Due to the lipid composition of the carriers, the picture depicts some particle collecting. Different particle shapes exist.The picture shows some gathering of particles due to the lipid nature of carriers. Some particle shapes are different from spherical due to the drying process of sample treatment.

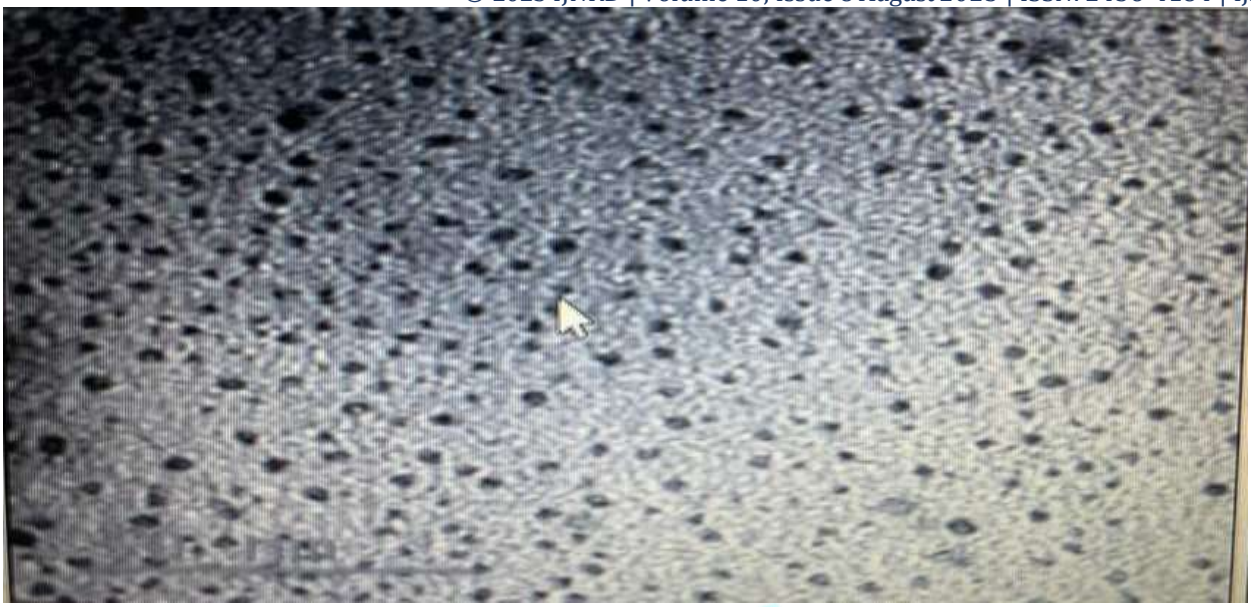

Figure 11: TEM Image showing spherical droplet in nano range of f9 formulations

CHAPTER 6
RESULT & DISCUSSION

Spherical droplets in the nanometer range may be seen in the NLC gel microscopy. Selected nanostructure lipid carrier formulations (1gm) were diluted with 500ml of phosphate buffer pH 7.4. On a film that was coated with a copper grid, a drop of the emulsion was placed, and any surplus droplets were rapidly wiped away with filter paper. After drying in the air at a predetermined air temperature, the grid was then placed into the microscope.

To create the TEM images, the material that had been drying overnight was placed onto a copper grid inside the TEM's vacuum chamber. The results showed that the particles were spherical, and the particles in Fig. 11 did not appear to contain any drug crystals. The image shows the following because of the lipid makeup of the carriers.

The outcomes of the experiments demonstrated that the various surfactants had a significant impact on entrapment effectiveness. Poor entrapment efficiency was seen in formulations F1 to F7, which may be related to inadequate NLC formation. The formulations (F8 and F9) with the best entrapment efficiency were chosen. Evaluation of the HPMC plain gel at various concentrations led to the conclusion that 2.5% HPMC plain gel is compatible with the best-prepared NLCs and was utilised to make NLC gel.

The selection of these three formulations was made because when ionic and nonionic surfactants are combined, transparent dispersion with good stability is the result. It's possible that non-ionic surfactant (Tween 80) stabilises the stearic component of the NLC gel dispersion while ionic surfactant (SLS) stabilises the electrostatic component.

Using a Franz diffusion cell, the in-vitro release profile of the gel formulations of acitretin NLCs was tested for 48 hours. Due to the combination of surfactant in the highest proportion, the F9 formulation demonstrated greater

drug release than other formulations. The Franz diffusion cell was used to conduct in-vitro permeation tests on the optimised NLCs gel (F9) utilising a cellophane membrane barrier.

According to the figure, the release kinetics of selective F9 were assessed for zero-order. The NLCs gel system's release pattern and sustained release over an extended length of time were revealed by the in-vitro release research of the optimised formulation (F9).

CHAPTER 7

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