DESIGN OF LIPOSOMAL TOPICAL DOSAGE FORM OF BEXAROTENE FOR THE EFFECTIVE TREATMENT OF CUTANEOUS T-CELLS LYMPHOMA

A Thesis

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By

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DECLARATION

I, declare that the thesis entitled "**Design of liposomal topical dosage form of bexarotene for the effective treatment of cutaneous t-cells lymphoma"** has been prepared by me under the guidance of **Dr. Surajpal Verma**, Associate Professor, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Jalandhar, India. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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ABSTRACT

The cutaneous T-cell lymphoma (CTCL) is most common form of Non-Hodgkin's lymphoma Bexarotene is a highly effective anticancer agent which has been proven for the treatment of CTCL. US-FDA in 1999 also approved the bexarotene topical gel for the treatment. Skin irritation, high log P value, poor aqueous solubility, low bioavailability, high dose, high molecular weight, short penetration properties and short half-life of drug make it the poor candidate for skin penetration. For that purpose radio therapy is recommended with bexarotene topical gel for effective treatment of CTCL. So improve the drawbacks for producing more therapeutic effect with lesser side effects liposomal and niosomal delivery of bexarotene can formulated to improve the topical delivery and increases the cell membrane penetration as well as treatment of cutaneous T-cell lymphoma.

The aim of the present research is to formulate bexarotene liposomes and niosomes and further incorporated in topical gel for improve the treatment and management of CTCL.

The liposomes and niosomes were developed by optimization modeling method. The mathematical modeling provides easiness to get the desired product. The suggested formula was used to prepare the liposomes and niosomes. Similar results were observed as the software suggested for the optimized formulation.

The promising liposome and niosomes further loaded in carbopol topical gel. The prepared topical gels were evaluated for their physical, chemical, cell apoptosis and biological properties. The prepared topical gels evaluations were found in the limit and preparations were also found stable.

The designs of the liposomes and niosomes containing bexarotene would be an effective way to deliver the drug through the skin via topical gel formulation. It was determined from the experimental results that, when bexarotene drug was encapsulated in liposomes and niosomes and further incorporated into topical gel formulation had shown effective treatment of CTCL as compared to simple bexarotene topical gel.

So, it was concluded that the promising formulations of liposomal and niosomal topical gel improves the treatment effectiveness of early stage CTCL which also provides patient compliance and convenience.

DEDICATED TO

LORD SHIVA

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believe in ourself and God. Education commences at the mother's knee, my father is the soul of my energy. Her blessings have always leaded me towards achieving my goals. I cannot repay her silent patience and constant encouragement. She stood by me whenever I felt let down, pepped me up when my spirit dampened.

Last but not the least, whatever endeavor I have put to prepare this work, it would not have been possible without the support of my in laws, who sacrificed a lot for my welfare and provided me the right impetus soulfully to undertake a challenge of this proportial.

I acknowledge all my other nears & dears. I shall be failing in my duty without expressing deep sense of faith to GOD, the 'Almighty' who graced me in finishing this task.

Needless to say, errors and omissions are mine.

Date:10/11/2020   **Neelam Sharma**

LIST OF ABBREVIATION

Short Form	Full Form
%	Percent
% RSD	Percent Residual Standard Deviation
°C	Degree Centigrade (unit of temperature)
°F	Degree Fahrenheit (unit of temperature)
^{0}	Degree (unit of angle)
ANOVA	Analysis of variance
AUC	Area Under the Curve
BP	British Pharmacopoeia
cm	Centimeter (unit of length)
C_{max}	Maximum Concentration
cps	Centipoise (unit of viscosity)
CTCL	Cutaneous T-Cell Lymphoma
DSC	Differential Scanning Calorimetry
EE	Entrapment Efficiency
et al.,	Latin term "et alia," meaning "and others."
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared Spectroscopy
g	Gram (unit of weight)
g/mol	Gram per moles
HL	Hodgkin Lymphoma
HPLC	High Performance Liquid Chromatography
hr	Hour (unit of time)
IC50	Inhibitory Concentration to half of total amount
IP	Indian Pharmacopoeia
kg	Kilogram (unit of weight)
$K_{o/w}$	Oil/water Partition Coefficient
LOD	Limit of Detection
LOQ	Limit of Quantification
LUV	Large Unilamellar Vesicles

MF	Mycosis Fungoides
min	Minute (unit of time)
ml	Milliliter (unit of volume)
MLVs	Multilamellar Vesicles
mm	Millimeter (unit of length)
mV	Millivolt
MW	Molecular Weight
NHL	Non-Hodgkin Lymphoma
nm	Nanometer (unit of length)
PDI	Polydispersity Index
pKa	Dissociation Cofficient
RH	Relative Humidity
RXR	Retinoid X Receptors
s	Second (unit of time)
SD	Standard Deviation
SEM	Scanning Electron Microscopy
SLN	Solid Lipid Nanoparticle
SS	Sézary Syndrome
SUV	Small Unilamellar Vesicles
$t\frac{1}{2}$	Half Time
TEM	Transmission Electron Microscopy
t_{max}	Time at which Maximum Concentration
USP	United State Pharmacopoeia
UV	Ultraviolet Spectroscopy
w/v	Weight by Volume
w/w	Weight by Weight
WHO	World Health Organization
XRD	X-Ray Diffraction
XRPD	X-ray powder diffraction
λ_{max}	Absorption Maxima
μl	MicroLiter (unit of volume)
μm	MicroMeter (unit of length)

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CHAPTER- 1

Introduction

1.1 CANCER DISEASE

Cancer is a type of disease that is characterised by un-controlled cell division and the capacity of these cells to invade other tissues, through invasion or through implantation into distinct sites by metastasis. In normal cell cycle progression, growth regulating mechanism endeavors to keep homeostasis. The balance between proliferation, growth arrest, and apoptosis controls homeostasis within a cell. Imbalance between growth of cell and death of cells are consequences of cancer. Anomalies in the genetic material of the transformed cells may causes cancer.[1] The pharmaceutical company's interest in this field of cancer research is due to high treatment & drug cost, the need for repeated administration over an prolonged period of time and the exponential rise in the number of cancer patients. With regulatory approvals for several formulations for cancer treatment, liposomal and niosomal drug delivery systems are the most developed and promising dosage forms. Liposomes and niosomes are tiny spherical lipid vesicles with a phospholipid or surfactants composed bilayer membrane and also capable of encapsulating hydrophilic and lipophilic drugs. Several FDA approved liposomal and niosomal formulations like cytarabine liposomal injection (Depocyt), Doxorubicin PEGylated liposomal injection and Doxorubicin liposomal formulation (Doxil) are available for cancer treatment. Liposomal and niosomal formulations have greatest impact on oncology field because of their biodegradability, biocompatibility, immunogenicity and low toxicity. Administration through dermal route is one strategy studied by drug delivery specialists with the aim to improve the therapeutic efficiency of chemotherapy by targeting the drug directly to tumor site, especially in breast and skin cancers. [2,3]

1.1.1 Pathophysiology of Cancer

Cancer is primarily a disease of cell cycle control failure. In cancer, cells transform from normal to cancer cells primarily due to gene changes that govern the growth and differentiation of cells. Two broad groups are classified into the altered genes; Oncogenes (Her-2, c-Myc, etc.) and genes that inhibit tumours (p53 Rb).Oncogenes are responsible for the reproduction of cell and growth of cell. Second classes of genes are responsible to inhibit the division of cell. Cancerous transformation can

occur when new oncogenes are created, normal oncogenes are improperly over-expressed or tumour suppressor genes are disabled. At various levels and through various mechanisms, genetic changes can occur. The addition or destruction of a whole chromosome structure can occur in mitosis due to some errors. The division of cells is a genetic mechanism in which a cell transmits its genes to two daughter cells, one of which is a clone of its own. Often this orderly process unexpected changes will happens, the genes in a cell may undergo a mutation during cell division, certain errors may occur in DNA replication and recombination causes cancer.

1.1.2 Mechanism of Action of Anti-Cancer Drugs

The broad mechanism of anti-cancer drugs specific to cells is that they can function during a particular phase of the cell cycle. Figure 1.1 shows the specific mechanism of action of different anti-cancer drugs.
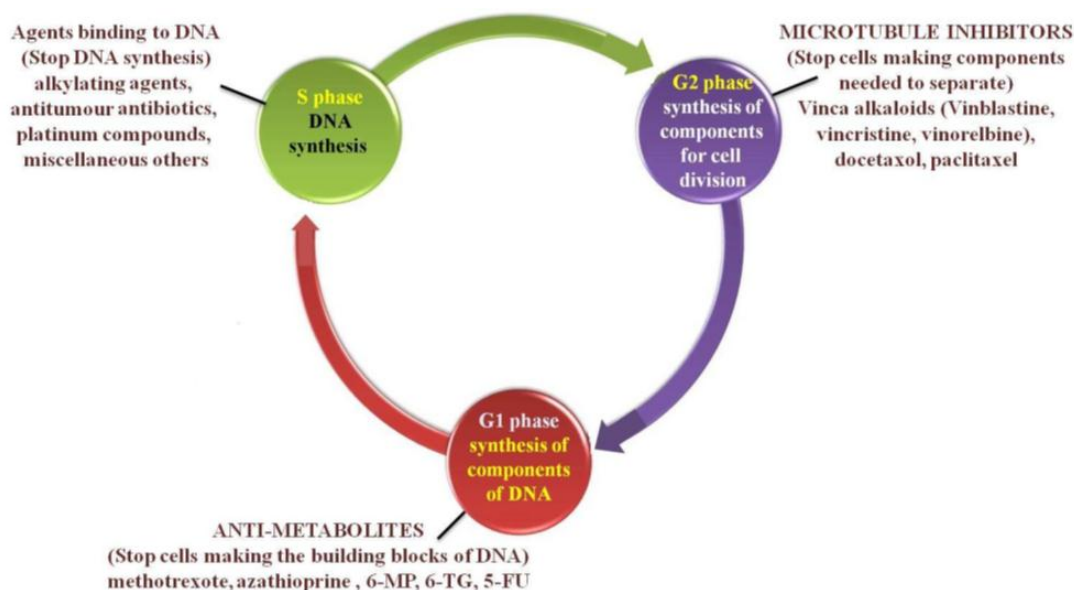
Figure 1.1: Effect of anti-cancer drugs on cell cycle[3]

1.2 LYMPHOMA

Body has an immune system, which helps to fight against infection. The immune system is made up of organs linked by a network of tiny lymphatic vessels containing lymph fluids, such as the thymus, bone marrow, spleen, and the lymph nodes (lymph glands). The lymphatic tissues are present in the organs like stomach, lungs and skin. The lymph nodes are found all over the body. These lymph nodes collect and sieve out anything that the body doesn't need or anything that is bad for

the body, as lymph fluid flows through the lymph nodes. In the bone marrow, where red blood cells are formed, lymphocytes start to develop there. There are two primary lymphocyte types in the body: B-lymphocyte(B -cells) and T-lymphocytes(T-cells). In the bone marrow, B-cells are mature and T-cells mature in the thymus gland. Both B-cells and T-cells guard the body from infection after maturation.

The most common type of blood cancer is lymphoma. Either B cells or T cells develop abnormally or uncontrollably in cutaneous cancer diseases. These developed cancerous lymphocytes, may migrate to several parts of the body including the spleen, bone marrow, lymph nodes and other organs. In these organs they start to accumulate and develop tumours.[4-6]

Dr. Thomas Hodgkin first reported lymphoma in 1832. The lymphatic system is mostly affected by in this type of cancer. Lymphoma can be further classified in two classes according to the WHO:

> A. Hodgkin Lymphoma (HL)
> B. Non-Hodgkin Lymphoma (NHL)

Both Hodgkin's lymphoma and non-Hodgkin's lymphoma are a type of cancer that occurs in a group called lymphocytes of white blood cells. Lymphocytes, which protect the body from germs, are an integral part of our immune system. In the particular lymphocyte involvement is the key distinction between Hodgkin's and Non-Hodgkin's lymphoma is. B-Lymbhocytes are found in the HL but in NHL B-lympocyte are not present. Lymhoma with the involvement of Natural Killer (NK) Cells & T-Cells known as Non-Hodgkin Lymphoma (NHL). By observing the cancer cells under a microscope may easily differentiate between the HL & NHL. If a particular type of abnormal cell called a Reed-Sternberg cell is present while analysing the cells, then the lymphoma is known as Hodgkin's. Lymphoma is known as non-Hodgkin's if the Reed-Sternberg cells are not present.
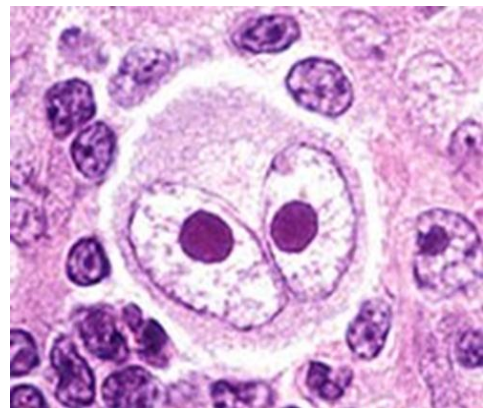
Figure 1.2: Reed-sternberg cell[4]

1.3 CUTANEOUS T-CELLS LYMPHOMA (CTCL)

A type of non-Hodgkin's lymphoma is popularly known as Cutaneous T-cell lymphoma (CTCL). CTCL, a general term for T-cell lymphomas involving the skin, is one of the most prevalent forms of T-cell lymphoma. Blood, lymph nodes, and other internal organs may also be affected by CTCL. Dryness of skin, red rashes, itching on skin and swollen lymph nodes can be sign of CTCL. This condition affects males more often than females and usually occurs in males in their age of 40 to 60. The majority of CTCL patients experience only skin symptoms. Some early-stage CTCL patients do not progress at all to later-stages, while others may progress quickly with the cancer spreading to lymph nodes or internal organs.[7]

1.3.1 Types of Cutaneous T-cells Lymphoma

With varied signs, findings, and treatment factors, CTCL explains several different conditions. Mycosis fungoide and Sézary syndrome are the two most common forms of CTCL.[8]

A. Mycosis Fungoides (MF)

Mycosis Fungoides is the most common form of CTCL, occurs in one-half of patients of all CTCLs. At each stage, MF looks distinct in each patient, with skin symptoms that can appear as plaques, patches or tumours. Patches are typically smooth, probably scaly, and look like rashes; plaques are darker, raised, typically itchy lesions that are often look like dermatitis, eczema & psoriasis; and bumps that may or may not ulcerate are raised tumours. Mycosis fungoides (MF) and its variants include indolent subtypes, primary cutaneous anaplastic large cell lymphoma, lymphomatoid papulosis, subcutaneous panniculitis-like T-cell lymphoma, and small or medium pleomorphic T-cell primary cutaneous CD4+lymphoma. For diagnosis of MF, a medical history of patient, physical exam and skin biopsy are necessary. Lymph glands, separate blood samples and other diagnostic tests, such as a chest X-ray or a CT scan, are examined by a physician to confirm MF. [9-10]

B. Sézary Syndrome (SS)

The presence of lymphoma cells in the blood is characterised for Sézary Syndrome (SS). Large, thin, red, itchy rashes typically appear on the skin in the SS. In some of the patients, tumors and patches will also appear. Patients may also have swollen lymph nodes and undergo changes in their hairs, nails and eyelids.

Sézary syndrome's aggressive subtypes include primary cutaneous natural killer (NK)/T-cell nasal type lymphoma, CD8+ T-cell lymphoma, γ / δ T-cell lymphoma, and unspecified primary cutaneous T-cell lymphoma. To assess if the cancer has spread to the lymph nodes or other organs, a series of imaging tests may be required. A PET scan, CT scan, and/or MRI scan may be used as identification of cancer.

1.3.2 Epidemiology

MF, which is nearly twice as common in men as in women, is the most common form of CTCL, although blacks are twice as prevalent as whites. Children and adolescents are rarely affected. Depending on the stage of the disease, survival ranges from only a couple of months to several decades. A sustained survival with little morbidity is encountered by most patients, although some people experience an asymptomatic path with rapid spread and death. [11-13]

1.3.3 Etiology

Alibert first used the word mycosis fungoides in 1832. [14] He identified an unusual eruption of the skin which developed into mushroom-shaped tumours. Since there is no correlation with a fungus, MF was known to be a misnomer, and MF was rather a skin manifestation of lymphoma. Several theories, including sensitivity to environmental, genetic and infectious agents, about the etiology of MF have been theorised. Subsequent research could not substantiate early epidemiological studies indicating a causative effect of environmental exposure to persistent antigenic stimulation (e.g. herbicides / pesticides, synthetic chemicals & metals). [15-17] The prevailing hypothesis is that, due to a multitude of factors in a stepwise mechanism involving mutations in oncogene and some DNA repair genes, MF is likely to evolve secondary to chronic antigenic stimulation. Many infectious agents, including human T-cell lymphotropic virus (HTLV) I / II, human immunodeficiency

virus (HIV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human simplex virus (HSV), have been examined for CTCL. [18]

1.3.4 Clinical Manifestations

Bazin identified three classical cutaneous phases of MF-patches, infiltrated plaques, and tumours in 1876. Through each of these stages, which often overlap or occur simultaneously, the disease can progress. Erythematous patches or slightly raised plaques with a fine scale are patch-stage lesions. The lesions are often found on the buttocks, thighs, and abdomen and may be single or multiple. [19]

Figure 1.3: MF Patch-stage.[19]

Due to epidermal hyperplasia or severe neoplastic lymphocytic destabilises, plaques of MF are raised. From pre-existing patches, or de-novo, these lesions can develop. They are typically red-brown and sharply demarcated, but they can coalesce, often with central clearing, to form arciform, annular or serpiginous in shapes. Leonine facies may result in infiltrative plaques occurring on the face, and those appearing in hairy areas may induce alopecia or cysts.
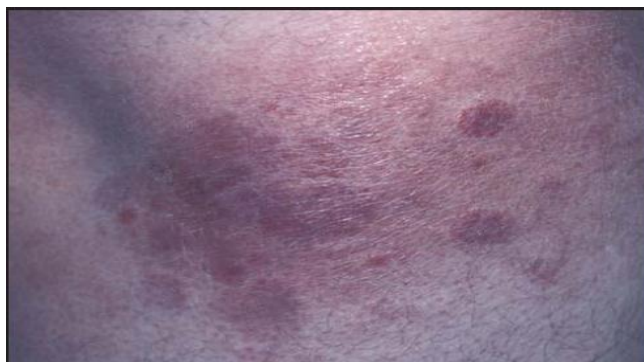
Figure 1.4: Plaques of MF on a limb.[19]

The tumour-stage MF lesions are usually mushroom-shaped tumours that preferentially influence the folds of the face and body. Ulceration or necrosis and secondary infections also occur in the lesions.

Figure 1.5: MF at Tumor-stage.[19]

Approximately 5 percent of new CTCL cases are accounted for by Sézary syndrome and represent the leukemic form of CTCL. The classic triad of generalised erythroderma, leukaemia, and lymphadenopathy recognises Sézary 's syndrome. There are malignant T cells circulating in the blood with hyperconvoluted cerebriform nuclei. Sézary cells in 90 percent of erythrodermal CTCL patients can be found in peripheral blood.

Despite the ongoing doubt regarding the degree of peripheral blood involvement, the main clinical symptom of SS is widespread pruritic erythroderma. Fever, chills, weight loss and malaise can be present in patients. Hepatomegaly, onychodystrophy, leonine facies, alopecia and palm oplantar keratoderma can be more characteristics.[20-22]

Figure 1.6: Sézary Syndrome[19]

1.3.5 Staging of CTCL

There have been proposals for a multitude of staging schemes for MF. Bunn et al, 1979, adopted the simplest and most widely used system, [23] incorporates the tumor nodemetastasis (TNM) system. This system of staging combines both clinical and histopathological viewpoints (Table 1.1), and a recent revision includes blood staging (TNMB). The revised staging is given by World Health Organization and European Organization for Research and Treatment of Cancer (WHO-EORTC).

Table 1.1: WHO-EORTC revision to the staging of MF and SS[23]

Classification	Description			
T: Skin				
T0	Lesions clinically and/or histopathologically suggestive of CTCL			
T1	Limited plaques, papules, or eczematous patches covering <10% of skin surface			
T2	Generalized plaques, papules, or erythematous patches covering ≥10% of skin surface			
T3	Cutaneous tumors			
T4	Generalized erythroderma			
N: Lymph Nodes				
N0	No palpable lymphadenopathy, lymph node pathology negative for CTCL			
N1	Palpable lymphadenopathy; lymph node pathology negative for CTCL			
N2	No palpable lymphadenopathy, lymph node pathology positive for CTCL			
N3	Palpable lymphadenopathy, lymph node pathology positive for CTCL			
M: Viscera				
M0	No visceral organ involvement			
M1	Visceral organ involvement, pathology present			
B: Blood				
B0	Atypical circulating cells not present (<5%)			
B1	Atypical circulating cells present (≥5%)			
B2	High blood tumor burden			
Stage	**T**	**N**	**M**	**B**
Early Stage				
IA	1	0	0	0-1
IB	2	0	0	0-1
IIA	1-2	1-2	0	0-1
Advanced Stage				
IIB	3	0-2	0	0-1
III	4	0-2	0	0-1
IIIA	4	0-2	0	0
IIIB	4	0-2	0	1
IVA1	1-4	0-2	0	2
IVA2	1-4	3	0	0-2
IVB	1-4	0-3	1	0-2

1.3.6 Treatment Options

Many factors, including the degree of skin involvement, the type of skin lesion, and whether the cancer has spread to the lymph nodes or other internal organs, are considered to determine the most suitable treatment for each patient. Treatment is either aimed at the skin (local) or the whole body (systemic) for MF. When treating their disorder, many people live normal lives, and others are able to stay in treatment for lengthy periods of time. Since Sézary's syndrome is systemic, one in which exposure of both blood and skin is noted, skin-directed treatments alone are normally not considered. To attain the best long-term benefit, medications can be administered alone or in combination. For earlier stage disease, Skin-Directed Remedies are commonly used and are evidence to help for lesions and limited signs. Topical medications like corticosteroids, retinoids, imiquimod, or chemotherapy also include therapies; localized ultraviolet light radiation. [24, 25]

Among these, in patients taking previous skin treatment, bexarotene gel (Targretin) and mechlorethamine gel (Valchor) have been authorised by the US-FDA as potential therapy for Stage 1A and 1B mycosis fungoides. In more advanced stages of disease and in those with earlier stage of disease in whom skin-directed therapies did not help, were not well tolerated, a systemic treatment may be a good option.[25]

Systemic remedies involve extracorporeal photopheresis (ECP) and single-agent or combined effect with chemotherapy regimens, which may include:

• Acitretin (Soriatane)

• Methotrexate tablets (Trexall)

• Bexarotene capsules (Targretin)

• Romidepsin (Istodax)

• Interferons (alpha or gamma)

• Vorinostat (Zolinza)

Table 1.2: Summary of treatment options for MF/SS[25]

Therapy	MF		Sézary syndrome/ erythrodermic MF	Comments
	Early-stage disease	Advanced-stage disease		
Topical corticosteroids	++++	++	+++	Symptomatic control
PUVA	++++	+	+++	Availability may be restricted in nonmetropolitan areas
UVB	+++	+	++	More readily accessible than PUVA
Topical chemotherapy	+			If limited number of lesions
Imiquimod	+			If small lesions and limited number of lesions
Photodynamic therapy	+			If limited number of lesions; limited availability
Retinoids	+	+	+	Usually second line; less used since bexarotene became available
Bexarotene	+++	+++	+++	Usually second line; can be used in combination with PUVA or IFN- α
Interferon-α	++	+++	++++	Second line
HDACi	+	+++	++++	Beyond second line
Oral MTX	+	+++	++	Low dose weekly
Localized radiotherapy	+	+++		If localized or large/plaques and tumor nodules
TSEB	+	++	+	For widespread disease
Systemic chemotherapy		++	++	Beyond second line
ECP		++++		If circulating clone detectable
Autologous transplantation		+	+	Very selected cases
Allogeneic transplantation		+	+	Very selected cases
Denileukin diftitox		++	++	Beyond second line
Alemtuzamab		+	+	Beyond second line; immunosuppressive
Proteasome inhibitors		+		Under investigation
Immunomodulatory agents (lenalidomide)		+		Under investigation

Combination therapies are typically reserved for when several single-agent therapies have not responded well to patients. Brentuximab vedotin (Adcetris), gemcitabine (Gemzar), pralatexate (Folotyn), and liposomal doxorubicin (Doxil) are alternatives for refractory disease (the disorder may not benefit from treatment), but none of these are officially FDA approved. Bexarotene, a new retinoid with a high retinoid X receptor affinity, was recommended for the treatment of patients with chronic MF by the US-FDA in 1999. Bexarotene is also available in a 1% w/w topical gel form and has been shown to generate significant responses in patients with early-stage MF. The gel is well absorbed, however its use has been reported to cause some local skin irritation.[27-29]

1.4 TOPICAL DRUG DELIVERY SYSTEM

The skin, vagina, eye and nose are the main routes that have been commonly identified for topical administration of drugs. Since the skin is the biggest and easy to access organ of the human body, anti-cancer drugs for skin cancer and breast cancer are relatively easy to administer via this path. In the topical delivery of anti-cancer drugs via the skin, the key challenge is to increase the penetration of anti-cancer drugs in therapeutic efficient concentrations through stratum corneum to kill tumour cells.

The application of the drug to the topical sites prevents the metabolism of the hepatic first pass, gastric pH changes and variations in plasma level, often found when a drug is delivered via the oral path. The epidermal region consists of the superficial layers of the stratum corneum, the special cellular structure of which provides a permeability obstacle to the passage of molecules > 500 Da in size, thus offering physical defence against invading microbes. The other benefits of the topical drug delivery model include the following:

- Application and delivery with ease and convenience,
- Highly accepted formulation by patients,
- Topical delivery is noninvasive and painless technique,
- Drug bioavailability may also increase,
- Improvement in drug bioavailability,
- Better response in pharmacological and physiological terms and
- Least clinical toxicity and drug penetration to nonpathogenic tissue / locations.

Skin disorders afflict millions of people daily. For the localized treatment of diseases, the topical drug delivery to the skin surface is continually being researched. In developing of the topical drug delivery system, however, resistance to absorption for most active moiety or drugs poses a major challenge. Attempts have been made to use drug delivery systems to ensure sufficient localization or penetration of the drug inside or via the skin in the formulation of topical delivery forms in order to improve localized and reduce systemic effects or to ensure proper percutaneous absorption. Liposomes, applied to the skin, can serve as a solublizing system for poorly water soluble drug, at a same time decreasing the adverse effect of the drug as well as increasing the penetration enhancer and local depot of drug. Compared to traditional formulations, topical liposome formulations may be more effective and less toxic. [30,31]

1.4.1 Drug Transport across the Skin

The thickness of the dermis varies from 3-5 mm and consists of a mixture of glycosaminoglycans, salts and water with fibrous proteins (collagen and elastin) and an interfibrillar gel. Forms I and II of collagen constitute approximately 75 percent of the dry weight of the dermis. The dermis embeds blood and lymph channels, free nerve endings, hair follicles, and sebaceous and sweat glands. On the skin's surface, the hair follicles and sweat glands ducts open directly towards the outside.[32-34]

The primary route of skin absorption is through the intact epidermis, and two main pathways have been identified: the intercellular pathway through the stratum corneum lipids and the corneocyte transcellular pathway. The drug must be diffused through the intercellular lipid matrix in both cases, which is known as the main determinant of the skin's drug absorption.

Drug delivery via skin involve several step: a) dosage from dissolution and drug; b) partitioning of drug into the stratum corneum; c) across the stratum corneum drug is diffused; d) drug partitioning into viable epidermis layers from the stratum corneum; e) diffusion across into the dermis from the viable epidermis layers, and f) absorption of drug by capillary vessels, which achieves to systemic circulation. [35]
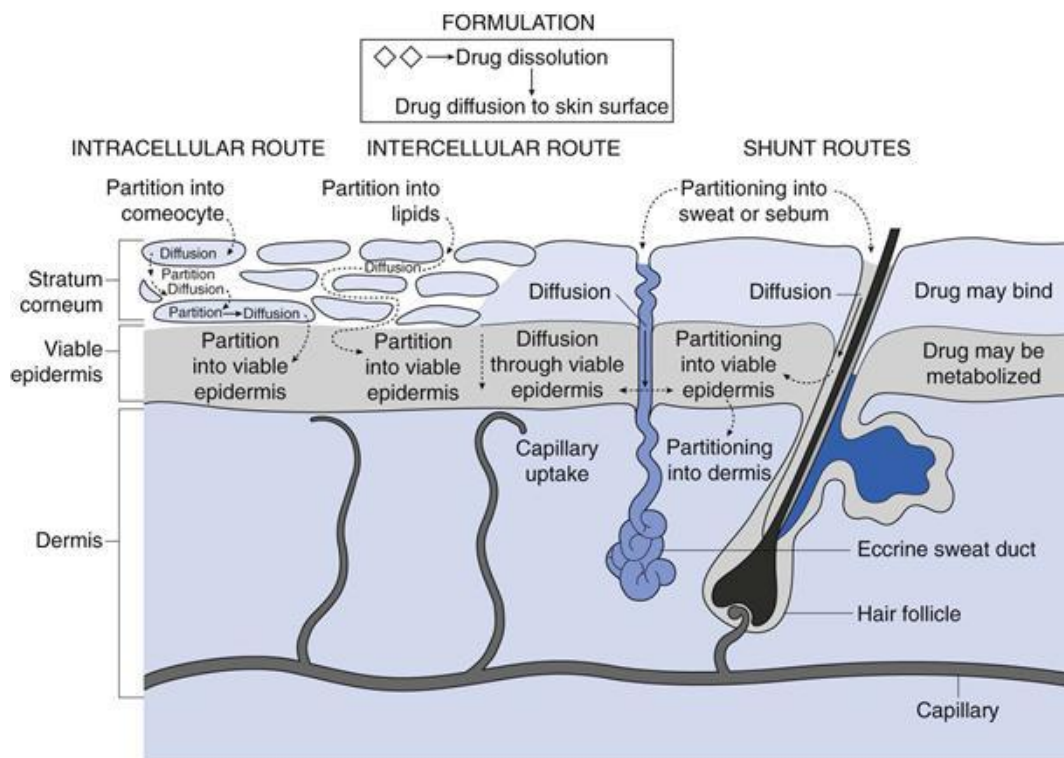
Figure 1.7: Drug transport mechanism through the skin[35]

Selection of drug candidates for skin permeation must be based on a variety of considerations, including physicochemical properties, membrane drug interactions, and pharmacokinetic properties of drug. The desired physicochemical properties of a drug molecule selected for topical delivery have low molecular weight (<500 Da), high diffusion coefficient value; solubility in water as well as in oils to achieve a high rate of diffusion via the skin; a high but balanced partition coefficient required because a very high partition coefficients may hinder drug clearance from the skin and increase drug retention; and low melting point (<250 ℃), which is related to an appropriate solubility. [36, 37]

1.5 GELS AS TOPICAL DOSAGE FORMS

In the late 1800s, the term 'Gel' was introduced to name some semisolid material according to its physiological features rather than molecular composition. Gels are described by the U.S.P. as a "semisolid system consisting of dispersion consisting of either small inorganic particles or large organic molecules that are enclosed by liquid

and interpenetrated. Gels are a cross-linked mechanism that is greatly diluted, which shows no flow when in the steady state." [38-39]

1.5.1 Classification of Gels

Gels are classified as [40, 41]:

1) *Nature of colloid phase*
        a. Inorganic gels
        b. Organic gels

2) *Based on nature of solvent*
        a. Hydro-gel
        b. Xero-gel
        c. Organo-gel

3) *Based on rheological properties*
        a. Plastic gels
        b. Pseudoplastic gels
        c. Thixotropic gels

4) *Based on physical nature*
        a. Elastic gel
        b. Rigid gel

1.5.2 Hydrogels

Hydrogels are defined as "gels that consist of an aqueous dispersion medium that is gelled with a suitable hydrophilic gelling agent. By definition, hydrogels are three-dimensional polymeric networks that are capable of imbibing high concentrations of water or biological fluids. In polymers forming hydrogel structures, their affinity to absorb water is due to the presence of hydrophilic groups such as-OH,-CONH,-$CONH_2$ and-SO_3H." Because of the network contribution of these groups, the polymer is thus hydrated to varying degrees, depending on the existence of the aqueous environment and the composition of the polymer.

Types of Hydrogels:

        pH – Sensitive Hydrogel

        Temperature Sensitive Hydrogel

        Nanohydrogels

        Glucose Sensitive Hydrogel

1.5.3 Organogels

Organogels may also be referred as oleaginous gels. They are made up of both polar and nonpolar groups, but the non-polar component ratio is quite high. As the gels appear to swell in water, they can contain 35 percent water. Organogelators are typically small molecules with low molecular weight that have the ability to thicken in organic solvents. With the discovery and synthesis of a very large number of different molecules, physical organogels have evolved rapidly, which can form gel at low concentrations with organic solvents.

1.5.4 Desirable Properties of Gels

The gelling agent must ideally be inert, safe and unable to interact with all other components of the formulation. [42-44]

- At the time of storage, the gelling agent should form a solid-like character that is readily disrupted when subjected to shear.
- It must be non irritating, non sticky and have an effective anti-microbial property.
- The ophthalmic gel may be sterile.
- With an increase in the gel's effective crosslink density, the apparent viscosity of gel strength increases. They show the mechanical properties of the solid state.

1.5.5 Characteristics of Gels

A. *Swelling*

If a gellant comes in contact with water, so these gellant absorbs a large amount of water and the volume may increase. This mechanism is called a swelling. As a result of solvent penetration into the matrix, this phenomenon occurs. [45]

B. *Syneresis*

On standing, several gels frequently contract spontaneously and exude some water. This effect is referred to as syneresis. As the gellant concentration decreases, the degree to which syneresis increases.[45]

C. *Ageing*

Slow aggregation is usually shown naturally by colloidal systems. This phemomena is called ageing of gel. In gels, ageing allows the gelling agent to form a denser network progressively. [45]

D. *Structure*

Because of the existence of a network formed by the interconnecting of molecules of the gelllant, results in the solidity of a gel. The nature of the particle and the applied shear or force, may straightening it out and reducing flow resistance. [45]

E. *Rheology*

Gellant solutions and flocculated solid dispersion are pseudoplastic in nature, are Non-Newtonian flow behaviour is followed, characterised by a reduction in viscosity with an increase in shear rate. [45]

1.5.6 Preparation of Gel

Gels are usually processed at room temperature on an industrial scale. However, before processing, few of the polymers require special care. Gels can be prepared using the methods like thermal change, flocculation technology and chemical reaction. [46]

1.5.7 Formulation Design

Main components of topical gels are:[47-50]

A. *Gel forming agent or polymer*
- For a polymer to be used in a topical device, the following conditions should be met:
- Diffusion and release must be made possible by molecular weight and chemical functionality.
- The polymer should allow a large quantity of medication to be incorporated.
- The polymer does not interfere with the drug, physically or chemically.
- The polymer can be processed into the desired product easily.The polymer must be stable and must not decompose in the presence of drug and other

excipients used in the formulation, at high humidity conditions, or at body temperature.

- Polymers and its degradation products must be nontoxic.

Gellants of Gel forming polymers are classified accordingly.

a) Natural Polymers

    i. Proteins –Collagen, Gelatin, Xanthin, Gellum Gum

    ii. Polysaccharides – Agar, Alginic acid, Sodium or potassium carrageenan, Tragacanth, Pectin, Guar Gum, Cassia tora

b) Semisynthetic Polymers

    Cellulose Derivatives – Carboxymethyl cellulose Methylcellulose, Hydroxypropyl cellulose, Hydroxypropyl methylcellulose, Hydroxyethyl cellulose

c) Synthetic Polymers

    i. Carbomer –Carbopol -940, Carbopol -934, Carbopol -941

    ii. Poloxamer

    iii. Polyacrylamide

    iv. Polyvinyl Alcohol

    v. Polyethylene and its copolymers

d) Inorganic Substances – Aluminium Hydroxide, bentonite

e) Surfactants – Cetosteryl alcohol, Brij-96

B. *Drug Substance*

In the successful development of a topical product, the drug substance plays a very significant role. The following are the significant drug properties that influence its diffusion through gels as well as through the skin.

Physicochemical properties

- The molecular weight of the drug should be lower than 500 Daltons.
- For topical delivery, highly acidic or alkaline drugs in the solution are not suitable.
- The drug must be sufficiently lipophilic.
- A pH should be 5 to 9 of a saturated aqueous solution of the drug.

Biological properties

- The medication does not irritate the skin.
- Drugs that degrade or inactivated by the hepatic first pass effect in the GIT are ideal for topical delivery.
- Under the zero order release profile of topical delivery, tolerance of the drug does not develop.
- The medication does not stimulate the skin to undergo an allergic response.
- Drugs that need to be given for a long time or that cause adverse effects on non-target tissue.

C. Penetration Enhancer

Penetration enhancers can increase the penetration of drugs into the skin by reversibly reducing the resistance of the skin barrier (also called accelerants or sorption promoters). Under the influence of a concentration gradient and its subsequent diffusion through the stratum corneum and underlying epidermis, through the dermis, and into the blood circulation, percutaneous absorption involves the transition of the drug molecule from the skin surface into the stratum corneum. As a passive barrier behavior of the skin resist the penetrant molecule for easy penetration. The stratum corneum provides the greatest penetration resistance and in percutaneous absorption it is the rate-limiting step. For altering the skin molecular structure, a penetration enhancer serves as to growing a drug's flux and is known as a main part of most topical formulations. Skin resistance (stratum corneum) to drug diffusion must be decreased in order to allow drug molecules to cross the skin and to maintain therapeutic levels in the blood in order to reach and maintain therapeutic concentrations of the drug in the blood. Either by communicating

with the formulation applied or with the skin itself, they may change the skin's barrier to penetration. [51-54]

D. *Other Ingredients used in Formulation of Gels*

a. Vehicle or Solvent

As a solvent, water is mostly used. Co-solvents may also be used to increase the solubility of the drug in the dosage form or to enhance drug penetration across the skin, for example Glycerol, PEG 400, etc.

b. Buffer

To maintain the pH of the gel, buffers can be involved in aqueous and hydroalcoholic-based gels like phosphate and citrate buffers.

c. Preservatives

Preservatives addition to the gel may increases shelf-life or stability. For that purpose Parabens, phenolic are added in the gel in very low amount.

d. Antioxidant:

In order to improve the chemical stability of therapeutic agents vulnerable to oxidative degradation, it may be integrated into the formulation. Since most gels are aqueous-based, like sodium metabisulphite, sodium formaldehyde sulfoxylate are water-soluble antioxidants are commonly used in gel formulations.

e. Flavors and Sweetening agents

Flavouring agents and sweetening agents are included only in gels intended for oral administration. It would not be out of scope to provide specifics of concentrations for various gelling substances. [55-57]

1.6 LIPOSOMES

"Liposomes are spherical-shaped vesicles composed of one or more lipid bilayers, involving an aqueous compartment" as shown in Figure 1.8. These are spontaneously moulded; first, by continuous stirring, the lipids are distributed in an aqueous medium, which creates vesicles that can vary in size from nanometers to microns in diameter. The lipid molecules consist of head molecules that are

drawn to hydrophilic molecules and shape themselves in a such way as to point toward the aqueous region, while the water molecules repel the lipophilic tails and point in the opposite direction. [58]
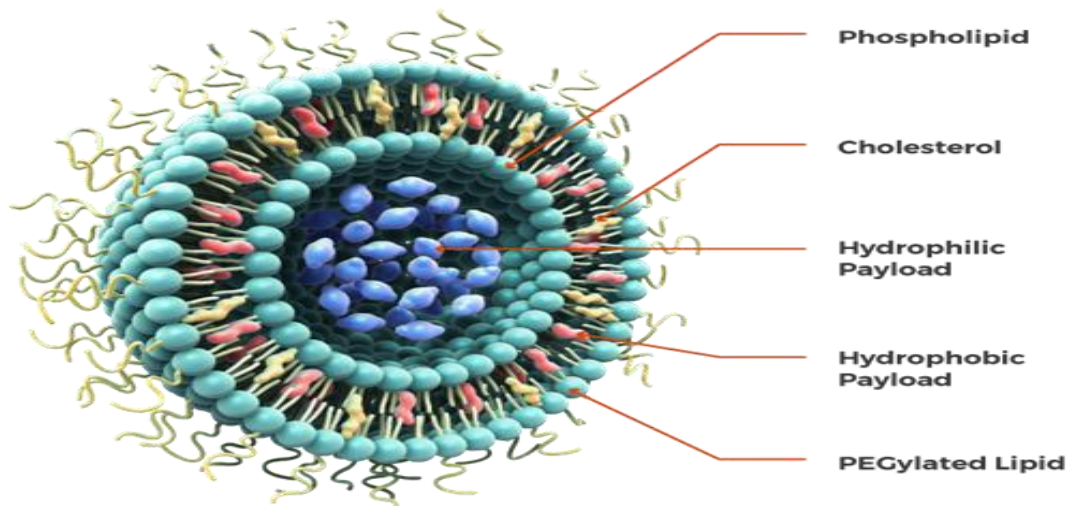
Figure 1.8: Schematic diagram of liposomes[58]

The inner layer's hydrophilic groups point at the side of the intravesicular fluid, with the tails pointing away from it. One layer's lipophilic tails point towards the hydrocarbon tails of the outermost layer, forming the usual bilipid membrane in turn.[59]

The lipids that are often used in the preparation of liposomes, most of them biodegradable and biocompatible in nature, are phospholipids and sphingolipids. These lipids derive either from natural sources either from synthetic sources. Lipids, on the other hand, are cylindrical molecular shapes that organise aqueous solutions into stable bilayers. Phosphatidylcholines are mostly used because of their stability and ability to counteract changes in pH or changes in salt concentrations in the product or biological environment.[60]

Liposomes are characterized by their size (small, intermediate or large), bilayers available (unilamellar and multilamellar), composition and drug delivery mechanisms. A single lipid bilayer with an average diameter ranging from 25to75 nm consists of small unilamellar vesicles. Wide unilamellar vesicles also consist of a single bilayer of lipids and are larger than 75 nm in vesicle size, whereas multilamellar vesicles (MLVs) consist of several concentric bilayers of lipids. [Figure1.9].
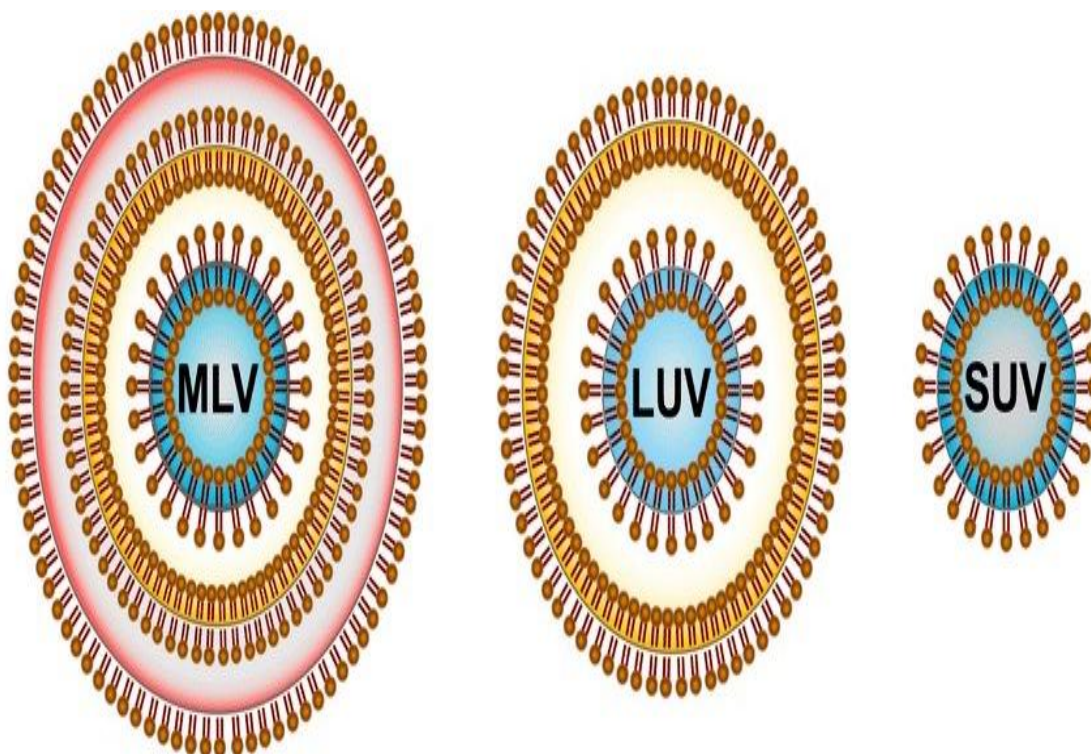
Figure 1.9: Types of liposomes[60]

Liposomes are small, spherical-shaped artificial vesicles that can be formed from cholesterol and nontoxic natural phospholipids. Liposomes are promising mechanisms for drug delivery due to their size and hydrophobic and hydrophilic character.[61]

1.6.1 Advantages of Liposome

- Liposomes have improved stability by drug encapsulation.

- For systemic and non-systemic administration, liposomes are non-toxic, versatile, biocompatible, fully biodegradable, and non-immunogenic.

- Liposomes decrease the encapsulated agent's toxicity.

- Liposomes help to minimize exposure of harmful drugs to sensitive tissues.

- Provide flexibility to combine with ligands unique to the site to achieve successful targeting.[62]

1.6.2 Liposomal Delivery of Drugs to the Skin Cancer Sites

The liposomal adrug delivery system currently provides a great opportunity to target melanocytes and other cancerous sites in the skin safely and effectively. The liposomes, which have emerged as an important method for delivering the therapeutic agent(s), directly to the site of cancer, have been tremendously helpful in improving the overall effectiveness of care for skin cancer by delivering it to the cancer site and permeating it. In liposomal formulations, including high MW (molecular weight) organometallic and medicinal organic / secondary metabolite products, a wide variety of cytotoxic agents, discovered from terrestrial plants and natural sources as well as synthetic products, have been trapped. Several liposomal formulations are currently being clinically used to treat a range of skin cancers and melanomas with enhanced cytotoxicity, decreased adverse effects, and near-precise drug targeting. By safeguarding the photophysical properties and preserving the photodynamic functions of the encapsulated photosensitizer with enhanced efficiency of payload delivery photosensitizer as a therapeutic tool in an alternative approach for different skin malignancies, the liposomes have also been known to be an effective nanocarrier for photosensitizers.[63-65]

Table 1.3: Characteristics and advantages of liposomal delivery[64]

Factors	Results	Example(s)
Solubility	Improved solubility of lipophilic and amphiphilic drugs	Amphotericin B, porphyrins, minoxidil, some peptides, and anthracyclines; hydrophilic drugs, such as anticancer agent doxorubicin (DOX) and acyclovir
Targeting of drug and their class	Passive targeting to the cells of the immune system, mononuclear phagocytic system	Antimonials, amphotericin B, porphyrins, vaccines, immunomodulators, anti-inflammatory drugs, anti-cancer, anti-infectious agents
Release of drug	Enhanced drug release, sustained release system of systemically or locally administered liposomes	DOX, cytosine arabinoside, cortisones, biological proteins or peptides such as vasopressin
Site-avoidance mechanism	Achieved	DOX and amphotericin B
Penetration	Improved penetration into tissues	Corticosteroids, anesthetics, and insulin
Clearance	Rapid clearance	Rapid clearance from circulation due to RES uptake

1.6.3 Liposomes and Skin Permeability

Skin permeability and drug penetration rates are an significant measure of the drug's transdermal delivery, in particular for the improvement of the delivery of anti-cancer drugs to the sites of skin cancer. The existence and concentration of desired delivery at the tumour site is another design criterion for liposomal formulation drug load and specificity of the site.

Ethosomes, micelles, and cationic liposomes have shown greater permeability on an experimental basis. The drug scope was demonstrated by analytical techniques through the ethosome and flexible stealth liposome. [66]

1.6.4 Methods of Liposome Preparation

All of the liposome preparation methods include four basic stages[67]:

 i. Drying of lipids in organic solvents.

 ii. Lipid dispersion in aqueous media.

 iii. Purification of the resulting liposome.

 iv. Final evaluation.

For liposome drug loading, the following techniques are used:

 i. Techniques for passive loading of drug.

 ii. Technique of fast loading of drug.

Three different methods include passive loading techniques:

1. Mechanical process for dispersion.

2. Solvent dispersion method.

3. Method of detergent removal.

-24-
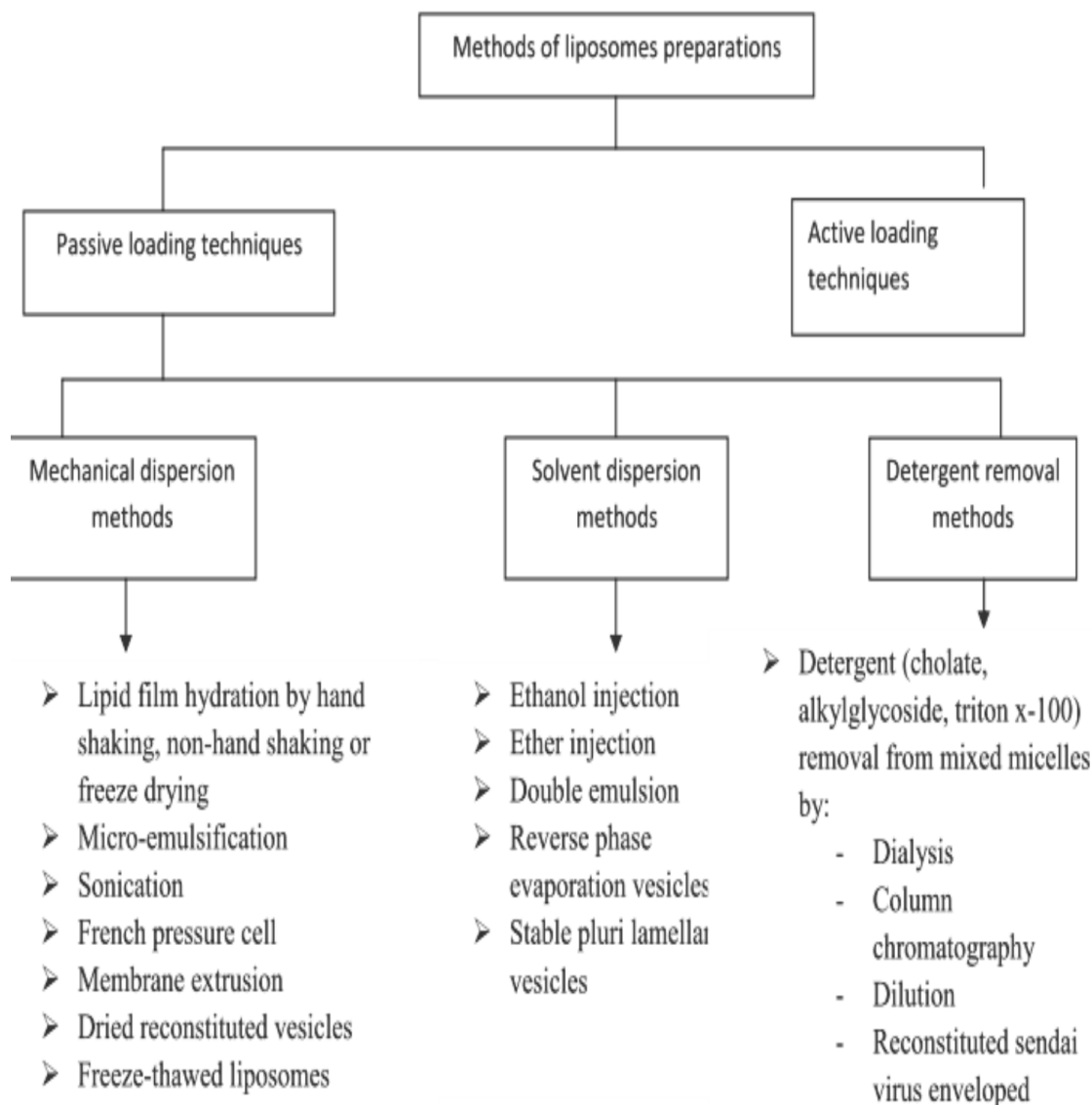
Figure 1.10: Preparation methods of liposomes[66]

A. Sonication

Sonication is the most commonly used technique for SUV preparation. Here, either with a bath sonicator or a probe sonicator used for liposome prepration. Very low internal volume, encapsulation effectiveness, potential degradation of phospholipids and compounds to be encapsulated, removal of large molecules, metal pollution from the tip of the probe, and the presence of MLV along with SUV are the main drawbacks of this approach.[68]

There are two sonication techniques:

a) Probe sonication

The tip of the sonicator is touched directly into the dispersion of the liposome. In this method, the energy input into lipid dispersion is very high. The energy coupling at the tip results in local warmth; thus, the vessel must be put into a water / ice bath. About 5% of the lipids can be de-esterified during the sonication for upto 1h. Also, titanium can slough off and pollute the solution while using probe sonicator.

b) Bath sonication

In a cylindrical vessel, the liposome dispersion is inserted into a bath sonicator. In this process, it is generally easier to monitor the temperature of lipid dispersion, in comparison to sonication by dispersal specifically using the tip. In a sterile vessel, differing from the probe systems, or under an inert environment, the substance being sonicated may be covered.

B. Lipid Film Hydration Technique

The lipids must first be dissolved and blended into an organic solvent to ensure a homogeneous mixture of lipids when preparing liposomes with a blended lipid composition. This procedure is generally conducted using chloroform or chloroform: methanol mixtures. The aim is to obtain a transparent lipid solution for full lipid mixing. Usually, lipid solutions are prepared with an organic solvent of 10-20 mg lipid per ml, although higher concentrations can be used if lipid solubility and blending are appropriate. The solvent is extracted to yield a lipid film until the lipids are thoroughly blended into the organic solvent. The solvent can be evaporated using a dry nitrogen or argon stream in a fume hood for small amounts of organic solvent (< 1 ml). The organic solvent can be extracted by rotary evaporation for larger amounts, forming a thin lipid film on the sides of a round bottom flask. To extract residual organic solvent, the lipid film is thoroughly dried by putting the vial or flask on a vacuum pump overnight. If chloroform is not used in prepration of liposomes, the dissolution of the lipid(s) in tertiary butanol or cyclohexane are used as an alternative. The lipid solution is transferred to glass containers and freezed by placing the containers on a dry ice block or by spinning the bottle in a bath of dry

ice-acetone or alcohol. When using the bath technique, care should be taken to allow the container to withstand sudden temperature variations without breaking. The frozen lipid cake is put on a vacuum pump and lyophilized until dry after freezing completely. It is possible to extract dry lipid films or cakes from the vacuum pump, tightly close and tap the container, and store them frozen until ready to hydrate. [69]
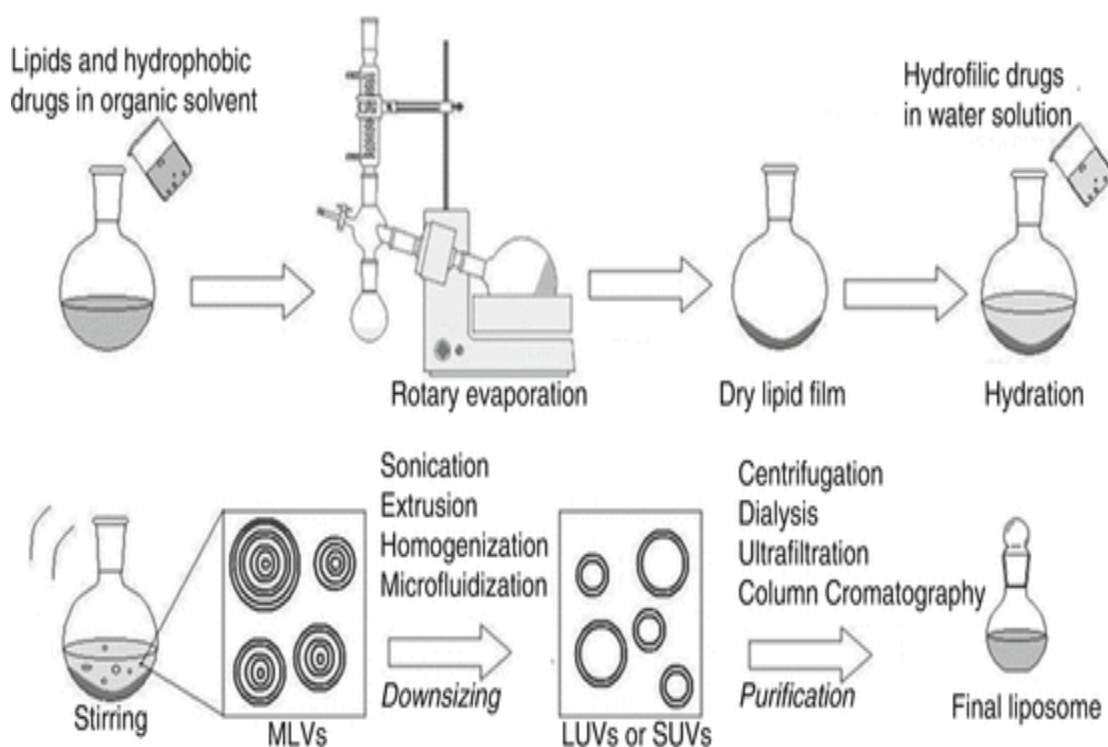
Figure 1.11: Liposomes prepared by thin layer evaporation technique[69]

1.7 NIOSOMES

Niosomes are vesicular nanocarriers and, due to their unique advantages, have received a lot of attention as potential drug delivery systems in the last 30 years. They have lamellar (bilayer) structures surrounded by an aqueous compartment composed of amphiphilic molecules. Both hydrophobic groups (tails) and hydrophilic groups (heads) contain these amphiphilic molecules, known as surfactants, and exhibit self-assembling features, aggregating into a number of shapes such as micelles or into a geometric bilayer lamellar. Sorbitan esters and analogues, sugar-based, polyoxyethylene-based, polyglycerol, or crown ether-

based surfactants, often in-addition to membrane additives, such as cholesterol or its derivatives, are surfactants that may be used as possible drug delivery systems. Nonionic surfactants are favoured because they are less likely to cause discomfort, which in cationic > anionic > nonionic order decreases.[70]

They are capable of encapsulating both hydrophilic and lipophilic compounds through the special structures of niosome as vesicular systems. Hydrophilic drugs are normally encompassed on the bilayer surfaces in the inner aqueous core or adsorbed, whereas lipophilic compounds are trapped in the lipophilic domain of the bilayers by their partitioning.
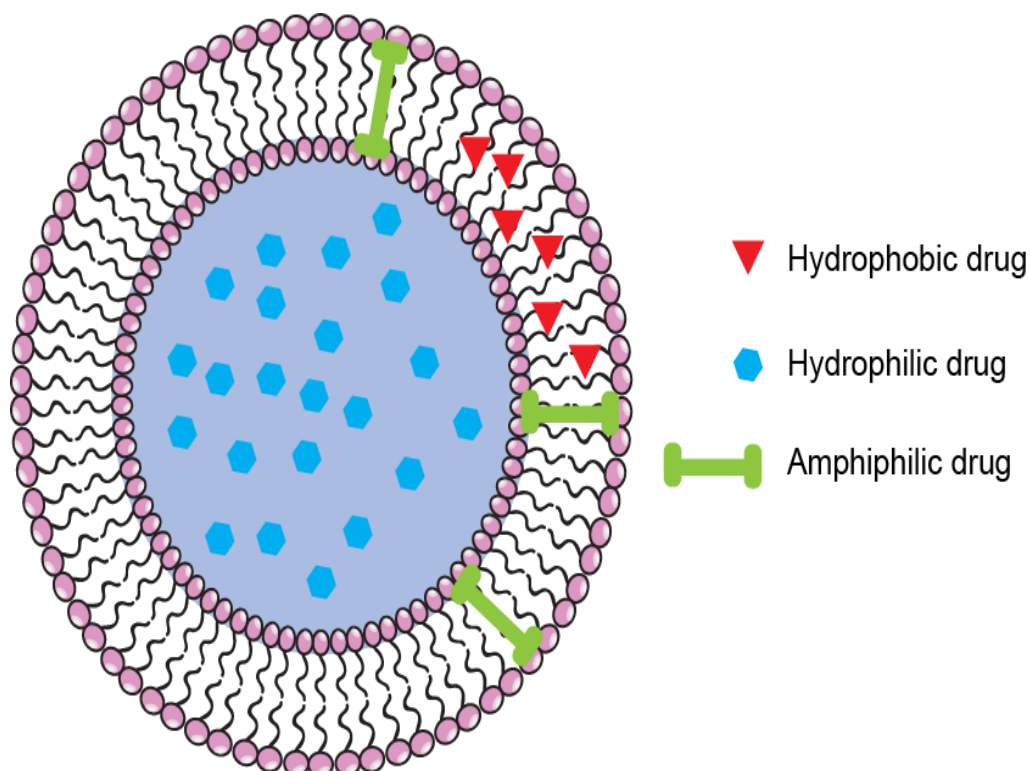
Figure 1.12: Schematic representation of a niosomal vesicle[70]

These vesicles have been widely used as drug delivery systems to achieve drug targeting, controlled release, and permeation improvement, due to their potential ability to carry a variety of therapeutics. In fact, in a controlled way, niosome can serve as a therapeutic reservoir for the delivery of a drug to improve bioavailability, achieving a therapeutic effect over a longer period of time.

1.7.1 Advantages of Niosomes

Niosomes have many advantages over other nanocarriers. [71]:

- Biodegradable, biocompatible and non-immunogenic surfactants are used to prepare niosomes.

- The handling and storage of niosomes does not require any special conditions because of the chemical stability of their structural composition.

- By adjusting their structural composition and the production process, the physicochemical properties of niosomes, such as their shape, form, fluidity, and vesicle size can be easily controlled.

- A significant amount of drug can be encapsulated in a small vesicle of niosomes.

- It is possible to use niosomes to distribute labile and responsive drugs.

- Niosomes enhance the therapeutic overall performance of drug molecules by delaying blood circulation clearance and limiting the impact on target cells.

- It is possible to administer the drugs via niosomes through various routes, such as oral, parenteral and topical.

- Compared to oily dosage formulations, the aqueous vehicle-based suspension formulation results in improved patient compliance; additionally, niosomal dispersion, being aqueous, may be emulsified in a nonaqueous process to control the rate of drug release.

- To achieve better patient adherence, compliance and satisfaction and also better performance than traditional oily formulations, niosomes have been used widely.

1.7.2 Niosome Percutaneous Absorption

When niosomes are applied to the skin, it is important to distinguish what form of effect is needed, i.e. a local effect within the skin or a systemic effect followed by skin permeation, as shown in Figure 1.13.

The goal of transdermal targeting is to enter the blood stream, and it is becoming a subject of concern for many pharmaceutical research groups researching diseases such as inflammation, cancer, psoriasis, alopecia, and acne. The transdermal route has many advantages over traditional drug administration routes: avoiding

serum peak and trough levels; avoiding first-pass hepatic metabolism and gastrointestinal degradation (pH, enzymatic activity, and food, beverage, and other orally administered drug interactions), leading to an improvement in bioavailability and efficacy of the drug. The accessibility of the skin, the relatively wide surface area for absorption, and the fact that it is noninvasive are other benefits of the transdermal route, making the patient more compliance.[72]
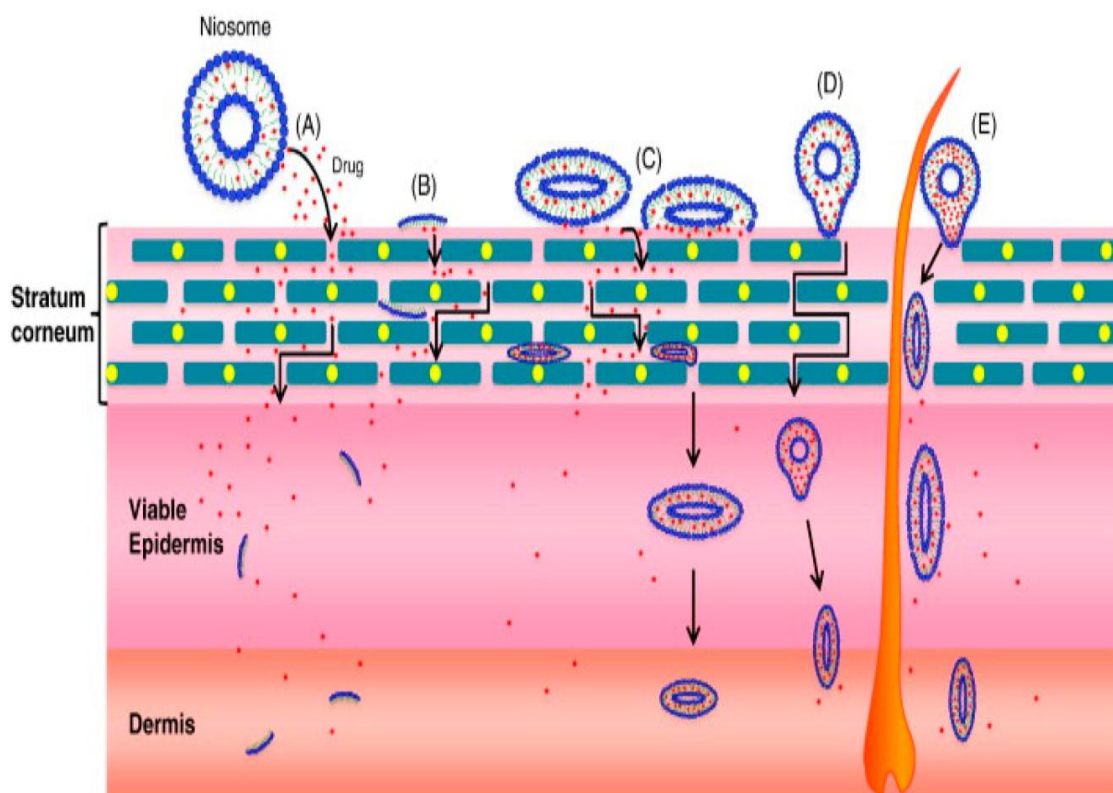
Figure 1.13: Mechanism of action of niosome transdermal applications[72]
"(A) drug molecules are released by niosomes;(B) niosome constituents act as penetration enhancer; (C) niosome adsorption and/or fusion with stratum corneum; (D) intact niosome penetration through the intact skin; (E) niosome penetration through hair follicles and/or pilosebaceous units."

1.7.3 Types of Niosomes

Based on the vesicle size, niosomes can be classified into three classes.These are –

- Small unilamellar vesicles: 0.025- 0.05 μm.

- Multilamellar vesicles :>0.05 μm.

- Large unilamellar vesicles: >0.10 μm.
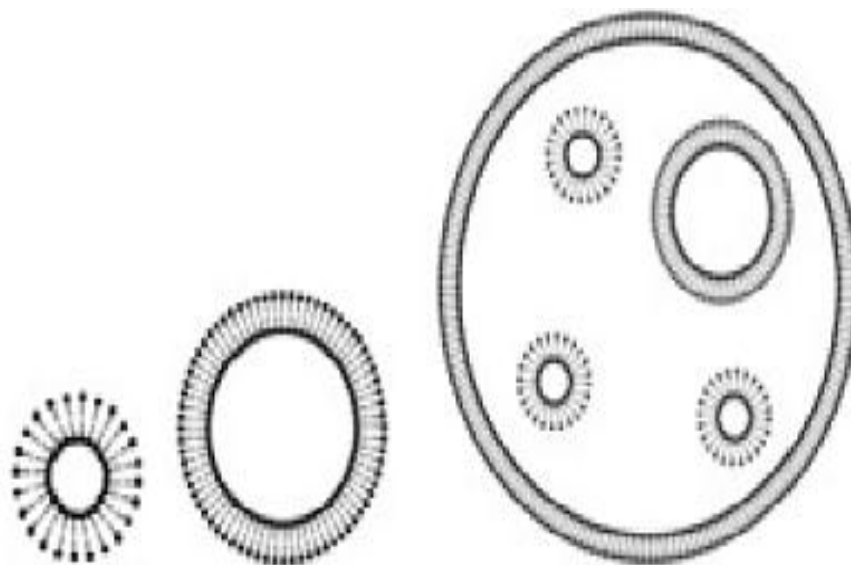
Figure 1.14: Types of niosomes[73]A. SUV, B. MUV, C. LUV

1.7.4 Methods of Preparation

A. Sonication

This method involves addition of surfactant/cholesterol in organic solvent and drug in aqueous solution. This aqueous solution of drug is mixed with surfactant solution and further homogenized for 3 minutes at a temperature of 60°C.[73]

B. Micro fluidization

The theory of submerged jet is followed for the formulation of niosomes. Inside the interaction chamber, the fluidized stream of drugs and surfactants is allowed to communicate at ultra-high speeds via the micro channel. The impingement of a thin liquid layer along a common front is structured so that within the region of niosome formation, the energy used by the device remains. The method results in greater uniformity, smaller size and better reproducibility of niosomes.[74]

C. Hand Shaking Method (Thin Film Hydration Technique)

In the rota flask, this method requires the creation of a thin dried layer. This process dissolves surfactants and cholesterol in organic solvents. Both solutions are mixed and, at a certain temperature, the mixture is evaporated under reduced pressure to form a thin layer on the inner wall of the flask. Hydration is carried out with sonication to form niosomes after evaporation.[75]

D. *Trans-membrane pH Gradient Drug Uptake Process*

This procedure utilises the technique of thin film hydration. A definite ratio of cholesterol and surfactant is dissolved in organic solvents like ether or chloroform in this technique and evaporated to form a thin dried film under reduced pressure. By vortex mixing, the resulting film is hydrated with 300 mM of citric acid (pH 4.0). The tiny vesicles are allowed many times to freeze and thaw and are sonicated to get niosomes. The aqueous drug solution is applied to the niosomal suspension above and is vortexed. With 1M disodium phosphate, the niosomal suspension pH is modified to 7.0-7.2, heated to 60° C for 10 minutes to form the desired multilamellar vesicles. [76]

E. *Reverse Phase Evaporation Technique*

In this technique, a definite ratio of cholesterol and surfactant is dissolved in organic solvents such as ether or chloroform and evaporated under reduced pressure to form a thin, dried film. The resulting film is hydrated with 300 mM of citric acid by vortex mixing (pH 4.0). Following the addition of a small amount of phosphate buffer solution, the formed gel is further sonicated and the organic phase present is extracted at 40 ° C, which forms high viscosity niosomes. This viscous niosome is diluted with a saline phosphate buffer that is maintained for 10-15 minutes at 60°C. [77]

F. *Ether Injection Method*

In the preparation of large unilamellar vesicles, this process is used where the definite ratio of cholesterol and surfactant is dissolved in organic solvents. Organic drug solution is applied to the above mixture and injected into the aqueous solution located at a magnetic stirrer maintained at 60°C with niosomal suspension forming continuous stirring.[78]

G. *Multiple Membrane Extrusion Method*

In this method surfactant and cholesterol dissolves in organic solvent, i.e. chloroform. Dicetyl phosphate is then added to the organic surfactant / cholesterol solution, and the resulting solution is evaporated to form a thin film under reduced pressure. The developed film is rehydrated with an aqueous drug solution. The

resulting suspension is extruded through a polycarbonate membrane. This method can regulate the size of the niosomal formulation. [79]

H. Emulsion Method

Separately, an aqueous solution of the drug is prepared and this solution is added to the surfactant and cholesterol solution dissolved in organic solvent, forming oil in the emulsion of water. The organic solvent is then evaporated, leaving the aqueous phase of niosomes dispersed.[79]

I. Lipid Injection Method

Along with surfactants, this approach uses lipids also. The lipids and surfactants are dissolved, and the mixture is injected into the heated aqueous drug solution. Another procedure requires the addition of lipid drugs and melting. The molten solution is pumped into a surfactant aqueous solution that is heated to form niosomes.[80]

J. The Bubble Method

This is a one-step technique for niosome preparation without organic solvent use. For temperature control, the bubbling unit has a round-bottomed flask with three necks positioned in a water bath. The water-cooled reflux is placed in the first neck, the thermometer is placed in the second and nitrogen supply is given in the third neck. Niosome dispersion is prepared by adding cholesterol and surfactant to the pH 7.4 phosphate buffer, which is maintained at $70^{o}C$ and the dispersion formed is mixed for 15 seconds using a high shear homogenizer, then it is bubbled at $70^{o}C$ using nitrogen gas.[81]

1.7.5 Factors Affecting Niosome Formation

A. Surfactants and Additives

For the formation of niosomal vesicles, non-ionic surfactants are used. Hydrophobic tail surfactants can consist of one or two groups of alkyls or perfluoroalkyls or, in some cases, a single steroidal group. Mono alkyl chain ether-type surfactants are more toxic than ester-type surfactants. Ester type surfactant is less stable than ether-type surfactants when stability is taken into account, and it is because ester-related surfactants are converted into triglycerides and fatty acids by esterase, in-vivo. The alkyl chain length surfactants from C12 to C18 are sufficient for the preparation of

niosomes. The development of niosome vesicles terminates the suitability of surfactants with an HLB value between 4 and 8.[82]

B. Surfactant and Lipid Level

The surfactant / lipid level that is necessary for niosomal formulation is usually maintained between 10-30 mM (1-3percent w/w). Alteration of the surfactant, water ratio during the hydration process, affects the niosomal dispersion. The total amount of drug encapsulated is also increased by increasing the surfactant / lipid ratio.

C. Composition of Membrane

By adding various additives to the surfactant mixture, stabilization of niosomes can be achieved. The leakage of drugs from the vesicles that can be regulated by the addition of cholesterol is one of the key advantages of niosome formulation. Cholesterol gives the membrane better rigidity and thus reduces the leakage of the drug.

D. Nature of Encapsulated Drug

Niosomal formulation is affected by the existence of the encapsulated medication. The physico-chemical features of the encapsulated drug affect the charge and the niosomal bilayer's rigidity. The presence of the head groups of the surfactant contributes to drug trapping in vesicles and induces charge increases. The charge formation generates the surfactant bilayer's mutual repulsion and thus increases the size of the vesicle. The degree of capture is also influenced by the HLB of the compound.

E. Temperature of Hydration

Hydration temperature determines the shape and size of the niosome. Temperature variation affects the assembly of surfactants into vesicles, and thus affects the formation of niosomal vesicles. The hydration temperature for the formation of niosomes should be above the device transition temperature of the gel to the liquid phase.

F. Cholesterol Content

The addition of cholesterol in the niosomal formulation increases the efficacy of trapping and thus provides the vesicles with rigidity. It also increases the niosomal vesicles' hydrodynamic diameter. The chain order of liquid state bilayers is also increased by cholesterol and the chain order of gel state bilayers is decreased. The bilayers of the gel state can be converted into a liquid state by increasing cholesterol concentration.[83]

G. Charge

The interlamellar distance between successive bilayers increases in the multilamellar vesicle structure due to charge presence. This results in a larger total amount of trapped volume.[84]

1.7.6 Factors Affecting Vesicle Size, Entrapment Efficiency and Release Rate

A. Drug

Drug loading in niosomes and liposomes may increases their size, apparently through the interaction of the solution with the head groups of surfactants, increases the charge and the reciprocal repulsion of the bilayers of surfactants, thereby increasing the size of the vesicles. Some drugs are attached in the long PEG chains in polyoxyethylene glycol (PEG) coated vesicles, thereby minimizing the tendency to increase in size. The drug's hydrophilic lipophilic balance determines the degree of entrapment.[85]

B. Amount and Type of Surfactant

With the rise in the HLB value of surfactants such as span 85 to span 20, the mean size of niosomes and liposomes increases proportionally since the surface free energy decreases with an increase in surfactant hydrophobicity. Depending on the temperature, the form of lipid or surfactant and the presence of other elements, like cholesterol, the bilayer of vesicles are either in the so-called liquid state or in the gel state. Alkyl chains are found in a well ordered structure in the gel state, and the structure of bilayers is more disordered in the liquid state. The gel-liquid phase transition temperature (Tc) characterizes the surfactant and lipids. Surfactant phase

transitiona emperature (Tc) also influences the efficiency of trapping, i.e. span 60, which has higher Tc, provides better entrapping. [85]

C. Cholesterol Contents and Charge

The presence of cholesterol in niosomes improves its performance in hydrodynamic diameter and trapping. In general, the cholesterol action is two folds; on the one side, cholesterol increases the chain order of bilayers of the gel state. The gel state is converted to a liquid-ordered process at a high cholesterol concentration. The increase in the cholesterol content of the bilayers resulted in a reduction in the rate of drug release and, subsequently, an increase in the rigidity of the attained bilayers. The presence of charge tends to increase the interlamellar gap in the multilamellar vesicle structure between successive bilayers and contributes to greater total trapped volume.[86]

D. Method of Preparation

Compared to the ether injection process (50-1000 nm), the hand shaking process shapes vesicles with a larger diameter (0.35-13 nm). It is possible to generate small sized niosomes and liposomes by the process of reverse phase evaporation. The method of microfluidization provides greater uniformity and vesicles of small sizes.

E. Resistance to Osmotic Stress

The addition of a hypertonic salt solution to the niosome and liposome suspension results in a decrease in diameter. In hypotonic salt solution, initial slow release can occur with mild vesicle swelling, which may be due to mechanical loosening of the structure of the vesicles under osmotic stress.[87]

1.7.7 Uses of Niosomes

Generally peptide drugs have stability problem so it is difficult to formulate in the form of tablets, parenteral. Therefore, using niosome as a drug carrier can improve the stability of peptide drugs.[88-90]

- Nowadays due to various disadvantages of oral drug delivery system, research is going on for transdermal drug delivery and this has achieved good response. Niosome as a drug carrier has good penetration capacity. Therefore, niosome can be used as transdermal drug delivery for various drugs.

- Niosomes may be used for the administration of anticancer drugs, like 5-FU, as an effective drug delivery method. It is also used to improve the potency of the medication by introducing it into the niosome.

- Niosomal suspension displays a visible spectrum that can be superimposed on that of free haemoglobin, so it can be used as a haemoglobin carrier.

- Drugs with a low therapeutic index and low water solubility can be encapsulated by the niosomal system and this can be retained in the circulation, indicating continuous release action.

- In diagnostic agents niosomal system plays an important role. Radiopharmaceuticals are encapsulated in the niosomes for diagnostic uses.

1.8 COHERENCE BETWEEN THESIS CHAPTERS

The present thesis is divided in the seven chapters. First chapter gives short description about cancer and lymphoma, hydrogels, liposomes and niosomes. Second chapter includes drug review, excipient review and review on liposomes and niosomes. The chapter content contained research work done in the past which revealed the problem statement of the present research work. In the chapter three the aim and objectives of the thesis work is explained related to the treatment of CTCL. The research problem and hypothesis and methodology described in the chapter. The chapter four materials and methods include the development of liposomes and niosomes of bexarotene drug. The design expert software is used for optimization of best formulation. Promised liposome and noisome are incorporated into topical gel. All evaluation parameters are well defined in the chapter. Chapter five covers the results and discussion of the formulated preparations. The statistical analysis is also included in the chapter. The discussion part interpretate and implicate the results. Chapters six summary and conclusion provide the important findings of research. Chapter seven consist the references used in the present research work.

CHAPTER- 2

Review of Literature
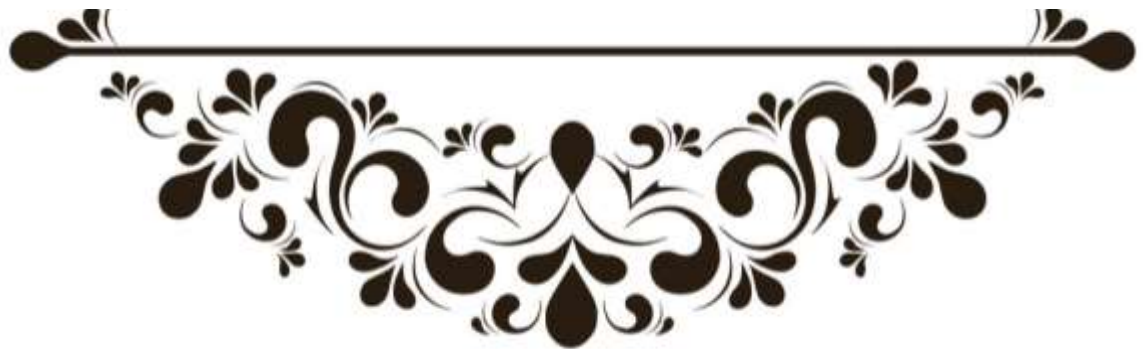
2.1 DRUG REVIEW

Bexarotene

Bexarotene (Targretin) belongs to synthetic retinoid class that selectively triggers the retinoid X receptor and shown potent antitumor action. Bexarotene is a potent anti-neoplastic drug used for treatment of cutaneous T cell lymphoma.[91]

IUPAC Name: 4-[1-(3, 5,5,8,8-pentamethyltetralin- 2-yl)ethenyl] benzoic acid or

4-[1- (3,5,5,8,8-pentamethyl-5,6,7,8- tetrahydro-2-naphtalenyl)vinyl] benzene carboxylic acid.

Bexarotene/CAS ID: 153559-49-0

Structural Formula:
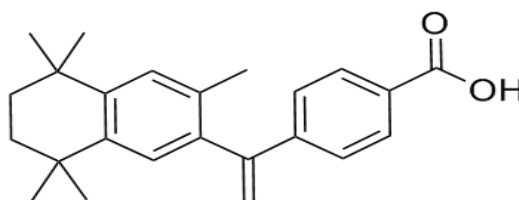
Figure 2.1: Chemical structure of bexarotene

Emperical Formula:	$C_{24}H_{28}O_2$
Proprietary Name:	Targretin
Molecular Weight :	348.478 g/mole
Appearance:	Off-white powder
Solubility :	Soluble in ethanol, DMSO, and insoluble in water.
Category :	Anti-cancer
Melting point:	230-231°C
Elimination Half life:	7 hrs
Dissociation cofficient(pKa):	4.2
Partition cofficient:	6.9
Dose:	Capsule 75mg, Gel 1% w/v dose is 300 mg/m^2/day

Mechanism of Action

Bexarotene belongs to retinoid family that activates retinoid-X-receptors (RXRs), as attached to the receptors retinoic acid. By so doing it brings cell differentiation and apoptosis and inhibits the drug resistance development. It also inhibits cancer metastasis because it has also antiangiogenic properties. The retinoic acid receptors

(RARs) regulate cell variation and propagation whereas RXRs control apoptosis. Bexarotene drug selectively attach with and triggers the types of retinoid-X-receptor. The retinoid-X-receptor classified in three subclasses; RXR_α, RXR_β, RXR_γ. The particular mechanism of action of bexarotene drug is not clear. In the CTCL treatment the drug bexarotene has shown their significant action in each and every clinical phase of CTCL.

Pharmacodynamics

In-vitro studies

The activity of bexarotene has not been explored in models of CTCL, since no pre-clinical models exist. Therefore the mechanism of action of drug bexarotene in the cutaneous T-cell lymphoma (CTCL) treatment is not known clearly. However, the following cell systems are known to respond to RAR-active ligands:

Promyelocytic leukaemia cell system

In the HL-60 promyelocytic leukaemia cell system bexarotene induced markers consistent with the induction of apoptosis, DNA laddering and morphological changes. At RXR/RAR-activating concentrations bexarotene was able to hinder the growth and induce differentiation of the cells and was also capable of inducing differentiation of leukaemia cells from patients, including those with acute promyelocytic leukaemia (APL). When tested against the APL cell line NB4 (which harbours the same t (5:17) chromosomal translocation detected in the majority of APL patients) bexarotene at concentrations $> 1 \mu M$ was able to induce cell cycle hold in G(1) and to induce the expression of the granulocyte diffentiation antigen CD11b on the cell surface.

HBE cells

Bexarotene inhibited transcription factor AP-1 (AP-1) activity in cultured human bronchial epithelial (HBE) cells. Other agents known to inhibit AP-1 activity also inhibited the expression of the differentiation markers. Bexarotene has demonstrated to have anti-AP-1 activity mediated via $RXR\alpha$. This effect may account for its ability to inhibit cellular proliferation.

Squamous cell carcinoma xenograft tumours in nude mice

9-*cis*-RA suppressed the differentiated squamous phenotype with the increased presence of basal tumour cells; bexarotene caused no change in the highly differentiated phenotype of the tumour. In addition, bexarotene inhibited the growth of two primary human HNSCC xenograft tumours grown in nude mice, but did not inhibit the growth of 1483 HNSCC xenograft tumours; bexarotene was not a potent antiproliferative agent in these cells.

Prostate cancer cell line (LNCaP)

Bexarotene was ineffective at inhibiting the growth of xenograft tumours derived from the human prostate cancer cell line or the transplantable Dunning prostate tumour in the rat.

Cultured AIDS-KS cells

Bexarotene inhibited cellular proliferation in culture in a dose-dependent manner and similar to ATRA and RAR ligand. These data suggested that bexarotene may have inhibitory activity for AIDS-related KS cells in-vivo.

Pharmacokinetics

Single-dose studies (*Oral administration*)

Plasma bexarotene concentration data from pharmacokinetic and toxicity studies in the rat and dog demonstrated that bexarotene was orally bioavailable. The increase in plasma AUC values appeared proportional at lower doses and less than dose-proportional at higher doses. From oil formulations, nonmicronised bexarotene was 30-40% bioavailable in rats and 7.5% bioavailable in dogs. Maximum plasma bexarotene concentrations (C_{max}) generally occurred a few hours post-dose (t_{max} = 2 to 4 hr).

Intravenous administration

Intravenous administration of bexarotene, clearance was 10 to 20 ml.min^{-1}kg^{-1}in the rat and 2 to 4 ml.min^{-1}kg^{-1}in the dog. I.V. kinetics in plasma appeared multiphasic in both species. The t½ of bexarotene was 2 to 3 hr in rats and 3 to 6 hr in dogs. bexarotene's volume of distribution was close to 1 l/kg, consistent with minimal retention of bexarotene in the extravascular tissues.

Repeat dose studies

In rats repeat dose C_{max} and AUC values increased with increasing dose but were less than dose proportional. Plasma bexarotene concentrations in rats were almost always dose-dependently lower after repeated dose administration than concentrations after a single dose. The extent of this reduction in concentrations generally did not increase beyond 28 days of dosing. The magnitude of the reduction tended to be greater in male rats than in female rats.

In all of the toxicity studies conducted in dogs, systemic exposure to bexarotene was dose-dependent. There was dose-dependent reduction in exposure associated with repeated dosing. In a 28 days toxicity studies in which bexarotene was administered in vegetable oil at 10 to 200 mg/kg/day, C_{max} and $AUC_{(0-6)}$ values were 2-fold and 4-fold lower respectively on day 28 compared to day 1 at 10 mg/kg and 3-fold and 6-fold lower at 30 mg/kg.

Absorption

The oral bioavailability of bexarotene was formulation-dependent. For micronised drug in rat and nonmicronised drug in dog, suspensions in oil tended to provide greater bioavailability than suspensions containing PEG. Bioavailability of nonmicronised bexarotene was also significantly greater from a PEG-based solution formulation than that from oil- or PEG-based suspension formulations in dogs. These data suggested that the drug absorption is dissolution rate-limiting for bexarotene absorption and that bexarotene particle size reduction could enhance bioavailability.

Distribution

A study in rats demonstrated that drug-related radioactivity distributed essentially throughout the body after an oral dose of radiolabelled bexarotene. At the 4-hr time point, radioactivity was present in all of the 26 tissues examined. Other than organs of the gastro-intestinal tract, tissue-to-plasma radioactivity ratios were greatest in liver, kidneys and adrenals over four time points examined (4, 8, 24 and 48 hr). No substantial retention occurred in any tissue; less than 1% of the radioactivity remained in the rats at 48 hrs.

Protein binding studies

Bexarotene was highly protein bound (>99.9%) at concentrations present in the rat and dog plasma in toxicity studies.

Metabolism

In the rat and dog bexarotene was metabolized primarily by P450-dependent oxidation and glucuronidation. Systemic exposure to these metabolites (6-or 7-oxobexarotene and 6-or7-hydroxybexarotene) was found to be less than that to parent compound after both single and multiple doses.

Excretion

In a rat oral-dose tissue distribution study using 100 mg/kg radiolabelled bexarotene, >90% of the radioactive bexarotene was excreted in the faeces within 48 hours.

Enzyme induction

A rat study revealed that 4 days of daily, oral bexarotene treatment (600 mg/m2) significantly increased (by 91%) gross hepatic microsomal cytochrome P450. Levels of some specific cytochrome P450 isozymes decreased significantly (90% for CYP1A2 and 41% for CYP2C11) while others increased significantly (CYP2B1/B2 by 28-fold, CYP3A by 8-fold and CYP4A by 71-fold). Rates of cytochrome P450 and glucuronyl transferase-mediated bexarotene metabolism were increased significantly in these microsomal preparations (by 252% and 77%, respectively), further supporting metabolic induction by bexarotene as a potential mechanism contributing to the reduction of systemic exposure after repeated dosing.

Side Effects

Headache, fatigue, tiredness, nausea, dry skin, vomiting, diarrhoea, trouble sleeping an increase in fats in the blood (blood lipids) such as cholesterol or triglycerides (blood tests will detect this), an underactive thyroid (blood tests will detect this)**.**

Storage

Avoid excessive humidity, Avoid extreme Temperatures, Protect from light, Store between 36-77 °C

Table 2.1 : Interaction of bexarotene with other drugs

Drug	Interaction
Abacavir	Decreased dolutegravir plasma concentrations
Dihydrocodeine	Decrease the dihydrocodeine level
Acetohexamide	Enhance insulin secretion resulting hypoglycemia
Amlodipine	Increases hepatic metabolism

2.2 EXCIPIENT REVIEW

2.2.1 Cholesterol

Cholesterol is a chemical substance that the body needs for construction of cell membranes and also helpful in composition of hormones like testosterone and estrogen. Mainly, the liver creates about 80% of the total body's cholesterol and the remains comes from daily diet food sources like dairy products, milk, curd, meat.[92]

Synonyms: Cholesterin, cholesterolum

Non-proprietary   BP: CholesterPhEur, Cholesterol

Names:

Structural formula:
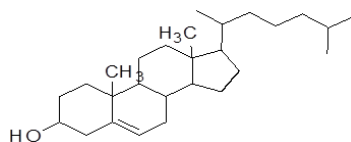
Figure 2.2: Chemical structure of cholesterol

Chemical name: Cholest-5-en-3β-ol

Chemical formula: $C_{27}H_{46}O$

Molar mass: 386.67 g/mole

Description: It is found in white to faintly-yellow in colour, mostly odourless and shiny granules. On light and air exposure changes its colour faint-yellow to tan colour.

Density: 1.052 g/cm^3

Functional category: Emulsifying agent and emollient

Boiling point: 360 °C

Melting point: 148-150 °C

Solubility: Soluble in benzene, acetone, ethanol and ether.

Applications in Pharmaceutical Formulation

Cholesterol is commonly used in topical formulations and mainly in cosmetics preparation, as an emulsifying agent. It improves the absorbing power of water to an ointment and provides the emollient to the skin on topical application.

Stability and Storage

Cholesterol is stable when stored in a well-closed air tight and amber colour container which protects it from light.

2.2.2 Soybean Lecithin (1-Palmitoyl-2-linoleoylphosphatidylcholine)[92]

Molecular formula:	$C_{42}H_{80}NO_8P$
Molecular weight:	758.1 g/mol
IUPAC name	[3-hexadecanoyloxy-2-[(9Z, 12Z)–octadeca-9,12dienoyl] oxypropyl]2-(tri methyl azaniumyl)ethyl phosphate
CAS number	6931-84-6
Physical description	Dry Powder; Liquid
Color & form	It is white in colour when freshly made, but changes to brown when comes in contact with air
Odor	Mostly odourless
Taste	Bland
Melting point	236-237 °C
Chemical structure	
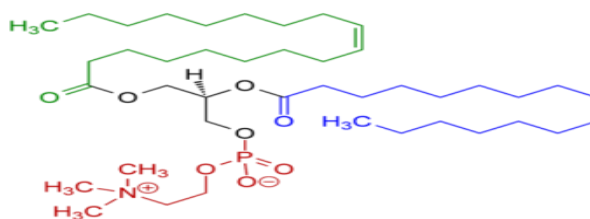
Figure 2.3: Chemical structure of syabean lecithin

Solubility	It is soluble in ether, chloroform, fatty acids and mineral oils. Slightly soluble in acetone; insoluble in water, vegetable oils and animal oils.
Stability/Shelf life	Stable under recommended storage conditions.
pH	6.6
Therapeutic uses	Surface-Active Agents

Uses

It is a natural originated digestible and edible surfactant and emulsifier. It is commonly used as emulsifier and antioxidant in foods and cosmetics; emollient and penetrating agent in cosmetics.

Storage Conditions

Keep stored in the air tight container in a dry and well-ventilated place. Stored at - 20°C temperature in deep freezer.

2.2.3. Span 60[92]

Synonym:	Sorbitan stearate
Chemical name:	Sorbitan monostearate , [2- [(2R,3R,4S)-3, 4-dihydroxyoxolan-2-yl] - 2 - hydroxyethyl]octadecanoate
Molecular formula:	$C_{24}H_{46}O_6$
Structural formula:	
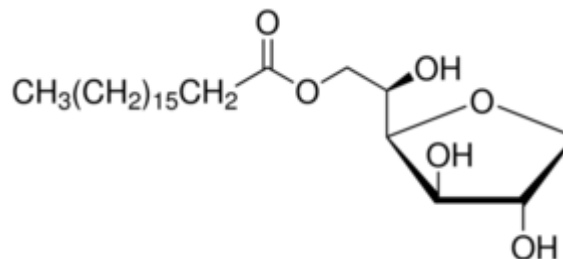
Figure 2.4: Chemical structure of span 60

Molecular weight:	430.6 g/mole
Description:	Sorbitan is a non-ionic surfactant. It is white in colour, odourless and has waxy texture. It has wetting, dispersing and emulsifying properties.
Melting point:	49-65 °C
Density:	1.0 g/ml
Boiling point:	579.01°C
Solubility:	Soluble in dioxane, toluene, ether, carbon tetrachloride, ethanol, and methanol; insoluble in petroleum ether and acetone; insoluble in cold water but dispersible in warm water.

Applications of Pharmaceutical Formulations

Sorbitan is mostly used as an emulsifier. It is used to prepare w/o or o/w emulsion and creams. Sorbitan is used in the food and phrmaceutical products industry.

Stability and Storage Conditions

Keep stored in air tight container at room temperature. Keep store away from oxidizing agents.

2.2.4 Span 40 [92]

Synonyms:	Sorbitan monopalmitate
Chemical name:	[(2*R*)- 2-[(2*R*, 3*R*, 4*S*)- 3,4 – dihydroxyoxolan -2 -yl] -2-hydroxyethyl]hexadecanoate
Appearance:	Yellowish brown wax
Molecular formula:	$C_{22}H_{42}O_6$
Molecular weight:	402.57g/mole
Chemical structure:	
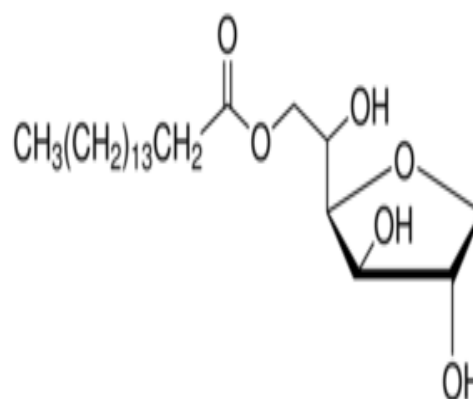
Figure 2.5: Chemical structure of span 40

Melting point:	46-47°C
Flash point:	113°C
Solubility:	Soluble in ethanol, methanol, ether, Insoluble in cold water but slightly soluble in warm water.

Pharmaceutical Applications

Span 40 is widely used as wetting agent, stabilizer, co-solvent and emulsifying agent. Span 40 is mostly used in production of food, medicines, pharmaceuticals and cosmetics. It also used in the preparation of inks, paints, oils and stabilizers.

2.2.5 Carbopol

Carbopol polymers are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. They are produced from primary polymer particles of about 0.2 to 6.0 μm average diameter. The flocculated agglomerates cannot be broken into the ultimate particles when produced. Each particle can be viewed as a network structure of a polymer chains inter connected with cross-linking. Carbomers were first prepared and patented in 1957. Since then, a number of extended release tablet formulations, which involve carbomer matrices, have been patented. Carbomers readily absorb water, get hydrated and swell. In addition to its hydrophilic nature, its cross-linked structure and it's essentially insolubility in water makes carbopol a potential candidate for use in controlled release drug delivery system.[92]

Structure
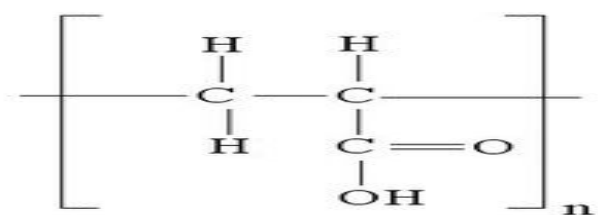
Figure 2.6: Chemical structure of carbopol

Carbopol polymers are manufactured by cross linking process . Depending upon the degree of cross linking and manufacturing conditions, various grades of carbopol are available. Each grade is having its significance for its usefulness in pharmaceutical dosage form.

Table 2.2: Grades of carbopol polymers

Grades	Viscosities	Properties
Carbopol-934	30K-39K	Used in preparation of thick formulations like emulsions, extended release formulations, topical and transdermal dosage forms.
Carbopol-940	40K-60K	Used as an emulsifying, stabilizing, suspending, thickening and gelling agent. Widely used as thickener.

2.2.6 Triethanolamine [92]

Chemical name:	2,2,2-Nitrilotriethanol
Empirical formula:	$C_6H_{15}NO_3$
Functional category:	Emulsifying agent, alkalizing agent
Molecular weight:	149.9 g/mole

Structure:
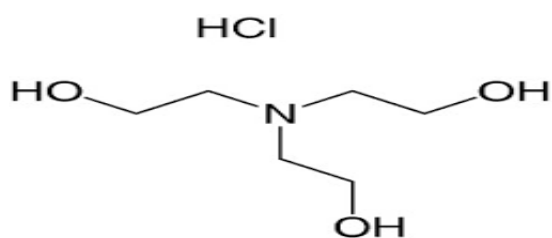
Figure 2.7: Chemical structure of triethanolamine

Colour:	clear, colourless to pale-yellow colour
Odour:	slight ammonical odour
Acidity/alkalinity:	pH =10.5(0.1N solution)
Boiling point:	335 °C
Flash point:	208 °C
Freezing point:	21.6 °C
Melting point:	21 °C
Moisture content:	0.09%

Applications

Triethanolamine is widely used in topical pharmaceutical formulations primarily in the formation of emulsions. Concentrations that are typically used for emulsification are 2–4% v/v of triethanolamine and 2–5 times that of fatty acids. Triethanolamine is also used in salt formation for injectable solutions and in topical analgesic preparations. It is also used in sun-screen preparations. Other general uses are as buffers, solvents, and polymer plasticizers, and as a humectant.

2.3 REVIEW ON LIPOSOME FORMULATION

Dong YD, et al., (2019), described the effect of liposome size on tendency for accumulation in tumor tissue requires preparation of defined populations of different sized particles. The extent of extravasation was clearly dependent on size of the liposomes, with the small liposomes showing tissue distribution beyond the vascular area compared to the large liposomes. The use of microfluidics to prepare smaller size distribution liposomes compared to sonication methods was demonstrated and allowed preparation of different size distribution drug carriers from the same lipid composition.[93]

Guan YM et al., (2019), prepared the liposomes of essential oil. In the experiment, the method for the determination of encapsulation efficiency of liposomes was established by ultraviolet spectrophotometer and dextran gel column. The encapsulation efficiency and particle size of liposomes were used as evaluation indexes for single factor investigation and Box-Behnken design-response surface method was used to optimize the design. Then the optimal formulation of volatile oil liposome was characterized using methyleugenol, elemin, β-asarone and α-asarone as index components. Finally, the in-vitro transdermal properties of liposomes were studied by modified Franz diffusion cell. The prepared volatile oil liposomes met the relevant quality requirements, providing a reference for further research on preparation of multi-component Chinese medicine essential oil liposomes.[94]

Zhang H, (2017), prepared liposomes by thin-film hydration method followed by extrusion. The method involves making a thin lipid film in a round-bottom flask by the removal of organic solvent. Upon the addition and agitation of the dispersion medium, heterogeneous liposomes are formed. Finally, after extrusion through polycarbonate membranes, homogeneous small liposomes were obtained.[95]

Yuan JJ et al., (2017), prepared liposomes containing water-soluble HT to improve the bioavailability and biocompatibility of the target drug. The preparation process factors (temperature, mass ratio of phospholipid (PL) and cholesterol (CH), Tween-80 volume, HT mass) were studied and response surface methodology (RSM) was applied to optimize the conditions. The results demonstrated that by using a

temperature of 63 °C, mass ratio of PL and CH 4.5:1, HT mass 5 mg and Tween-80 volume of 6 ml, HT liposomes with an encapsulation efficiency (EE) of 45.08% were prepared. It was found that the particle sizes of the HT liposomes were well distributed in the range of 100-400 nm. Compared to free HT, prepared HT liposomes had better stability and a distinct slow release effect in-vitro.[96]

Aburai K, et al., (2011), improved the trapping efficiency by addition of lipopeptides (LPs), and using a supercritical reverse-phase evaporation (SCRPE) process, along with incorporation of PEG-modified phospholipids to improve long-term stability. In this study, bovine serum albumin (BSA) was used as a model drug substance for entrapment by liposomes. Improvements in the entrapment efficiency and stability of liposomes were achieved by modification with LPs and use of a SCRPE preparation process. The BSA-entrapment efficiency of liposomes modified with cationic LPs with arginine residues, as a result of their ionic interactions, was six times that of liposomes prepared by the Bangham method. This study therefore suggests that there are opportunities for the development of novel DDS carriers with excellent performance and which address environmental concerns.[97]

Liu JJ., et al., (2013), developed and characterized a liposome delivery system co-encapsulating two cosmeceutical ingredients, avobenzone (AVO) and arbutin (AR). Two different liposome preparation methods, that is, thin film hydration and reverse-phase evaporation, were evaluated. The effects of liposome formulation and preparation method on particle size, entrapment efficiency (EE), and skin permeation rate were studied. The results of this study revealed that liposomes are a promising delivery system for co-encapsulated AR and AVO.[98]

Maalej CJ et al., (2010), prepared liposomes of a hydrophobic (beclomethasone dipropionate; BDP) and a hydrophilic (cytarabine; Ara-C) drugs for administered via the pulmonary route. The effects of critical process and formulation parameters have been investigated. The drug-loaded liposomes were prepared and characterized in terms of size, zeta potential, encapsulation efficiency, release study, cell uptake, and aerodynamic behavior. Small multilamellar vesicles, with sizes ranging from about 80

to 170 nm, were successfully obtained. The elaborated liposomes seem to be promising carriers for both Ara-C and BDP pulmonary delivery.[99]

Niu G et al., (2010), developed stealth liposomes (SLs), containing polyethylene glycol-conjugated lipid, which can form a hydro-layer around liposomes bilayer, have a long circulation time and hence result in enhanced drug efficiency. Doxorubicin (DOX), an effective anticancer drug, can be loaded into liposomes by transmembrane pH gradient method to get high encapsulation efficiency with high drug/lipid ratio. They also described the preparation of SLs via extrusion, DOX loading by transmembrane pH gradient method and characterization analysis, including phospholipid concentration, size, transmission electronic microscopy graph, encapsulation efficiency and in-vitro drug release.[100]

2.4 REVIEW ON NIOSOME FORMULATION

Witika BA, (2019), prepared and optimized niosomes of Nevirapine (NVP) by using thin layer hydration. The impact of cholesterol and surfactant content, hydration time and temperature on manufacture was investigated. Critical quality attributes (CQA) in respect of particle size (PS), entrapment efficiency (EE), polydispersity index (PDI) and the amount of NVP released at 48 hours was also assessed. They made a simple, cheap, rapid and precise method of manufacture of NVP niosomes was developed, validated and optimised using DoE and RSM and the product exhibited the target CQA.[101]

Silva LD et al., (2019), prepared niosomes from a mixture of sorbitan monostearate 60, cholesterol, and synthesized D-α-tocopherol, polyethylene glycol 1000, succinate-diethylene triaminepentaacetic acid (synthesis confirmed by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy). Parameters affecting the radiolabeling efficiency such as concentration of stannous chloride ($SnCl_2 \cdot H_2O$), pH, and incubation time were evaluated. In-vitro stability of radiolabeled niosomes was studied in 0.9% saline and human serum at 37°C for up to 8 hours. Experimental data revealed that 30 µg/ml of $SnCl_2 \cdot H_2O$ was the optimal concentration of reducing agent required for the radiolabeling process. The pH and incubation time required to obtain high radiolabeling efficiency was pH 5 and 15 minutes, respectively. ^{99m}Tc-labeled

niosomes exhibited high radiolabeling efficiency (>90%) and showed good in-vitro stability for upto 8 hours. The formulated ^{99m}Tc-labeled niosomes possessed high radiolabeling efficacy, good stability in-vitro, and show good promise for potential use in nuclear imaging in the future.[102]

Eid RK et al., (2019), investigated the effect of incorporation of essential oils in niosomes on felodipine transdermal delivery. Rigid niosomes comprising Span 60 with cholesterol (2:1, w/w) were used with clove, eucalyptus or lemon oil being incorporated in the vesicles at increasing concentrations. The vesicle size and shape was monitored using scanning electron microscopy. The results correlated with the fluidizing and penetration enhancing effects of oils. The study introduced essential oils as potential niosomes fluidizing agents for enhanced transdermal drug delivery.[103]

Ghafelehbashi R et al., (2019), optimized cephalexin-loaded niosomal formulations based on span 60 and tween 60 were prepared as a promising drug carrier system. The niosomal formulations were characterized using a series of techniques such as scanning electron microscopy, fourier transform infrared spectroscopy, dynamic light scattering, and zeta potential measurement. The prepared niosomes showed negligible cytotoxicity for HepG2 cells, measured by MTT assay. The findings of the study shown that the improvement of cephalexin bioavailability and prolonged drug release profile could be obtained by niosomal formulation as a favorable antibiotic drug delivery system.[104]

Ahad A et al., (2018), prepared telmisartan loaded niosomes using thin film hydration method by varying the Span 60 and cholesterol at several molar ratios and characterized for vesicles size, polydispersity index, zeta potential, entrapment efficiency. The optimized niosomes formulation NS6 presented vesicles size of 618.47 nm, polydispersity index of 0.86, with entrapment efficiency of 83.83% and possesses negative charge. Results of present study confirm the potential of developed niosomes as suitable carriers for improved oral delivery of telmisartan.[105]

Zidan AS et al., (2017), formulated niosomes from proniosome gels of methotrexate (MTX). Box-Behnken's design was employed to prepare a series of MTX proniosome gels of Span 40, cholesterol (Chol-X_1) and Tween 20 (T20-X_2). The responses investigated were niosomal vesicles size (Y_1), MTX entrapment efficiency percent (EE%-Y_2) and zeta potential (Y_3). MTX loaded niosomes were formed immediately upon hydration of the proniosome gels with the employed solvents. The optimized formula of MTX loaded niosomes showed vesicle size of 480 nm, high EE% (55%) and zeta potential of -25.5 mV, at Chol and T20 concentrations of 30% and 23.6%, respectively, when G was employed as the solvent. Hence, G was the solvent of choice to prepare MTX proniosomal gels with a maintained stability and highest entrapment.[106]

Pando D et al., (2015), formulated resveratrol (RSV) entrapped niosomes for topical use. Niosomes were formulated with Gelot 64 (G64) as surfactant, and two skin-compatible unsaturated fatty acids (oleic and linoleic acids), commonly used in pharmaceutical formulations, as penetration enhancers. Niosomes were prepared by two different methods: a thin film hydration method with minor modifications followed by a sonication stage (TFH-S), and an ethanol injection modified method (EIM). Niosomes prepared with the EIM method were in the range of 299-402 nm, while the TFH-S method produced larger niosomes in the range of 293-496 nm. The EIM method, which yielded the best RSV-entrapped niosomes, seems to be the most suitable for scaling up.[107]

Zhang Y et al., (2015), prepared Span 40-based niosomes as nanocarriers to improve cutaneous absorption of salidroside. The niosomal formulation with a molar proportion of Span 40 to cholesterol of 4:3 showed the highest transdermal flux and skin deposition of salidroside. The transdermal flux of the 4:3 niosomal formulation was significantly greater than that of the aqueous solution. Salidroside-loaded niosomes showed good biocompatibility with skin tissue, human epidermal immortal keratinocytes (HaCaT), and human embryonic skin fibroblasts (CCC-ESF).[108]

Bayindir ZS et al., (2015), prepared candesartan cilexetil-loaded niosomes and mixed niosomes to enhance the aqueous solubility of the drug, thus improving its oral

bioavailability. The formulations were prepared using various types and combinations of surfactants, copolymers, and charge-inducing agents. The in-vitro drug release from niosomes was improved after niosomal entrapment compared to pure candesartan cilexetil. Oral drug delivery of candesartan cilexetil by Span 60/Pluronic P85-mixed niosomes seems feasible due to enhanced drug release and stability.[109]

Ramlingam N et al., (2013), formulated ofloxacin niosomes by thin film hydration technique using rotary flash evaporator were evaluated for their particle size, zeta potential, surface morphology, entrapment efficiency, in-vitro drug release and in-vivo pharmacokinetic studies. The designed ofloxacin niosomes with span 60 showed good physicochemical properties, good stability, improved pharmacokinetic parameters, prolonged action and improved bioavailability than the commercially available conventional dosage form which might be a potential carrier system to improve the patient compliance and reduce the side effects.[110]

Hasan AA et al., (2013), prepared of metformin hydrochloride (MH) loaded niosomes by the reverse phase evaporation technique. Span 40 and cholesterol were used as polymer and Dicetyl phosphate (DCP) and 1,2-dioleoyl-3-trimethylammonium-propane chloride salt (DOTAP) were used to obtain negatively and positively charged vesicles, respectively. The mean particle size ranged from 223.5 to 384.6 nm and the MH-loaded niosomes' surface was negatively charged in the absence of charge inducing agents (-16.6 ± 1.4 mV) and also with DCP (-26.9 ± 1.0 mV), while it was positively charged ($+8.7 \pm 1.2$ mV) with DOTAP. It could be concluded that MH-loaded niosome is promising extended-release preparation with better hypoglycemic efficiency.[111]

Hashim F et al., (2010), prepared ribavirin niosomes and evaluated the influence of niosomal encapsulation on drug liver targeting in rats. Ribavirin niosomes were prepared by the thin film hydration method using span 60, cholesterol, and dicetyl phosphate in molar ratios of (1:1:0), (4:2:0), (1:1:0.1), and (4:2:1). The prepared niosomes were characterized in-vitro for vesicle size, drug entrapment, drug release profiles and vesicular stability at refrigerator temperature. The obtained results show that the niosomal formulation significantly increased ribavirin liver concentration (6-

fold) in comparison with ribavirin-free solution. Niosomes improved the efficacy of low doses of ribavirin and minimize its toxic side-effects at higher doses.[112]

2.5 REVIEW ON NANOFORMULATIONS IN TOPICAL GEL

Shukla R et al., (2020), assessed topical potential of ethosomes of Melatonin (MLT). Melatonin was encapsulated using different ratios of ethanol, soya lecithin and cholesterol. Prepared ethosomes were characterized for SEM, zeta potential, %EE, in-vitro drug release kinetics. Then, optimized formulation was incorporated in gel and evaluated for viscosity, pH, extrudability, homogeneity, skin irritation study, spreadability, in-vitro skin permeation study, flux, and stability. Ethosomes were spherical in structure as confirmed by SEM and zeta potential was in range of -12.4 mV to -27.4 mV. %EE of the vesicles was in the range of 49.61-78.047%. An ethosomal gel was prepared by using carbopol 934 and compared with plain gel formulation. This research suggested that MLT loaded ethosomes can be potentially used as a topically drug delivery system.[113]

Agrawal YO et al., (2020), Methotrexate-loaded nanostructured lipid carriers (MTXNLCs) were formulated and characterized to determine in-vitro drug release and evaluate the role of MTXNLC gel in the topical treatment of psoriasis. A solvent diffusion technique was employed to prepare MTXNLCs, which was optimized using 3^2 full factorial designs. The diameter and surface morphology of MTXNLCs was evaluated. MTXNLCs were integrated in 1% w/w Carbopol 934 P gel base, and in-vitro skin deposition studies in human cadaver skin (HCS) were carried out. The optimized MTXNLCs were rod-shaped, with an average particle size of 253 ± 8.65 nm, a zeta potential of -26.4±0.86 mV, and EE of 54.00±1.49%. Developed formulation of MTXNLC gel demonstrated better anti-psoriatic activity and also displayed prolonged and sustained release effect, which shows that it can be a promising alternative to existing MTX formulation for the treatment of psoriasis.[114]

Chandra A et al., (2019), developed an ethosomal gel of methotrexate (MTX)-incorporated ethosomes and salicylic acid (SA) and to evaluate and study its ethosomal gel potential in Imiquimod-induced psoriasis animal model to treat

symptoms of psoriasis. MTX-SA ethosomal gel was prepared by the cold method and optimized by comparing it with MTX ethosomal gel and drug solution. Particle size, zeta potential, entrapment efficiency, and ex-vivo study were selected as the critical quality checking attributes. Optimized MTX-SA exhibited a particle size of 376.04 ± 3.47nm, EE(Entrapment efficiency) of $91.77 \pm 0.02\%$. At the end of 24h, MTX-SA ethosomal gel exhibited a slow and prolonged release of MTX ($26.13 \pm 1.61\%$ versus $6.97 \pm 0.06\%$) compared to MTX drug solution. The developed MTX-SA ethosomal gel formulation can be a promising alternative to existing MTX formulation in topically treating psoriasis.[115]

Albash R et al., (2019), prepared transethosomes (TEs) for enhancing the transdermal delivery of Olmesartan medoxomil (OLM) to avoid its oral problems. TE formula were prepared utilizing full factorial design using various surfactants (SAAs) and different phospholipid-to-SAA ratios. The formula were characterized regarding their entrapment efficiency percentage (EE%), particle size (PS), polydispersity index (PDI), zeta potential (ZP), and the amount of drug released after 6 hours (Q6h). The optimum formula (TE14) had an EE% of $58.50\% \pm 1.30\%$, PS of 222.60 ± 2.50 nm, PDI of 0.11 ± 0.06, ZP of -20.80 ± 0.30 mV, and Q6h of $67.40\% \pm 0.20\%$. Moreover, TE14 showed superiority in dermatokinetic study when compared with drug suspension.[116]

Lac D et al., (2019), developed a unique, stable, hydrophilic topical gel formulation with fully solubilized minocycline (MNC-H). Minocycline delivered in our hydrophilic gel remained more stable *in situ*, resulting in less degradation product (4-epiminocycline) than a lipophilic formulation (MNC-L). The hydrophilic nature of formulation enabled 2-3 fold increase in delivery into the skin *ex-vivo* compared to a lipophilic counterpart, mostly seen in the epidermis and pilosebaceous units. The lipophilic formulation also appeared to be more occlusive, resulting in higher sebum production in minipigs, which may exacerbate acne vulgaris. These findings suggest that topical hydrophilic minocycline gel may provide a novel tool for topical acne therapy.[117]

Ansari H and Singh P, (2018), developed solid lipid nanoparticles (SLN) of lopinavir and formulated a topical gel for improved systemic bioavailability of

lopinavir. SLNs were prepared using high-pressure homogenization technique and optimized. The nanoparticles were characterized by SEM to confirm their spherical shape. Differential Scanning Calorimetry (DSC) analysis was carried out to ensure the entrapment of drug inside the SLNs. A comparative evaluation was done between SLN based gel and plain gel of drug by performing ex-vivo skin permeation studies using Franz diffusion cell. The optimized formulation composed of Compritol 888ATO (0.5 %) as a lipid, Poloxamer 407 (0.25 %) as a surfactant and Labrasol (0.25 %) as a co-surfactant gave the maximum entrapment of 69.78 % with mean particle size of 48.86nm. Lopinavir SLN based gel was found to have modified drug release pattern providing sustained release as compared to plain drug gel. This indicates that Lopinavir when given topically has a good potential to target the HIV as compared to when given orally.[118]

Kaur N et al., (2018), developed and evaluated nanostructured lipid carrier based topical hydrogel of mometasone furoate for the treatment of psoriasis. NLCs were developed by microemulsion technique. The optimized formulation was characterised for droplet size, zeta potential, entrapment efficiency and morphology was studied using Transmission Electron Microscopy. Ex-vivo permeation studies were carried out using Wistar rat skin. The optimized formulation (F4) has droplet size of 163.2 ± 0.522 nm, zeta potential - 0.086 ± 0.099 mV and entrapment efficiency of $60.0\pm0.187\%$. Transmission electron microscopy confirmed spherical shape of nanostructured lipid carrier. Carbopol 940 was used to convert NLC dispersion into NLC based hydrogel to improve its viscosity for topical administration. Drug permeation studies showed prolonged drug release from the NLC based gel as compared to marketed formulation following Higuchi release kinetics. The prepared NLC based formulation has proved to be a promising carrier system for the treatment of psoriasis.[119]

Deshkar SS et al., (2018), formulated and evaluated solid lipid nanoparticles (SLN) loaded gel of Dapsone (DS). These were prepared by microemulsion technique and evaluated for its in-vitro characteristics. The effect of DS concentration in lipid phase (X1), Gelucire:Precirol ratio (X2) and lipid:Smix ratio (X3) on entrapment efficiency (Y1) and drug release (Y2) from SLN was studied using Box-Behnken design. The

result of dependent variables was used to generate polynomial equations and the surface response and counterplots. The optimized SLN formulation was incorporated into the gel using 1% carbopol-934 as a gelling agent. The SLN loaded gel was characterized for pH, viscosity, percent drug content, in-vitro drug release and ex-vivo permeation through rat skin. It was concluded that DS SLN gel as a possible alternative to a conventional topical formulation for the treatment of acne.[120]

Karman M et al., (2016), "formulated, optimized and evaluate the transdermal potential of novel vesicular nano-invasomes, containing above anti-hypertensive agent. Olmesartan with β-citronellene as potential permeation enhancer were developed and optimized using Box-Behnken design. The physicochemical characteristics e.g., vesicle size, shape, entrapment efficiency and skin permeability of the nano-invasomes formulations were evaluated. The optimized formulation was further evaluated for in-vitro drug release, confocal microscopy and in-vivo pharmacokinetic study. The optimum nano-invasomes formulation showed vesicles size of 83.35±3.25nm, entrapment efficiency of 65.21±2.25%. The pharmacokinetic study presented that transdermal nano-invasomes formulation showed 1.15 times improvement in bioavailability of olmesartan with respect to the control formulation in Wistar rats. It was concluded that the response surface estimated by Design Expert(®) illustrated obvious relationship between formulation factors and response variables and nano-invasomes were found to be a proficient carrier system for transdermal delivery of olmesartan.[121]

Avasatthi V et al., (2016), developed a nanogel composed of methotrexate (MTX)-loaded nanostructured lipid carrier (MTX-NLC) and to evaluate its potential in imiquimod-induced psoriasis model to ameliorate symptoms of psoriasis. MTX-NLC nanogel was prepared by hot-homogenization method and optimized by Design of Experiments. Particle size, polydispersity index (PDI) and entrapment efficiency were selected as the critical quality attributes. Antipsoriatic potential of MTX-NLC nanogel was evaluated by Psoriatic Area and Severity Index (PASI) score and histopathological examination in the imiquimod-induced psoriasis model. Optimized MTX-NLC exhibited particle size of 278 ± 10 nm, PDI of 0.231 ± 0.05 and EE of 22.29 ± 1.23%. At the end of 48 hr, MTX-NLC gel exhibited slow and prolonged

release of MTX (47.32 ± 0.94% versus 94.23 ± 0.79%) compared to MTX gel. The developed MTX-NLC gel formulation can be a promising alternative to existing MTX formulation in treating psoriasis.[122]

Asthana GS et al., (2016), investigated the delivery potential of Etodolac (ETD) containing topical niosomal gel. Niosomal formulations were prepared by thin film hydration method at various ratios of cholesterol and Span 60 and were evaluated with respect to particle size, shape, entrapment efficiency, and in-vitro characteristics. Mean particle size of niosomal formulation was found to be in the range of 2 μm to 4 μm. Niosomal formulation N2 (1: 1) ratio of cholesterol and surfactant displayed good entrapment efficiency (96.72%). TEM analyses showed that niosomal formulation was spherical in shape. Niosomal formulation (N2) displayed high percentage of drug release after 24 hr (94.91) at (1:1) ratio of cholesterol:surfactant. The present study suggested that topical niosomal gel formulations provide sustained and prolonged delivery of drug.[123]

Rajnikanth PS and Chellian J, (2016), developed a nanostructured lipid carrier (NLC)-based hydrogel of 5-fluorouracil (5-FU). Precirol® ATO 5 and Labrasol® were selected as the solid and liquid lipid phases, respectively. Poloxamer 188 and Solutol® HS15 were selected as surfactants. The developed lipid formulations were dispersed in 1% Carbopol® 934 gel medium in order to maintain the topical application consistency. The average size, zeta potential, and polydispersity index for the 5-FU-NLC were found to be 208.32±8.21 nm, -21.82±0.40 mV, and 0.352±0.060, respectively. Transmission electron microscopy study revealed that 5-FU-NLC was <200 nm in size, with a spherical shape. In-vitro drug permeation studies showed a release pattern with initial burst followed by sustained release, and the rate of 5-FU permeation was significantly improved for 5-FU-NLC gel (10.27±1.82 μg/cm^2/hr) as compared with plain 5-FU gel (2.85±1.12 μg/cm^2/hr). These results show that the prepared 5-FU-loaded NLC has high potential to improve the penetration of 5-FU through the stratum corneum, with enormous retention and with minimal skin irritation, which is the prerequisite for topically applied formulations.[124]

Aggarwal G et al., (2016), formulated tazarotene nanosponge and niosomes based gel for topical application. Nanosponge and niosomes of tazarotene were prepared by

emulsion solvent evaporation technique and thin film hydration method respectively. The prepared formulations were characterized for drug content, morphology, size distribution, PDI, viscosity, % swelling and in-vitro permeation. The nanosponge and niosome formulations were incorporated into carbomer 940 (gel matrix) to convert them into nanosponge and niosome based gel. The gel formulations were subjected to drug content determination, pH determination, spreadability, viscosity, rheological behaviour. The study showed that nanosponge and niosome based gel formulation can be a possible alternative to conventional formulations of tazarotene with enhanced bioavailability and skin retention characteristics for topical application.[125]

Nagaich U and Gulati N, (2016), nanostructured lipid carrier (NLC)-based gel was developed for clobetasol propionate (CP). The characterizations of the prepared NLC formulation were assessed by means of SEM, particle size distribution, zeta potential analysis, drug entrapment efficiency and in-vitro drug release studies to select the optimized NLC formulation. The optimized NLC formulation encompasses particle size of 137.9 nm with -20.5 mV zeta potential and 0.224 polydispersity index which indicates good stability of NLC dispersion. NLC formulation showed a good entrapment efficiency of 78.5 % ± 0.03 with cumulative in-vitro release 85.42 % up to 24 hr. The optimized NLC formulation was suitably gelled and characterized for rheology, drug content, ex-vivo drug permeation studies, and drug release kinetics studies. The anti-inflammatory activity of NLC gel via paw oedema technique showed a rapid onset of action, as well as a prolonged duration of action as compared with the marketed gel.[126]

Khurana S et al., (2013), developed nanostructured lipid carriers (NLC) based gel for of meloxicam (MLX). NLC gel containing MLX was prepared and characterized for particle size, polydispersity, zeta potential, pH, rheology, entrapment efficiency, occlusion factor, and thermal behaviour. In-vitro drug release, in-vitro skin permeation and deposition studies were carried out. MLX-NLC gel demonstrated sustained release and enhanced the skin permeation and deposition of meloxicam especially into the dermis in comparison to meloxicam gel (control). The results suggest that NLC gel could be a promising carrier for the transdermal delivery of meloxicam.[127]

Dasgupta S et al., (2013), solid lipid nanoparticle gel formulations of the dispersions were compared with the marketed gel of aceclofenac. The SLNs were prepared by high speed homogenization and ultra-sonication method with fixed amount of aceclofenac (10%) and pluronic F68 (1.5%). The particle size, zeta potential and span concentration of developed formulations was found to be within the range of 123 nm to 323 nm, -12.4 to -18.5 and 0.42 to 0.86 respectively as the lipid concentration was increased from 7.5% to 40%. The highest entrapment efficiency was found to be 75% with the formulation having lipid concentration of 30% and 0.85% of phospholipon 90G. The drug release of SLN gel formulations was better controlled as compare to SLN dispersions. The results indicated the superiority of SLN based formulations for topical delivery of aceclofenac.[128]

Patel D, et al., (2012), prepared nanostructured lipid carriers (NLC)-based topical gel of aceclofenac for the treatment of inflammation and allied conditions. NLCs were prepared by melt-emulsification, low-temperature solidification, and high-speed homogenization methods. Characterization of the NLC dispersion was carried out through particle size analysis, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and an in-vitro release study. The anti-inflammatory effect of the NLC gel was assessed by the rat paw edema technique and compared to marketed aceclofenac gel. The NLC dispersions exhibited d(90%) between 233 nm and 286 nm. All of the NLC showed high entrapment efficiency ranging from 67% to 82%. The particle size of NLC was further confirmed by the SEM study. The anti-inflammatory activity of NLC gel showed a rapid onset of action, as well as a prolonged duration of action as compared with the marketed gel.[129]

Labouta HI and El-Khosrdagui LK, (2010), polymethacrylate microparticles (MPs) incorporating verapamil hydrochloride (VRP) as a model hydrophilic drug using Eudragit RS100 and Eudragit L100 were prepared for the formulation of a composite topical gel. The effect of initial drug loading, polymer composition, particularly the proportion of Eudragit L100 as an interacting polymer component and the HLB of the dispersing agent on MPs characteristics was investigated. MPs showed high % incorporation efficiency and % yield. Composition of the hybrid polymer matrix was a main determinant of MPs characteristics, particularly drug release. The developed MPs gel showed controlled VRP release and reduced skin retention compared to a free drug gel.[130]

Rationale, Aim & Objectives

3.1 RATIONALE OF THE STUDY

The cutaneous T-cell lymphoma (CTCL) is most common form of Non-Hodgkin's lymphoma that involves in the skin, blood, lymph nodes and other internal organs. In early stage of disease only skin symptoms are observed which includes itching, skin dryness, rashes and enlargement of lymph nodes. Various epidemiological studies have proven that CTCL disease is promoted or triggered by environmental and external exposure too. Bexarotene is a highly effective anticancer agent which has been proven for the treatment of CTCL. Third generation selective retinoid X-receptor agonist drug bexarotene activates the retinoid receptor thereby prompting cell proliferation, differentiation of cells and apoptosis that mechanism to be found helpful in CTCL treatment. US-FDA in 1999 also approved the bexarotene topical gel for the treatment. The previous studies and literature reveals that there are certain limitations to use the drug direct on skin. Some of them related to skin irritation, low bioavailability, high dose, high molecular weight, short penetration properties and short half-life of drug. For that purpose in addition radio therapy is also recommended with bexarotene topical gel for effective treatment of CTCL. However, the topical delivery of bexarotene obstructs due to its high log P value (6.9) and poor aqueous solubility. So, it is difficult to penetrate the different layer of skin and produces local and systematic effect. For improvement in the drawbacks of present dosage form may easily cover-up by using nanotechnology dosage form. These dosage form are more therapeutic effect with less side effects. Now a day's industry is also focus on the nano-formulations. The present research work may meet the industrial needs.

3.2 AIM OF THE STUDY

The aim of the research is to formulate liposome and noisome of bexarotene. The promised liposomes and noisome are incorporated in topical gel for improvement the treatment and management of CTCL.

3.3 OBJECTIVES OF THE STUDY
- Analytical methods development and validation bexarotene drug.
- Development of liposomes and niosomes by using optimization modeling techniques.
- Incorporation of the optimized liposomes and niosomes in topical gel.
- In-vitro and ex-vivo characterization of developed topical gel.
- Determination of cytotoxicity and cell apoptosis on CTCL specified cell lines.
- Skin irritation studies of promising topical gels.
- Stability study of developed formulation.

CHAPTER- 4

Material & Methods
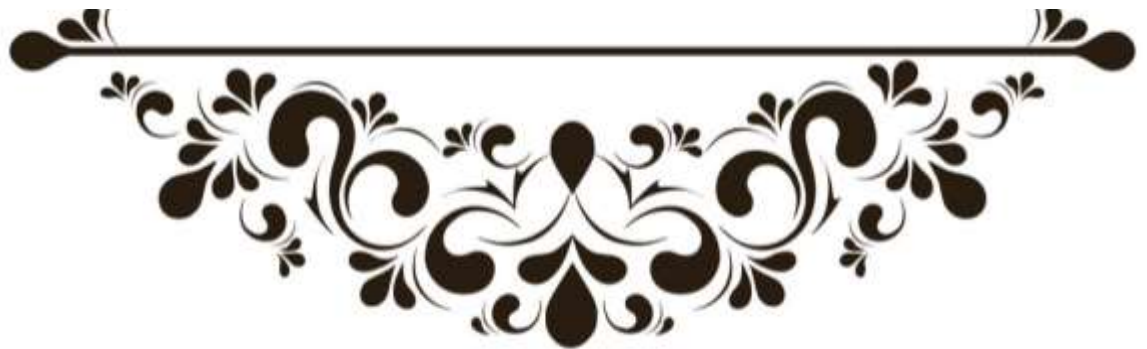
4.1 MATERIALS USED

Table 4.1: Materials used in research work

S. No.	Name	Company
1.	Acetone	S. D. Fine Chemicals Ltd., India
2.	Acetonitrile	Merk, India
3.	Bexarotene	Apicore Pharmaceuticals Pvt. Ltd, Gujarat
4.	Carbopol 934	Lubrizol Pvt. Ltd., India
5.	Carbopol 940	Lubrizol Pvt. Ltd., India
6.	Cellophane membrane, MTT	Himedia, India
7.	Chloroform	Loba Chemicals Ltd., India
8.	Cholesterol	Himedia, India
9.	Di-Sodium hydrogen phosphate,	S. D. Fine Chemicals Ltd., India
10.	Ethanol	Finar Ltd., India
11.	Methanol	Finar Ltd., India
12.	n- octanol	Merk, India
13.	Phospholipon 100S	Himedia, India
14.	Phospholipon 90H	Himedia, India
15.	Potassium dihydrogen phosphate	S. D. Fine Chemicals Ltd., India
16.	Soya phosphatidylcholine	Himedia, India
17.	Span 40	Loba Chemicals Ltd., India
18.	Span 60	Loba Chemicals Ltd., India
19.	Triethanolamine	Merk, India

4.2 EQUIPMENTS USED

Table 4.2: Equipments used in research work

S.No.	Name	Company
1.	Brook field viscometer	DV-E, Brook Field, USA
2.	24 and 96 Well Plates	Corning Incorporated, Corning NY, USA
3.	Diffusion cell apparatus	Orchid Scientific and Innovative, India
4.	Digital weighing balance	Shimadzu, Japan
5.	DSC instrument	SDT-Q600® TA Instruments, Tokyo, Japan
6.	Elisa plate reader	APR4 Microplate Reader, Germany
7.	FE-SEM	SEM, LEO43 SVP, Cambridge
8.	FTIR apparatus	ALPHA FTIR, Bruker, Germany
9.	Homogeniser	IKA, Germany
10.	Hot air oven	Narang Scientific Works, India
11.	HPLC	Shimadzu, Japan
12.	Humidity chamber	Narang Scientific Works, India
13.	Incubator	Narang Scientific Works, India
14.	Magnetic stirrer	Remi's Equipment Pvt. Ltd., India
15.	Melting point apparatus	Remi's Equipment Pvt. Ltd., India
16.	Optical microscope	Motic, Xiamen, China
17.	Particle size analyzer	Malvern, UK
18.	pH meter	Equiptronics, India
19.	Rotary evaporator	Heildolph Pvt.Ltd. Germany
20.	Sonicator (bath)	PCI Analytics, India
21.	TEM	Hitachi, H-7650,japan
22.	UV spectrophotometer	Shimadzu, Japan
23.	Vortex mixer	Remi's Equipment Pvt. Ltd., India
24.	XRD instrument	Malvern, UK
25.	Zeta potential analyser	Malvern, UK

4.3 PREFORMULATION STUDIES

Before the formulation of any dosage forms from a new drug or combination of drugs, a contender drug should come across a phase known as a preformulation. Preformulation is a physiochemical depiction of the solid and liquid properties of the chemical compounds. Preformulation testing incorporates all examinations approved on the new medicinal compound with a specific extreme objective to create valuable information for succeeding formulation of biopharmaceutically sensible and stable dosage forms.

The preformulation studies should not be completed on a checklist basis. Rather, drug and polymers are closely investigated for their physiochemical characterization to find out the compatibility to each other. The preformulation stage provides a basic knowledge about applicant polymers and drugs. The choice made on the data generated during this stage can profoundly affect the resulting improvement of compounds. Accordingly, it is crucial that the preformulation sought to be executed carefully as possible to empower reasonable choices to be made.

4.3.1 Physical Appearance

The organoleptic characters of the drug were noted by using sensible organ observations. The drug was characterized for its color, odor, taste and surface morphology.

4.3.2 Melting Point Determination

Two techniques were used to evaluate the melting point of bexarotene.

A. Capillary Fusion Method

Melting point (MP) of the bexarotene was evaluated by taking small quantity of bexarotene in a one sided closed glass capillary tube and positioned in the MP apparatus and the temperature at which bexarotene start to melts was noted in triplicate.

B. Differential Scanning Colorimetry (DSC)

DSC has been widely utilized thermal method in pharmaceutical drug excipients compatibility assessment. DSC is a fast and simple analytical tool for evaluating any physical or chemical interactions occurs between excipients and drug of the formulation stage. The sample (4 mg of bexarotene) was put in aluminum crucibles of

DSC/TG equipment (SDT-Q600, T A Instruments, Japan). The temperature with a increasing heating rate of 10ºC/min was used in range of 20–400 ºC, [131,132]

4.3.3 Fourier Transform Infrared Spectral Assignment

Infrared spectroscopy of any chemical entity or drug provides information about the functional groups available on the individual chemical entity. The infrared spectral assignment of bexarotene was carried out using FTIR spectrophotometer analytical tool. The FTIR spectra (ALPHA FTIR, Bruker, Germany) acquired for the pure drug bexarotene and for the mixtures with polymers were obtained in a scan range of 4000–400 cm^{-1}.[133]

Approximately 0.2 gm of drug or drug-excipient sample was put on the scanner for scanning. The major functional groups were identified by their significant peaks observed in the spectra. The obtained IR spectra of the sample was compared with reference spectra to identify the peaks.[134]

4.3.4 X-ray Diffraction Studies

X-ray diffraction (XRD) is a simple and fast analytical tool primarily utilized to identify the nature of compound (amorphous or crystalline) and provides the dimensions of unit cell. [134,135]

Samples analyzed included pure drug bexarotene and drug-polymer mixtures. The samples were sensibly put in the sample holder and kept at 5 rpm during the analyses of samples.

4.3.5 Solubility Studies

The solubility may defined as a "the maximum quantity of solute that can dissolve in a certain quantity of solvent or quantity of solution (mixture of two or more solvent) at a specified temperature. In the other words the solubility can also define as the ability of one substance to form a solution with another substance."

Factors which may affect the solubility of drug molecule are their particle size, polymorph stage, nature of solute and temperature. [136-138]

A. *Qualitative Solubility of Bexarotene*

The solubility studies was carried out in various solvents like purified water, DMSO, ethanol, chloroform, vegetable oils and phosphate buffer (pH 7.4). Bexarotene was

added into distinct glass test tubes, which may contain solvent (10 ml). The entire glass tubes were shaken on temperature regulated waterbath shaker for 5-10 min. Then the solubility of bexarotene was examined visually.

B. Quantitative Solubility of Bexarotene

The quantitative solubility studies of bexarotene in different solvents were determined by an equilibrium solubility method. Various solvents used in study were purified water, DMSO, ethanol, chloroform; vegetable oils and PBS (pH 7.4).

The method followed as:

10mg of drug was placed in screw capped glass tubes

↓

10ml of different solvents were added, in each glass tubes

↓

Glass tubes were kept in water bath with shaking at 37°C for 24 hr

↓

Drug was added to the glass tubes, if required, to obtain saturated solution

↓

After shaking for 24 hr the mixture was filtered

↓

Drug in the supernatant solution was determined spectrophotometriacally at the absorption maxima

4.3.6 Absorption Maxima (λ_{max}) Determination in UV Spectoscopy

When solution of the organic molecules was exposed to UV-light region, these molecules may absorb the light at specific wavelength. At this, particular wavelength molecule was shown the electronic transitions. The molecule absorbs some light and remain light will be transmitted. For determining the absorption maxima (λ_{max}), scanning was carried out in the UV-vis range of 200-400 nm. A standard solution of bexarotene (100μg/ml) was developed by dissolving 10mg of bexarotene in 100 ml methanol. This solution after dilution (05μg/ml) was examined in the wavelength range of 200-400 nm and the absorption maxima were determined spectrophotometrically by Shimadzu 1700 spectrophotometer. The scanned absorption maxima (λ_{max}) values recorded.[139]

4.3.7 Calibration Curve Preparation

The standard curve of bexarotene was developed in different media [Distilled water and ethanol; PBS (pH 7.4)]. Accurately weighed bexarotene (50mg) was dissolved in media solution (500 ml) which produced a stock solution of 1000µg/ml. From this developed stock solution, quantity of 0.1 to 1.0 ml were taken in a volumetric flask (10 ml) and diluted by media upto 10ml. The resulting aliquots had a concentration range of 1to10µg/ml. Then these samples were analyzed at fixed wavelength of 264nm spectrophotometrically. For calculation of statistical parameters computer program (Microsoft Excel) was used.[140]

4.3.8 Partition Coefficient

For passive transportation of drug through the cell membrane is influenced by the lipophillicity of a drug molecule. Specific and sufficient lihophillicity value is required for entering of drug molecule through cell membrane; if the value is high then drug molecule trapped in the cell membrane and not diffused to blood stream and if the value is at lower side then difficult to cross the cell membrane.

The lipohillicity is determined by the partition coffecient of the drug. An oil/water partition coefficient ($K_{o/w}$) is determined to evaluate the property of drug penetration into the cell membrane. The partition coefficient of Bexarotene was evaluated in oil phase (n-octanol) and water phase (PBS pH 7.4) (1:1) system by using a glass separating funnel. Partition coefficient of bexarotene was estimated as the ratio of amount of bexarotene in oil and water phase as shown in the equation. Bexarotene (10 mg) was precisely weighed and taken in the glass stopper vials. The bexarotene drug was mixed with 2 immiscible phases, oil (*n*-octanol) phase and water phase (PBS pH 7.4). These glass vials were put in the waterbath shaking apparatus for a day. The mixture was shaken until equilibrium was attained. The phase separation was done in a separating funnel. The lower side solution of bexarotene in water phase (PBS pH 7.4). The amount of bexarotene was estimated in obtained sample after dilution with PBS pH 7.4. This diluted sample was analyzed by using UV-Vis spectrophotometrically at 264 nm after filtration through 0.5µ filter. [136, 141]

The partition coefficient was estimated as:

$$PC = \frac{C_o}{C_w}$$

Where, PC = Partition Coefficient;

Co = Concentration of drug in n-octanol phase; Cw = Concentration of drug in distilled water"

4.3.9 HPLC Analysis

A. Determination of Retention Time by HPLC

The drug molecules in optimized mobile phase showed the characteristic retention time under standard set of conditions e.g. column, temperature and flow rate. The 0.002%w/v solution of bexarotene drug in mobile phase (Acetonitrile : Ammonium acetate buffer) (75:25). The mobile phase was passed via C-18 column at the fixed flow rate of 0.02ml/min for period of 16 minutes. The retention time was recorded at which highest peak was observed.[142]

B. Preparation of Calibration Curve of Bexarotene

Bexarotene (2mg) drug was firstly liquefied in a smallest quantity of HPLC grade methanol. A stock solution of bexarotene drug (10 μg/ml) was prepared by diluting the methanolic solution of bexarotene by mobile phase (Acetonitrile: Ammonium acetate buffer) (75:25) From this stock solution, samples of 1, 2, 3 ......,10 ml were taken out in a volumetric flasks (10 ml) and diluted with mobile phase upto 10 ml. This provides a bexarotene solution concentration range of 10-100 μg/ml. In HPLC analysis the small amount (20 μL) of prepared samples were injected into the port. The calibration curve of bexarotene was prepared between an area under curve of peak obtained at specific retention time and concentration of bexarotene solution.

4.3.10 Development of HPLC Method

A. Instrumentation

Analysis was performed by Shimadzu binary RP-HPLC (LC-20AD) system; Analytical column was LC Solution C18, 250 mm × 4.6 mm, with a Rheodyne injector (20 μL) loop was used for sample injection. LC Solution software was used in the method development. The mobile phase (Acetonitrile and Ammonium Acetate Buffer) in the ratio of 75:25 was developed and used as binary phase mobile solvent system. The mobile phase and sample solutions were degassed by 1.5 LH ultrasonic bath sonicator and filtered through a 0.45 μm membrane before use. The identification of compound was established by comparing the retention time of test and standard. Chromatography was performed in an ambient environment maintained at 20±1°C.[143]

B. Development of Stock Solution of Bexarotene

A standard stock solution (1000 µg/ml) of the bexarotene drug was developed by dissolving bexarotene drug (10 mg) in volumetric flask (10 ml) containing mobile phase (5 ml). The prepared stock solution was sonicated for 15 min and then diluted with mobile phase upto 10ml. The working stock solution (100µg/ml) was devloped from stock solution (1000µg/ml) of the bexarotene by withdrawn 1ml aliquot of this solution and further diluted with mobile phase. [144]

C. in Validation Studies Calculations

Percentage recovery was estimated by following formula:

$$\% \, Recovery = \frac{[Peak \, Area]_{Sample}}{[Peak \, Area]_{Standard}} \, X \, 100$$

Percentage relative standard deviation (%RSD) was estimated as

$$\% \, RSD = \frac{Standard \, Deviation}{Average} \, X \, 100$$

D. Method Development

In the bexarotene analysis method design, appropriate chromatographic conditions were used. Methodical studies on different factors/conditions have been undertaken for development of a method. At a time one factor was changed and other parameters kept constant to study the effect of particular factor on method development.

Table 4.3: Optimized chromatographic conditions for estimation bexarotene

Chromatographic Mode	Chromatographic Condition
HPLC System	Shimadzu binary RP-HPLC (LC-20AD) system
Data Processor	LC solution
Detector	Photo Diode Array Detector
Column	LC-Solution C18 column
Mobile Phase	Acetonitrile and Ammonium Acetate Buffer (75:25)
Mobile phase pH	4.1
Column Temperature	Ambient
Wavelength (nm)	264
Injection Volume (µl)	20
Flow Rate (ml/min)	1.0

E. Linearity (Calibration Curve)

When sample concentration was plotted against the peak area forms a calibration curve also known as linearity. Mainly the peak area find proportional to the sample concentration. From the lower concentration to upper concentration in which the peak observes in linearity known as the range. Linearity, accuracy, precision and other validation parameters always performed in the range of analyte concentration. The ICH guidelines also identify the specified range for the validation of parameters which lies between 80–120% of the standard analyte concentration.

Linearity was evaluated by developing working stock solutions of bexarotene (100 µg/ml) in different concentration levels. 20 µl of prepared analyte concentration was introduced in triplicate into the RP-HPLC system. The responses were recorded at 264nm. From the chromatograms, the highest peak areas were taken and linearity curves were prepared between mean peak areas and analyte concentration. The calibration curve was plotted between an analyte concentration range of 10-100 µg/ml for bexarotene drug. The regressions equation (y=mx+c) and regression coefficient (r^2) were estimated. [145]

F. Determination of LOD & LOQ

According to the ICH guidelines, the limit of detection (LOD) and the limit of quantification (LOQ) for the bexarotene were estimated by using the ration of signal-v/s-noise. The equations used were:[146]

$$LOD = 3.3 \, X \, {}^{\sigma}/_{S} \qquad\qquad LOQ = 10 \, X \, {}^{\sigma}/_{S}$$

Where, σ = Standard deviation of the response, S = Slope of the standardcurve accuracy

G. Accuracy

The accuracy of the developed method was confirmed by determining the nearness of the true value to the observed measured value. Accuracy of method was evaluated from the recovery of bexarotene from spiked solutions. Known amount of working stock solution of bexarotene were utilized at 50, 100, 150 percent level to 60 µg/ml sample solution of bexarotene.

H. Precision

Precision is known as "the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as Intra-day precision and Inter-day precision." Precision of the developed method was estimated through computing % RSD of three developed concentration of 100% target concentration of bexarotene. The recommended % RSD is NMT 2.0% of target concentration. Precision of method was assessed by repeatability (intraday) and intermediate precision (interday) in triplicate analysis of the working stock analytes (30, 60 and 90 µg/ml) of bexarotene drug.

I. Robustness

Robustness is the analysis of sensitivity of method by done some minor alterations in the experimental factors/conditions. For the evaluation of robustness of method bexarotene concentration 60µg/ml was utilized. The alter in flow rate of mobile phase (0.98 & 1.02 ml/min), alter in wavelength (detection) (262 & 264nm), alter in proportion of mobile phase components (70:30 & 80:20) and alter in pH of mobile phase (3.9 & 4.3) were studied to evaluate robustness of analytical method. The triplicate injections of analyte were injected and changes in retention time and mean peak area were observed and %RSD was computed.

J. Ruggedness

Ruggedness is an important validation parameter of developed analytical method. In this environmental conditions may alter (change of working laboratory or RP-HPLC system) or change of working analytical person. To determine ruggedness of the method, two different working analysts executed the assay method of bexarotene drug in same conditions. To evaluate the ruggedness triplicates of working stock solution (60µg/ml) of bexarotene were used by another person to determine the person to person variation.

4.4 DRUG-POLYMER COMPATIBILITY STUDIES

While designing liposomes, niosomes or gel, it was imperative to give consideration to the compatibility of drug and polymer used within the systems. It was therefore necessary to confirm the interaction between polymer and drug under experimental

conditions (40 ± 5^{0}C and $75 \pm 5\%$RH) for a month. At the regular intervals the physical changes like discoloration, liquefaction and clumping of material were. The infrared absorption spectra of 4 week aged physical mixture of polymer and bexarotene are run between 400-4000 cm^{-1}. The FTIR spectra of mixture of polymers and bexarotene drug (1:1) were used to determine the compatibility with each other.[147]

4.5 DEVELOPMENT OF LIPOSOMES

4.5.1 Preparation of Liposomes

Liposomes of bexarotene were developed by using thin film hydration method in rotary evaporator (Heidolph Instruments, Germany). Firstly, the drug was mixed in mixture of chloroform and ethanol blend in the ratio of (3:2). The thin film formed by using mechanical dispersion of bexarotene, different phospholipids like phospholipon 90H , phospholipon 100S, soya phosphatidylcholine (1-4% w/v) and cholesterol (1 % w/v) were mixed in chloroform in a RBF (250 ml) as given in Table 4.4. The organic solvents were removed by rotating evaporator under reduced pressure to create a thin film around the inside surface of flask at 50ºC and 90 rpm. For removal of residual solvent the prepared films were subjected to vacuum for 1h. The dried film was then hydrated with Phosphate buffer pH 7.2 for 30 min. The ready mixture was vortexed, and sonicated in bath sonicator (Ultra Sonic, India) for 15 min. and prepared solution of liposome was stored in refrigerator for further evaluations.[148] The precautions were taken regarding temperature, solvent/excipient content and stirring time and speed of rotatory evaporator to ensure the reproducibility of dosage form.

Figure 4.1: Rotary evaporator used for preparation of dried film

Table 4.4: Composition of liposome formulation of bexarotene

Form. Code	Lipid(% w/w)			Cholesterol (% w/w)	Drug (% w/w)
	Phospholipon 90H	Soya PC (Himedia 35%)	Phospholipion 100S		
LIPO F1	1	-	-	1	1
LIPO F2	2	-	-	1	1
LIPO F3	3	-	-	1	1
LIPO F4	4	-	-	1	1
LIPO F5	-	1	-	1	1
LIPO F6	-	2	-	1	1
LIPO F7	-	3	-	1	1
LIPO F8	-	4	-	1	1
LIPO F9	-	-	1	1	1
LIPO F10	-	-	2	1	1
LIPO F11	-	-	3	1	1
LIPO F12	-	-	4	1	1

4.5.2 Characterization of Liposomes

A. Particle Size Determination

Dynamic light scattering method was employed to determine the particle size of bexarotene liposomes. The liposomes suspension was diluted with water (10 times, milliQ®). This diluted liposome suspension was put in cell of zeta sizer (Malvern Zetasizer NanoZS90, UK) and particle size was determined according to standard operating procedure. The temperature of cell was kept fixed on 25°C and three values were obtained at scattering angle of 175°.[149]

B. Zeta Potential Determination

Polydispersity index was determined by zeta potential which could be a measure of uniformity. Sample for zeta potential determination was prepared similarly as the sample prepared for determination of particle size. The sample was then examined three times from -200 mV to +200mV.[150]

C. Morphological Evaluation

Surface morphology of liposomes was evaluated by using transmission electron microscopy (TEM) JEM 2100 (Hitachi, H-7650, Japan). The dilute liposomal dispersion was fixed on the copper grids (formvar®) for morphological studies. Diluted liposome suspension was stained with solution of uranyl acetate (2%w/v) for proper imaging of samples.[151]

Figure 4.2: Transmission electron microscope (Hitachi, H-7650, Japan)

E. Drug Loading and Entrapment Efficiency

For estimation of entrapment efficiency (%EE) the ultrafiltration centrifugation method was applied. The 2 mL formulation was centrifuged at the rate of 10000 RPM for 30 minutes utilizing cooling centrifuge AmiconUltra-4 (Ultracel-10) centrifugal filter (Merck Millipore Ltd., Germany). Both pellet and supernatant were collected and the amount of free drug within the supernatant liquid was determined utilizing RP-HPLC assay method. The entrapment efficiency in liposomes was calculated as

$$\% \text{Entrapment } efficiency = \frac{Ct - Cr}{Ct} \times 100$$

Where, Ct = Total drug concentration, Cr = Free drug concentration.

F. In-vitro Drug Release

Dialysis method was employed to study the in-vitro drug release of drug from liposomes. Ten milliliter of the bexarotene liposomes were placed in a dialysis bag (SpectraPor S/P 2 dialysis membrane, 12K–14K molecular weight), which was dipped in 40mL dialysis medium (PHB, pH 7.4). The temperature was set at 37°C and stirring rate kept constant at 50 rpm. Aliquots of two milliliter of release medium were withdrawn for analysis at one hour interval and changed with fresh medium. Drug release studies were continued for 12 hours. The obtained samples were analyzed by using RP-HPLC method.[152]

4.5.3 Optimization of Liposomes

For preparation of bexarotene liposomes various lipids were screened in preliminary studies. These include PL-100S, PL-90H and SPC. The parameters kept fixed such as sonication time, cholesterol-lipid ratio and drug loading during the experiment. Bexarotene liposomes were optimized by using Response Surface Methodology by 3^2 full factorial design methods. The two factors at three level design was employed for making a second-order polynomial models (quadratic) using Design Expert 11(Version Trial; Stat-Ease Inc, Minneapolis, Minnesota) software. Total nine formulations as shown in Table 4.5 were developed according to experimental design. The selected independent variables were amount of phospholipon-100S (X1) and time for rotation (X2). The entrapment efficiency (%) and vesicle size (nm) were selected as dependent variables.[153]

Table 4.5: Design matrix 3^2 factorial design for bexarotene liposomes

Batch	X1: Amount of Phospholipon-100S (%w/w)	X2: Time of Rotation (Minutes)	Y1 Entrapment Efficiency (%)	Y1 Vesicle Size (nm)
L1	-1	-1	60.34±1.33	170.92±10.32
L2	-1	0	65.13±1.24	182.45±10.56
L3	-1	+1	63.16±2.23	176.84±15.52
L4	0	-1	78.34±2.34	242.56±16.25
L5	0	0	81.25±2.48	213.24±13.33
L6	0	+1	79.46±2.89	225.28±25.49
L7	+1	-1	86.52±3.79	620.46±16.49
L8	+1	0	89.96±2.32	639.27±18.33
L9	+1	+1	90.56±3.49	656.59±25.33
Coded Value		-1 (Low)	0 (Intermediate)	+1 (High)
X1	Amount of Phospholipon-100S (%w/w)	1	2	3
X2	Time of Rotation (Minutes)	10	15	20

The responses were calculated by using interactive and polynomial terms of the mathematical model.

$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1X_1 + b_{22}X_2X_2 + b_{12}X_1X_2$

In the equation, Y denotes the dependent variable, b_0 is the arithmetic mean. b_1 and b_2 were coefficient for the factor X_1 and X_2. Optimized formulation was validated and analysis of variance (ANOVA) was used to analyses the results. ANOVA was applied to find out the significance of factors.[154]

A. Check Point Batches

The check point batches were developed and matched with values obtained from the software utilizing the equations of the model validity. Check point batches were

prepared and evaluated for percent entrapment efficiency and vesicle size. The predicted value and observed values were compared residual value was determined (Table 4.6 & 5.17)

B. Optimized Batch

Final optimized Bexarotene liposome batch FL1, was prepared as per the suggested quantity of X1 and X2 factors which was set targeted for entrapment efficiency (85%) and vesicle size (625 nm) shown in Table 5.17. The method of preparation of the liposome was same as mentioned in previous.

Table 4.6: Check point batches and optimized batch of bexarotene liposomes

Form. Code	Lipid(% w/w) Phospholipion 100S	Rotation Time (Minutes)
LC1	-0.5 (1.5)	-0.5 (12.5)
LC2	-0.5 (1.5)	0.5 (17.5)
LC3	0.5 (2.5)	-0.5 (12.5)
LC4	0.5 (2.5)	0.5 (17.5)
FL1	1.0 (3.0)	0.1 (~15.5)

4.6 DEVELOPMENT OF NIOSOMES

4.6.1 Preparation of Niosomes

Niosomes of bexarotene were developed by using thin film hydration method in rotary evaporator (Heidolph Instruments, Germany). First the, drug was dissolved in mixture of chloroform and ethanol blend in the ratio of (3:2). The thin film formed by using mechanical dispersion of bexarotene, different surfactants like span 60, tween 60 and tween 80 (1-3% w/v) and cholesterol (1 % w/v) were mixed in chloroform in a 250 ml round bottom flask (RBF) as given in Table 4.7. The organic solvents were removed by rotating evaporator under reduced pressure to create a thin film around the inside surface of flask at 50ºC and 90 rpm. For removal of residual solvent the prepared films were subjected to vacuum for 1h. Then, the dried film was hydrated with Phosphate buffer pH 7.2 for 30 min. The ready mixture was vortexed, and sonicated in bath sonicator (Ultra Sonic, India) for 15 min. and prepared solution of niosome was stored in refrigerator for further evaluations.[148]

Table 4.7: Composition of niosome formulation of bexarotene

Form. Code	Surfactant (% w/w)			Cholesterol (% w/w)	Drug (% w/w)
	Span 60	**Tween 80**	**Tween 60**		
NIO F1	1	-	-	1	1
NIO F2	2	-	-	1	1
NIO F3	3	-	-	1	1
NIO F4	-	1	-	1	1
NIO F5	-	2	-	1	1
NIO F6	-	3	-	1	1
NIO F7	-	-	1	1	1
NIO F8	-	-	2	1	1
NIO F9	-	-	3	1	1

4.6.2 Characterization of Niosomes

A. *Particle Size Determination*

Dynamic light scattering method was employed to determine the particle size of bexarotene niosome. The niosome suspension was diluted with water (10 times, milliQ®). This diluted niosome suspension was put in cell of zeta sizer (Malvern Zetasizer NanoZS90, UK) and particle size was determined according to standard operating procedure. The temperature of cell was kept fixed on 25°C and three values were obtained at scattering angle of 175°.[149]

B. *Zeta Potential Determination*

Polydispersity index of prepared niosomes were determined by using zeta potential which could be a measure of uniformity. Sample for zeta potential determination was prepared similarly as the sample prepared for determination of particle size. The sample was then examined three times from -200 mV to +200mV.[150]

C. *Morphological Evaluation*

Surface morphology of niosomes was evaluated by using transmission electron microscopy (TEM) JEM 2100 (Hitachi, H-7650, Japan). The dilute niosome dispersion was fixed on the copper grids (formvar®) for morphological studies. Diluted niosome suspension was stained with solution of uranyl acetate (2%w/v) for proper imaging of samples.[151]

E. Drug Loading & Entrapment Efficiency

Entrapment efficiency of developed niosomes were evaluated by the ultrafiltration centrifugation method. The 2 ml of niosome suspension formulation was centrifuged at the rate of 10000 RPM for 30 minutes utilizing cooling centrifuge AmiconUltra-4 (Ultracel-10) centrifugal filter (Merck Millipore Ltd., Germany). Both pellet and supernatant were collected and the amount of free drug within the supernatant liquid was determined utilizing RP-HPLC assay method. The entrapment efficiency in niosomes was calculated as

$$\% \, Entrapment efficiency = \frac{Ct - Cr}{Ct} \times 100$$

Where, Ct = Total drug concentration, Cr = Free drug concentration.

F. In-vitro Drug Release

Dialysis method was employed to study the in-vitro drug release of drug from niosomes. Ten milliliter of the bexarotene niosomes were placed in a dialysis bag (SpectraPor S/P 2 dialysis membrane, 12K–14K molecular weight), which was dipped in 40ml dialysis medium (PHB, pH 7.4). The temperature was set at 37°C and stirring rate kept constant at 50rpm. Samples of two milliliter of release medium were withdrawn for analysis at one hour interval and changed with fresh medium. Drug release studies were continued for 12 hours. The obtained samples were analyzed by using RP-HPLC method.[152]

4.6.3 Optimization of Niosomes

For preparation of bexarotene niosomes various surfactants were screened in preliminary studies. These include span-60, tween-60 and tween-80. The parameters kept fixed such as sonication time, cholesterol-surfactant ratio and drug loading during the experiment. Bexarotene niosomes were optimized by using Response Surface Methodology by 3^2 full factorial design methods. The two factors at three level design was employed for making a quadratic second-order polynomial models using Design Expert-11(Trial-Version; Stat-Ease Inc, Minneapolis, Minnesota) software. Total nine formulations as shown in Table 4.8 were prepared according to experimental design. The selected independent variables were amount of span-60

(X1) and time for rotation (X2). The entrapment efficiency (%) and vesicle size (nm) were selected as dependent variables.[153]

Table 4.8: Design matrix 3^2 factorial design for bexarotene niosomes

Batch		X1: Amount of Span 60 (%w/w)	X2: Time of Rotation (Minutes)	Y1 Entrapment Efficiency (%)	Y1 Vesicle Size (nm)
N1		-1	-1	71.68±2.56	665.87±11.67
N2		-1	0	93.76±1.24	569.98±10.56
N3		-1	+1	82.90±3.73	440.16±13.88
N4		0	-1	68. 26±1.33	749.33±10.65
N5		0	0	71.98±3.56	612.36±14.45
N6		0	+1	65.44±5.29	580.03±21.99
N7		+1	-1	74.31±2.24	892.27±16.33
N8		+1	0	69.09±7.56	772.45±10.51
N9		+1	+1	90.56±3.49	672.05±18.30
Coded Value			-1 (Low)	0 (Intermediate)	+1 (High)
X1	Amount of Span 60 (%w/w)		1	2	3
X2	Time of Rotation (Minutes)		10	15	20

The responses were calculated by using interactive and polynomial terms of the mathematical model.

$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1X_1 + b_{22}X_2X_2 + b_{12}X_1X_2$

In the equation, Y denotes the dependent variable, b_0 is the arithmetic mean. b_1 and b_2 were coefficient for the factor X_1 and X_2. Optimized formulation was validated and analysis of variance (ANOVA) was used to analyses the results. ANOVA was applied to find out the significance of factors.[154]

A. Check Point Batches

The check point batches for niosome formulations were developed and matched with values obtained from the software utilizing the equations of the model validity. Check point batches were prepared and evaluated for percent entrapment efficiency & vesicle size. The predicted value and observed values were compared residual value was determined. (Table 4.9 & 5.20).

B. Optimized Batch

Final optimized bexarotene niosome batch FN1, was prepared as per the suggested quantity of X1 and X2 factors which was set targeted for entrapment efficiency (85%) and vesicle size (625 nm) shown in Table 5.20. The method of preparation of the liposome was same as mentioned in previous.

Table 4.9: Check point batches and optimized batch of bexarotene niosomes

Form. Code	Surfactant (% w/w) Span 60	Rotation Time (Minutes)
NC1	-0.5 (1.5)	-0.5 (12.5)
NC2	-0.5 (1.5)	0.5 (17.5)
NC3	0.5 (2.5)	-0.5 (12.5)
NC4	0.5 (2.5)	0.5 (17.5)
FN1	-1.0 (1.0)	0.1 (~15.5)

4.7 DEVELOPMENT OF TOPICAL GEL

4.7.1 Preparation of Topical Gel

For the preparation of liposomal and niosomal gel, firstly the carbopol 934P (0.5 to 1.75% w/w) was splashed in distilled water (Table 4.10). The gel was formed by swelling and hydration for 3-4 hr. The acidic nature of carbopol suspension (pH 3.1) was neutralized altered to pH 7.4 by using of triethanolamine for crosslinking and gel formation. Then isopropylalcohol, propyl paraben and methyl paraben were added and blended with gel base. The liposomal suspension (FL1) or niosomal suspension (FN1) was blended with this carbopol gel base by mechanical blending at speed of 1000-1500 RPM for 1 hr to make the liposomal and niosomal gel dispersion.[155]

Table 4.10: Formulation of topical gel

Ingredients	Formulation Code		
	G1	**LG2**	**NG3**
Drug in	Simple	Liposomes	Niosomes
Drug (% w/w)	1	1	1
Carbopol 934 (% w/w)	1.5	1.5	1.5
Propylene Glycol (ml)	2	2	2
Ethanol (ml)	5	5	5
Triethanolamine (ml)	q.s. to neutralize the gel base		
Water (upto ml)	100	100	100

4.7.2 Characterization of Topical Gel

The topical gels were characterized for its physical and chemical characters like:

A. *Visual examination*

The prepared topical gels were checked for their physical activities like color, transparency, purity, uniformity and separation of phase.[156]

B. *pH of topical gel*

The pH of the prepared topical gels were evaluated by using digital pH meter. The topical gel was dissolved in triple distilled water and the electrode of pH meter was plunged inside the topical gel solution and the pH was recorded until the constant reading achieved.[157]

C. *Extrudability estimation*

To degree the extrudability was determined by using a closed aluminum collapsible tube filled with prepared topical gel was squeezed immovably at the creased conclusion and a holder was associated to expect any roll-back of topical gel. The aluminum collapsible tube mouth was opened by removing of cap and the topical gel was removed out from aluminum collapsible tube. The extrudability estimation was observed by the easiness in comes out of topical gel from the tube.[158]

D. *Spreadability*

Parallel plate method was used to determine the spreadability of prepared topical gel. Topical gel (1 gm) was put on a transparent glass slide and another transparent slide of glass was put upon the slide which containing topical gel. Initially, spreaded topical

gel diameter was recorded by the weight of glass slide only. After the stress given by weight ranging from 5g to 100g for 30 seconds time interval to the topical gel, the spread diameter was calculated in after addition of each weight. [158]

Spreadability was estimated by given formula:

$$S = m \ X \ \frac{l}{t}$$

where, "S" is spreadability, "m" is weight tied to upper slides (20g), "l" is length of the glass slide (7.5 cm), "t"is time taken in s.

E. Consistency

Viscosity of prepared topical gel was determined with the help of Brookfield viscometer (S-62, LVDV-E) at the room temperature (25 ℃) at fixed rotation speed of 12 RPM.

F. Drug content

Drug content of prepared topical gel was estimated by shaken 100 gm of topical gel with adequate quantity of methanol to extract the bexarotene and then estimated by utilizing RP-HPLC method..

G. Drug release study by In-vitro methods

The in-vitro diffusion of drug from prepared topical gel and plain drug loaded topical gel was examined utilizing Franz diffusion cell apparatus kept at 37 ± 1^0C. The actual permeation diameter of the diffusion cell apparatus was 2.30 cm^2. The receptor compartment consist 6.5 ml phosphate buffer (pH 7.4), and stirred at 100 rpm. Cellophane membrane (MW;12K–14K dalton) was fixed between the compartments of the donor and the receptor. Formulation was placed in the donor compartment. The aliquots were taken from the Franz diffusion cell at different time intervals and after each sampling the diffusion media already kept at 37 ± 1^0C was exchanged into the receptor compartment.[159]

H. Drug deposition study

The rodent skin which was used in skin permeation studies was carefully expelled. Skin was washed with methanol and distilled water (1:1) combinations for eliminating

traces of drug formulation in outer part of skin portion. The washed skin was divided into small portions by cutting and dipped in 10 ml volume of 0.05% trypsin solution. Sample tests were put on mechanical stirrer for 24h at 100 rotations per minutes and 37 ± 1^0C. Samples were analyzed after filtration by using RP-HPLC method. [160]

4.8 SKIN IRRITATION STUDY

The designed protocol for animal studies was approved by IAEC (PBRI/IAEC/PN-19016). The animals were subdivided into 7 groups of 36 animals (Table 4.11). Animals of Group I served as control group, Animals of Group II received five minute ultraviolet light exposure two times in a day and served as irradiated control group. The test group III received drug free gel topically. The test groups IV and V received both UV radiations and drug free liposomal and niosomal topical gel. Test group VI and VII received both UV radiations and bexarotene loaded liposomal (LG2) and niosomal (NG3) topical gels, respectively. The treatment was provided to animals four hour prior to ultraviolet radiation exposure as per the protocol guidelines. All the animals were kept in a locally prepared wooden ultraviolet simulator or chamber. Ultraviolet lamp (300W Waton® bulb, Germany) was fixed inside on a top of chamber so the animals received radiations from the distance of 40 cm. The bulb produces the full spectrum of ultraviolet radiation from 260 - 400 nm. Ultraviolet radiation exposure was controlled by exposure time. The animals were treated for a month to study the changes obtained on dorsal skin [161, 162]

Table 4.11: Protocol design for skin irritation studies

Group	Treatment	Dose	No. of Animals
Group-I	Control	-	03
Group-II	UV treated	-	03
Group-III	UV treated + Drug free gel topically	50 mg/cm^2	06
Group-IV	UV + Drug free Liposomal gel topically	50 mg/cm^2	06
Group-V	UV + Drug free Niosomal gel topically	50 mg/cm^2	06
Group-VI	UV + Drug Liposomal gel topically	50 mg/cm^2	06
Group-VII	UV + Drug Niosomal gel topically	50 mg/cm^2	06

4.9 IN-VITRO CELL PROLIFERATION STUDIES

A well-developed MTT assay was used to assess the proliferative ability of cells treated with bexarotene loaded topical gel and bexarotene loaded liposomal and

niosomal topical gel. Around 5×10^3 Hut78 cells per well were inoculated in 96 well format. Serum medium RPMI was changed serum free RPMI medium after one day incubation period. After completion of a day, cells were treated with bexarotene loaded topical gel and bexarotene loaded liposomal (LG2) and niosomal topical gel (NG3) at gradient concentrations ranging from 0-150μM/mL in PBS (pH 7.4) for three days. In the last of treatment, 5mg/mL MTT was added in each well, and the plates were incubated at 37°C in dark for 5h. The formazan product was then mixed in 100μl of DMSO after removing the medium or each well content. [163]

4.10 STABILITY STUDIES

In the development of new dosage form stability or shelf life of prepared product always take place on a priority. The effectiveness, quality and safety are only ensured by the stability studies of formulations. Assurance of quality product is only determined by the stability studies. The level of acceptance any product is governed by the quality after spending some time.

4.10.1 Stability Studies Protocol

The stability study of optimized topical gel dosage form was implemented according to ICH and WHO guidelines. The optimized bexarotene simple topical gel (G1), bexarotene liposomal topical gel (LG2) and bexarotene niosomal topical gel (NG3) were placed in an air tight wide mouth container and stored at room temperature (25±2°C & 60±5% RH), and accelerated temperature (40±2°C & 75±5% RH) for 90 days and tested for appearance, pH, viscosity, and drug content.[164]

CHAPTER-5

Results & Discussion
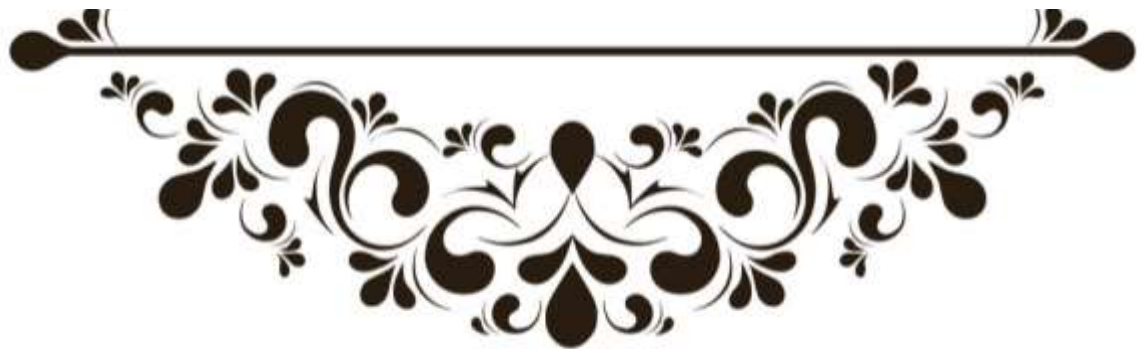
5.1 PREFORMULATION STUDIES

5.1.1 Physical Appearance

Physical appearance of bexarotene was examined for its organoleptic properties like

Color: White   Odor: Odorless.   State: Crystalline Powder

5.1.2 Determination of Melting Point

Melting point of the bexarotene was evaluated by capillary fusion methods, and the temperature was observed between 230-231°C which is concordant with the Jean-Claude Bradley open melting point dataset and US national library of medicines at 230^0 C. Melting point of the bexarotene was also evaluated by DSC. The thermograph of bexarotene was confirmed that melting point was 224^{0}C (Figure 5.1).

Table 5.1: Melting point determination of bexarotene

Sample	Reference Melting Point (^{0}C)	Observed Melting Point (^{0}C)	Observed DSC Range (^{0}C)
Bexarotene	230	230-231	218-235 (224^{0}C)
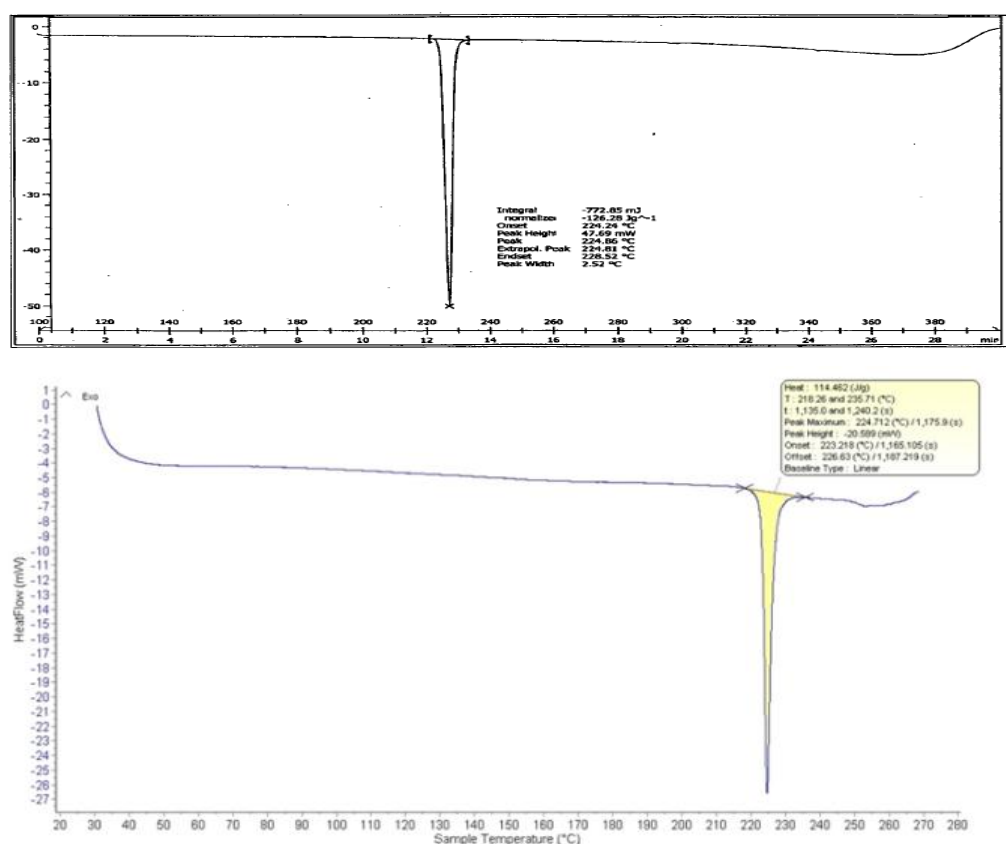
Figure 5.1: DSC thermograph of bexarotene (A) Reference[91] (B) Sample

5.1.3 Fourier-Transform Infrared Spectral Assignment

The FTIR spectrum of the bexarotene was determined for its identification. For carrying out FTIR, a sample of approximately 2 mg was kept in FTIR spectrometer (ALPHA FTIR, Bruker, Germany) and the spectrum is recorded. The observed peaks were compared with the reference spectrum (Figure 5.2).

(A)

(B)
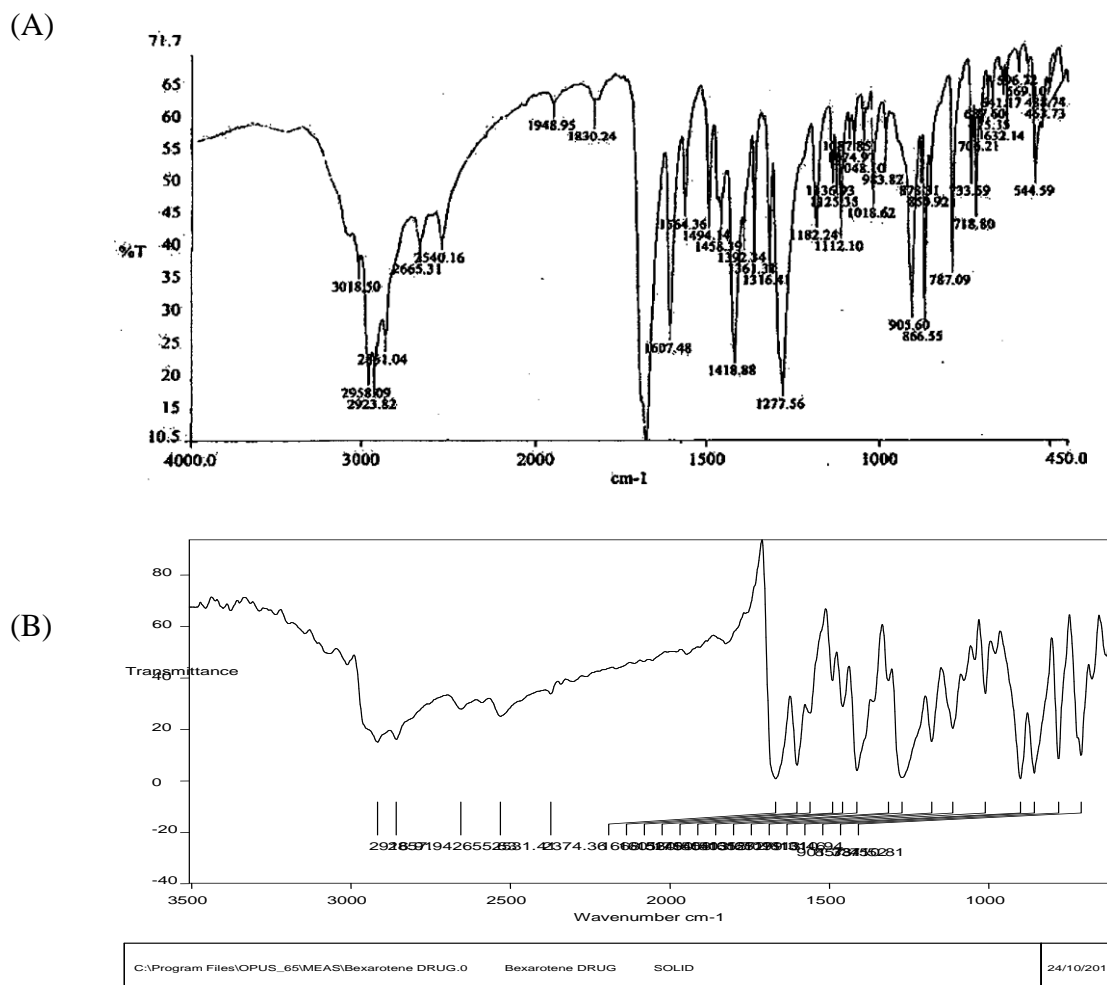
Figure 5.2: FTIR spectrum of bexarotene (A) Reference[91] (B) Sample

5.1.4 X-ray Powder Diffraction (XRPD)

X-ray diffraction (XRD) is a simple and fast analytical tool primarily utilized to identify the nature of compound (amorphous or crystalline) and provides the dimensions of unit cell. The XRD graph of bexarotene (Figure 5.3) shown sharp specific peaks notably at 2θ diffraction angles of $16^0, 19^0$ & 23^0 proved that bexarotene was available in crystalline form.
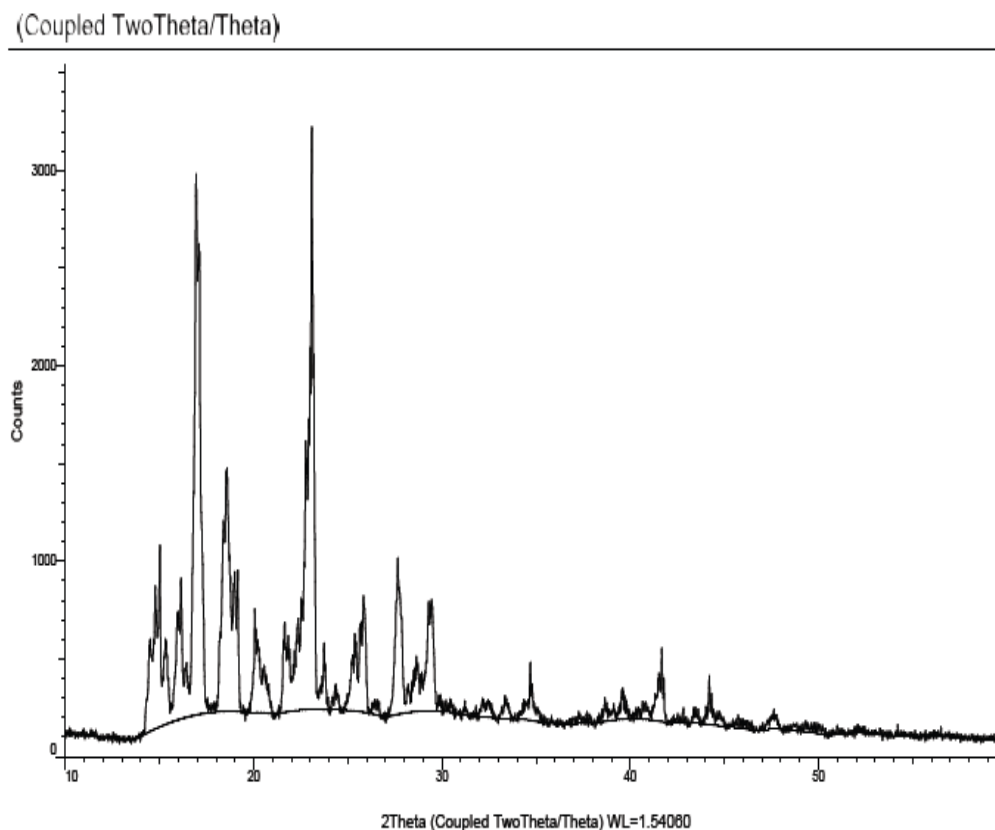
Figure 5.3: XRD spectrum of bexarotene drug

5.1.5 Solubility Studies

A. *Qualitative Solubility of Bexarotene*

The qualitative solubility was carried out in different solvents like purified water, DMSO, ethanol, chloroform, vegetable oils and phosphate buffer (pH 7.4). The results were obtained by visual inspection of solution (Table 5.2).

Table 5.2: Qualitative solubility data of bexarotene

Solvents	Solubility
Purified water	-
0.1 N HCl	-
Vegetable oils	+
Phosphate buffer (pH 7.4)	+
Ethanol	++
DMSO	++
Chloroform	++

*(-) practically insoluble, (+) slightly soluble, (++) soluble

B. Quantitative Solubility of Bexarotene in Different Solvents

A small amount of the drug was put in screw capped glass tubes and dissolved in solvent (10 ml). These screw capped glass tubes were kept in waterbath shaker for one day at room temperature (25°C). The concentration of drug solubilized was examined by UV spectrophotometric method. The quantitative solubility shown that drug bexarotene was highly soluble in DMSO, chloroform, and ethanol. In vegetable oils 0.1 N HCl bexarotene was found slightly soluble. Bexarotene was found practically insoluble in water.

Table 5.3: Quantitative solubility data of bexarotene

Solvents	Quantitative Solubility (mg/ml)
Distilled water	-
0.1 N HCl	0.09 mg/ml
Vegetable Oils	2.13 mg/ml
Phosphate buffer (pH 7.4)	2.10 mg/ml
DMSO	10.02 mg/ ml
Ethanol	09.05 mg/ml
Chloroform	12.51 mg/ml

5.1.6 Ultraviolet Absorption Maxima (λ_{max}) Determination

The UV-absorption maxima (λ_{max}) of bexarotene (05µg/ml) in the different solvents were found 264 nm and 305 nm which were concordant with the Clarke Analysis.

Table 5.4: **Absorption Maxima (λ_{max}) of bexarotene in different solution**

Solvents	Absorption Maxima λ_{max} (nm)
Distilled water	264, 304
Ethanol	264
Phosphate buffer (pH 7.4)	264

5.1.7 Preparation of Calibration Curve

50 mg of bexarotene was solubilized in 50 ml of Distilled water / Ethanol/ Phosphate buffer (pH 7.4) (1000μg/ml) (A small portion of ethanol 1-2 mL was used as co-solvent in case of distilled water and Phosphate buffer). Aliquots of 1-10μg/mL bexarotene were prepared in same dissolving solvent. Absorbance is estimated by UV-Visible spectrophotometer at 264 nm.

Table 5.5: Calibration data of bexarotene drug in different solvent

Bexarotene Conc. (μg/mL)	Absorbance (at 264 nm)		
	In Distilled Water	**In Ethanol**	**In Phosphate Buffer (pH 7.4)**
0	0	0	0
1	0.062	0.072	0.091
2	0.134	0.139	0.183
3	0.246	0.243	0.268
4	0.322	0.318	0.386
5	0.417	0.405	0.498
6	0.524	0.511	0.587
7	0.612	0.613	0.684
8	0.734	0.709	0.781
9	0.815	0.808	0.895
10	0.937	0.912	0.996
Equation	y = 0.0942x - 0.0356	y = 0.0925x - 0.032	y = 0.1001x - 0.0127
R^2 Value	0.9969	0.9966	0.9992

$y = 0.0942x - 0.0356$
$R^2 = 0.9969$

(A)

$y = 0.0925x - 0.032$
$R^2 = 0.9966$

(B)

$y = 0.1001x - 0.0127$
$R^2 = 0.9992$

(C)
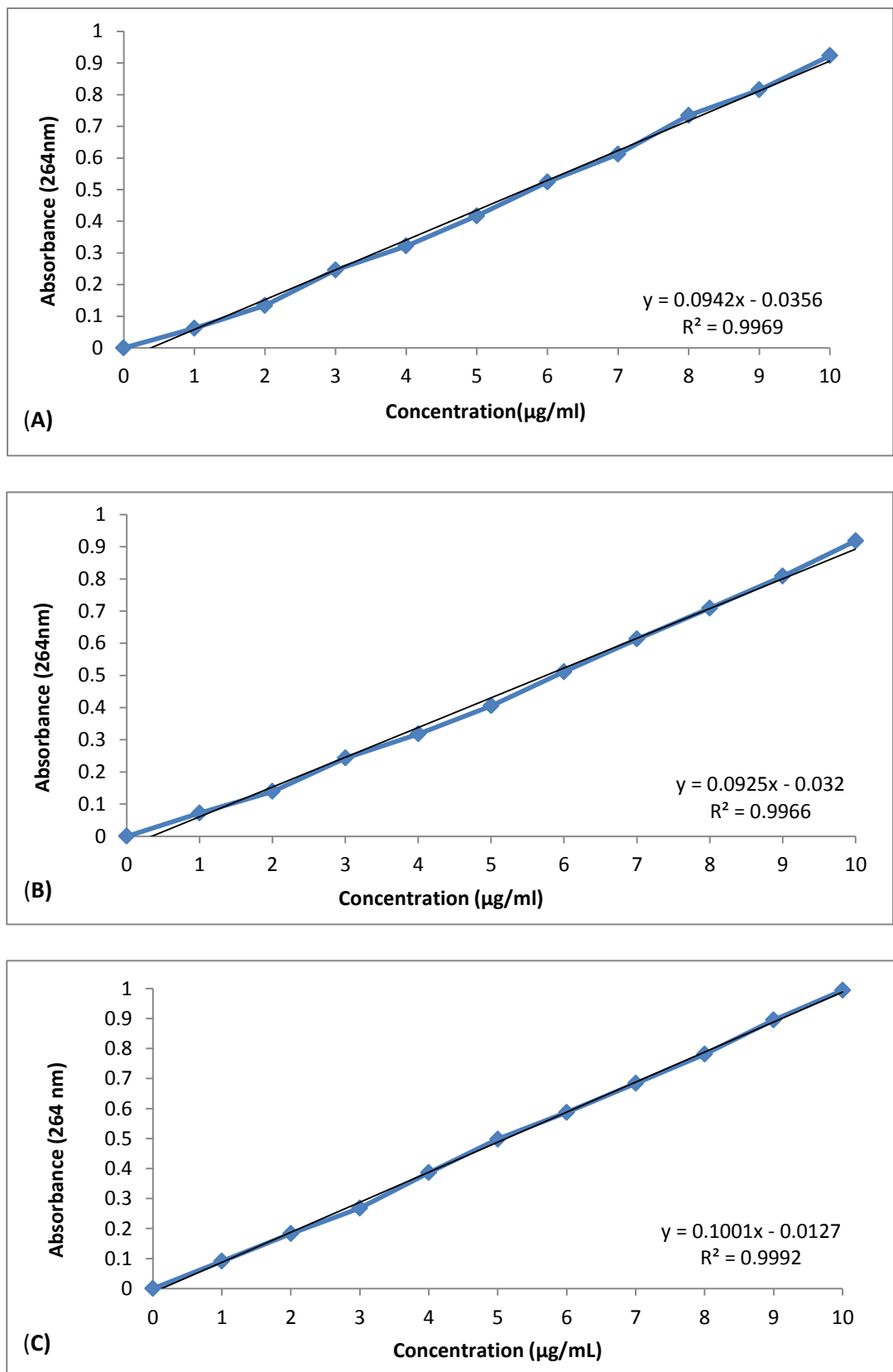
Figure 5.4: Calibration curve of bexarotene drug in

A) Distilled Water B) Ethanol C) Phosphate Buffer

5.1.8 Partition Coefficient

Partition coefficient of bexarotene was estimated as ratio of bexarotene amount present in oil (*n*-octanol) phase and water phase (PBS pH 7.4). The partition coefficient value was calculated and compared with literature value and is reported in Table 5.6.

Table 5.6: Partition coefficient of bexarotene

Method Used	Experimental Value	Literature Value
n-octanol: PBS pH 7.4	6.9±0.1	6.9

5.1.9 HPLC Analysis

A. Determination of Retention Time by HPLC

The retention time of drug bexarotene in acetonitrile and ammonium acetate buffer solution was in between 7 to 9 minutes.
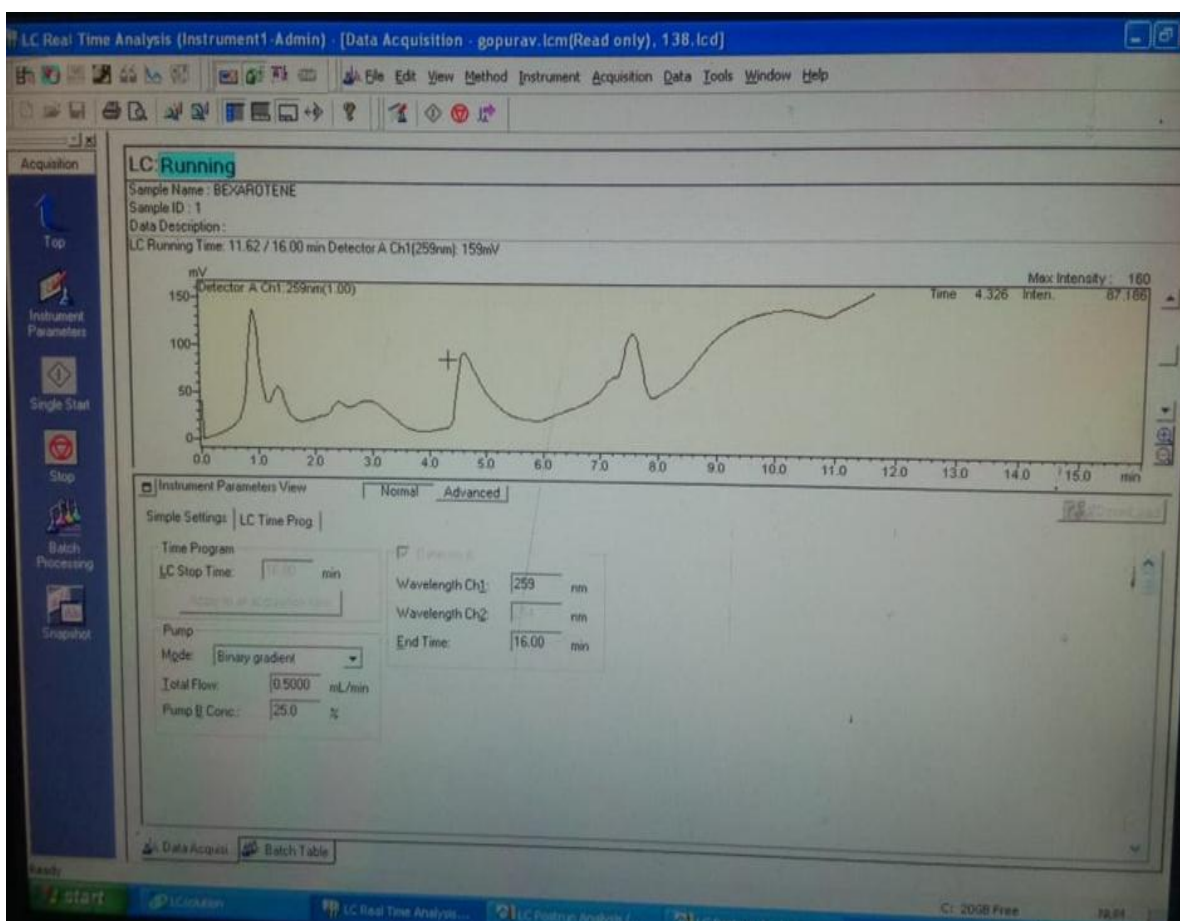
Figure 5.5: Retention time of bexarotene drug
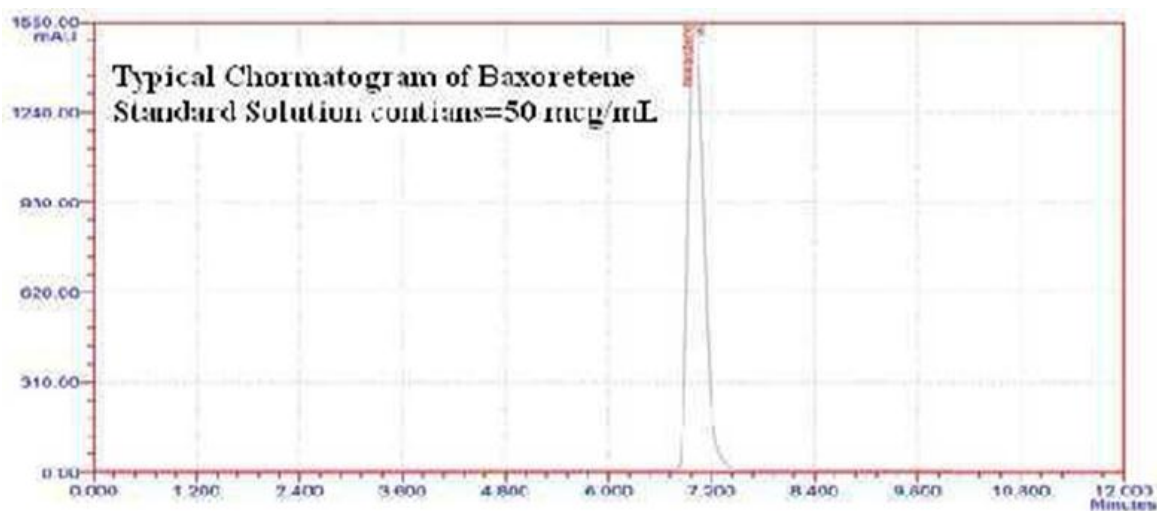
Figure 5.6: Chromatogram of bexarotene standard solution

5.1.10 Development of HPLC Method

RP-HPLC method has been developed and validated for assay of Bexarotene as per ICH guideline.

A. Linearity

The calibration curve of bexarotene was developed in the range of 10-100µg/ml. It shows the linear correlation coefficient(r) 0.998. LOD value of 0.01 µg/ml and LOQ value of 0.03µg/ml, suggest that the developed method was reliable and sensitive.

Table5.7: Calibration curve data of bexarotene by HPLC

Concentration of Bexarotene (µg/ml)	Mean Peak Area
10	5635
20	10489
30	16239
40	22106
50	27394
60	32848
70	38129
80	42559
90	47128
100	51866

Table 5.8: Linearity data for bexarotene

Parameters	Results
Detection Wavelength (nm)	264
Beer's Law Range (µg/ml)	10-100
Regression Coefficient (r)	0.998
Regression Equation (y=mx+c)	523.6x + 579.6
Slope	523.6
Intercept	579.6
Limit of Quantification (µg/ml)	0.03
Limit of Detection (µg/ml)	0.01
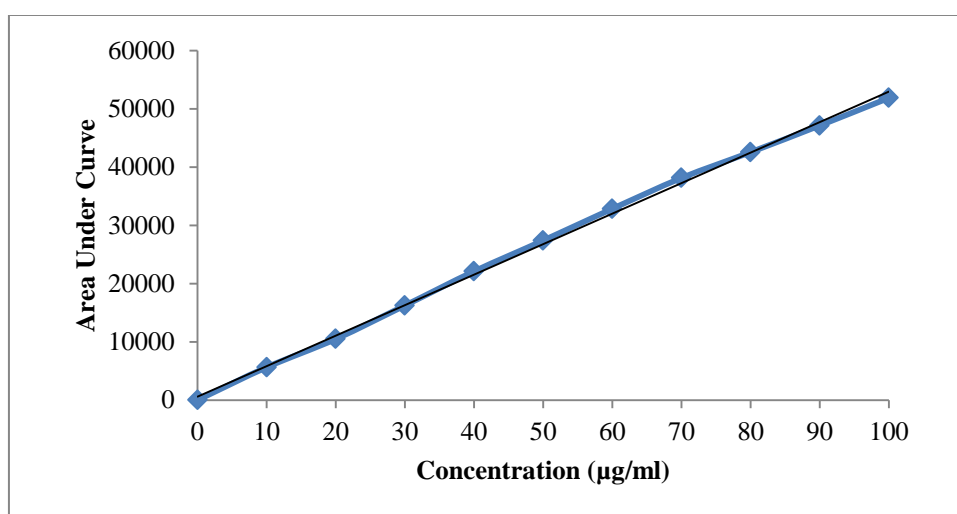
Figure 5.7: Calibration curve of bexarotene drug by HPLC

B. Accuracy

Accuracy of the developed method is determined by comparison with bexarotene reference standard and from recovery data of spiked samples for 50%, 100%, 150% levels and average recovery of 99-100% is desirable to each level as per ICH Guideline. The percent recovery results (Table 5.9) were found in the limit.

Table 5.9: Accuracy data for bexarotene

Percentage Level of Standard	Peak Area	SD	%RSD	Amount of Standard (µg/ml)		%Recovery
				Spike	Found	
50	16182	65.744	0.406	30	29.495	98.319
100	32792	61.857	0.188	60	61.451	102.411
150	47179	114.7359	0.243189	90	89.129	99.032

C. Precision

Precision of the developed method was validated by analysis of the repeatability of proposed method. Interday and intraday precision of the method was verified. The results are shown in Table 5.10 and 5.11 which shows high precision of the developed method.

Table 5.10: Precision intraday data for bexarotene

Concentration Levels		Low	Medium	High
Concentration (µg/ml)		30	60	90
Peak Area	Injection 1	16236	32735	47312
	Injection 2	16109	32858	47108
	Injection 3	16202	32785	47119
Average Peak Area		16182	32793	47180
SD		65.744	61.857	114.736
%RSD		0.4063	0.1886	0.2432

Table 5.11: Precision interday data for bexarotene

Concentration Levels		Low	Medium	High
Concentration (µg/ml)		30	60	90
Peak Area	Day 1	16183	32792	47179
	Day 2	16452	32616	47642
	Day 3	16438	33328	47875
Average Peak Area		16358	32912	47565
SD		151.428	371.419	354.277
%RSD		0.925	1.128	0.744

D. Robustness

Robustness of the method was carried out by making slight deliberate changes in mobile phase flow rate (±0.2 ml/min), detection wavelength (±2 nm), composition of mobile phase (ratio) and mobile phase pH of (±0.2). The %RSD of the robustness determination was observed less than 2%, which indicate that the method is robust to deliberate the changes. The validation data for robustness is summarized in Table 5.12.

Table 5.12: Robustness data for bexarotene

Change in Flow Rate

Flow Rate- 0.98 ml/min

Injection	Peak Area	Retention Time (min)
1	32188	6.85
2	31675	6.97
3	31977	6.95
Average Peak Area	31947	6.92
SD	257.842	0.064
%RSD	0.807	0.929

Flow Rate- 1.02 ml/min

Injection	Peak Area	Retention Time (min)
1	32008	6.92
2	32186	6.99
3	32119	7.02
Average Peak Area	32104	6.98
SD	89.902	0.051
%RSD	0.280	0.736

Change in Wavelength

Wavelength – 262nm

Injection	Peak Area	Retention Time (min)
1	32561	7.02
2	33110	6.99
3	32568	7.01
Average Peak Area	32746	7.01
SD	314.964	0.015
%RSD	0.962	0.218

Wavelength – 266nm

Injection	Peak Area	Retention Time (min)
1	31789	6.98
2	32091	6.98
3	32410	6.99
Average Peak Area	32097	6.98
SD	310.539	0.006
%RSD	0.968	0.083

Change in Mobile Phase

Mobile Phase- (70:30)

Injection	Peak Area	Retention Time (min)
1	32009	7.01
2	32106	7.02
3	32115	7.01
Average Peak Area	32077	7.01
SD	58.774	0.006
%RSD	0.183	0.082

Mobile Phase- (80:20)

Injection	Peak Area	Retention Time (min)
1	32175	7.02
2	32188	7.02
3	32098	6.99
Average Peak Area	32154	7.01
SD	48.645	0.017
%RSD	0.151	0.247

Change in pH of Mobile Phase

Mobile Phase pH 3.9

Injection	Peak Area	Retention Time (min)
1	32009	7.01
2	32106	7.02
3	32115	7.01
Average Peak Area	32077	7.01
SD	58.774	0.006
%RSD	0.183	0.082

Mobile Phase - pH 4.3

Injection	Peak Area	Retention Time (min)
1	31989	6.98
2	31784	7.02
3	33156	7.01
Average Peak Area	32310	7.00
SD	740.079	0.021
%RSD	2.291	0.297

E. Ruggedness

The ruggedness of the method was determined by estimating %RSD of Bexarotene standard solution by two different analysts of different days. %RSD of the preparation was found to be 0.30 to 1.10%. This proves that there is no variation in day to day and intrapersonal variation.

Table 5.13: Ruggedness data for bexarotene

Analyst	Amount of Standard (μg/ml)	Avg. Peak Area	% Drug Found	SD	%RSD
I	60	32180	60.122	0.185	0.308976
II	60	32558	60.987	0.671	1.101054

5.2 DRUG-POLYMER COMPATIBILITY STUDIES

Compatibility study of drug and polymer was performed to ensure that drug is not interacting with the polymer used under experimental conditions (40 ± 2^{0}C and $75\pm5\%$ RH) for four weeks. No interaction was seen between drug and polymer. The obtained IR spectra of polymer and drug mixtures showed all the substantial peaks of functional groups which present in the standard FT-IR spectra of bexarotene moiety.

Table 5.14: Drug-polymer interactions

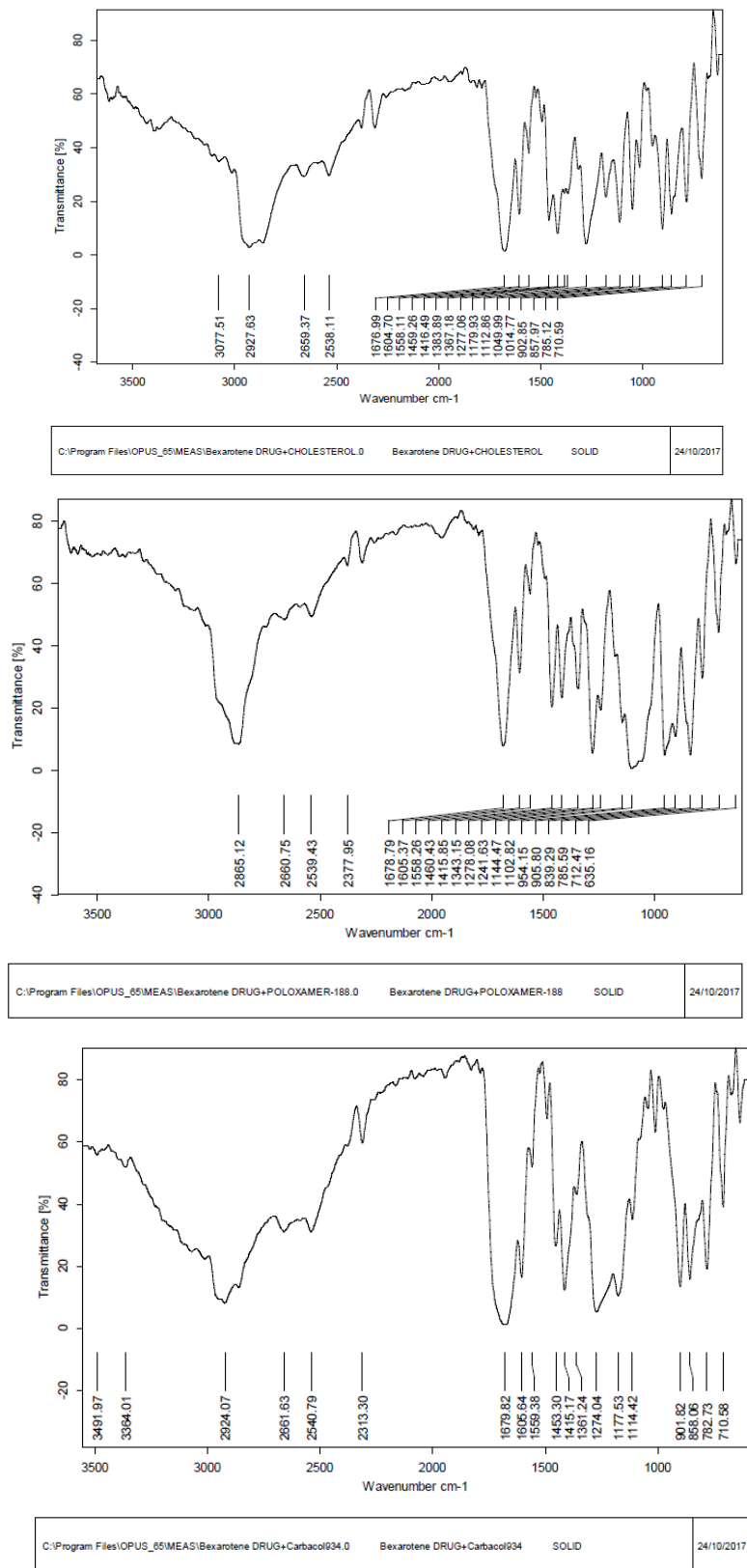| Mixture	Physical Changes				IR Peaks (cm^{-1})
	Week 1	Week 2	Week 3	Week 4	
Drug	–	–	–	–	C-H stretch = 2921.13 C=C stretch (Alkene) = 1604.07 C=O stretch = 1681.65 O-H = 3393.38 C=C aromatic stretch = 1456.29
Drug + Cholesterol	–	–	–	–	2927.63 , 1604.70, 1676.99, 1459.26, 3077.51
Drug + surfactant	–	–	–	–	2924.07 , 1605.64 , 1679.82 3364.01, 1453.30
Drug + Carbopol 934	–	–	–	–	2919.16, 1603.56, 1671.82, 1459.62, 3012.19

Figure 5.8: FTIR spectra of bexarotene with cholesterol, poloxamer and carbapol

5.3 DEVELOPMENT OF LIPOSOMES

Liposomes were developed by thin film hydration method by using three lipids phospholipon 90H, phospholipon 100S and soya phosphatidylcholine. Prepared liposome's vesicle size was found in the range of 182 to 995nm. Liposomes prepared using Phospholipon 100S (182.4±2.4 nm, 0.123) shown smaller size and size distribution and PDI (0.116) when compared to phospholipon 90H (835.5 ±5.4nm, 0.519), soya phosphatidylcholine (779.3±3.7 nm, 0.579) as shown in Table 5.15.

Table 5.15: Evaluation of liposomes

Form. Code	Visual Appearance	Shape	Vesicle size (nm)	EE (%)	PDI
LIPO F1	Rough layer formation	Vesicular	835.5 ±5.4	61.1 ±2.1	0.519
LIPO F2	Rough layer formation	Vesicular	901.3 ±6.8	72.3 ±2.8	0.573
LIPO F3	Rough layer formation	Vesicular	983.5 ±8.2	82.1 ±3.8	0.641
LIPO F4	Rough layer formation	Vesicular	995.4 ±9.3	73.3 ±6.5	0.654
LIPO F5	Rough layer formation	Vesicular	779.3 ±3.7	61.1 ±1.9	0.522
LIPO F6	Rough layer formation	Vesicular	895.4 ±4.8	69.3 ±2.8	0.571
LIPO F7	Rough layer formation	Vesicular	885.4 ±8.3	71.2 ±2.5	0.481
LIPO F8	Rough layer formation	Vesicular	991.4 ±7.4	83.5 ±2.6	0.594
LIPO F9	Smooth layer formation	Vesicular	182.4 ±2.4	65.1 ±1.5	0.123
LIPO F10	Smooth layer formation	Vesicular	213.2 ±2.9	81.2 ±1.8	0.271
LIPO F11	Smooth layer formation	Vesicular	639.2 ±3.6	89.9 ±1.9	0.116
LIPO F12	Smooth layer formation	Vesicular	715.2 ±3.7	74.2 ±2.4	0.317

Liposomes with phospholipon 100S shown lower PDI values indicating smaller size distribution compared to others. Further to smaller size of liposomal formulations prepared by Phospholipon 100S also exhibited higher drug loading (L11, 89.9 ±1.9%) compared to other formulations. The higher vesicle size of the formulated preparation is due to high concentration of polymer, stirring time of rotation and less evaporation

rate. Proper check of evaporation rate, temperature and stirring speed may give positive results

5.3.1 Optimization of Liposomes

The liposomes have stabilized by using the cholesterol in their structure. Rigidity improves and leakage of drug from bi-lipid layer prevent by the use of cholesterol. It also reduces the flexibility of the liposomes which increases the stability. The entrapment efficiency have improved due to prevention in leakage and flexibility. The cholesterol-lipid ratio was optimized for 1:1, 1:2, 1:3 and 1:4. It was witnessed that by increasing the amount of cholesterol and lipid the percent entrapment efficiency of drug was increased gradually. The vesicle size and PDI has also increased as the ratio of cholesterol-lipid was increased. In the preliminary studies it was found that if %EE is >85% then there is no effect of sonication time. Hence sonication time was optimized to 15 minutes on the basis of results of vesicle size, PDI and %EE.

A 3^2 full factorial design was employed in the optimization of liposome development. In the used design two factors are evaluated, each at three levels. All nine possible combinations were performed in experimental trial. The amount of Phospholipon-100S (X_1) and the time for rotation (X_2) were selected as independent variables. The entrapment efficiency (%) and vesicle size (nm) were selected as dependent variables. ANOVA was applied to detect insignificant factors. Fit of model was dependent upon the lower p value, high F value, high level of adjusted R^2 and predicted R^2 (Table 5.16). From the data of entrapment efficiency (%) of the factorial formulations L1 to L9, polynomial equation for entrapment efficiency (%) has been derived using Design Expert 11 software. The coefficients for entrapment efficiency (%) (Y1) and Vesicle Size (nm) (Y2) of the factorial formulations are shown in Table 5.16. In case of entrapment efficiency (%) (Y1) the positive sign for coefficients of X1 indicate that as the amount of Phospholipon-100S increases, entrapment efficiency (%) increases. The negative sign for coefficients of the time for rotation (X2), indicate that as the time increases, entrapment efficiency (%) decreases.

In case of vesicle size (nm) (Y2) the positive sign for coefficients X1 and X2 indicate that as the concentration of Phospholipon-100S and the time for rotation (X2), increases, Vesicle Size (nm) increases.

Table 5.16: ANOVA response and coefficient table for independent variables

	Y1	Y2
	Entrapment Efficiency (%)	**Vesicle Size (nm)**
ANOVA Response		
F Value	20.71	188.24
P Value	0.0156	0.0006
R^2	0.9718	0.9968
Adjusted R^2	0.9249	0.9915
Predicted R^2	0.6687	0.9663
Adeq Precision	14.13	39.2413
Coefficients		
b_0	73.222	376.333
b_1	9.833	196
b_2	-10.833	76.5
b_{12}	-0.25	20
b_{11}	2.166	49
b_{22}	-0.833	-12.5

Validity of the optimization model was verified by using four extra design check point formulations (LC1 to LC4) and determining their entrapment efficiency (%) and vesicle size (nm). The nearness of the predicted and observed values for (LC1 to LC4) in the method indicated by low value of percent residual value so it was valid that derived equation for the dependent variable entrapment efficiency (%) and Vesicle Size (nm) (Table 5.17). The software generated contour plots of response surface optimization method for the dependent variable are shown in Figure 5.9 (A & B) respectively.

Table 5.17: Check point batches and optimized batch

| Form. Code	Lipid(% w/w) Phospholipion 100S	Rotation Time (min)	Entrapment Efficiency (%)			Vesicle Size (nm)		
			Predicted Value	**Observed Value**	**% Residual Value**	**Predicted Value**	**Observed Value**	**% Residual Value**
LC1	1.5	12.5	73.99	74.23 ±12.35	0.32	254.20	248.12 ±12.39	2.45
LC2	1.5	17.5	63.28	61.26 ±6.31	3.29	320.78	333.10 ±18.21	3.69
LC3	2.5	12.5	83.95	86.91 ±11.25	3.40	440.20	448.31 ±15.28	1.80
LC4	2.5	17.5	72.99	75.84 ±10.56	3.75	526.70	551.45 ±19.84	4.48
FL1	3	15.5	84.99	89.9 ±1.9	4.67	623.26	639.2 ±4.9	2.49
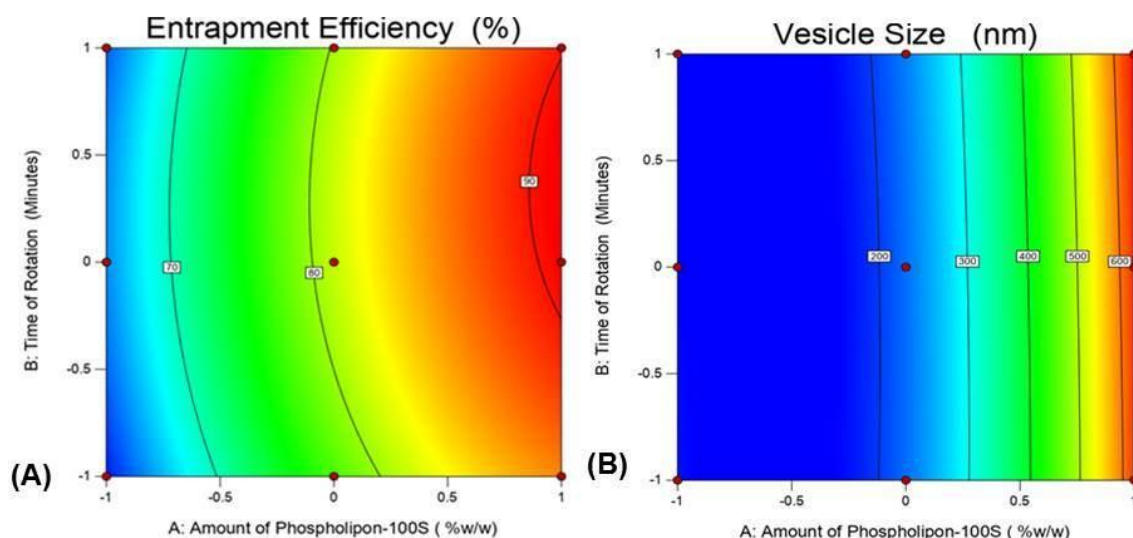
Figure 5.9: Contour plots indicating the relationship between the factors A and B on the response variables, (A) entrapment efficiency (%) (B) vesicle size (nm)

The optimized batch (FL1) was prepared for the entrapment efficiency (85%) and vesicle size (625 nm). The software suggest the amount of Phospholipon-100S (3 %w/w) and time of rotation (15.5 minutes) to get optimized results. Here, very low deviation was observed in predicted value and observed value. Average particle size of optimized batch (FL1) was found below 639nm and PDI was found below the wanted value of 0.15 (Figure 5.11). Zeta potential of optimized formulation was observed to be -19.3 mV (Figure 5.12). TEM results showed the lamellarity and morphology of optimized bexarotene liposomes. (Figure 5.13).

Figure 5.10: Optimized bexarotene liposome formulation (FL1)

Results

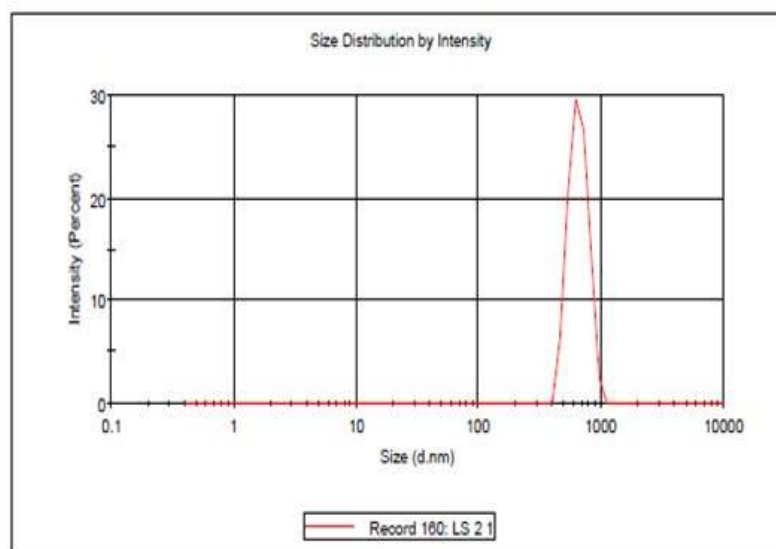|	Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 639.2	Peak 1: 652.1	100.0	115.6
PdI: 0.116	Peak 2: 0.000	0.0	0.000
Intercept: 0.771	Peak 3: 0.000	0.0	0.000
Result quality : Good			

Figure 5.11: Particle size of optimized bexarotene liposome formulation (FL1)

Results

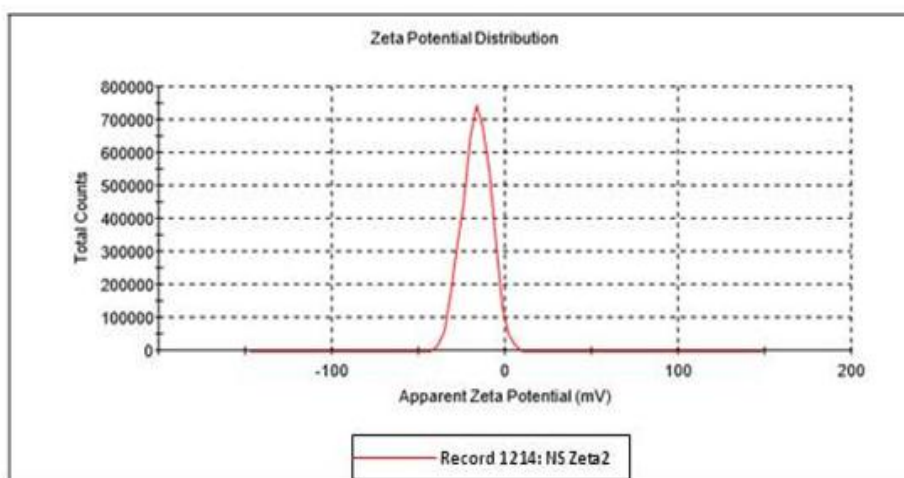|	Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -19.3	Peak 1: -19.3	100.0	6.19
Zeta Deviation (mV): 6.19	Peak 2: 0.00	0.00	0.00
Conductivity (mS/cm): 0.542	Peak 3: 0.00	0.00	0.00
Result quality: Good			

Figure 5.12: Zeta potential of optimized bexarotene liposome formulation (FL1)
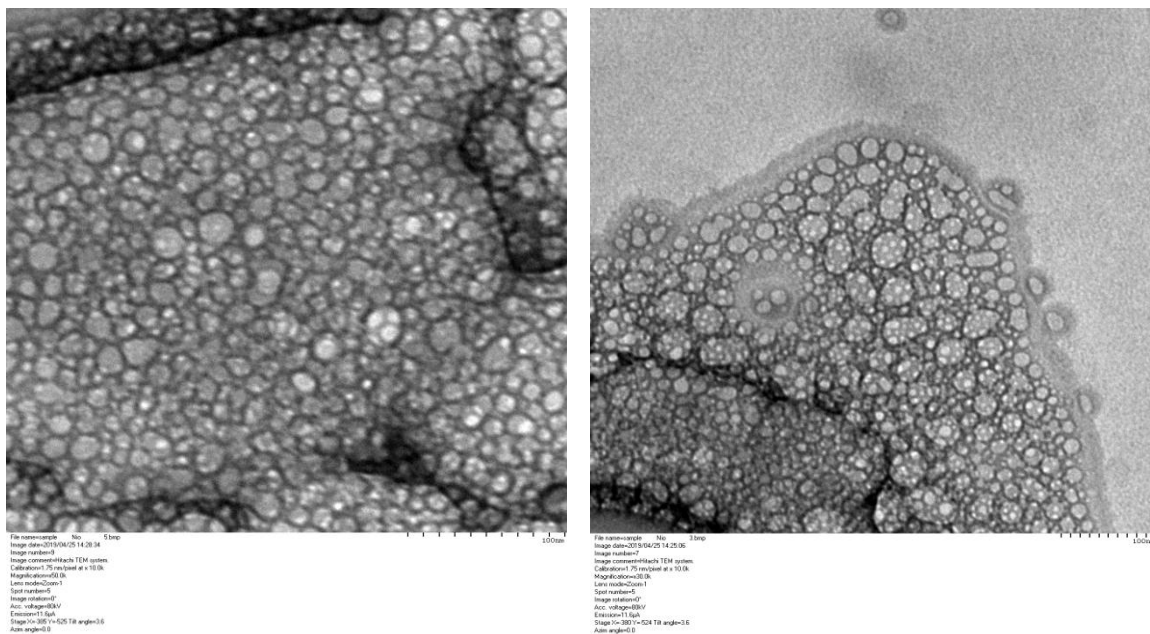
Figure 5.13: TEM of optimized bexarotene liposome formulation (FL1)

For release studies of bexarotene drug solution and bexarotene liposome formulation the withdrawn samples from dialysis apparatus were dilute and analyzed. For the drug solution dialysis, complete release was perceived within 11h (Figure 5.14) whereas from the liposome formulation drug release was found in controlled manner and 60% of drug was released within 12 h.
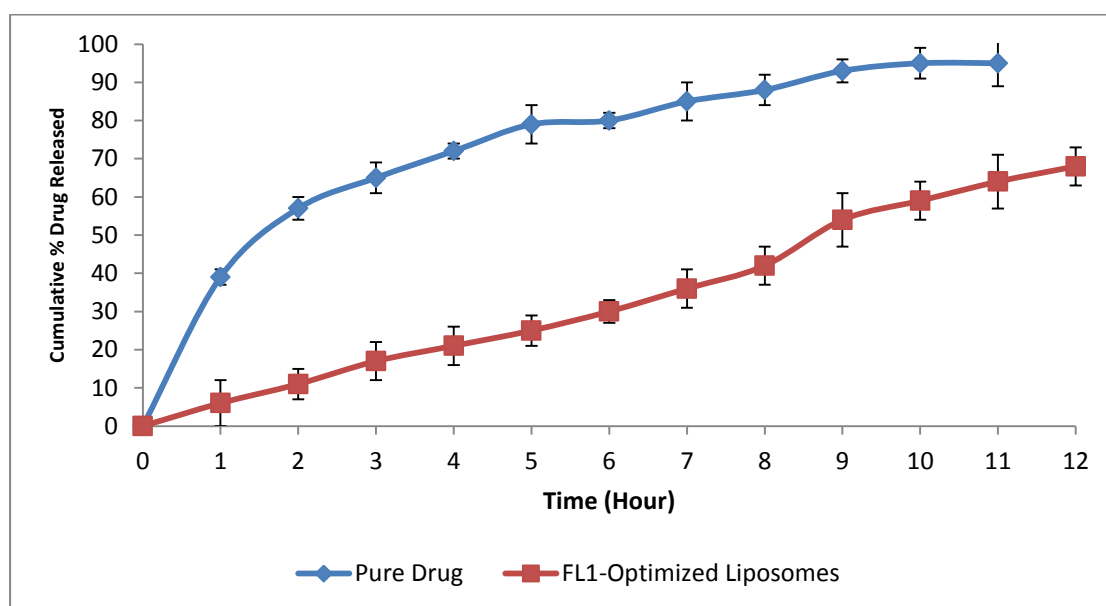

Figure 5.14: In vitro dialysis of pure bexarotene and liposome formulation

5.4 DEVELOPMENT OF NIOSOMES

Niosomes were developed by using thin film hydration technique by utilizing three surfactants span 60, tween 60 and tween 80. The vesicle sizes of prepared niosomes were found in range of 570 to 783 nm. Niosomes prepared using Span 60 (569.8±8.6 nm, 0.328) shown smaller size and size distribution and PDI when compared to Tween 60 (615.6±8.5 nm, 0.592), Tween 80 (671.8±5.8 nm, 0.548) as shown in Table 5.18.

Table 5.18: Evaluation of niosomes

Form. Code	Visual Appearance	Shape	Vesicle size (nm)	EE (%)	PDI
NIO F1	Smooth texture	Spherical	769.5±12.7	82.6±1.4	0.411
NIO F2	Smooth texture	Spherical	651.7±11.7	89.3±1.9	0.429
NIO F3	Smooth texture	Spherical	569.8±8.6	93.6±1.6	0.328
NIO F4	Smooth texture	Spherical	816.7±12.5	72.1±0.9	0.531
NIO F5	Smooth texture	Spherical	782.5±11.6	79.2±0.8	0.492
NIO F6	Smooth texture	Spherical	671.8±5.8	81.4±1.3	0.548
NIO F7	Smooth texture	Spherical	782.7±10.6	71.6±1.1	0.492
NIO F8	Smooth texture	Spherical	671.9±9.5	74.9±1.9	0.422
NIO F9	Smooth texture	Spherical	615.6±8.5	79.4±1.3	0.592

Niosomes with Span 60 shown lower PDI values indicating smaller size distribution compared to others. Further to lower particle size, niosomal formulations containing span-60 also shown higher % EE (N3, 93.6±1.6) compared to other formulations.

A 3^2 full factorial design was employed in the optimization of niosomes development. In the used design two factors are evaluated, each at three levels. All nine possible combinations were performed in experimental trial. The amount of span-60 (X_1) and the time for rotation (X_2) were selected as independent variables. The entrapment efficiency (%) and vesicle size (nm) were selected as dependent variables. ANOVA was applied to detect insignificant factors. Fit of model was dependent upon the lower p value, high F value, high level of adjusted R^2 and predicted R^2 (Table 5.19). From the data of entrapment efficiency (%) of the factorial formulations OL1 to OL9, polynomial equation for entrapment efficiency (%) has been derived using Design

Expert 11 software. The coefficients for entrapment efficiency (%) (Y1) and Vesicle Size (nm) (Y2) of the factorial formulations are shown in Table 5.19. In case of entrapment efficiency (%) (Y1) the positive sign for coefficients of X1 indicate that as the amount of span-60 increases, entrapment efficiency (%) increases. The negative sign for coefficients of the time for rotation (X2), indicate that as the time increases, entrapment efficiency (%) decreases.

In case of vesicle size (nm) (Y2) the positive sign for coefficients X1 and X2 indicate that as the concentration of Span-60 and the time for rotation (X2), increases, vesicle size (nm) increases.

Table 5.19: ANOVA response and coefficient table for independent variables

	Y1	Y2
	Entrapment Efficiency (%)	**Vesicle Size (nm)**
ANOVA Response		
F Value	6.663	31.81
P Value	0.0006	0.0084
R^2	0.9762	0.9815
Adjusted R^2	0.9875	0.9505
Predicted R^2	0.6687	0.9216
Adeq Precision	12.2654	39.2413
Coefficients		
b_0	70.39	673.23
b_1	-2.40	110.13
b_2	4.11	-102.54
b_{12}	1.26	1.37
b_{11}	11.82	21.56
b_{22}	-2.75	15.02

Validity of the optimization model was verified by using four extra design check point niosome formulations (NC1 to NC4) and determining their entrapment efficiency (%) and vesicle size (nm). The nearness of the predicted and observed values for (NC1 to NC4) in the method indicated by low value of percent residual value so it was valid that derived equation for the dependent variable entrapment efficiency (%) and Vesicle Size (nm) (Table 5.20). The software generated contour plots of response surface optimization method for the dependent variable are shown in Figure 5.15 (A & B) respectively.

Table 5.20: Check point batches and optimized batch

| Form. Code	Amount of Span 60 (%w/w)	Time of Rotation (Minutes)	Entrapment Efficiency (%)			Vesicle Size (nm)		
			Predicted Value	Observed Value	% Residual Value	Predicted Value	Observed Value	% Residual Value
NC1	1.5	12.5	72.12	74.87 ±6.13	3.81	642.91	650.12 ±10.11	1.12
NC2	1.5	17.5	75.60	76.21 ±2.42	0.80	539.69	541.88 ±12.49	0.40
NC3	2.5	12.5	69.09	66.91 ±10.25	0.31	752.36	748.09 ±11.07	0.56
NC4	2.5	17.5	73.83	75.11 ±9.03	0.17	650.50	651.32 ±9.14	0.12
FN1	1	15.5	84.12	85.6 ±1.3	1.75	564.58	569.9 ±3.1	0.94
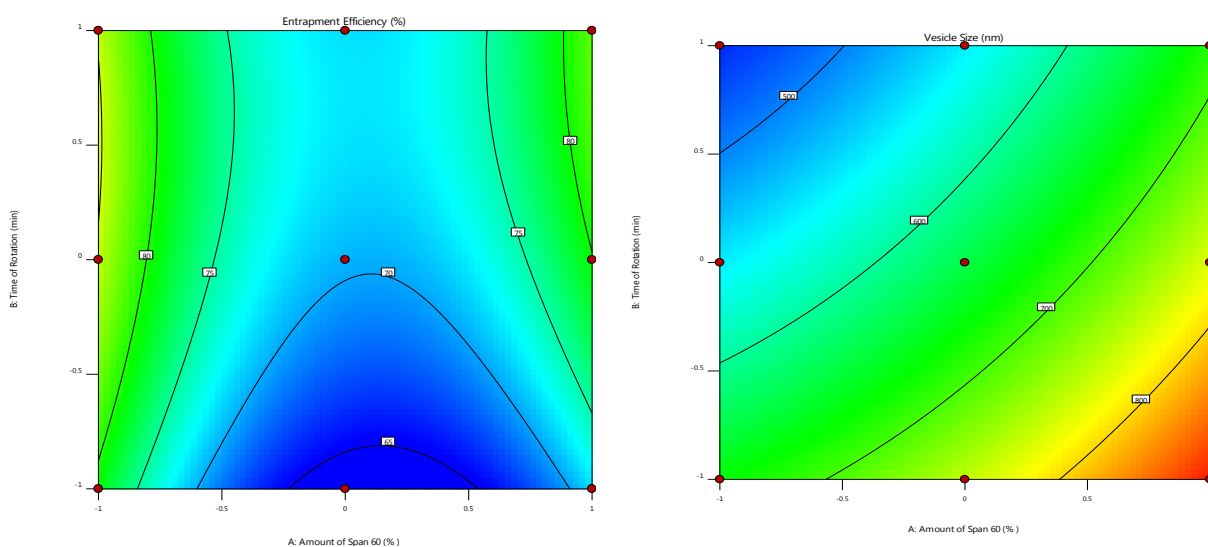
Figure 5.15: Contour plots indicating the relationship between the factors A and B on the response variables, (A) entrapment efficiency (%) (B) vesicle size (nm)

The optimized batch (FN1) was prepared for the entrapment efficiency (85%) and vesicle size (565 nm). The software suggest the amount of span-60 (1 %w/w) and time of rotation (15.5 minutes) to get optimized results. Here, very low deviation was observed in predicted value and observed value. Average particle size of optimized batch (FN1) was found below 570 nm and PDI was found below the wanted value of 0.328 (Figure 5.17). Zeta potential of optimized formulation was observed to be -19.3 mV (Figure 5.18). TEM results showed the lamellarity and morphology of optimized bexarotene niosomes. (Figure 5.19).
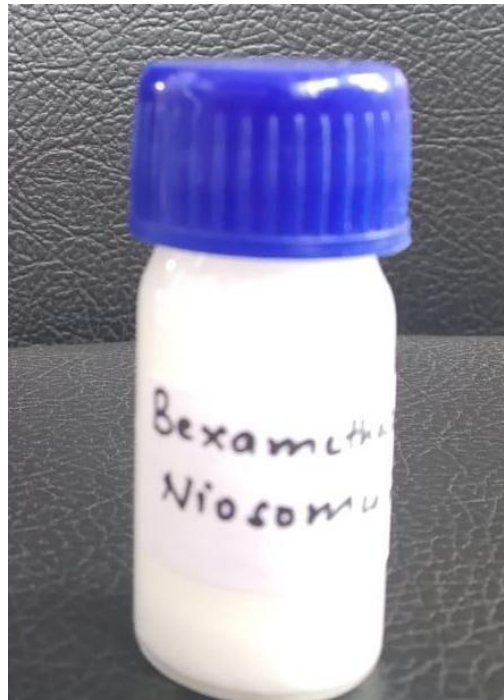
Figure 5.16: Optimized bexarotene niosome formulation (FN1)

Figure 5.17: Particle size of optimized bexarotene niosome formulation (FN1)

Figure 5.18: Zeta potential of optimized bexarotene niosome formulation (FN1)

Figure 5.19: TEM of optimized bexarotene niosome formulation (FN1)

For release studies of bexarotene drug solution and bexarotene niosome formulation the withdrawn samples from dialysis apparatus were dilute and analyzed. For the drug solution dialysis, complete release was perceived within 11h (Figure 5.20) whereas from the niosome formulation drug release was found in controlled manner and 70% of drug was released within 12 h.

Figure 5.20: In vitro dialysis of pure bexarotene and niosome formulation

5.5 DEVELOPMENT OF TOPICAL GEL

5.5.1 Characterization of Topical Gel

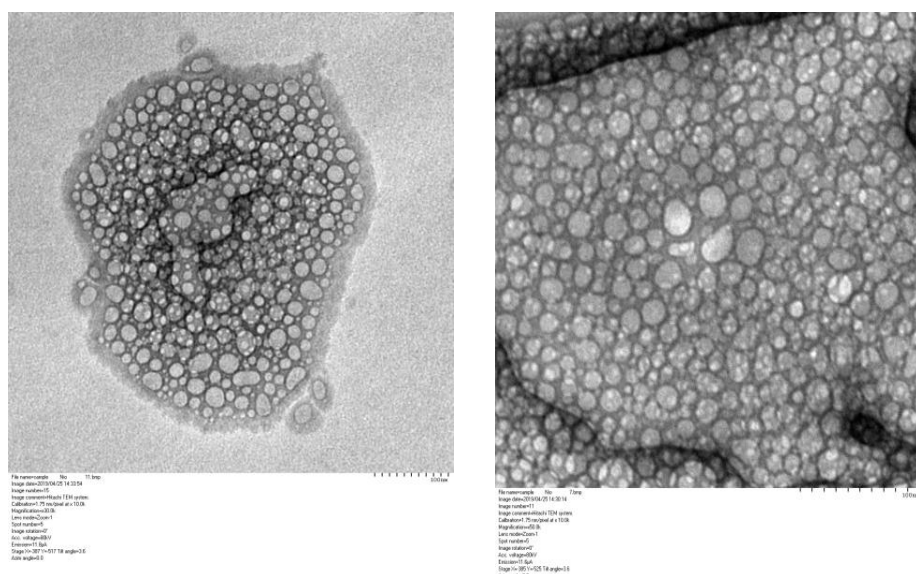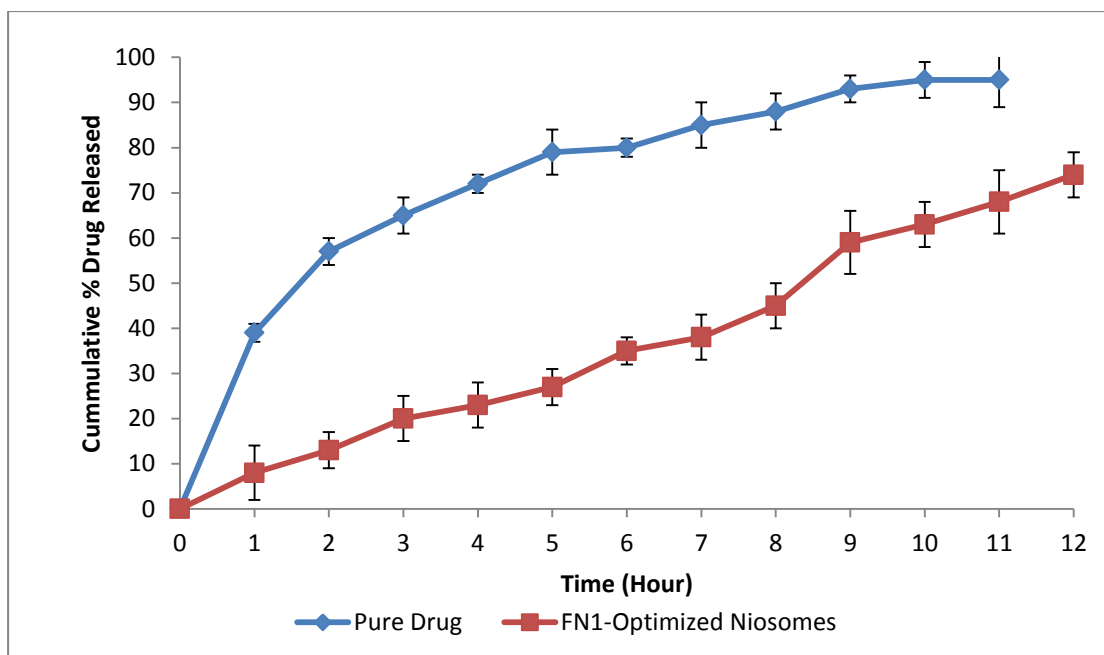The liposomal and niosomal topical gel formulations were evaluated for quality control parameters like pH, extrudability, viscosity and drug content. The pH of liposomal and niosomal were found to be in range of 6.7to 6.8, which similar to the pH of skin and this reveals that topical gel was non-irritant to the skin. All the formulations were tested for extrudability to evaluate the ease with the formulation came out of the collapsible tube. Optimized formulation (LG2) and (NG3) showed better extrudability. The viscosity of final batches of drug loaded topical gel, liposomal and niosomal topical gel formulations were 4508.4±231, 4286.9±112, 4128.3±301 respectively and shows good viscous nature like other colloidal dispersions for good retention of drug on affected area. The spreadability of optimized batch of drug loaded gel, liposomal and niosomal gel formulations were 4.8 ±0.32, 4.6 ±0.24 and 4.6 ±0.31 respectively. And it means it's good enough in consistency for easily application. Drug content in the formulation was determined for check how much drug loaded in nano-vesicular system and fulfill the aim of preparing these system for controlled and targeted delivery of drug so by determine the drug content of dosage forms and the values were 99.07 ±2.6, 93.78 ±1.4, 96.81 ±1.2

respectively and its represent that the method of preparation was suitable for vesicular system.

Table 5.21: Characterization of topical gel

Code	Appearance	Homogeneity	Grittiness	Extrudability	Spreadibility (cm)	pH	Viscosity (cps)	Drug Content (%)
G1	Non greasy, Homogenous	+	-	***	4.8 ±0.32	6.8 ±0.1	4508.4 ±231	99.07 ±2.6
LG2	Non greasy, Homogenous	+	-	***	4.6 ±0.24	6.8 ±0.2	4286.9 ±112	93.78 ±1.4
NG3	Non greasy, Homogenous	+	-	***	4.6 ±0.31	6.7 ±0.3	4128.3 ±301	96.81 ±1.2

" +" implies presence; "-" implies absence; "***" implies excellent

5.5.2 Skin Permeation and Deposition Studies

The drug permeation data was fitted and compared with all formulations. From the bexarotene topical gel (G1) the entire drug was diffused within 10h whereas for liposome formulation (LG2) 70% and in niosomal topical gel (NG3) 60% of bexarotene was diffused in sustained and controlled manner in time period of 12 h. In case of bexarotene diffused from liposomal gel was observed that the more than 50% and in case of niosomal topical gel 46% drug diffused through the cellophane membrane in time period of 8h. Same results were observed in ex-vivo permeation models the liposomal and niosomal topical gel same as shown in in-vitro diffusion studies. These results demonstrate that the drug was diffused in a sustained or controlled manner for an extended period of time which is essential for CTCL where one or two applications a day found sufficient thus improving patient compliance . Drug diposition studies were performed to determine the penetrative properties of topical gel formulation; the dermal uptake was carried out using the extent of drug was deposited in the skin, diffused from skin was calculated. It was observed that in formulation LG2 and NG3 shown 32% and 40% respectively restoration of drug in skin. It shows that the high concentration of drug was available in skin for a long time period for local application of the drug in effective management of CTCL.

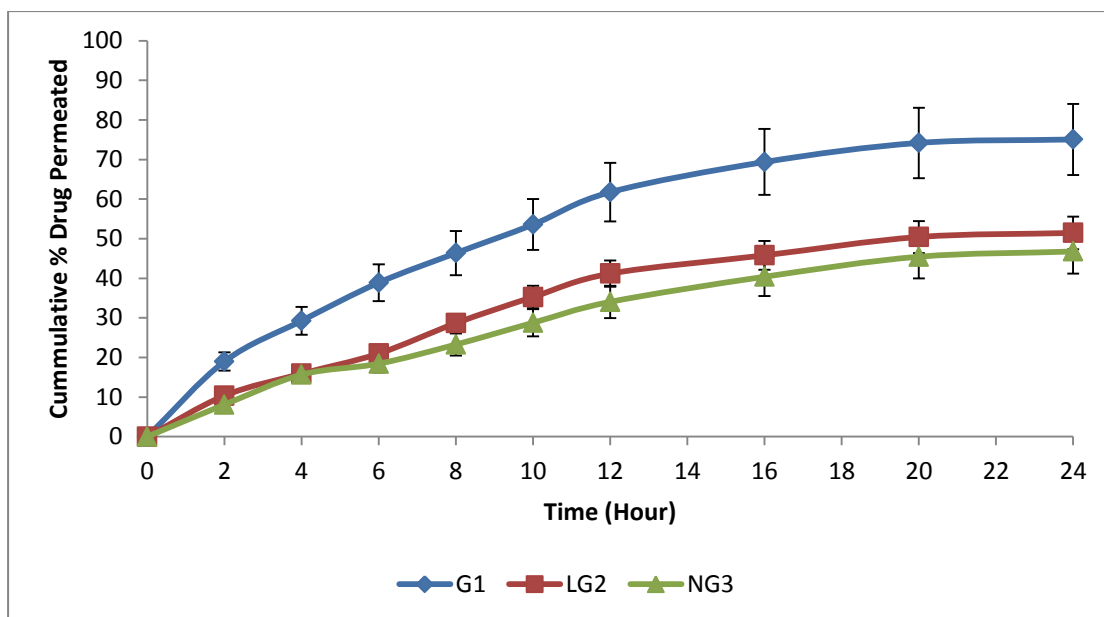*Figure 5.21: Drug permeation studies through topical gel*

Table 5.22: Skin permeation and deposition studies

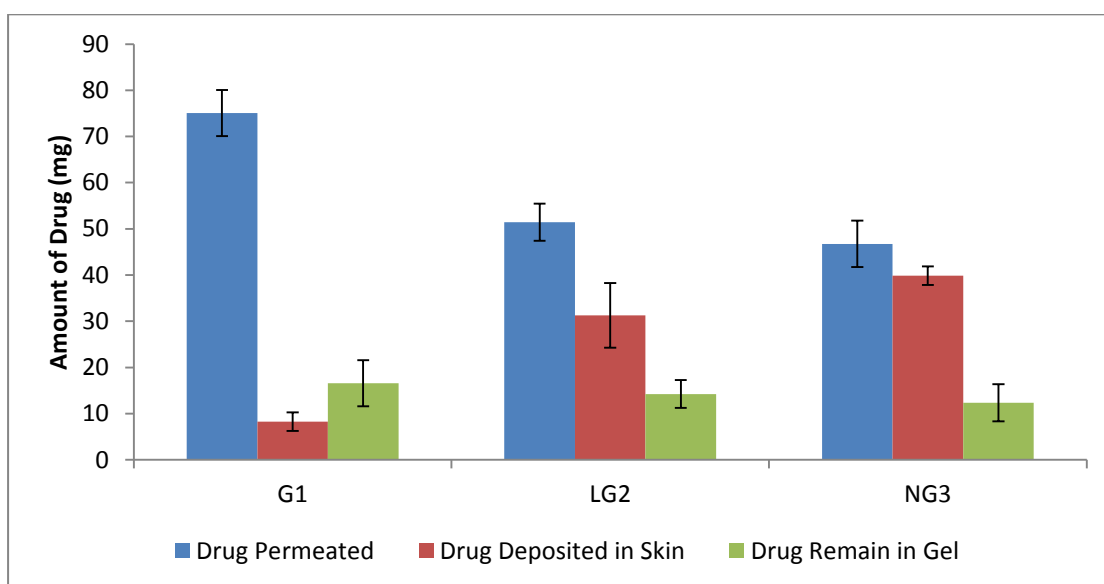|	G1	LG2	NG3
Drug Permeated	75.06±5.05	51.45±4.18	46.76±5.01
Drug Deposited in Skin	8.25±.86	31.29±7.45	39.87±2.74
Drug Remain in Gel	16.59±5.11	14.25±3.16	12.35±4.02

Figure 5.22: Drug diposition studies through topical gel

5.6 SKIN IRRITATION STUDIES

The liposomal topical gel (LG2) and niosomal topical gel (NG3) is topically applied and used with UV radiations for more effective treatment and management of CTCL. The skin irritation studies were observed under the UV light exposure. It was found that the liposomal gel reduces the wrinkle score on the skin. The results clears that the gel does not produce any irritation to skin while in the presence of the UV light (Table 5.23).

Table 5.23: Data for skin irritation studies

Treatment	Dose	No. of Animals	Wrinkle Score
Control	-	03	0 ± 0
UV treated		03	2.91 ± 0.116*
UV treated + Drug free gel topically	$50\ mg/cm^2$	06	2.83 ± 0.408^{NS}
UV + Drug free Liposomal gel topically	$50\ mg/cm^2$	06	2.33 ± 0.516^{NS}
UV + Drug free Niosomal gel topically	$50\ mg/cm^2$	06	2.50 ± 0.548^{NS}
UV + Drug Liposomal gel topically	$50\ mg/cm^2$	06	2.28 ± 0.753^{NS}
UV + Drug Niosomal gel topically	$50\ mg/cm^2$	06	2.17 ± 0.816^{NS}

Values are expressed as MEAN±SD One way Anova followed by Bonferroni test,
*P<0.050 significant and ^{NS}P<0.050 non significant compared to the UV treated group.

5.7 IN-VITRO CELL PROLIFERATION STUDIES

Minimum inhibitory concentration (MIC) of optimized drug loaded gel, liposomal and niosomal topical gel formulation cytotoxic activity were evaluated using the normal MTT cell viability assay and the outcomes of MTT cell viability method depicted IC50 value of IC 50: > 150 μg/ml, IC50: 128 μg/ml*,   IC50: 109 μg/ml*by drug loaded gel, liposomal gel and niosomal gel formulation against *Hut 78 cell line associated with CTCL* respectively. In comparison, drug loaded gel, liposomal gel, and niosomal gel of bexarotene showed the value of IC 50: > 150 μg/ml, IC50: 128 μg/ml*,   IC 50: 109 μg/ml* reveals that amount of drug needed for prevent cell line proliferation is maximum by bexarotene loaded gel than other nano-vesicular formulations as shown in Table 5.25. The absorbance was observed at 263 nm by using a plate reader (BioRad). ANOVA Test was employed to find the statically significance of the observed value.

Table 5.24: In vitro cell proliferation studies

Concentration (µg/ml)	% Cell Viability		
	G1	**LG2**	**NG3**
0	99.1±0.08	98.6±0.9	99.3±0.02
50	96.4±2.1	95.6±3.6	95.8±2.3
70	90.6±3.4	85.3±4.5	82.6±4.6
90	86.8±4.5	71.9±3.9	68.5±5.2
110	73.7±3.7	54.3±4.1	51.6±3.9
130	64.9±3.9	48.3±1.2	42.9±1.5
150	55.2±2.7	33.4±1.7	30.1±3.7
	IC 50: > 150 µg/ml	**IC50: 128 µg/ml***	**IC50: 109 µg/ml***

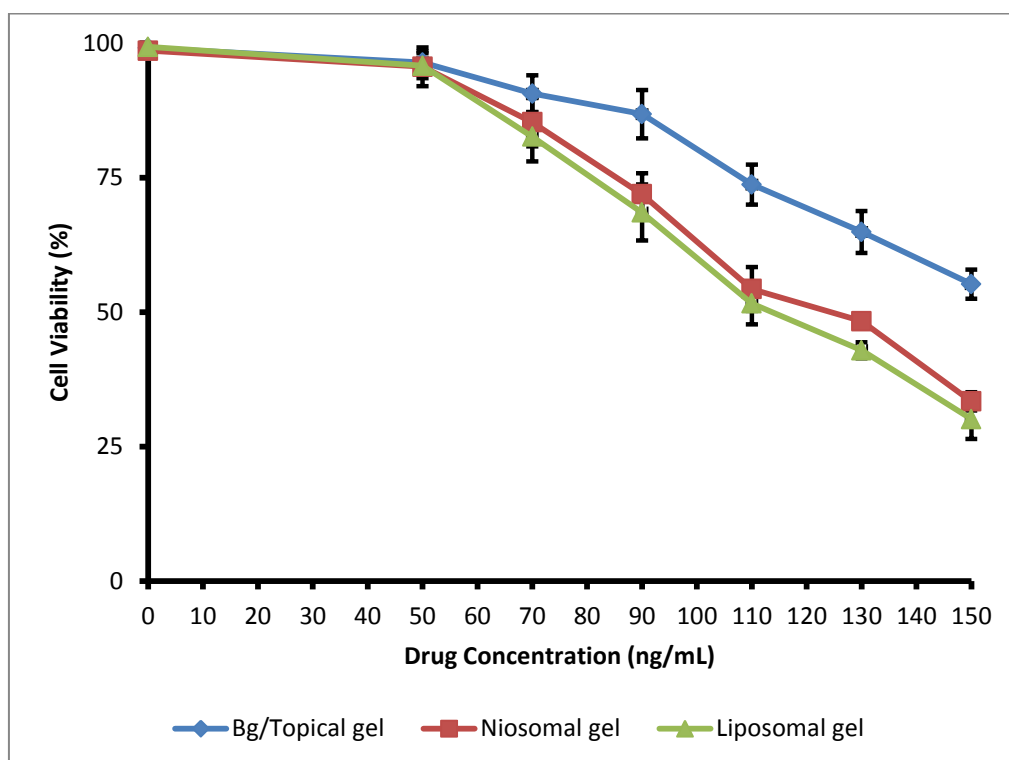n=6, StatTM Software, P<0.05 (*implies Significant Changes Observed)

Figure 5.23: Plot of In vitro cell proliferation assay

On statistical analysis of obtained data multiple comparisons were calculated by using one-way analysis of variance (ANOVA) by on Graph Pad In StatTM software (GraphPad Software, Inc., La Jolla, CA). Statistical significance was measured at a level of $p < 0.05$.

5.8 STABILITY STUDIES

The promising formulations G1, LG2 and NG3 were kept for 90 days for stability studies. The topical gels were stored at room temperature (25±2°C and 60±5% RH), and accelerated temperature (40±2°C and 75±5% RH) for physiochemical parameters like color, odor, viscosity, pH and drug content. Evaluations were done in triplicate with samples of 30g each. The result of stability study is shown in table 5.25 and 5.26. The visual appearance of the drug loaded gel, liposomal topical gel, and niosomal topical gel of bexarotene formulation did not changed and it did not showed any sign of physiochemical instability, such as phase separation or cracking or bleeding of the topical gel base during the entire time of study. There was no significant difference was observed in any parameter. So, it was concluded that prepared topical gels were found stable for more than two years.

Table 5.25: Stability studies at room temperature for topical gel formulations

Code	Before				After 90 Days			
					25±2°C and 60±5% RH			
	Appearance	pH	Viscosity (cps)	Drug Content (%)	Appearance	pH	Viscosity (cps)	Drug Content (%)
G1	Transparent, Homogeneous	6.8 ±0.1	4508.4 ±231	99.07 ±2.6	Transparent, Homogeneous	6.7 ±0.1	4479.4 ±221	98.67 ±1.7
LG2	Transparent Homogeneous	6.8 ±0.2	4286.9 ±112	93.78 ±1.4	Transparent Homogeneous	6.8 ±0.3	4197.7 ±353	93.55 ±2.2
NG3	Transparent Homogeneous	6.7 ±0.3	4128.3 ±301	96.81 ±1.2	Transparent Homogeneous	6.8 ±0.3	4065.9 ±282	96.57 ±1.1

Table 5.26: Stability studies at 40^0C /75% RH for topical gel formulations

Code	Before				After 90 Days			
					$40\pm2°C$ and $75\pm5\%$ RH			
	Appearance	pH	Viscosity (cps)	Drug Content (%)	Appearance	pH	Viscosity (cps)	Drug Content (%)
G1	Transparent, Homogeneous	6.8 ±0.1	4508.4 ±231	99.07 ±2.6	Transparent, Homogeneous	6.8 ±0.2	4671.8 ±119	98.88 ±2.1
LG2	Transparent Homogeneous	6.8 ±0.2	4286.9 ±112	93.78 ±1.4	Transparent Homogeneous	6.7 ±0.2	4311.8 ±265	93.66 ±1.6
NG3	Transparent Homogeneous	6.7 ±0.3	4128.3 ±301	96.81 ±1.2	Transparent Homogeneous	6.7 ±0.2	4267.8 ±241	96.48 ±2.5

CHAPTER-6

Summary & Conclusion
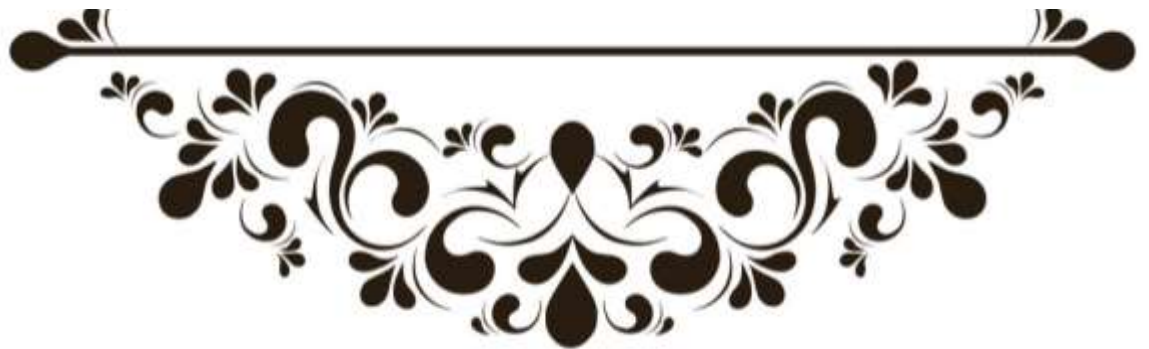
The objective of present research was to develop topical gel of bexarotene for the management and treatment of CTCL. For this purpose the bexarotene drug was loaded into the liposome and niosome formulations for long lasting effect and improved treatment of CTCL. CTCL is the utmost form of Non-Hodgkin lymphoma which involves in the skin, blood, lymph node and other organs. Bexarotene is an most effective anti-cancer drug for the management and treatment of CTCL. USFDA approved the bexarotene drug in the year 1999 for the treatment of early stages of CTCL. The topical delivery of bexarotene obstructs due to its high log P value and poor aqueous solubility, low bioavailability, high dose, high molecular weight and short half-life, which makes it difficult to penetrate the skin easily. Liposomal and niosomal delivery of bexarotene can improve the topical delivery and increases the penetration through the skin, which finally improves the treatment of CTCL.

The complete research work was presented in six chapters such as introduction, literature review, aim and objective, material and methods, result and discussion & summary and conclusion. In the preformulation studies, bexarotene was characterize for its physical and chemical properties. The obtained sample of bexarotene drug was white in color, odorless and free-flowing crystalline powder. The melting point of bexarotene was found to be 230-231°C. The drug was found soluble in chloroform, methanol and DMSO and insoluble in water. The quantitative estimation of drug was established by UV-Visible spectroscopy and HPLC method. In UV-spectroscopy the absorption maxima (λ_{max}) was observed at 264 nm. Linearity was absorbed in the from 0-10 µg/ml for the bexarotene drug. A novel simple and rapid RP-HPLC method has been developed and validated for estimation of bexarotene. The developed method was validated as per guidelines of ICH. RP-HPLC was derived out on a Shimadzu binary C18 column (250mm x 4.6mm, 5µm) with a mobile phase of Acetonitrile and Ammonium Acetate Buffer (pH 4.1) in the ratio of 75:25 v/v. The retention time is 7.0 minutes for Bexarotene. Linearity was found in the range of 10 to 100µg/ml with correlation coefficient of 0.998, which was very near to one. The percent recoveries found were observed in the range of 85.33-101.87%. The method was found to be linear and suitable in the specified range for the assay of drug. Accuracy of the method was established. The method also found to be specific,

robust, stable and precise. Hence the developed method was proved, validated and can be used for bexarotene sample analysis.

In drug polymer compatibility studies samples were taken in the ratio of 1:1 (drug: polymer) and kept at 40 ± 2 ˚C and 75 ± 5 % RH for period of 4 weeks and examined for physical and chemical changes. FTIR spectroscopy was carried out to obtain drug-polymer interaction studies. There were no physical and chemical changes observed in physical evaluation and FTIR analysis. It concludes that the drug and polymers were found compatible with each other and found suitable for designing of dosage forms.

The liposome and niosome formulations were formulated by using thin film hydration method. In the preparation of liposome and niosome following steps were taken

1. Preparation of preliminary trial batches:

2. Optimization of liposomes and niosomes.

3. Preparation of optimized liposome and niosomes.

4. Evaluation of prepared liposomes and niosomes.

Three lipids (Phospholipon-90H, Soya-PC, Phospholipon-100S) in different concentrations were screened in preliminary trial batches preparation of liposomes and three surfactants (Span 60, Span 80 and Tween 60) were used in niosomes preliminary trial batches preparation. Additionally rotation time was also studied as main effect of formulation of liposomes and niosomes. On the basis of the physical evaluations optimum concentration of lipid or surfactant and rotation time was screened out and fit to next step.

The evaluation summarized that liposomes prepared by using Phospholipon-100S and niosomes with Span 60 shown best results than other lipids and surfactants respectively. In second step, 3^2 full factorial designs were employed to optimize the liposome and niosome formulations. The lipid or surfactant concentration and time of rotation were used as an independent factor while percent entrapment efficiency and vesicle size were used as dependent factors. The final formulation was optimized for

85% entrapment efficiency and 625 nm vesicle size (in liposome formulation) and in the case of niosome formulation 85% entrapment efficiency and 565 nm vesicle size. The predicted liposomal formulation (FL1) and niosomal formulation (FN1) by the design expert software also had shown similar particle size and percent entrapment efficiency when evaluated experimentally. TEM was used to determine the surface structure, lamellarity and morphology of bexarotene liposome(FL1) and niosome (FN1). In- vitro drug release studies proved that the bexarotene was released from liposomes and niosomes for an extended period of time in sustained manner which was essential for effective treatment of CTCL.

Then the optimized formulation of liposomes (FL1) and niosomes (FN1) were incorporated in carbopol topical gel. These topical gels were evaluated for their visual examination, pH, extrudability, spreadability, in-vitro drug permeation studies and for drug diposition studies. Liposomal and niosomal topical gel fulfill all the evaluation parameters and had shown high drug permeation and diposition in skin as compare to simple topical gel (G1) formulation.

In skin irritation studies it was found that liposomal and niosomal topical gel formulations were effective to reduce the wrinkles on the mice skin in the presence of UV light radiation. Standard MTT cell viability assay was used to evaluate the cytotoxic activity of prepared topical gel. The Hut-78 cell lines were used in in-vitro cell proliferation studies. It was found that low MIC value was found in niosomal topical gel (IC50 -109 μg/ml) than liposomal topical gel (IC50 – 128 μg/ml) and simple topical gel (IC50 - >150 μg/ml).

The optimized topical gel formulations were kept for stability studies at accelerated stressed condition and room temperature for a period of 3 months. No significant changes were observed before and after evaluation in appearance, pH, viscosity and drug content in liposomal and niosomal topical gel. The close similarity between the before and after evaluation results, it was concluded that the prepared formulations were found stable for more than 2 years.

The designs of the liposomes and niosomes containing bexarotene would be an effective way to deliver the drug through the skin via topical gel formulation. It was

determined from the above results that, when bexarotene drug was encapsulated in liposomes and niosomes and further incorporated into topical gel formulation had shown effective treatment of CTCL as compared to simple bexarotene topical gel.
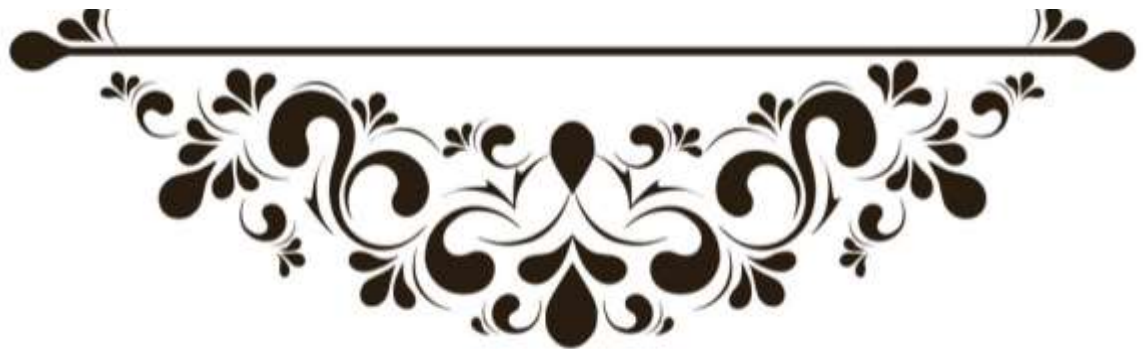
As a drug carrier system for topical treatment, liposomes and niosomes have noted to be superior over conventional topical dosage form. These novel formulations product fulfills the need of patient convenience and compliance. In future, the *in vivo* evaluations in CTCL patients may be evaluated for the exact pharmacokinetics and pharmcodynamic properties of the future formulations. *In vitro* and *in vivo* correlations may also establish for future changes in dosage form. Pilot plant batches may also prepared to transfer the formula to industrial scale. Patent on the present research work is filed and also published on 06 December 2019.

So, it was concluded that the promising formulations of liposomal and niosomal topical gel improves the treatment effectiveness of early stage CTCL which also provides patient compliance and convenience.

CHAPTER-7

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Appendices

LIST OF PUBLICATIONS

SNO.	TITLE OF PAPER WITH AUTHOR NAMES	NAME OF JOURNAL / CONFERENCE	PUBLISHED DATE	ISSN NO/ VOL NO, ISSUE NO
1.	Current and future prospective of liposomes as drug delivery vehicles for the effective treatment of cancer **Neelam Sharma** **Surajpal Verma**	International Journal of Green Pharmacy	Sept., 2017	11(3)
2.	Bexarotene: a novel retinoid in cutaneous T-cell lymphomas (CTCL) treatment **Neelam Sharma** **Surajpal Verma** **Manish Vyas**	Journal of Emerging Technology and Innovative Research	Jan., 2019	6(1)
3.	Development and Validation of RP-HPLC method for the analysis of Bexarotene as bulk drug substance **Neelam Sharma** **Surajpal Verma** **Shailesh Sharma**	Indian Drugs	Accepted	Jan, 2020
4.	A novel gel formulation of bexarotene **Neelam Sharma** **Surajpal Verma**	PATENT	06 Dec., 2019	201911048239
5.	**01 International Conference**	AIMST Univ. Malasiya	14to 15 Sept. 2019	
6.	**04 National Conference** IPGA National Convention IPGA:COP-BELA 69th IPC 70th IPC	ISFCP COP-BELA Chitkara Univ. Amity Univ.	28-29 Oct., 2017 18 Nov., 2017 22-24 Dec., 2017 21-23 Dec., 2018	

(12) PATENT APPLICATION PUBLICATION		(21) Application No.201911048239 A
(19) INDIA		
(22) Date of filing of Application :26/11/2019		(43) Publication Date : 06/12/2019

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(54) Title of the invention : A NOVEL GEL FORMULATION OF BEXAROTENE

(51) International classification	:A61K 9/00 A61K 31/00 A61K 47/00	(71)**Name of Applicant :** **1)Lovely Professional University Jalandhar-Delhi G.T. Road, Phagwara, Punjab-144 411, India** Address of Applicant :Lovely Professional University Jalandhar-Delhi G.T. Road, Phagwara, Punjab-144 411, India Punjab India
(31) Priority Document No	:NA	(72)**Name of Inventor :**
(32) Priority Date	:NA	**1)Neelam Sharma**
(33) Name of priority country	:NA	**2)SurajPal Verma**
(86) International Application No	:NA	
Filing Date	:NA	
(87) International Publication No	: NA	
(61) Patent of Addition to Application Number	:NA	
Filing Date	:NA	
(62) Divisional to Application Number	:NA	
Filing Date	:NA	

(57) Abstract :
The present invention discloses a novel formulation of bexarotene for effective treatment of cutaneous T-Cell lymphoma. The formulation is a topical gel. The topical gel of the formulation consists of either drug loaded liposomes or niosomes.

No. of Pages : 25 No. of Claims : 5

Current and future prospective of liposomes as drug delivery vehicles for the effective treatment of cancer

Neelam Sharma[1], Surajpal Verma[2]

[1]Department of Pharmaceutics, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, Bela, Punjab, India, [2]Department of Quality Assurance, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

Abstract

Liposomes, as the leading drug delivery system, have played a significant role in the formulation of anticancer drug to improve therapeutic effect. This system improves the pharmacokinetic and pharmacodynamic profiles of the therapeutic payload, promote controlled and sustained release of anticancer drugs, and exhibit very less systemic toxicity as compared to the free anticancer drug. The mechanism giving rise to therapeutic advantages of liposomes such as the ability of long-circulating liposomes to preferentially accumulate at disease sites such as tumors, site of infection, and site of inflammation. In the studies, liposomal anthracyclines have shown highly efficient drug encapsulation, resulting in significant anticancer activity with reduced cardiotoxicity. There are several methods for liposomes preparation based on lipid drug interaction and liposomes disposition mechanism including the incubation of rapid clearance of liposomes by controlling particle size and surface hydration. The liposomes are characterized with respect to physical, chemical, and biological parameters. This review discusses the recent advances in the preparation methods of liposome for the treatment of cancer.

Key words: Characterization, drug carrier, liposome, phospholipids, targeted site

INTRODUCTION

Nowadays, liposomes can be frequently used to recover existing cancer treatment due to their rise in the solubility of water-insoluble antitumor drugs. The long-circulating liposomes also act to decrease the mononuclear phagocyte system's uptake due to a passive directing toward cancer.[1,2] These approaches reduce the degradation of drug and drug inactivation on administration, as well as an increase in the bioavailability of antitumor drug and the fraction of delivered drug within the cancer area, thus increasing in efficacy of delivered drug and minimizing drug toxicity and side effect too.

DEFINITION, STRUCTURE, AND CLASSIFICATION OF LIPOSOMES

"Liposomes are spherical-shaped vesicles composed of one or more lipid bilayers, involving an aqueous compartment" as shown in Figure 1. These are molded spontaneously;

first, the lipids are dispersed in an aqueous medium by continuous stirring, which produce vesicles may in size range from nanometers to microns in breadth.[3] The lipid molecules consist head moiety which are attracted to hydrophilic molecules and shape themselves in such a way as to point toward the aqueous zone, whereas the lipophilic tails are repelled by the water molecules and point in the opposite way.

The hydrophilic groups of the inner layer point in the side of the intravesicular fluid, with the tails pointing away from it. As such, the lipophilic tails of one layer point toward the hydrocarbon tails of the outermost layer, in turn forming the normal bilipid membrane.[4-6]

Phospholipids and sphingolipids are the choice of lipids that are mostly used in the preparation of liposomes. Both

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Bexarotene: a novel retinoid in cutaneous T-cell lymphomas (CTCL) treatment

Neelam Sharma, Surajpal Verma*, Manish Vyas

School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India-144411

ABSTRACT

The present paper is an exhaustive review on an active pharmaceutical ingredient-Bexarotene (rexinoids) that specifically enact retinoid X receptors (RXRs), which used for the management of cutaneous T-cell lymphomas (CTCL). It is available in the form of topical gel and oral drug product. The earlier one having a high efficacy in the patients with settle and plaque organizes CTCL. Its preeminent toxicities are neighborhood erythema at the area of application. This review article focuses about its chemistry, mechanism of action, uses and side effects.

KEYWORDS

Bexarotene, rexinoids, retinoid X receptors, Cutaneous T-cell lymphoma

INTRODUCTION

Cutaneous T-cell lymphomas (CTCLs) are the special types of cancers that begin within the T-cell lymphocytes. Mycosis fungoides (MF) and Sezary Syndrome (SS) are two types of lymphomas of skin. It can emerge in other parts of the body as well such as lymph hubs, membrane of GIT, or the spleen. [1,2,3]

CTCLs also include cutaneous CD30+ T-cell lymph proliferative disarranges, subcutaneous panniculitis-like T cell lymphoma (SPTL), and fringe T cell lymphoma.[4]

In early, gentle, and gradually dynamic stages, treatment is basically focused on at decreasing side effects and topical treatments (corticosteroids, BXT), topical chemotherapy (nitrogen mustard), radiation or electron bar treatment, phototherapy and topical imiquimod.[5]

CTCL is an indolent, extra nodal non-Hodgkin's T-cell lymphoma.[6,7] The most common and sluggish shape of CTCL is MF.[8] Diagnostically, single-cell epidermatropism of lymphocyte along the cellar layer are more commonly famous than the normal intraepithelial collections called pautrier's smaller scale abscesses.[9, 10,11]

Patients with organize IA (T1, patches and/or plaques), arrange IB (T2, patches and/or plaque, no lymphadenopathy), organize IIA (clinically unusual lymph hubs), prominent arrange III (T4, generalised erythroderma),and long time in patients with arrange IV (pathologically included lymph hubs or visceral involvement) disease.[12]

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Articles Accepted

Development and Validation of RP-HPLC method for the analysis of Bexarotene as bulk drug substance
by Dr. SURAJPAL VERMA, 09 Jun 2020

A novel simple and rapid reverse phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for estimation of Bexarotene, an antineoplastic drug used orally and topically for effective treatment of Cutaneous–T-Cell Lymphoma. Bexarotene is a member of a subclass of retinoids that selectively activate retinoid X receptors (RXRs). The developed method was validated as per guidelines of ICH. RP-HPLC was derived out on a Shimadzu binary C18 (250 mm x 4.6 mm, 5µm) column with a mobile phase of Acetonitrile and Ammonium Acetate Buffer (pH 4.1) in the ratio of 75:25 v/v. The 20 µl standard and sample was injected with the flow rate of 1.0 ml/minute. The detection wavelength at PDA detector was carried out at 264 nm. The retention time is 7.0 minutes for Bexarotene. The linearity was found in the range of 10-100 µg/ml with correlation coefficient of 0.998. The percent recoveries found were in the range of 85.33-101.87%.

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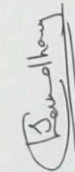
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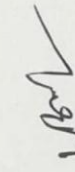
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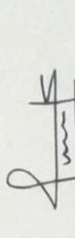
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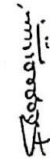22nd – 24th December, 2017
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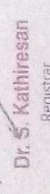
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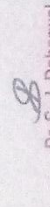
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Subject: Letter of Candidacy for Ph.D.

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As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. Programme of the University, and amendments, if any, made from time to time.

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