DEVELOPMENT OF LIPID BASED TETRAHYDROCURCUMIN NUTRICOSMETICS: INVESTIGATION OF ESSENTIAL OIL AS PRESERVATION SYSYEM

A Dissertation submitted to

THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY,

Chennai - 600 032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted by

JAYAKUMAR.K.S

REGISTRATION NO. 261511102

Under the guidance of

Dr.S.M. HABIBUR RAHMAN M.Pharm,Ph.D.,

Department of Pharmaceutics

PSG COLLEGE OF PHARMACY

PEELAMEDU

COIMBATORE 641 004

October 2017

CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT OF LIPID BASED TETRAHYDROCURCUMIN NUTRICOSMETICS: INVESTIGATION OF ESSENTIAL OIL AS PRESERVATION SYSYEM"** is a bonafide work submitted by **University Reg. No. 261511102** to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for **Master of Pharmacy** in **Pharmaceutics** and has been conducted under the guidance of **Dr. S.M. Habibur Rahman, M.Pharm, Ph.D.,** Department of Pharmaceutics, PSG College of Pharmacy, Peelamedu, Coimbatore in the academic year of 2016-2017 (October 2017).

Guide

Dr. S. M. HABIBUR RAHMAN, M.Pharm, Ph.D.,

Head of the Department

Dr.V.SANKAR, M.Pharm, Ph.D.,

Principal,

Dr. M. RAMANATHAN, M. Pharm, Ph.D.,

Dr. S.M. HABIBUR RAHMAN,M.Pharm, Ph.D.,
Associate Professor,
Department of Pharmaceutics,
PSG College of Pharmacy,
Coimbatore - 641 004. (T.N)

CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT OF LIPID BASED TETRAHYDROCURCUMIN NUTRICOSMETICS: INVESTIGATION OF ESSENTIAL OIL AS PRESERVATION SYSYEM"** submitted by **University Reg. No. 261511102** to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** at the Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore, during the academic year 2016-2017.

Place: Coimbatore
Date:

Dr. S.M. Habibur Rahman, M.Pharm, Ph.D.,
Associate Professor

Dr.V. SANKAR, M.Pharm, Ph.D.,
Head of the Department,
PSG College of Pharmacy,
Coimbatore - 641 004. (T.N)

CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT OF LIPID BASED TETRAHYDROCURCUMIN NUTRICOSMETICS: INVESTIGATION OF ESSENTIAL OIL AS PRESERVATION SYSYEM"** submitted by **University Reg. No. 261511102** to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** and has been conducted under the guidance of **Dr. S.M. Habibur Rahman, M.Pharm, Ph.D.,** Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore, during the academic year 2016-2017

Place: Coimbatore **Dr.V.Sankar, M.Pharm, Ph.D.,**

Date: **Head of the Department**

Dr. M. RAMANATHAN, M.Pharm, Ph.D.,
Principal,
PSG College of Pharmacy,
Coimbatore - 641 004. (T.N)

CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT OF LIPID BASED TETRAHYDROCURCUMIN NUTRICOSMETICS: INVESTIGATION OF ESSENTIAL OIL AS PRESERVATION SYSYEM"** submitted by **University Reg. No. 261511102** is a bonafide work carried out by the candidate under the guidance of **Dr. S.M. HABIBUR RAHMAN, M.Pharm, Ph.D.,** and submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics**, at the Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore, during the academic year 2016-2017.

Place: Coimbatore
Date:

Dr. M. Ramanathan, M.Pharm, Ph.D.,
Principal

DECLARATION

I do hereby declare that the dissertation work entitled **"DEVELOPMENT OF LIPID BASED TETRAHYDROCURCUMIN NUTRICOSMETICS: INVESTIGATION OF ESSENTIAL OIL AS PRESERVATION SYSYEM"** submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics,** was done by me under the guidance of **Dr. S.M. HABIBUR RAHMAN, M. Pharm, Ph. D.,** Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore, during the academic year 2016-2017.

Reg. No. 261511102

EVALUATION CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT OF LIPID BASED TETRAHYDROCURCUMIN NUTRICOSMETICS: INVESTIGATION OF ESSENTIAL OIL AS PRESERVATION SYSYEM"** submitted by **University Reg. No. 261511102** to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by the candidate at the Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore and was evaluated by us during the academic year 2016-2017.

Examination Center: PSG College of Pharmacy, Coimbatore.

Date:

Internal Examiner **External Examiner**

ACKNOWLEDGEMENT

I, the project student Department of Pharmaceutics in PSG College of Pharmacy take the pleasure of retracing our steps back to thank our pioneers in my project work.

To start with, My first and foremost thankful to my highly respected beloved sir, who gives me immense pleasure to express my deep sense of gratitude to my esteemed guide **Dr. S. M. Habibur Rahman, M. Pharm, (Ph.D)., Associate Professor, Department of Pharmaceutics**, PSG College of Pharmacy for his unflagging interest, constant source of inspiration and guidance throughout the course of the study. He by his endeavour showed us cumbersome work at this very narrow and short period of time.

I would be failing in my duties if I did not record my sincere thanks to respected **Dr. V. Sankar, M. Pharm, Ph.D., Professor and Head, Department of Pharmaceutics**, PSG. College of Pharmacy for his benevolent help in the completion of the study.

I deeply thank our beloved sir, **Dr. M. Ramanathan, M.Pharm., Ph.D., Principal,** P.S.G College of Pharmacy who provided us all the essential and necessary facilities in bringing out this dissertation.

A special note of thanks to **Mr. Siva Selva Kumar, Assistant Professor, Department of Pharmaceutical Analysis ., Mr. R. Hariprasad, Assistant Professor, Department of Pharmaceutical Analysis., Mr. Karthikeyan, Assistant Professor, Department of Pharmaceutics,** PSG College of Pharmacy for supporting and encouraging the work.

I heartly thank you Dr. S. Subramaniam, Associate professor, Mr. C. Vaiyana Rajesh, Assistant Professor, Ms. R. Nithya, Assistant Professor, Department of Pharmaceutics, PSG. College of Pharmacy, who were very generous in sharing their time and knowledge with me and at the same time for providing much needed assistance which helped me to complete the study successfully.

It is our privilege to extend my special thanks to my Guru **Mr. Siram Karthik, Research Scholar**, **Department of Pharmaceutics,** PSG College of Pharmacy, without his unconditional loves and support this process of our learning would have been incomplete. And they are the backbone for all successful endeavours in our life.

I thank Mr. Arjun A.J, Dr. Ranjith, Mr. Mrinmoy Gautam, Research scholar, PSG college of Pharmacy for supporting to complete my work.

I am highly indebted to Non Teaching staffs **Karthik kumar, Chitra, Aasath, Murugan, Nithya. N, Jagadeshwari. S, Priya, Mary Arokia Jacqueline**, **Kayalvizhi,** PSG College of Pharmacyfor the necessary support and valuable suggestions from time to time for the conduct of the project.

I am overwhelmed by the general help and encouragement offered by my friends **Manivaasagam. B, Vijayalakshmi. M** and my dear juniors which gave me enthusiasm and motivation for the successful completion of the work.

Words give way to gratitude and love to my beloved **parents and brothers** who, in their perseverance and affection, been a constant inspiration and support to us throughout times of hardship and success. Above all we bow to our **God almighty** who led our ways.

Words can't express our sincere gratitude and obligation to our dear batch mates who directly helped during our work.

Above all we humbly submit our desertion work, into the hands of Almighty, who is the source of all wisdom and knowledge for the successful completion of our thesis.

We owe more than words can express, and for which nothing in this world can ever pay off

"No work is accomplished with optimum refinement;

Without the support and indulgence of great people"

CONTENTS

LIST OF TABLES

20.	Preparation of cream with combination of Bees wax and lanolin	95
21.	Stability studies	100

LIST OF FIGURES

LIST OF ABBREVIATION

ABS	-	Absorbance
ADME	-	Absorption Distribution Metabolism Excretion
THC	-	Tetrahydrocurcumin
SLN	-	Solid Lipid Nanoparticle
NLC	-	Nano Lipid Carrier
FT-IR	-	Fourier Transform Infrared spectroscopy
LFCS	-	Lipid Formulation Classification System
LBDDS	-	Lipid based drug delivery system
UV	-	Ultra Violet
SEM	-	Scaning Electron Microscopy
AFM	-	Atomic Force Microscopy
PCM	-	Phase contrast Microscopy

1. INTRODUCTION

As the number of poorly soluble compounds coming out of drug discovery increases, conventional formulation strategies may no longer suffice in providing acceptable bioavailability and targeted clinical profiles. In modern molecular pharmaceutics, advanced delivery technologies, such as Lipid based drug delivery systems (LBDDS) become an essential approach in the development of new chemical entities. LBDDS are a group of technologies that utilize lipid molecules; alone or in combination with other biocompatible materials to present drugs in a more harmonious to the biological systems.

Lipid based drug delivery systems are nowadays popular as they are expected to be the promising carriers because of their potential to increase solubility and improve bioavailability of poorly water soluble and/ or lipophilic drugs (Bharti Gaba *et al*., 2015).

The most frequent role of lipid based formulations has traditionally been to improve the solubility of sparingly water soluble drugs especially Bio pharmaceutical classification system (BCS) classes II and IV drugs. However, the spectrum of applications for lipid based formulations has widened as the nature and type of active drugs under investigation vary. Lipid based formulations may also protect active compounds from biological degradation or transformation that in turn can lead to an enhancement of drug potency. In addition, lipid based particulate DDS have been shown to reduce the toxicity of various drugs by changing the bio distribution of the drug away from sensitive organs (Anthony A. Attama *et al*., 2012).

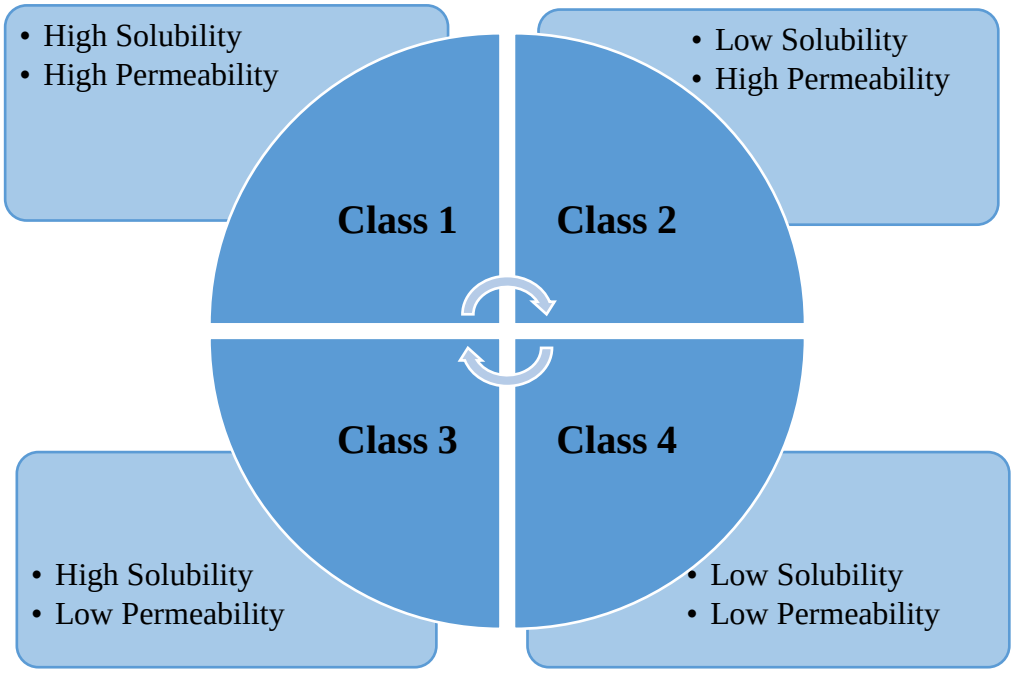


Fig 1: Biopharmaceutical classification system (BCS)

1.1. Merits of LBDDS

A well researched and commercially proven technology, lipid based formulations have seen shown to improve solubility and bioavailability. LBDDS include: lipid solutions, emulsions, self emulsifying systems (SMEDDS and SNEDDS), liposomes and solid lipid particles. These formulations provide greater bioavailability due to increased solubilisation of a compounds and increased surface area resulting in emulsification and micro- emulsification of the lipid formulation in the GI tract.

Lipid drug delivery is also an attractive option for controlled drug release and targeted drug delivery. Using molecular assembly and coating technologies, formulations can be designed to tailor a desired release profile and target it to a specific area in the GIT.

The introduction of advanced generation of lipid nanoparticles named as NLCs has overcome the general limitation associated with conventional lipid based formulations and solid lipid nanoparticles(SLNs). Earlier, SLN attracted lot of attention as a drug delivery system for they offer the advantages of biocompatibity, drug targeting, modified release and ease of large scale production. However, depending on the drug, various potential problems can occur, such as drug leaking during storage and insufficient drug loading. Later on, NLCs are designed by mixing the solid lipid with the liquid lipid, which leads to special nanostructures with improved properities for therapeutic loading, alteration of the drug

release profile and stability. The major advantage of this type of carrier/ delivery system is its ability to incorporate large quantities of drugs as a result of formation of a less ordered lipid matrix with many imperfections. NLCs ensure close contact to the stratum corneum owing to its unique lipid composition and smaller particle size, thereby enhancing drug flux through the skin. Also, because of solidified lipid matrix, a controlled release of the therapeutic moiety from these carriers is possible. NLCs for the reason that they formed a less ordered lipid matrix with many imperfections have the ability to incorporate large quantities of drugs. They are also found to significantly increase skin hydration and exhibits occlusive properities due to reduction in the tranepidermal water loss (Jana Pardeike *et al*., 2009).

Rapid advances in the ability to produce nanoparticles of uniform size, shape, and composition have started a revolution in science. The development of lipid-based drug carriers has attracted increased attention over the last decade. Lipid nanoparticles (e.g. solid lipid nanoparticles, SLNs) are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research, as well as in other varied sciences. Due to their size-dependent properties, lipid nanoparticles offer the possibility to develop new therapeutics that could be used for secondary and tertiary level of drug targeting. Hence, lipid nanoparticles hold great promise for reaching the goal of controlled and site specific drug delivery and have attracted wide attention of researchers. At the turn of the millennium, modifications of SLN, nanostructured lipid carriers (NLC) and lipid drug conjugate (LDC)-nanoparticles were introduced in addition to liquid crystal DDS. These carrier systems overcome observed limitations of conventional SLN and more fluid lipid DDS. Compared to liposomes and emulsions, solid particles possess some advantages, e.g. protection of incorporated active compounds against chemical degradation and more flexibility in modulating the release of the compound. This will focuses on the different lipid based nano systems, their structure and associated features, stability, production methods, drug incorporation and other issues related to their formulation and use in drug delivery (Anthony A. Attama *et al*., 2012).

The following advantages among others could be ascribed to lipid nanocarriers:

- Ability to control and target drug release
- Ability to improve stability of pharmaceuticals
- Ability to encapsulate high drug content (Compared to other carrier systems e.g. Polymeric nanoparticles).
- The feasibility of carrying both lipophilic and hydrophilic drugs
- Most of the lipids used are bio degradable and as such they have excellent.
- Biocompatibility are non toxic, non allergenic and non irritating
- They can be formulated by water based technologies and thus can avoid organic solvents.
- They are easy to scale up and sterile
- They are less expensive than polymeric/ surfactant based carriers
- They are easy to validate

1.2. General routes of LBDDS

Routes like oral, parentral, ocular, intranasal, dermal/ transdermal and vaginal can be for the administration of the lipid based drug delivery systems (LBDDS). However, oral route is the most preferred route because of the properities like non-invasiveness, less expensive and less side effects, such as injection-site reactions. It is also considered as the easiest and the most convenient method of drug delivery for chronic therapies. But, at a very early stage of development, formulation strategies based on a rational and systematic approach need to be developed to avoid erratic and poor in vitro/ in vivo correlations and thus increase the chances of success in formulation development. Various useful guidelines regarding routes and formulation strategies have been published by several authors (Hina Shrestha *et al*., 2014).

Points to be considered for the formulation

Main factors affecting the choice of excipients for lipid based formulations are as follows:

i. Solubility
ii. Dispersion
iii. Digestion
iv. Absorption

Other factors are as follows:

i. Regulatory issues-irritancy, toxicity, knowledge and experience.
ii. Solvent capacity
iii. Miscibility
iv. Morphology at room temperature (i.e., melting point)
v. Self- dispersibility and role in promising self- dispersion of the formulation.
vi. Digestibility and fate of digested products
vii. Capsule compatibility
viii. Purity, chemical stability
ix. Cost of goods (Hina Shrestha *et al*., 2014).

1.3. LIPID DRUG DELIVERY SYSTEMS

Lipid based DDS are an accepted, proven, commercially viable strategy to formulate pharmaceuticals for topical, oral, pulmonary or parentral delivery. Lipid formulations cab be tailored to meet a wide range of product requirements. One of the earliest lipid DDS liposomes have been used to improve solubility. Currently, some companies have established manufacturing processes for the preparation of large scale batches of sparingly soluble compounds, often at drug concentrations several orders of magnitude higher than the nominal aqeous solubility because of the introduction of novel lipid based DDS (Anthony A. Attama et al., 2012).

1.4. TETRAHYDROCURCUMIN

Tetrahydrocurcumin (THC), one of the major metabolites of curcumin, which has same physiological and pharmacological properties as that of curcumin. THC is insoluble in water and soluble in alcohol, acetone and glacial acetic acid. The pharmacological effect of THC is limited due to its low aqueous solubility. In addition, a relative short gastric emptying time can result in an incomplete release of THC from the dosage form at the site of absorption which cause diminished efficacy of the administered dose (Bharti Gaba *et al.*, 2015)

The regular curcuminoids once ingested reach to intestine, where they need to pass through the absorption barrier to enter the biological system. Further the reductase system at the cellular level then converts the curcumin to tetra hydocurcumin. Tetrahydrocurcuminis an active metabolite of the curcumin as investigation in its activity reveals.

THC has been identified in the intestinal and hepatic cytosol from humans and rats. The reduction of curcumin to THC was proposed to occur via a reductase enzyme in the cytosolic compartment either in intestine or hepatic cells. THC has also been demonstrated that it has anti-cancer and anti-angiogenic effect and prevents type II diabetes. It was also proved that it is more effective than curcumin in preventing azoxymethane induced colon carcinogenesis.

THC is categorized as BCS class IV drugs owing to its poor aqueous solubility and poor GI absorption.

In the present context, development of nutricosmetics with natural preservation system will be beneficial for the broad application and development of nutricosmetics. The present investigation is focused on the development and optimization of natural preservation system and development of nutricosmetic loaded with nano lipid carrier with nutraceutical.

1.5. NUTRACEUTICALS

Nutraceuticals can be defined as a food or part of a food that provides medical or health benefits including the prevention or treatment of a disease.

When food is being cooked or prepared using “scientific intelligence” with or without knowledge of how or why it is being used, the food is called “functional food”. Thus, the functional food provides the body with the required amount of vitamins, fats, proteins, carbohydrates, etc, needed for its healthy survival.

When functional food aids in the prevention or treatment of diseases or disorders other than anemia, it is called a nutraceuticals. Thus, a functional food for one consumers can act as a nutraceutical for another consumer. Examples of nutraceuticals include fortified dairy products (eg- milk) and citrus fruits (eg- orange juice).

Several Criteria for nutraceuticals.,

- Is a product (other than tobacco) that is intented to supplement the diet that bears or contains one or more of the following dietary ingredients : a vitamin, a mineral, an herb or other botanicals, an amino acid, a dietary substancefor use by man to supplement the diet by increasing the total daily intake, or a concentrate, metabolite, constituents, extract, or combination of these ingredients.
- Is intended for ingestion in pill, capsule, tablet or liquid form.

- Is not represented for use as a conventional food or as the sole item of a meal or diet.
- Is labelled as a dietary supplement.

Thus, nutraceuticals differ from dietary supplements in the following aspects:

- nutraceuticals must not only supplement the diet but should also aid in the prevention and treatment of diseases.
- Nutraceuticals are represented for use as a conventional food or as the sole item of meal or diet.

1.6. TOPICAL DRUG DELIVERY SYSTEM

Over the last decades the treatment of illness have been accomplished by administering drugs to human body via various roots namely oral, sublingual ,rectal ,parentral ,topical ,inhalation etc.topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorder or the cutaneous manifestations of a general disease (eg:psoriasis) with the intent of containing the pharmacological or the effect of drug to the surface of the skin or within the skin semi-solid formulations in all their diversity dominate the system for topical delivery , but foams ,spray , medicated powders ,solutions and even medicated adhesive systems ae in the use. (Kotta Kranthi Kumar *et al*. 2011).

Localized drug delivery by semi-solid dosage forms continues to be a major area of research.advances in formulation approache shave led to increased drug stability as well as improve ment in the aesthetic appeal of semisolid dosage forms (R.E. Ugandar *et al.* 2013).

One or more drug substances dissolved or dispersed in a suitable base,usually an oil in water emulsion or aqueous microcrystalline dispersion of long- chain fatty acids or alcohols that are water washable and are cosmetically and aesthetically acceptable. Semisolid dosage forms for dermatological drug therapy are intended to produce desired therapeutic action at specific sites in epidermal tissue. A drug ability to penetrate the epidermis, dermis and subcutaneous fat layers of skin depends on the properties of drug and the carrier base although some drugs are meant primarily for surface action on the skin , the target area for most dermatological disorders lies in the viable epidermis or upper dermis.

1.7. COSMETICS

"cosmetics are external preparation meant for apply on external part of the body i.e., nails, skin, hair for coloring, covering, softening, cleaning nourishing waving ,setting ,mollification, preservation ,removal and protection".we can also define it as "A cosmetic is an item intended to be rubbed, poured, sprinkled or sprayed on, introduced in to or otherwise applied to the human body or any part of there for cleansing, beautifying, promoting attractiveness or altering the appearance "

Throughout the recorded history of man, cosmetics have been used with essentially the same three goals in mind namely to enhance appeal through decoration of the body. To camouflage flaws in the integument and to alter or improve upon nature .consider several historical vignettes showing the role of cosmetics down through the ages.

In daily life, human skin is exposed to variety of environmental factors and conditions that have detrimental effects on dermal integrity. The consequences are dry skin ,wrinkles ,cracks and eventually sepsis. The most protective and preventive step taken against dry skin formation and related disorders is the use of emollients or moisturizing creams and lotions preferably with antiseptic properties.

1.8. COSMETIC DELIVERY SYSTEM.

Skin act as a selective barrier to the penetration of compounds, hence cosmetic products should be compatible o the skin barrier which is attainable by using compatible carriers emulsions, fluorocarbon gels, liposomes, cyclodextrins, microcapsules etc, which are stable and allow controlled & sustained release of actives and bioavailability enhancers.the penetration of active compound through the skin is of fundamental importance in cosmetic science, to this end, the formulation of appropriate carriers is a must both for cosmetic and dietary supplements.

Expectations from a cosmetic product

While many age related changes are inevitable ,some can be reduced with healthy lifestyle and good skin care, free radical damage, inflammatory responses due to UV exposure ,pollution etc., are the main factors responsible for skin damage .cosmetics should help skin retain and enhance its natural form for a longer time by target specific mechanism of action and perfect delivery system.

1.9. Cosmeceuticals

Cosmeceuticals refer to the combination of cosmetics and pharmaceuticals ,they contain biologically active ingredients that are known to be beneficial to humans .the term cosmeceutical is used in the professional skin care arena to portray a product that has measurable biological accomplishment in the skin, like a drug, but is regulated as a cosmetic as a cosmetic since it claims to have an effect on appearance and is used for the treatment of conditions ranging from photo aging ,wrinkles, hyper pigmentation to hair damage. Their widespread use as products for skin, lips and nail care is also worth mentioning. Applying nanotechnology in the development of cosmeceuticals offers numerous advantages like targeting of the active therapeutic component to the desired site; Improvement in the stability of cosmetic ingredients ; greater aesthetic appearance and sustained release of active drug for long-lasting effect. Some of the nanotechnology-based novel carriers of cosmetics include nanoemulsion, nanocapsule, liposome, niosome, nanocrystal, solid lipid nanoparticle, carbon nanotube, fullerence and dendrimers.

Cosmeceutical cream

Cream consist of medicaments dissolved or suspended in water removable or emollient bases. Cream are classified as water-in-oil or oil-in-water therefore, combining immiscible compounds is possible by mechanical agitation or heat. The wet gum, dry gum, bottle, and beaker method are employed, more recently. The term has been restricted to products consisting of oil-in-water emulsions or aqueous microcrystalline dispersions of long chain fatty acids or alcohols that are water washable and more cosmetically and aesthetically acceptable.

1.10. TYPES OF COSMECEUTICAL CREAM

Most commonly available creams classified on the basis of their function.

- Cleansing & cold cream or lotion
- Vanishing & Foundation cream
- Night & massage cream
- Hand & body cream
- All purpose cream
- Moisturizing cream (Kotta Kranthi Kumar *et al*. 2011).

A cream is a topical preparation usually for application to the skin. Creams for application to mucous membranes such as those of the rectum or vagina are also used. Creams may be considered pharmaceutical products as even cosmetic creams are based on techniques developed by pharmacy and unmedicated creams are highly used in a variety of skin conditions (dermatoses). The use of the finger tip unit concept may be helpful in guiding how much topical cream is required to cover different areas.

Creams are semi-solid emulsions of oil and water. They are divided into two types: oil-in-water (O/W) creams which are composed of small droplets of oil dispersed in a continuous phase, and water-in-oil (W/O) creams which are composed of small droplets of water dispersed in a continuous oily phase. Oil-in-water creams are more comfortable and cosmetically acceptable as they are less greasy and more easily washed off using water. Water-in-oil creams are more difficult to handle but many drugs which are incorporated into creams are hydrophobic and will be released more readily from a water-in-oil cream than an oil-in-water cream. Water-in-oil creams are also more moisturising as they provide an oily barrier which reduces water loss from the stratum corneum, the outermost layer of the skin.

A base in which the pharmaceutical active ingredient is incorporated should be stable, non- irritating to the body, chemically and physiologically inert, compatible with a variety of drugs, stable during storage, without pharmacological activity or interfere with the release of drug substance and be able to provide products of esthetical value and easy to process during manufacturing.

1.11. COMPOSITIONS IN PREPARATION OF CREAM

There are four main ingredients of the cosmeceutical cream

- Water
- Oil
- Emulsifier
- Thickening agent

Advantages

- Avoidance of first pass metabolism
- Convenient and easy to apply
- Avoid of risk
- Inconveniences of intravenous therapy and the varied conditions of absorption like pH Changes presence of enzymes gastric emptying time etc.,
- Achievement of efficacy with lower total daily dosage of drug by continuous drug input
- Avoid fluctuation of drug levels inter and intra patent variations

Disadvantages

- Skin irritation of contact dermatitis may occur due to the drug and excipients
- Poor permeability of some drugs through the skin
- Possibility of allergic reactions
- Can be used only for drugs which require very small plasma concentration for action
- Enzyme in epidermis may denature the drugs
- Drugs of larger particle size not easy to absorb through the skin

1.12. NUTRICOSMETICS

The vital anti-aging synergy of cosmetics and nutricosmetics. Nutricosmetics stimulate cell mechanisms in the skin's inner (the dermis) and help in its renewal. They also boost the skin's natural defences. They provide it with nutrients from within, which strengthen the antioxidant defence system and improve skin hydration and the structural elements which both encourage cell regeneration and stimulate production of collagen, elastin and keratin… thus offering in depth protection against tissue damage.

Anti-aging cosmetics essentially act on the skin's outer layers (the epidermis) to improve its hydration, appearance and conditions. They contain ingredients which help restore hydration and stimulate collagen production on the skin's surface and in its outer layers. They combat the signs of photo-aging, improve softness and firmness and gradually reduce reduce the most visible damage, fines lines and wrinkles. Nutricosmetics and cosmetics thus act in perfect synergy to delay the ravaging effects of time on the skin and restore its radiance and youthful appearance.

Specifically, Nutricosmetics are formulated to:

- Increase ROS scavenging activity
- Reduce chronic inflammation
- Stimulate immunity
- Support healthy skin cell renewal and strong dermal structure
- Potencially repair photo- damaged skin
- Promote healthy hair and nails
- Support weight management

Consumers are becoming more interested in “natural” alternatives to skin health and beauty. It is coincides with the ecofriendly and organic beauty brands. Nutricosmetics ingredients are often naturally sourced, making them appealing to this type of consumer, particular within the channels of distributions where this demographic resides. Technical ingredients or formulations may not work best within this sector.

2. REVIEW OF LITERATURE

2.1. Nanotechnology

Nanotechnology is considered to be the most prospective technology of the 21st century, and can be defined as the nano-scale formulation, characterization, and application of compositions, devices, and structures by domineering shape and size. This approach is being used to amend the penetration of the incorporated active components and is achieved by the variation of several aspects, for manipulating the release profile. The development of nanotechnology-based innovative formulations shows a good deal of potential for skin administration. In the present day, this escalating technology plays a significant role in rising above the traditional drawbacks related to cosmetics and allied products (Bangale Ms1 et al., 2012).

2.2. Solid Lipid Nanoparticle (SLN):

SLNs were developed in the 1990s as an alternative carrier system to the existing traditional carriers, such as emulsions, liposomes and polymeric nanoparticles (Attama et al., 2012). They are a comparatively stable colloidal carrier system in which melted lipid is dispersed in an aqueous surfactant by high-pressure homogeni-zation or micro-emulsification (Müller et al., 2000). They are generally made up of a solid hydrophobic core containing the drug dissolved or dispersed. SLNs exhibit certain potential advantages over polymeric nano-particles. They are safely taken up by brain and exhibit the least toxicity due to the biodegradable nature of the carrier lipid (Mohammed et al., 2012; Blasi et al., 2007; Kaur et al., 2000). Smaller size (around 10 to 200 nm) and narrow size range (100 to 200 nm) allows them to cross tight endothelial cells of the blood-brain barrier, escape from the reticuloendothelial system (RES), and bypass the liver (Attama et al., 2012). They have comparatively higher drug entrapment efficiency, render the drug more stable in their lipid matrix, and provide a controlled release. Their production can be scaled up with excellent reproducibility. Surface coating of SLNs with hydrophilic polymers or surfactants, such as poly ethylene glycol (PEG) minimizes their uptake in liver cells and results in improved bioavailability (Salome Amarachi Chime* and Ikechukwu V. Onyishi, et al.,2013).

2.3. Nano Structured lipid carriers (NLC)

NLC are colloidal carriers characterized by a solid lipid core consisting of a mixture of solid and liquid lipids, and having a mean particle size in the nanometer range (Attama et al., 2012). They consist of a lipid matrix with a special nanostructure (Attama et al., 2012; Ravani et al., 2013). This nanostructure improves drug loading and firmly retains the drug during storage. NLC system minimizes some problems associated with SLNs such as low payload for some drugs; drug expulsion on storage and high water content of SLNs dispersions. The conventional method for the production of NLC involves mixing of spatially very different lipid molecules, that is blending solid lipids with liquid lipids (oils). The resulting matrix of the lipid particles shows a melting point depression compared with the original solid lipid but the matrix is still solid at body temperature. Depending on the method of production and the composition of the lipid blend, different types of NLC are obtained (Attama et al., 2012).

The suitability of Nano structured lipid carriers (NLCs) for the encapsulation of lipophilic drug was assessed for oral and topical administration. The hot high pressure homogenization (HPH) technique was used as production method for SLN. Mechanical approaches are capable of producing nanoparticles, typically in the 100–1000 nm range, whereas chemical methods tend to produce 10–100 nm particles.(A.C. Silva et al.,2011)

2.4. FUNCTIONAL PROPERTIES OF LIPIDS USED IN FORMULATING LIPID DRUG DELIVERY SYSTEMS

a) Crystallinity and polymorphism of lipids

Many pharmaceutical solids exist in different physical forms. It is well recognised that drug substances and excipients can be amorphous, crystalline or anhydrous, at various degrees of hydration or solvated with other entrapped solvent molecules, as well as varying in crystal hardness, shape and size. Amorphous solids consist of disordered arrangements of molecules and do not possess a distinguishable crystal lattice. In the crystalline state (polymorphs, solvates/hydrates, co-crystals), the constituent molecules are arranged into a fixed repeating array built of unit cells, which is known as lattice. Possession of adequate crystallinity is a prerequisite for a good lipid particulate DDS. Triglycerides, which are mainly used as lipid matrices crystallize in different polymorphic forms. The most important forms are the α and β forms. Since the formulation of lipid particulate DDS may involve

melting at some point, recrystallization from the melt results in the metastable α-polymorph, which subsequently undergoes a polymorphic transition into the stable β-form via a metastable intermediate form (β'). The β-polymorph especially consists of a highly ordered, rigid structure with low loading capacity for drugs. The formation of all these polymorphic forms has been proved amongst solid triglyceride nanoparticles (Anthony A. Attama *et al*., 2012).

b) Melting characteristics of lipid matrices

A pure triacylglycerol has a single melting point that occurs at a specific temperature. Nevertheless, certain lipids contain a wide variety of different triacylglycerols, with different melting points and as a result, they melt over a wide range of temperatures, producing a wide endothermic transition in differential scanning calorimeter. High purity lipids with sharp melting transitions exclude drugs on recrystallization. In addition to the solidity or melting point of each individual triglyceride, in drug delivery, we are interested and concerned with the combination of triglycerides throughout the fat mixture. This impacts the plasticity and the melting point range. In the development of lipid nanoparticles, lipids with melting points well above the body temperature are preferred. This will enable among others, sustained release of the encapsulated drug.

c) Crystallinity and polymorphism vs drug loading capacity and drug release

Crystallinity and polymorphism have a lot of influence on some properties of lipid matrices used in lipid DDS. Parameters like drug loading capacity and drug release depend highly on the crystallinity and the polymorphic form of the lipids. The crystalline order and density increase from α to β forms and are highest for the β-forms of polymorphic lipids. An increasing crystalline order has a great impact on the drug loading capacity, since an increase in order reduces the ability to incorporate different molecules including drugs. Hence, the drug loading capacity of the poorly organized polymorphic forms is high.

However, this advantage goes along with the particles being in a metastable form which are able to transform into the stable β-polymorph upon storage. As a consequence of this transformation, often drug expulsion occurs. The increasing order of the matrix also reduces the diffusion rate of a drug molecule within the particle and hence reduces the rate of drug release.

2.5. STRATEGIES TO IMPROVE DRUG LOADING IN LIPID PARTICULATE DRUG DELIVERY SYSTEMS

The high crystallinity of SLN leads to a rather low drug loading capacity for many drugs, a problem still being addressed. However, for lipophilic drugs the incorporation into the particles is much easier and often results in rather high drug loading. In order to overcome the disadvantage of low loading capacity, many investigations have been done. In these formulations, lipids of highly ordered crystalline structure are combined chemically different lipids of amorphous structure, giving rise to structure matrices that accommodate more drug. .Friedrich *et al* reported a different method to increase the drug payload by incorporating amphiphilic phospholipids into the lipid matrix. This resulted in a much higher solubility of the drug in the matrix, which was attributed to the formation of a solidified reverse micellar solution within the matrix. In this case, the nanoparticles were prepared by cold homogenization which may have prevented a redistribution of the lecithin to the surface of the particles or into the aqueous phase. Such behaviour could be observed for a similar system after high pressure homogenization of the molten lipids. Another mechanism of increasing the drug loading capacity of SLN has been recently developed. In these works, the researchers used mixtures of solid lipids of natural origin possessing fatty acids of different chain lengths. In the analytical characterization of the lipid mixtures using differential scanning calorimetry (DSC), X-ray diffraction and isothermal microcalorimetery, it was observed that the mixtures were able to form matrices of imperfect structure composed of mixed crystals and mixtures of crystals, which enhanced drug incorporation compared with the single lipids (Anthony A. Attama *et al*., 2012).

2.6. INGREDIENTS USED IN THE FORMULATION OF LIPID BASED PARTICULATE DRUG DELIVERY SYSTEM

Emulsifiers

Emulsifiers are essential to stabilize lipid nanoparticle dispersions and prevent particle agglomerations. The choice of the ideal surfactant for a particular lipid matrix is based on the surfactant properities such as charge, molecular weight, chemical structure and respective hrdrophilic – lipophilic balance (HLB). The HLB of an emulsifier is given by the balance between the size and strength of the hydrophilic and the lipophilic groups. Table 1 shows some of the emulsifiers employed in the production of lipid nanoparticles. The choice of the emulsifiers depends on the route of administration of the formulation, for e.g. for parentral

formulations, there are limits of the emulsifiers to be used. For topical and ocular route the issue of skin sensitization has to be considered, while for oral route, the emulsifier should not produce any physiological effect at the use concentration. Emulsifiers could be used in combination to produce synergistic effect and better stabilize the formulation (Anthony A. Attama *et al*., 2012).

Table 1. Some emulsifiers used for the production of lipid nanoparticles

S.no	Emulsifier/ Coemsulifier	HLB
1.	**Lecithin**	4-9
2.	**Poloxamer 188**	29
3.	**Poloxamer 407**	21.5
4.	**Polysorbate 20**	16.7
5.	**Polysorbate 65**	10.5
6.	**Polysorbate 85**	15
7.	**Cremophor EL**	12-14
8.	**Solutol HS 15**	15

LIPIDS

The carboxylic acid group of a fatty acid molecule provides a convenient place for linking the fatty acid to an alcohol, via ester linkages. If the fatty acid becomes attached to an alcohol with a long carbon chain, the resultant substance is called a wax. When glycerol and a fatty acid molecule are combined, the fatty acid portion of the resultant compound is called an acyl group, and the glycerol portion is referred to as a glyceride. A triacylglyceride thus has three fatty acids attached to a single glycerol molecule. Sometimes, this name is shortened to triglyceride. Triglyceride substances are commonly referred to as fats or oils, depending on whether they are solid or liquid at room temperature. A lipid is thus a fatty or waxy organic compound that is readily soluble in non polar solvents (e.g. ether), but not in polar solvent (e.g. water). Examples of lipids are waxes, oils, sterols, cholesterol, fat soluble vitamins, monoglycerides, diglycerides (fat) and phospholipids. Fatty acids(including fats)

are a subgroup of lipids. Hence it will be inaccurate to consider the terms synonymous (Anthony A. Attama et al., 2012).

Classification of solid lipids for delivery of bioactives

Lipids can be grouped into the following categories based upon their chemical composition

Homolipids

Homolipids are esters of fatty acids with alcohols. They are lipids containing only carbon (C), hydrogen (H) and oxygen (O), and as such are referred to as simple lipids. The principal materials of interest for oral delivery vehicle are long chain and medium chain fatty acids linked to a glycerol molecule known as triacylglycerols. The long chain fatty acids ranging from C14 to C24 appear widely in common fat while the medium chain fatty acids ranging from C6 to C12 are typical components of coconut oil or palm kernel oil. Examples of homolipids include: cerides (waxes e.g. beeswax, carnauba wax etc), glycerides (e.g. fats and oils) and sterides (e.g. the esters of cholesterol with fatty acids).

Heterolipids

Heterolipids are lipids containing nitrogen (N) and phosphorous (P) atoms in addition to the usual C, H and O e.g. Phospholipids, glycolipids and sulfolipids. They are also known as compound lipids. The emphasis here will be on the phospholipids only. Two main classes of phospholipids occur naturally in qualities sufficient for pharmaceuticals applications. There are the phosphoglycerides and phosphosphingolipids such as ceramide are used mainly in topically dosage forms. Phospholipids can be obtained from all types of biomass because they are essential structural components in all kinds of membranes of living organisms.

Complex lipids

The more complex lipids occur closely linked with proteins in cell membranes and subcellular particles. More active tissues generally have higher complex lipid content. They may also contain phospholipids. Complex lipids in this context include lipoproteins, chylomicrons, etc. lipoproteins are spherical lipid protein complexex that are responsible for the transport of cholesterol and other their within the body. Structurally, lipoprotein consists of an a polar core composed of cholesterol esters or triacylglycerols, surrounded by monolayer of phospholipid in which cholesterol and one or more specific apoproteins are embedded e.g. chylomicrons and lipoproteins.

Bilayer lipids used in drug delivery

Some lipids are capable of adopting a certain orientation depending on the processing condition. Compounds that have approximately equal-sized heads and tails e.g. phospholipids tend to form bilayers instead of micelles associate tail to tail, thus minimizing the contact of the hydrophobic portion with water and maximizing hydrophilic interaction. The phospholipid molecules can move about in their half the bilayer, but there is a significant energy barrier preventing migration to the other side of the bilayer.

Non-bilyer lipids used in drug delivery

In many biological systems, the major lipids are non-bilayer lipids, which in purified form cannot be arranged in a lamellar structure in the presence of aqueous systems. The structural and functional roles of these lipids in drug delivery are mainly in their utilization as matrix-forming lipids. They include such lipids as homolipids e.g. triglycerides and waxes. Their functional properties in lipids nanotechnology differ depending partly on their melting points, crystallinity and polymorphic characteristics. However they may have absorption promoting properties especially for liphophilc drugs. Table 2 shows some of the non-bilayer lipids used in the formulation of lipids micro- and nanoparticles.

Table 2. Some non-bilayer lipids used in the formulation of lipid nanoparticles

Hard fats e.g.	**Natural Hard fats e.g.**
Stearic acid Palmitic acid Behenic acid	Goat fat Theobroma oil
Triglycerides e.g.	**Waxes e.g.**
Trimyristin (Dynasan 114) Tripalmitin (Dynasan 116) Tristearin (Dynasan 118) Trilaurin	Bees wax Cetyl palmitate Carnauba wax
Mono, di and triglycerides mixtures e.g.	
Witepsol bases Glyceryl monostearate (Imwitor 900) Glyceryl behenate (Compritol 888 ATO) Glyceryl palmitostearate (Precirol ATO 5) Softisan 142 and softisan 154	

2.7. Lipid nanoparticulate drug delivery systems

Lipid nanoparticles show interesting nanoscale properties necessary for therapeutic application. Lipid nanoparticles are attractive for medical purposes due to their important and unique features, such as their surface to mass ratio that is much larger than that of other colloidal particles and their ability to bind or adsorb and carry other compounds. Lipid nano formulations produce fine dispersions of poorly water soluble drugs and can reduce the inherent limitations of slow and incomplete dissolution of poorly water soluble drugs (e.g. BCS II & IV drugs), and facilitate formation of solubilised phases from which drug absorption occurs. In any vehicle mediated delivery system (whether the vehicle is an emulsion, liposome, noisome or other lipidic systems), the rate and mode of drug release from the system is important in relation to the movement of the delivery system *in vivo* (Anthony A. Attama et al., 2012).

Lipid particulate DDS abound depending on their architecture and particle size. Due to the large number of administration routes available, these delivery systems perform differently depending on the formulation type and route of administration. Figure 2 shows some of the different lipid nanoparticulate drug delivery system available.

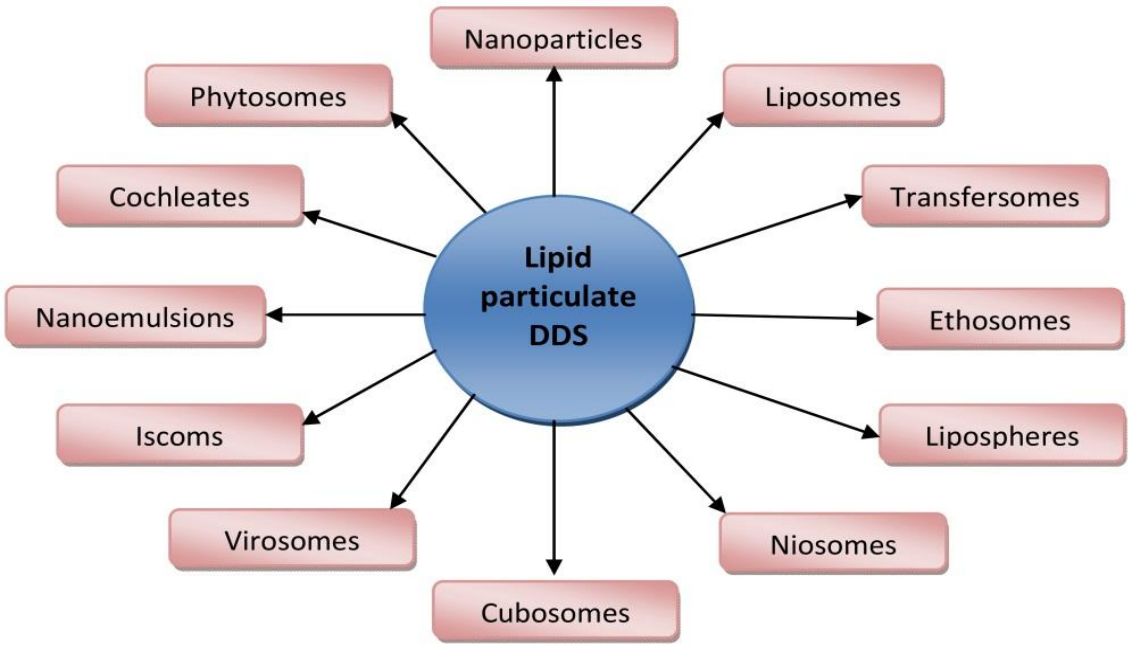


Fig 2: Different lipid nanoparticulate Drug delivery system

Solid lipid nanoparticles (SLN)

SLN are particulates structurally related to polymeric nanoparticles. However, in contrast to polymeric systems, SLN can be composed of biocompatible lipids that are physiologically well tolerated when administered *in vivo* and may also be prepared without organic solvents. The lipid matrices can be composed of fats or waxes (homolipids) that provide protection to the incorporated bioactive from chemical and physical degradation, in addition to modification of drug release profile. Typical formulations utilize lipids such as paraffin wax or biodegradable glycerides (e.g. Compritol 888 ATO) as the structural base of the particle.

SLN were developed in the 1990s as an alternative carrier system to the existing traditional carriers, such as emulsions, liposomes and polymeric nanoparticles. SLN are prepared either with physiological lipids or lipids with generally regarded as safe (GRAS) status. Under optimized conditions they can incorporate lipophilic or hydrophilic drugs and seem to fulfil the requirements for an optimum particulate carrier system. SLN have a potential wide application spectrum- parenteral administration and brain delivery, ocular delivery, rectal delivery, oral delivery, topical delivery and vaccine delivery systems etc., in addition to improved bioavailability, protection of sensitive drug molecules from the outer environment and even controlled release characteristics. Common disadvantages of SLN are particle growth, unpredictable gelation tendency, unexpected dynamics of polymorphic transitions and inherent low incorporation rate due to the crystalline structure of the solid lipid.

Nanostructured lipid carriers (NLCs)

NLC are colloidal carriers characterized by a solid lipid core consisting of a mixture of solid and liquid lipids and having a mean particle size in the nanometer range. They consists of a lipid matrix with a special nanostructure. This nanostructure improves drug loading and firmly retains the drug during storage. NLC system minimizes some problems associated with SLN such as low payload for some drugs; drug expulsion on storage and high water content of SLN dispersions. The conventional method for the production of NLC involves mixing of spatially very different lipid molecules, i.e. blending solid lipids with liquid lipids (oils). The resulting matrix of the lipid particles shows a melting point depression compared with the original solid lipid but the matrix is still solid at body temperature. Depending on the method of production and the composition of the liquid blend,

different types of NLC are obtained. The basic idea is that by giving the giving the lipid matrix a certain nanostructure, the payload for active compounds is increased and expulsion of the compound during storage is avoided. Ability to trigger and even control drug release should be considered while mixing lipids to produce NLC. Newer methods of generating NLC are being developed.

Lipid drug conjugates (LDC)-nanoparticles

A major problem of SLN is the low capacity to load hydrophilic drugs due to partitioning effects during the production process. Only highly potent low dose hydrophilic drugs may be suitably incorporated in the solid lipid matrix. In order to overcome this limitation, LDC nanoparticles with drug loading capacities of up to 33% were developed. An insoluble drug-lipid conjugate bulk is first prepared either by salt formation (e.g. with a fatty acid) or by covalent linking (e.g. to ester or ethers). The obtained LDC is then processed with an aqueous surfactant solution to nanoparticle formulation by high pressure homogenization (HPH). Such nanoparticles may have potential application in brain targeting of hydrophilic drugs in serious protozoal infections.

Liposomes

Liposomes are closed vesicular structures formed by bilayers of hydrated phospholipids. The bilayers are separated from one another by aqueous domains and enclose an aqueous core. As a consequence of this alternating hydrophilic and hydrophobic structure, liposomes have the capacity to entrap compounds of different solubilities. Additionally, the basic liposome structure of hydrated phospholipid bilayers is amenable to extensive modification or 'tailoring' with respect to the physical and chemical composition of the vesicle. This versatility has resulted in extensive investigation into the use of liposomes for various applications such as in radiology, cosmetology and vaccinology. Liposomes used in drug delivery typically range from 25 nm to several micrometers and are usually dispersed in an aqueous medium. There are various nomenclatures for defining liposome subtypes based either on structural parameters or the method of vesicle preparation. These classification systems are not particularly rigid and a variation exists in use the of these terms, particularly with respect to size ranges. Liposomes are oftendistinguished according to their number of lamellae and size. Small unilamellar vesicles (SUV), large unilamellar vesicles and large multilamellar vesicles or multivesicular vesicles are differentiated. SUVs with low particle

sizes in the nanometer range are of interest as liposomal nanocarriers for drug and antigen delivery.

Transfersomes

Transfersome technology was developed with the intention of providing a vehicle to allow delivery of bioactive molecules through the dermal barrier. Transfersomes are essentially ultra-deformable liposomes, composed of phospholipids and additional 'edge active' amphiphiles such as bile salts that enable extreme distortion of the vesicle shape. The vesicle diameter is in the order of 100 nm when dispersed in buffer. These flexible vesicles are thought to permeate intact through the intact dermis under the forces of the hydrostatic gradient that exists in the skin. Drug or antigen may be incorporated into these vesicles in a manner similar to liposomes.

Niosomes

Niosomes are vesicles composed mainly of non-ionic bilayer forming surfactants. They are structurally analogous to liposomes, but the synthetic surfactants used have advantages over phospholipids in that they are significantly less costly and have higher chemical stability than their naturally occurring phospholipid counterparts. Niosomes are obtained on hydration of synthetic non-ionic surfactants, with or without incorporation of cholesterol or other lipids. Niosomes are similar to liposomes in functionality and also increase the bioavailability of the drug and reduce the clearance like liposomes. Niosomes can also be used for targeted drug delivery, similar to liposomes. As with liposomes, the properties of the niosomes depend both on the composition of the bilayer and the method of production. Antigen and small molecules have also been delivered using niosomes.

Liquid crystal drug delivery systems

The spontaneous self assembly of some lipids to form liquid crystalline structures offers a potential new class of sustained release matrix. The nanostructured liquid crystalline materials are highly stable to dilution. This means that they can persist as a reservoir for slow drug release in excess fluids such as the gastrointestinal tract (GIT) or subcutaneous space, or be dispersed into nanoparticle form, while retaining the ‘parent' liquid crystalline structure. The rate of drug release is directly related to the nanostructure of the matrix. Lyotropic liquid crystal systems that commonly consist of amphiphilic molecules and solvents can be classified into lamellar (Lα), cubic, hexagonal mesophases, etc. In recent years, lyotropic

liquid crystal systems have received considerable attention because of their excellent potential as drug delivery vehicles. Among these systems, reversed cubic (QII) and hexagonal mesophases (HII) are the most important and have been extensively investigated for their ability to sustain the release of a wide range of bioactives from low molecular weight drugs to proteins, peptides and nucleic acids.

Nanoemulsions

Lipid-based formulations present a large range of optional delivery systems such as solutions, suspensions, self-emulsifying systems and nanoemulsions. Among these approaches, oral nanoemulsions offer a very good alternative because nanoemulsions can improve the bioavailability by increasing the solubility of hydrophobic drugs and are now widely used for the administration of BCS class II and class IV drugs. Oral nanoemulsions use safe edible materials (e.g., food-grade oils and GRAS-grade excipients) for formulation of the delivery system. Nanoemulsions possess outstanding ability to encapsulate active compounds due to their small droplet size and high kinetic stability. Nanoemulsions have sizes below 1 μm and have been extensively investigated as novel lipid based DDS together with microemulsions.

2.8. PREPARATION OF LIPID NANOPARTICULATE DRUG DELIVERY SYSTEMS

There many methods for the preparation of lipid nanoparticulate DDS. The method used is dictated by the type of drug especially its solubility and stability, the lipid matrix, route of administration, etc. Liposomal preparation follows a different method as described by Mozafari. In this section, emphasis was laid on the production of SLN, NLC and LDC nanoparticles, with methods that can also be applied to the formulation of liquid crystal DDS (Anthony A. Attama et al., 2012).

1. **High pressure homogenization**

High pressure homogenisation (HPH) is a suitable method for the preparation of SLN, NLC and LDC-nanoparticles and can be performed at elevated temperature (hot HPH technique) or at and below room temperature (cold HPH technique). The particle size is decreased by impact, shear, cavitation and turbulence. Briefly, for the hot HPH, the lipid and drug are melted (approximately 10°C above the melting point of the lipid) and combined with an aqueous surfactant solution at the same temperature. A hot pre-emulsion is formed by homogenisation (e.g. using Ultra-Turrax). The hot pre-emulsion is then processed in a

temperature-controlled high pressure homogenizer at 500 bar (or more) and predetermined number of cycles. The obtained nanoemulsion recrystallizes upon cooling down to room temperature forming SLN, NLC or LDC-nanoparticles. The cold HPH is a suitable technique for processing heat-labile drugs or hydrophilic drugs. Here, lipid and drug are melted together and then rapidly ground under liquid nitrogen forming solid lipid microparticles. A pre-suspension is formed by homogenisation of the particles in a cold surfactant solution. This pre-suspension is then further homogenised in a HPH at or below room temperature at predetermined homogenisation conditions to produce SLN, NLC or LDC-nanoparticles. The possibility of a significant increase in temperature during cold homogenisation should be borne in mind. Both HPH techniques are suitable for processing lipid concentrations of up to 40% and generally yield very narrow particle size distributions. A schematic representation of HPH method of lipid particle preparation is shown in Figure 3.

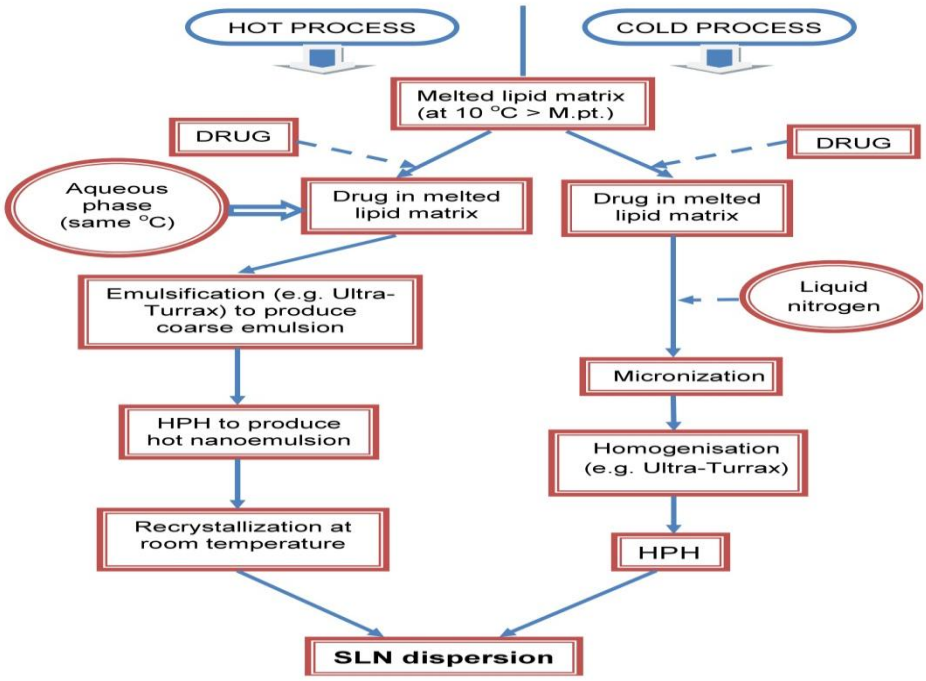


Fig 3: A schematic representation of HPH method of lipid particle preparation

2. Production of SLN via microemulsions

Gasco developed and optimised a suitable method for the preparation of SLN via microemulsions. In a typical process, a warm microemulsion is prepared and thereafter, dispersed under stirring in excess cold water (typical ratio about 1:50) using a specially developed thermostated syringe. The excess water is removed either by ultra-filtration or by lyophilisation in order to increase the particle concentration. Experimental process

parameters such as microemulsion composition, dispersing device, effect of temperature and lyophilisation on size and structure of the obtained SLN should be optimized. The removal of excess water from the prepared SLN dispersion is a difficult task with regard to the particle size. Also, high concentrations of surfactants and cosurfactants (e.g. butanol) are necessary for the formulation, but less desirable with respect to regulatory purposes and application.

3. SLN prepared by solvent emulsification/evaporation

For the production of nanoparticle dispersions by solvent emulsification/evaporation, the lipophilic material is dissolved in water immiscible organic solvent (e.g. cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent, nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. Siekmann and Westesen produced cholesterol acetate nanoparticles with a mean size of 29 nm using solvent emulsification/evaporation technique.

Other methods of lipid nanoparticle preparation include phase inversion and supercritical fluid (SCF) technology.

2.9. CHARACTERIZATION OF LIPID NANOPARTICLE QUALITY

Quantitative analysis of particle characteristics such as morphological features can be very informative as biophysical properties are known to influence biological activity, biodistribution and toxicity. Several techniques are often used to assess nanoparticle characteristics such as lamellarity, size, shape and polydispersity. Adequate and proper characterization of the lipid nanoparticles is necessary for quality control. However, characterization of lipid nanoparticles is a serious challenge due to the colloidal size of the particles and the complexity and dynamic nature of the delivery system. The important parameters which need to be evaluated for the lipid nanoparticles are particle size, size distribution kinetics (zeta potential), degree of crystallinity and lipid modification (polymorphism), coexistence of additional colloidal structures (micelles, liposome, super cooled melts, drug nanoparticles), time scale of distribution processes, drug content (encapsulation efficiency and loading capacity), *in vitro* drug release and surface morphology (Anthony A. Attama et al., 2012).

Particle size and size-distribution may be studied using photon correlation spectroscopy (PCS) otherwise known as dynamic light scattering (DLS), static light scattering (SLS), transmission electron microscopy (TEM), scanning electron microscopy

(SEM), atomic force microscopy (AFM), scanning tunneling microscopy (STM), freeze fracture electron microscopy (FFEM) or cryoelectron microscopy (Cryo-EM). These microscopy techniques are also used to study the morphology of nanoparticles.

Among the imaging techniques, AFM has been widely applied to obtain the size, shape and surface morphological information on nanoparticles. It is capable of resolving surface details down to 0.01 nm and producing a contrasted and three-dimensional image of the sample. Xray diffraction and differential scanning calorimetric analysis give information on the crystalline state and polymorphic changes in the nanoparticles. Confocal laser scanning microscopy (CLSM) gives information on interaction of nanoparticles with cells. Nuclear magnetic resonance **(**NMR) can be used to determine both the size and the qualitative nature of nanoparticles. The selectivity afforded by chemical shift complements the sensitivity to molecular mobility to provide information on the physicochemical status of components within the nanoparticle.

An important characterization technique for lipid nanoparticles is determination of solid state properties. This is very important in order to detect the possible modifications in the physicochemical properties of the drug incorporated into the lipid nanoparticles or the lipid matrix. It has been proven that although particles were produced from crystalline raw materials, the presence of emulsifiers, preparation method and high shear encountered (e.g. HPH) may result in changes in the crystallinity of the matrix constituents compared with bulk materials. This may lead to liquid, amorphous or only partially crystallized metastable systems. Polymorphic transformations may cause chemical and physical changes (e.g. shape, solubility, melting point) in the active and auxiliary substances. The solid state analysis of lipid nanoparticles is usually carried out using the following procedures: DSC, X-ray diffraction, hot stage microscopy, Raman spectroscopy and Fourier-transform infrared spectroscopy.

2.10. APPLICATIONS OF LIPID PARTICULATE DRUG DELIVERY SYSTEMS

During the last decade, different substances have been entrapped into lipid nanoparticles ranging from lipophilic to hydrophilic molecules and including difficult compounds such as proteins and peptides.

1. Lipid nanoparticles as carriers for oral drug delivery

Lipid nanoparticles such as SLN can be administered orally as dispersion, SLN-based tablet, pellets or capsules or even as lyophilized unit dose powders for reconstitution for oral delivery. The stability of the particles in the GIT has to be thoroughly tested, since low pH and high ionic strength in the GIT may result in aggregation of the particles. In order to prove this, an investigation of the effect of artificial gastric fluids on different lipidic nanoparticle formulations was performed. The authors showed that a zeta potential of at least 8-9 mV in combination with a steric stabilization hinders aggregation under these conditions. Additionally, for oral drug delivery, a release upon enzymatic degradation has to be taken into account.

The routes for particle uptake after oral application are transcellular (via the M cells in the Peyer's patches or enterocytes) or paracellular (diffusion between the cells). However, the uptake via M cells is the major pathway, resulting in the transport of the particles to the lymph. Uptake into the lymph and the blood was demonstrated by means of TEM and gamma counting of labelled SLN. It was found that uptake to the lymph was considerably higher than to the plasma and as such, a reduced first pass effect concludes, as the transport via the portal vein to the liver is bypassed. SLN containing the antituberculosis drugs rifampicin, isoniazid and pyrazinamide have been studied in animals model and it was found that administration every 10 days could be successful for the management of tuberculosis.

2. Lipid nanoparticles for parenteral drug delivery

Lipid nanoparticles can be formulated for subcutaneous, intramuscular or intravenous administration. For intravenous administration, the small particle size is a prerequisite as passage through the needle and possibility of embolism should be considered. SLN offer the opportunity of a controlled drug release and the possibility to incorporate poorly soluble drugs. Additionally, especially for intravenous application, drug targeting via modification of the particle surface is possible, and for SLN formulation with a controlled release, higher plasma concentrations over a prolonged period of time can be obtained. Such systems form

an intravenous depot. Further studies with different drugs such as idarubicin, doxorubicin, tobramycin, clozapine or temozolomide also showed a sustained release.

3. **Lipid nanoparticles as carriers for peptides and proteins drugs**

Lipid nanoparticles have been extensively studied for the delivery of proteins and peptides. Therapeutic application of peptides and proteins is restricted by their high molecular weight, hydrophilic character and limited chemical stability, which cause low bioavailability, poor transfer across biological membranes and low stability in the bloodstream. Most of the available peptides and proteins are delivered by injection, but their short half life demands repeated doses that are costly, painful and not well tolerated by patients. Lipid nanoparticles could be useful for peptide and protein delivery due to the stabilizing effect of lipids and to the absorption promoting effect of the lipidic material that constitute this kind of nanoparticles. The use of niosomes and liposomes as adjuvants for the delivery of Newcastle disease virus to chicks has been reported.

4. **Lipid nanocarriers for nasal vaccination**

The use of lipid nanocarriers provides a suitable way for the nasal delivery of antigenic molecules. Besides improved protection and facilitated transport of the antigen, nanoparticulate delivery systems could also provide more effective antigen recognition by Immune cells. These represent key factors in the optimal processing and presentation of the antigen, and therefore in the subsequent development of a suitable immune response. In this sense, the design of optimized vaccine nanocarriers offers a promising way for nasal mucosal vaccination.

5. **Lipid nanoparticles as carriers in cosmetic and dermal preparation**

Lipid nanoparticles can be incorporated into a cream, hydro gel or ointment to obtain semisolid systems for dermal applications. Another possibility is to increase the amount of lipid matrix in the formulation above a critical concentration, resulting in semisolid formulations. The substances used for the preparation of dermal SLN are rather innocuous since they are mostly rated as GRAS and many of them are used in conventional dermal formulations. This resulted in the first dermal formulations containing SLN for cosmetic purposes entering the market.

Due to the adhesiveness of small particles, SLN adhere to the stratum corneum forming a film as this films have been shown to possess occlusive properties. It was shown

that the degree of crystallinity has a great impact on the extent of occlusion by the formulation. With increasing crystallinity the occlusion factor increases as well. This explains why liquid nanoemulsions in contrast to SLN do not show an occlusive effect and why the extent of occlusion by NLC compared to SLN is reduced. Other parameters influencing the occlusion factor are the particle size and the number of particles. Whilst with increasing size the factor decreases, an increase in number results in an increase in the extent occlusion. The occlusive effect leads to reduced water loss and increased skin hydration. Highly crystalline SLN can be used for physical sun protection due to scattering and reflection of the UV radiation by the particles. A high crystallinity was found to enhance the effectiveness and was also synergistic with UV absorbing substances used in conventional sunscreens. Similarly synergism was observed on the sun protection factor and UV-A protection factor exhibited by the incorporation of the inorganic sunscreen, titanium-dioxide in NLC of carnauba wax and decyl oleate.

6. Lipid nanoparticles for ocular application

The eye possesses unique challenges with respect to drug delivery especially with respect to the posterior segment and treating vision threatening diseases. Poor bioavailability of drugs from ocular dosage form is mainly due to the pre-corneal loss factors which include tear dynamics, non-productive absorption, transient residence time in the cul-de-sac, and relative impermeability of the corneal epithelial membrane. Due to the adhesive nature of the small nanoparticles, these negative effects can be reduced. For ocularly administered SLN an increase in bioavailability was observed in rabbits by Cavalli *et al.* using tobramycin ion pair as the model drug. Various *in vitro* studies show a prolonged and enhanced permeation when the drug is incorporated in lipid nanoparticles. For systems containing phospholipids, a further improved permeation of diclofenac sodium was observed.

7. Pulmonary application of lipid nanoparticles

Pulmonary drug application offers the advantage of minimizing toxic side effects if a local impact is intended. Systemic delivery can be achieved through pulmonary delivery, offering the advantage of bypassing the first pass effect, as well as offering a large absorptive area, extensive vasculature, easily permeable membrane and low extracellular and intracellular enzyme activities. A problem of this method of administration is the low bioavailability. SLN can easily be nebulised to form an aerosol of liquid droplets containing nanoparticles for inhalation. *In vivo* studies showed that the administered drugs (rifampicin,

isoniazid and pyrazinamide for the treatment of tuberculosis) resulted in a prolonged mean residence time and a higher bioavailability than the free drug.

8. Application of liquid crystal drug delivery systems

The spontaneous self assembly of some lipids to form liquid crystalline structures offers a potential new class of sustained release matrix. The nanostructured liquid crystalline materials are highly stable to dilution. This means that they can persist as a reservoir for slow drug release in excess fluids such as the GIT or subcutaneous space, or be dispersed into nanoparticle form, while retaining the parent liquid crystalline structure. The rate of drug release is directly related to the nanostructure of the matrix. The particular geometry into which the lipids assemble can be manipulated through either the use of additives to modify the assembly process, or through modifying conditions such as temperature, thereby providing a means to control drug release.

Liquid crystal depot could be injected as a low-viscosity solution. Once in the body, itself assembles and encapsulates the drug in a nanostructured, viscous liquid crystal gel. The drug substance is then released from the liquid crystal matrix over a time period, which can be tuned from days to months. The liquid crystal depot system is capable of providing *in vivo* sustained release of a wide range of therapeutic agents over controlled periods of time. Liquid crystal nanoparticles can be combined with controlled-release and targeting functionalities. The particles are designed to form *in situ* at a controlled rate, which enables an effective *in vivo* distribution of the drug. The system has been shown to give more stable plasma levels of peptides in comparison to competing microsphere and conventional oil depot technologies.

Oral liquid crystal DDS are designed to address the varied challenges in oral delivery of numerous promising compounds including poor aqueous solubility, poor absorption, and large molecular size. Compared with conventional lipid or non-lipid carriers, these show high drug carrier capacity for a range of sparingly water-soluble drugs. For drugs susceptible to *in vivo* degradation, such as peptides and proteins, liquid crystal nanoparticles protect the sensitive drug from enzymatic degradation. The system also addresses permeability limitations by exploiting the lipid-mediated absorption mechanism. For water soluble peptides, typical bioavailability enhancements range from twenty to more than one hundred times. In an alternative application large proteins have been encapsulated for local activity in the GIT. Liquid crystal nanoparticle systems can be designed to be released at different

absorption sites (e.g., in the upper or lower intestine) which is important for drugs that have narrow regional absorption windows.

With regards to topical application, liquid crystal systems form a thin surface film at mucosal surfaces consisting of a liquid crystal matrix, whose nanostructure can be controlled for achieving an optimal delivery profile. The system also provides good temporary protection for sore and sensitive skin. Their unique solubilizing, encapsulating, transporting, and protecting capacity is advantageously exploited in liquid and gel products used to increase transdermal and nasal bioavailability of small molecules and peptides.

Advantages of Lipid based drug delivery system (LBDDS)

(1) Drug release in controlled and targeted way.
(2) Pharmaceutical stability.
(3) High and enhanced drug content (compared to other carriers).
(4) Feasibilities of carrying both lipophilic and hydrophilic drugs.
(5) Biodegradable and biocompatible.
(6) Excipients versatility.
(7) Formulation versatility.
(8) Low risk profile.
(9) Passive, noninvasive formation of vesicular system which is available for immediate Commercialization.

2.11. NUTRACEUTICALS

When a functional food aids in the prevention and/or treatment of disease(s) and/or disorder(s) (except anemia), it is called a nutraceutical. The proposed definition can help form distinction between functional foods, nutraceuticals, and dietary supplements.

The term "nutraceutical" was coined from "nutrition" and "pharmaceutical" in 1989 by Stephen DeFelice, MD, founder and chairman of the Foundation for Inno-vation in Medicine (FIM), Cranford, NJ.1 According to DeFelice, nutraceutical can be defined as, "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a dis-ease."1 However, the term nutraceutical as commonly used in marketing has no regulatory definition (Ekta K. Kalra et al., 2014)

2.12. Tetrahydrocurcumin bioavailability

Curcumin, a polyphenolic compound extracted from the rhizomes of turmeric (Curcuma longa Linn.), has a wide biological and pharmacological profile. It has also been reported to possess anti-oxidative, anti-inflammatory, anticarcinogenic, and gastroprotective effects (Ruby AJ et al.,). Tetrahydrocurcumin(THC), one of the major metabolites of curcumin *invivo* (Pan MH et al.,) was reported to exhibit the same physiological and pharmacological properties of curcumin. THC has been widely used in pharmaceutical and cosmetic preparations. THC, in a white to off-white powder form, has a molecular weight of 372.41 Da and a melting point of 85–100°C. THC is insoluble in water and soluble in alcohol, acetone, and glacial acetic acid. However, the pharmacological effect of THC is limited due to its low aqueous solubility. In addition, a relative short gastric emptying time can result in an incomplete release of THC from the dosage form at the site of absorption and lead to a diminished efficacy of the administered dose.

Based on the available literatures the therapeutic potential of THC can be achieved by making it as NLC formulation with improved bioavailability. The present investigation is focused on the development of THC nanostructure Lipid carrier with enhanced bioavailability.

2.13. ESSENTIAL OIL AS NATURAL PRESERVATIVES

Infections due to bacterial species also remain a serious clinical problem. Emerging resistance of bacterial species is seriously decreasing the number of effective antimicrobials. Because of increasing pressure of consumers and legal authorities, the food industry has tended to reduce the use of chemical preservatives in their products to either completely nil or to adopt more natural alternatives for the maintenance or extension of product shelf life. Plants and their essential oils are potentially useful sources of antimicrobial compounds. Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, including food-borne pathogens. The main constituents of essential oils – mono- and sesquiterpenes including carbohydrates, phenols, alcohols, ethers, aldehydes and ketones – are responsible for the biological activity of aromatic and medicinal plants as well as for their fragrance. Due to these properties, spices and herbs have been added to food since ancient time, no only as flavouring agents but also as preservatives (Marina Soković, Jasmina Glamočlija et al.,2010)

2.14. NATURE'S ALTERNATIVES TO SYNTHETICS

It's important for an educated consumer to understand what elements make an effective product. All skin care products (synthetic, organic and everything in between) contain the functional ingredients that serve as emollients, humectants, emulsifiers, surfactants and preservatives in some combination or another in order to produce effective products.

There is no reason we need to rely on chemicals to make a product work or even to preserve it. There are hundreds of natural ingredients that outperform synthetics and generally render synthetic chemicals obsolete! (Abhishek Jain, Pinky Radiya et al., 2014)

1. EMOLLIENTS

Emollients serve two functions; they prevent dryness and protect the skin acting as a barrier and healing agent. Water is the best emollient, but it doesn't work well for products because it evaporates quickly. In order for a product to be an effective moisturizer some sort of emollient oil needs to create emulsion so the skin can absorb the product.

Natural Emollients

- Natural emollients actually nourish the skin are recognized and metabolized by the skin's own enzymes and absorbed into it. These ingredients are provided by nature, thus readily biodegradable. There are no contamination concerns and these ingredients are of edible quality as pure as food.
- Some examples of natural emollients are Plant Oils (eg. Jojoba, Avocado, Rosehip) and Shea, Cocoa and Jojoba Butters

Synthetic Emollients

- Synthetic Emollients are occlusive, meaning they coat the skin and do not allow it to "breathe" or respire (much like plastic wrap), which can cause skin irritation. Some synthetic emollients have been proven to accumulate in the liver and lymph nodes of the body. Finally, synthetic emollients are not biodegradable and create a negative environmental impact.
- Some examples synthetic emollients are PEG Compounds, as in PEG- 45; Synthetic Alcohols, as in anything that contains the phrase benzyl –, butyl-, cetearyl-, cetyl -, glyceryl-, isopropyl-, myristyl propyl-, propylene-, or stearyl-); Hydrocarbons, as in

mineral oil, petrolatum, paraffin; Silicone oils, as in dimethicone, cyclomethicone, copolyol.

2. HUMECTANTS

The main purpose of a skin cream is to keep the skin moist and that function is served by the product's humectants.

Natural Humectants

- Natural phospholipids, from lecithin, are fantastic humectants. An important benefit of phospholipids is that they are hygroscopic (attract water from the surrounding air) and hold water where an increased level of hydration is needed.
- Therefore, phospholipids increase the hydration levels of the skin without being occlusive (forming a film to prevent water loss, and preventing normal cellular function).
- A recent study proved the value of topically applied phospholipids in skin care. It found that environmental factors (sun, wind, pollution) and the detergents and solvents found in most skin cleansers, actually stripped the natural phospholipid content from the top layer of skin.
- This loss resulted in a rough feel and a pitted appearance under a microscope. Importantly, the phospholipids in the uppermost skin layers cannot be replaced by natural cell function, as the top layer of cells no longer metabolize; they serve only as a protective barrier.
- Remarkably, the study showed that topically applied plant phospholipids restore the barrier function of the skin, protecting it from substances such as bacteria and harmful synthetic chemicals.
- Some examples of natural humectants are Lecithin, Panthenol (pro-vitamin B5), Glycerin

Synthetic Humectants

- Many conventional creams using synthetic humectants form a suffocating film on the skin to prevent moisture loss.
- Some examples of synthetic humectants are Propylene Glycol, Ethylene/Diethylene Glycol, PEG compounds (eg. Polyethylene Glycol), Synthetic alcohols (eg Glyceryl Coconate, Hydroxystearate, Myristate, Oleate)

3. EMULSIFIERS

Emulsifiers blend and hold together ingredients that don't normally mix. An emulsifier can either be a physical substance (like wax) or a physical action (like "shake well before use").

Natural Emulsifiers

- Natural emulsifiers are obtained from various nuts, berries and leaves
- Some examples of natural emulsifiers are Plant Waxes (eg. Candelilla, Carnauba, Jojoba, Rice Bran), Xanthan Gum, Quince Seed

Synthetic Emulsifiers

- Synthetic emulsifiers are usually petroleum/hydrocarbon derivatives
- Some examples of synthetic emulsifiers are Alkoxykated Amides (eg TEA, DEA, MEA, MIPA compounds), PEG compounds, Sorbitan Stearate, Laurate, Palmitate, Oleate, Ceresin, Silicone, Isopropyl Stearate.

4. SURFACTANTS

Surface-active-agents are substances capable of dissolving oils and holding dirt in suspension so it can be rinsed away with water. They are used in skin cleansers and shampoos.

Natural Surfactants

- Natural saponins (foaming agents) are a superior surfactant choice for shampoos and body washes as they gently cleanse the hair and scalp without stripping the natural oils. Natural cleansers create the perfect balance of mildness, effective cleansing, scalp and skin nourishment and protection.
- Some examples of natural surfactants are Castile Soap, Yucca Extract, Soapwort, Quillaja Bark Extract

Synthetic Surfactants

- Most synthetic surfactants are inexpensively produced, excessively harsh, stripping and irritating to skin and scalp.
- Another serious problem with ethoxylated surfactants (those that utilize ethylene or propylene oxide in the chemical reaction) is that they can be contaminated with dioxane, a potent carcinogen. These surfactants are listed on labels as ingredients

ending with –eth, (like laureth) or containing the phrase PEG (PolyEthylene Glycol), or PPG (PolyPropylene Glycol).

- Another potentially unsafe class of synthetic surfactants are amides. These are listed on labels containing the term TEA – TriEthanolAmine, DEA – DiEthanolAmine and MEA, MonoEthanolAmine. All compounds containing TEA, DEA and MEA can undergo nitrosation with other synthetic chemicals to form nitrosamines, which are carcinogenic.
- Some examples of synthetic surfactants are Sodium or Ammonium Lauryl or Laureth Sulphate, Sodium Methyl Cocoyl Taurate, Sodium Lauroyl or Cocoyl Sarcosinate Cocomidopropyl Betaine, TEA (Triethanolamine) compounds, DEA (Diethanolamine) compounds, MEA (Monethanolamine) compounds, PEG (Polyethylene Glycol) compounds, Quaternium -7, 15, 31, 60 etc, Lauryl or Cocoyl Sarcosine Disodium Oleamide or Dioctyl Sulfosuccinate.

5. PRESERVATIVES

Preservatives are used to protect products from bacteria and mold and give them a longer shelf life. In all matter, the decaying process is natural and will eventually occur with or without preservatives of any kind. Skin care products do not, and should not, last forever.

Natural Preservatives

- Another Miessence exclusive is the all natural and organic preservation system we feature in our products. This proprietary formulation is a combination of 6 different herbal, fruit and flower extracts, which varies from product to product.
- Just like food, all natural skin care products will eventually deteriorate and go rancid, you will just have to pay mind to the Miessence “fresh by dates” and “use by dates”. Read more on our Preserving Products Naturally page.
- Miessence has also developed distinctive airless vacuum pumps for many of our bottles, highly desirable for naturally preserved products due to the fact that it keeps air and airborne contaminants out of the bottles. This feature significantly assists the function of our natural preservative system and shelf life of our products.
- Some examples of natural preservatives are Tea Tree Essential Oil, Thyme Essential Oil, Grapefruit Seed Extract, Bitter Orange Extract

Synthetic Preservatives

- Synthetic chemical preservatives are toxic by nature since it is their chemical purpose to kill bacteria and mold for years and years. All synthetic preservatives are considered toxic in high doses, but manufacturers argue the small amounts in products are harmless. Since human safety testing does not exist for 89% of synthetic chemicals used in personal care products, we will have to wait for conclusive evidence.
- Some examples of synthetic preservatives are Imidiazolidinyl Urea (formaldehyde donor, releases formaldehyde if temperature over 50 degrees Fahrenheit), DMDM Hydantoin (also contains formaldehyde), Methyl, Propyl, Butyl and Ethyl Parabens, Benzalkonium Chloride, Chloromethylisothiazolinone and Isothiazolinone, Methylisothiazolinone and Methylchloroisothiazolinone, Butylated Hydroxytoluene (BHT) and Butylated hudroxyanisole (BHA).

3. AIM AND OBJECTIVES

Lipid based formulations usually contain lipid (soild or liquid) dispersed in aqueous solution of surfactant (or co-surfactant). Nano structured lipid carriers are proved to be suitable carriers with various advantages like (i) controlled release of the drug (ii) increased drug stability (iii) high drug loading (iv) no bio toxicity of the carrier (v) avoidance of organic solvents and (vi) no problems with respect to large scale production and sterilization.

But, they often show poor stability due to microbial growth. Moreover, the problem of bacterial resistance to conventional preservatives has been observed in recent years. Traditionally used chemical preservatives often cause skin irritation and lead to allergenic reactions. All these factors have contributed to a search for alternative preservative systems. Hence, there is a lacuna to identify a natural preservative which can enhance the stability of lipid based formulations.

These natural lipids (oils) have high oxidative resistance and are biocompatible, with no deleterious effects on the skin. The exotic fats used in skin care are known to exhibit their effects through restoration of a sufficient layer of skin lipids and skin elasticity, boost natural skin regeneration and increased skin hydration by forming an inert, epicutaneous occlusive membrane. Besides these facts, cocoa butter, mango butter, mineral and petroleum which is one of several exotic fats is viewed as an ingenious replacement for chemicals based lipid fat because of its appreciable contents which are very important as source of skin active ingredients.

Furthermore, using of natural surfactants is viewed as alternative to replace helping to reduce contamination of the environment with drug particles. Like conventional properties offers exceptional surfactant blends with a balanced combination and dermatological properties in cosmeceuticals products. Therefore, the suitable use of a base surfactant or a co-surfactant in cosmetic cleansing preparations is attractive in the recent past. Natural surfactants show a lower irritation potential in comparison to other surfactants and confirming the excellent suitability.

An idea of natural preservatives seems to be very promising and practical in terms of availability and eco friendly nature. Nowadays much attention has been focused on essential oils (EOs) that demonstrate antimicrobial activities and have been proposed as natural preservatives. Recent studies provided evidence that different mechanisms are involved in antibacterial activity of essential oils (EOs). EOs is usually considered safe due to their

natural origin. Although most EOs is regarded as safe, some of them may cause risk of irritation, sensitization, phototoxicity or allergic reactions including anaphylaxis. EOs are also effective in controlling bacterial and fungicidal activity, which is an important criteria of a preservative.

Growing demands for more natural and preservative- free cosmetics promoted an idea of the replacement of synthetic preservatives with essential oils (EOs) of antimicrobial properties. The antimicrobial effect of essential oil depends on content, concentration and interactions between the main active compounds. Effective preservatives should be characterized by a broad spectrum of antimicrobial activity at a minimum concentration.

Based on these facts, the aim of this work is to develop a natural preservative system using essential oils with antimicrobial activity to enhance the preservation of nutricosmetics which are prepared using natural lipids and surfactants. For this purpose, nanostructured lipid carriers of tetrahydrocurcumin would be prepared with using essential oils with enhanced stability. The project would be carried out with the following objectives:

- To screen out various essential oils for their antimicrobial activity and evaluation of their minimum inhibitory concentration against different bacterial strains.
- To develop nano structural lipid carriers (NLC's) and lipid based cream of Tetrahydrocurcumin (THC).
- To characterize and optimize the NLCs and lipid based cream of Tetrahydrocurcumin.
- To evaluate the stability of the NLCs and lipid based cream.
- To evaluate the antimicrobial activity of NLCs and lipid based cream of Tetrahydrocurcumin.

4. PLAN OF WORK

- Preformulation Studies
  - Development of analytical method by UV spectroscopy
  - IR spectroscopic Analysis
  - Evaluation of antibacterial activity of essential oils against various bacterial strains.
  - Determination of minimum inhibitory concentration of essential oils.
  - Determination of the stability of drug in different pH
- Formulation development
  - Formulation of nanostructured lipid (NLC) carrier using Ultra probe sonication technology
  - Preparation of base cream using natural excipients
  - Incorporation of NLC into Base Cream
- Characterization studies of the NLCs prepared
  - Determination of particle size, polydispersity index and zeta potential by Zeta sizer
  - Measurement of entrapment efficiency
  - Antimicrobial activity of NLCs and cream.
  - Visualization of morphology by Phase Contrast Microscopy (PCM)
  - Visualization of size, morphology and roughness by Atomic Force Microscopy(AFM)
  - Visualization of morphology by Scanning Electron Microscopy (SEM)
  - *In vitro* skin penetration study using pig ear skin
  - Evaluation of texture properties by Texture Analyzer.
  - Stability studies as per ICH guidelines

5. MATERIALS USED

Table 3: Materials Used

SL.NO.	MATERIALS	SOURCE
1.	Tetra Hydro Curcumin	Sami labs, Bangalore
2.	Cocoa Butter	National chemicals, vadodara, Gujarat
3.	Soya lecithin	Applichem
4.	Lanolin	Hi- Pure, Chennai
5.	Bees wax	Loba Chemie Pvt. Ltd., Mumbai
6.	Cinnamon oil pure	Sangrose Laboratories Pvt. Ltd., Mavelikara
7.	Methanol	Himedia Lab., Mumbai
8.	Sodium hydroxide	Himedia Lab., Mumbai
9.	Potassium dihydrogen phosphate	Fisher scientific, Mumbai
10.	Glacial acetic acid	Fisher scientific, Mumbai
11.	Sodium chloride	Himedia Lab., Mumbai
12.	Beef extract	Himedia Lab., Mumbai
13.	Agar media	Himedia Lab., Mumbai
14.	Muller Hinton Agar	Himedia Lab., Mumbai
15.	Peptone extract	Himedia Lab., Mumbai
16.	Distilled Water	Himedia Lab., Mumbai

EQUIPMENTS USED

Table 4: Equipments Used

SL.NO.	EQUIPMENT	MODEL/COMPANY
1.	Digital Weighing Balance	Shimadzu AY 220
2.	Magnetic Stirrer	Remi Equipments Ltd
3.	Bath Sonicator	RP 120 Ralsonics, Mumbai
4.	Ultra Probe Sonicator	SONICS vibracell
5.	UV Visible Spectrophotometer	UV-1650 PC Shimadzu
6.	FT IR Spectrophotometer	8400 S Shimadzu
7.	IR Hydraulic Pellet Press	Model M15 Technosearch Instruments
8.	ELIZA reader	Thermo Scientific
9.	Scanning Electron Microscope	JEOL, Japan- JSM 6360
10.	Atomic Force Microscope	Multimode Scanning probe microscope (NTMDT, NTEGRA prima, Russia)
11.	Zeta Sizer Nano ZS90	Malvern UK
12.	Texture Analyzer	TA-XT Plus
13.	Phase Contrast Microscope	NIKON Inverted Fluorescent Microscope
14.	Gel doc	Gel Doc System, Syngene

6. DRUG PROFILE

TETRAHYDROCURCUMIN (THC)

Chemical name : 1,7- bis (4-hydroxy 3 methoxy phenyl)-3,5 heptanedione

Formula : $C_{21}H_{24}O_6$

Structure :

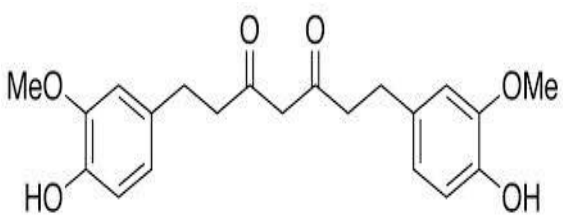


Fig 4: Drug profile of Tetrahydrocurcumin

Molecular weight : 372.41 Da

Physical state : Solid

Melting point : 95-97^{o}C

Solubility : Water solubility- 0.0056 g/ l, freely soluble in acetone, glacial acetic acid

Log P : 3.51

Log S : -4.82

Pka (strongest acidic) : 9.31

Pka(strongest basic) : -4.6

Hydrogen acceptor count : 6

Hydrogen donor : 2

Polar surface area : 93.06 A^2

Pharmacological actions : THC exhibits many of the pharmacological actions as that of curcumin. It has got many potent pharmacological actions like anti-oxidant property, anti-cancerogenic and anti-angiogenic and prevents type II diabetes. It was also proved to be more effective than curcumin in preventing azoxymethane-induced colon carcinogenesis.

COCOA BUTTER

Synonyms : Cocoa oil, Cocoa fat, Cocoa bean oil

Chemical name : 1,3-dipalmitoyl- 2-oleoyl-glycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS), and 1,3-stearoyl-2-oleoyl-glycerol

Empirical formula : $CH_3(CH_2)_{16}COOH$

Molecular weight :

Structural formula :

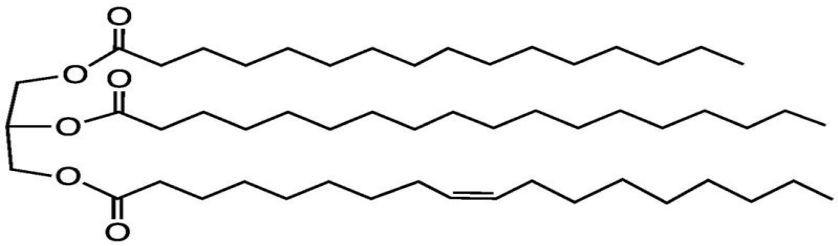


Fig 5: Structure of Cocoa butter

Melting point : 34.1°C (93.4° F)

Solidify : Solidify at 20°C

Refractive index : 1.44556- 1.44573

Iodine value : 32.11- 35.12

Acid value : 1.68

Saponification value : 191.214

Functional category : It is used in lotions and skin care products like cream, facial washes, etc.,

SOYA LECITHIN

Synonyms : Lecithin from Soybean

Chemical name : (2-nonanoyloxy-3-octadeca-9,12-dienoyloxypropoxy)-[2-(trimethylazaniumyl)ethyl]phosphinate

Empirical formula : $C_{35}H_{66}NO_7P$

Molecular weight : 643.887 g/mol

Structural formula :

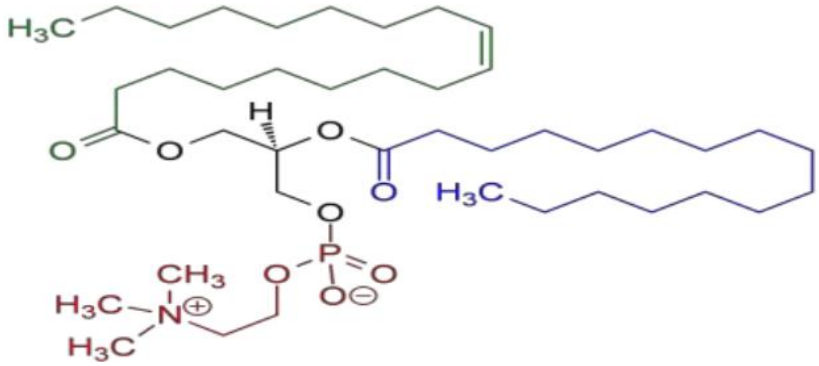


Fig 6: Structure of Soya Lecithin

Solubility : Soluble in ethanol and hot water

Boiling point : More than 80^{o}C

Functional category : It acts as a wetting, stabilizing agent and a choline enrichment carrier, helps in emulsifications and encapsulation, and is a good dispersing agent. It can be used in manufacture of intravenous fat infusions and for therapeutic use.

Storage : Soya lecithin should be stored at - 20˚C in a well-closed container, protected from light, in a cool, dry place.

CINNAMON OIL

Synonyms	**:**	Cassia oil, Chinese cinnamon,Cassia bark oil, Oil of cassia
Chemical name	**:**	2-methoxy-4-prop-2-enylphenol;[(E)-prop-1-enyl]benzene
Empirical formula	**:**	$C_{19}H_{22}O_2$
Molecular weight	**:**	282.383 g/mol
Structural formula	**:**	

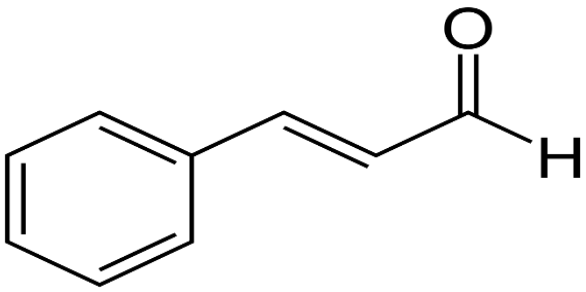


Fig 7: Structure of Cinnamon oil

Boiling point	**:**	194-234 °C
Solubility	**:**	Insoluble in water, soluble in alcohol
Description	**:**	It is of a golden-yellow colour, with the characteristic odour of cinnamon and a very hot aromatic taste. The pungent and scent come from cinnamaldehyde (about 90% of the essential oil from the bark) and, by reaction with oxygen as it ages, it darkens in colour and forms resinous compounds.
Functional catergory	**:**	Effective at treating skin conditions such as rashes, acne and infections, you can mix cinnamon essential oil with a carrier oil (like coconut oil) and apply it to the skin to take advantage of its antimicrobial activity

BEES WAX

Synonyms	**:**	Triglyceryl beeswax
Chemical name	**:**	hexadecan-1-ol;2-methyloxirane;oxirane
Empirical formula	**:**	$C_{21}H_{44}O_3$
Molecular weight	**:**	344.58 g/mol
Structural formula	**:**	

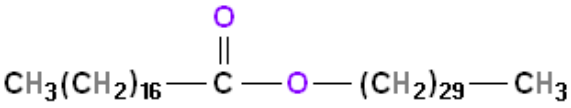


Fig 8: Structure of Bees wax

Boiling point	**:**	98 to 99 °C°C
Melting point	**:**	62 °C to 64 °C
Flash point	**:**	204.4 °C
Density	**:**	15 °C is 958 kg/m³ to 970 kg/m³
Description	**:**	When natural beeswax is cold it is brittle, during on room temperature it is tenacious, its fracture is dry and granular, it also softens at human body temperature
Functional catergory	**:**	Purified and bleached beeswax is used in the production of food, cosmetics, and pharmaceuticals.

LANOLIN

Synonyms	**:**	Wool wax or Wool grease, Anydrous lanolin
Melting point	**:**	38 °C to 42° C
Density	**:**	0.932-0.945 g/cm3
Description	**:**	It is odourless, tasteless and yellow in colour
Solubility	**:**	Practically insoluble in water. Sparingly sol in cold, more in hot alcohol; freely sol in chloroform, ether.
Storage	**:**	Store in a tightly closed container. Store in a cool, dry, well-ventilated area away from incompatible substances.
Functional catergory	**:**	Lanolin is used as emulsifing agent, binder, skin conditioner and increasing viscosity agent in cosmetic purpose

7. PREFORMULATION STUDIES

Preformulation studies involve physical, chemical and biological characterization of new drug substances in order to develop stable, safe and effective dosage form. Preformulation testing encompasses all studies enacted on a new drug compound in order to produce useful information for subsequent formulation of a stable and bio-pharmaceutically suitable drug dosage form.

7.1. Analytical methods for Tetrahydrocurcumin (THC)

- UV Spectrophotometric estimation of Tetrahydrocurcumin (European Pharmacopoeia 6.0)

7.1.1. Determination of lambda max:

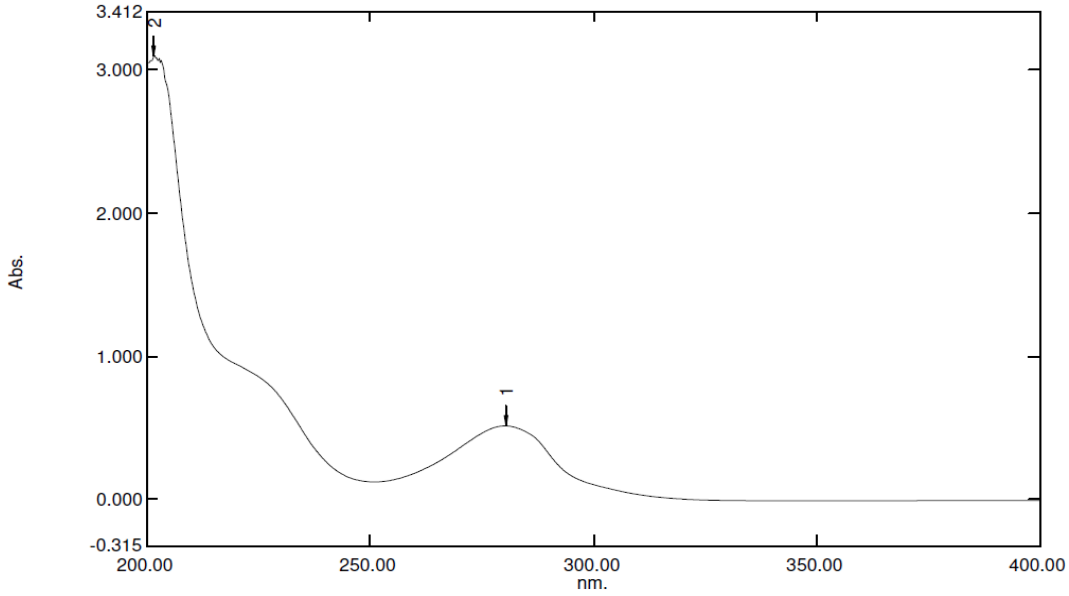
Tetrahydrocurcumin:

A concentration of 100µg/ml solution of THC, were prepared and scanned under UV Visible Spectrophotometer from 200 to 400nm. The corresponding peak with the highest absorbance was taken as the lambda max for quantifying THC.

Tetrahydrocurcumin standard solution (100µg/ml) showed highest peak at 281nm. From the UV-Vis spectrum (Graph 1) the lambda max of the THC was optimized as 281nm and used for further studies.

7.1.2. Lambda max of Tetrahydrocurcumin:

Fig 9 : UV Spectrum of Tetrahydrocurcumin



7.2. Compatibility studies

The sample of tetrahydrocurcumin of about 10mg was mixed with 100mg of KBr to make the pellet and scanned under FT-IR spectroscopy from 400 – 4000 cm^{-1}.

7.2.1. IR Spectrum of Tetrahydrocurcumin

Characteristic peaks for carbonyl group C=O stretching was observed in 1601 cm^{-1}, C-C Stretching at 1515 cm^{-1} and C-O stretching at 1233 which is evident that three peaks are confirming the purity of tetrahydrocurcumin molecule.

7.2.2. IR Spectrum of Cocoa Butter

Characteristic peaks for carbonyl group C=H stretching was observed in 1350 cm^{-1}, O-C=O Stretching at 2902 cm^{-1} and Triglycerides stretching at 1738 cm^{-1} which is evident that three peaks are confirming the purity of tetrahydrocurcumin molecule.

7.2.3. IR spectra of physical mixture of Tetrahydrocurcumin and Cocoa Butter

The characteristic peaks for CH_3 stretching at 2946 cm^{-1}, C=H stretching at 1350 cm^{-1}, O-C=O stretching at 2902 cm^{-1}, Carbonyl stretching at 1757 cm^{-1} and OH stretching at 3420 cm^{-1} was observed in the IR spectra of physical mixture of drug and lipid. This confirms

that the molecule under study, THC has not undergone any structural changes. Thus no incompatibility issue is observed with the lipid used.

Fig 10 : IR Spectra of Tetrahydrocurcumin

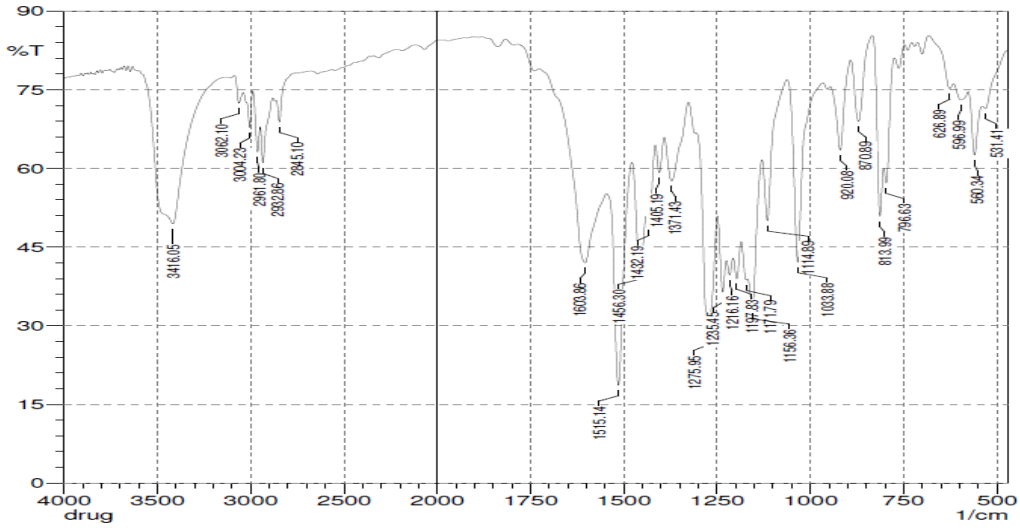


Fig 11 : IR Spectra of Cocoa Butter

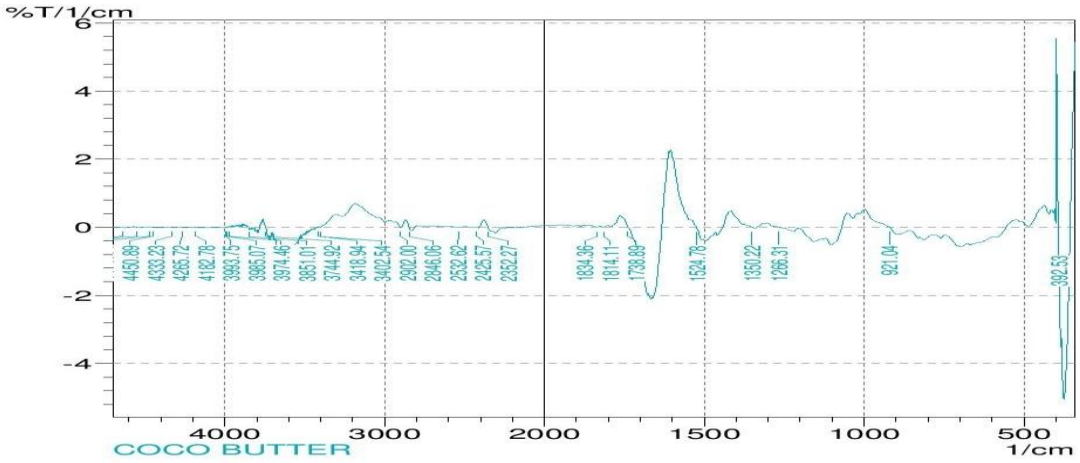
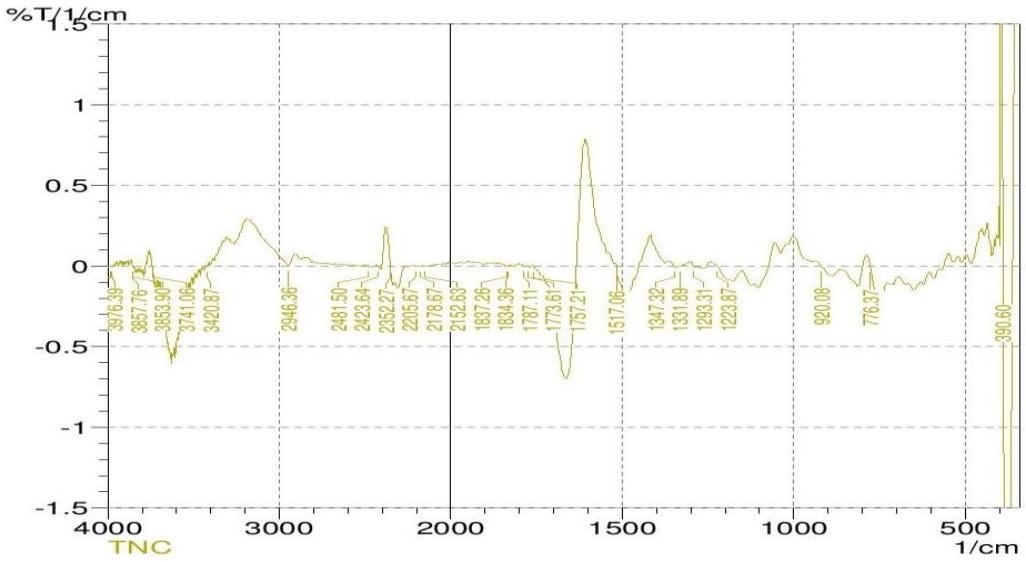


Fig 12: IR spectra of physical mixture of THC and Cocoa Butter



7.3. Calibration Curve

Phosphate buffer pH 7.4 was used as the buffering medium in the preparation of standard solution of Tetrahydrocurcumin

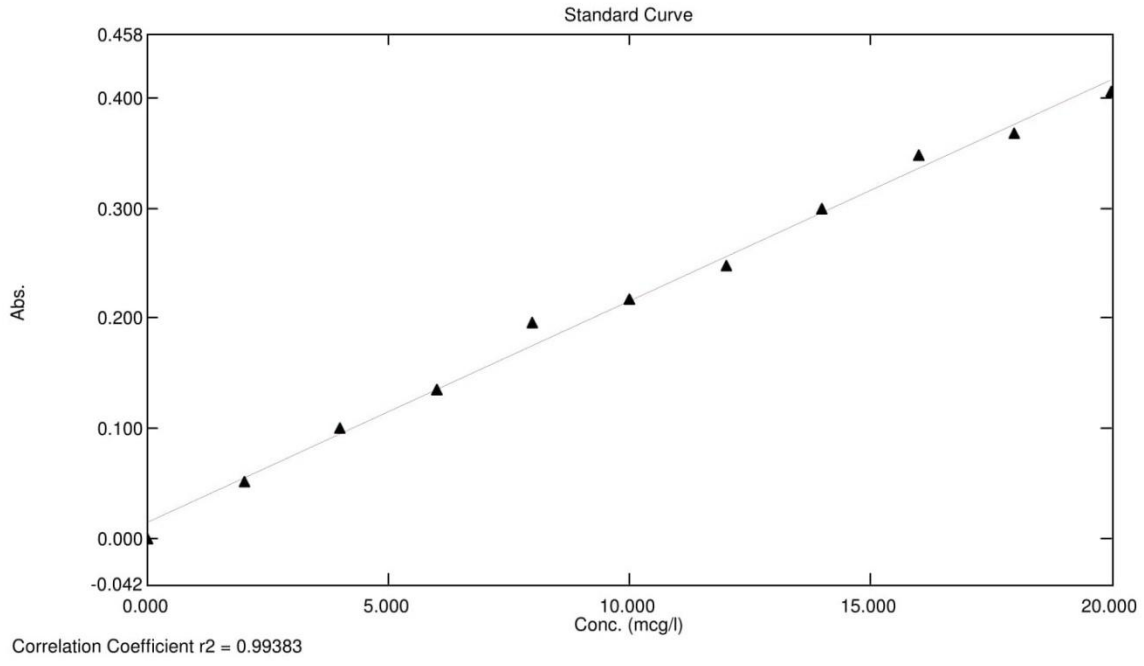
Standard graph for THC

Primary stock solution of THC was prepared by dissolving 10mg of THC is dissolved in methanol in a 100ml of volumetric flask until it soluble and make up with water. Aliquots of THC was prepared from stock solution in the concentration range of 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml, 12 µg/ml, 14 µg/ml, 16 µg/ml, 18 µg/ml, 20 µg/ml in 10ml volumetric flask using Phosphate buffer pH 7.4 in buffer as solvent. The absorbance of THC standard solutions was measured at 281 nm (lambda max of THC) against Phosphate buffer pH 7.4 in buffer solution as blank. The standard graph was prepared with concentration of solution (in µg/ml) on X-axis and absorbance on Y-axis. The results are shown in fig 8.

Table 5: Standard Table for Tetrahydrocurcumin

Sl.no	Concentration (µg/ml)	Absorbance at 281nm
1.	2	0.051
2.	4	0.100
3.	6	0.135
4.	8	0.196
5.	10	0.218
6.	12	0.248
7.	14	0.300
8.	16	0.359
9.	18	0.368
10.	20	0.406

Fig 13: Calibration Curve of Tetrahydrocurcumin

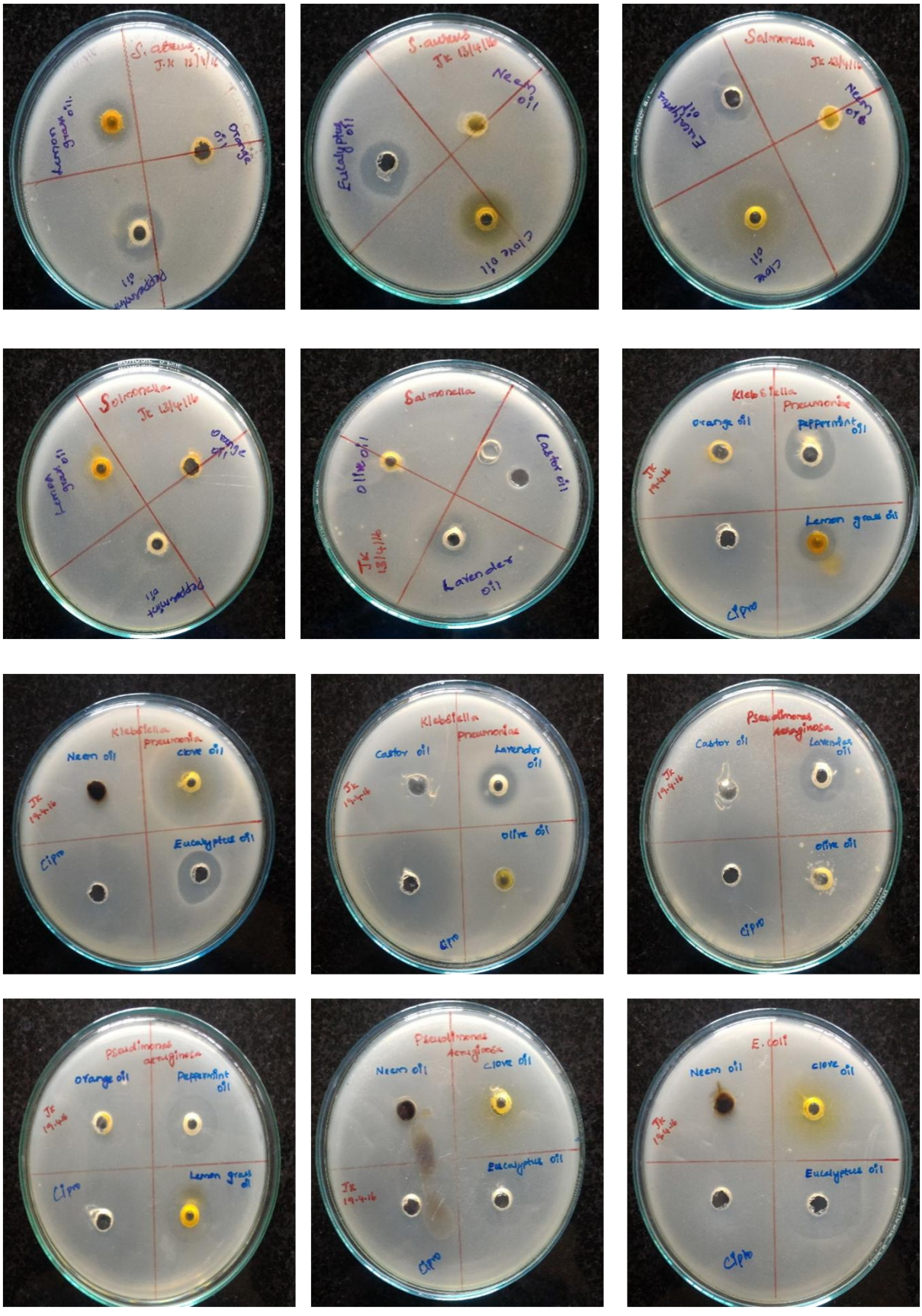


7.4. SELECTION OF OILS

Various Essential oils have been widely used for bactericidal, virucidal, fungicidal, ant parasitical, insecticidal, and other medicinal properties such as analgesic, sedative, anti-inflammatory, spasmolytic, and locally anesthetic remedies. Essential oils such as Neem oil,Clove oil,Eucalyptus oil,Orange oil,Peppermint Oil,Lemon grass Oil,Castor oil,Lavender Oil, Olive oil, Cinnamon oil tested for antibacterial activity against 7 Bacterial Strains. (Staphylococcus aureus, Enterococcus faecalis, Streptococcus aeruginosa, Salmonella typhimurium, E.coli, Klebseilla pneumonia,Candida albicans).

Table 6: Antibacterial activity of Essential Oils

Various Essential oils	**Strains**						
	Staphylococcus Aureus	**Solmonella typhimurium**	**Enterococcus faccalis**	**Pseudimonas aeroginosa**	**E.coli**	**Klebsiella pneumonia**	**Candida albicans**
Neem oil	-	-	-	-	-	-	-
Clove Oil	✓	✓	-	✓	✓	✓	-
Eucalyptus Oil	✓	✓	-	✓	✓	✓	-
Orange Oil	-	-	-	-	-	-	-
Peppermint Oil	✓	✓	-	✓	✓	✓	-
Lemon Grass Oil	-	✓	-	✓	✓	✓	✓
Castor Oil	-	-	-	-	-	-	-
Lavender Oil	✓	✓	-	✓	✓	✓	✓
Olive Oil	-	-	-	-	-	-	-


S. aureus
Neem oil
Eucalyptus oil
Clove oil
Salmonella
Orange oil
Lemon grass oil
Peppermint oil
Olive oil
Castor oil
Lavender oil
Klebsiella Pneumoniae
Cipro
Pseudomonas aeruginosa
E. coli

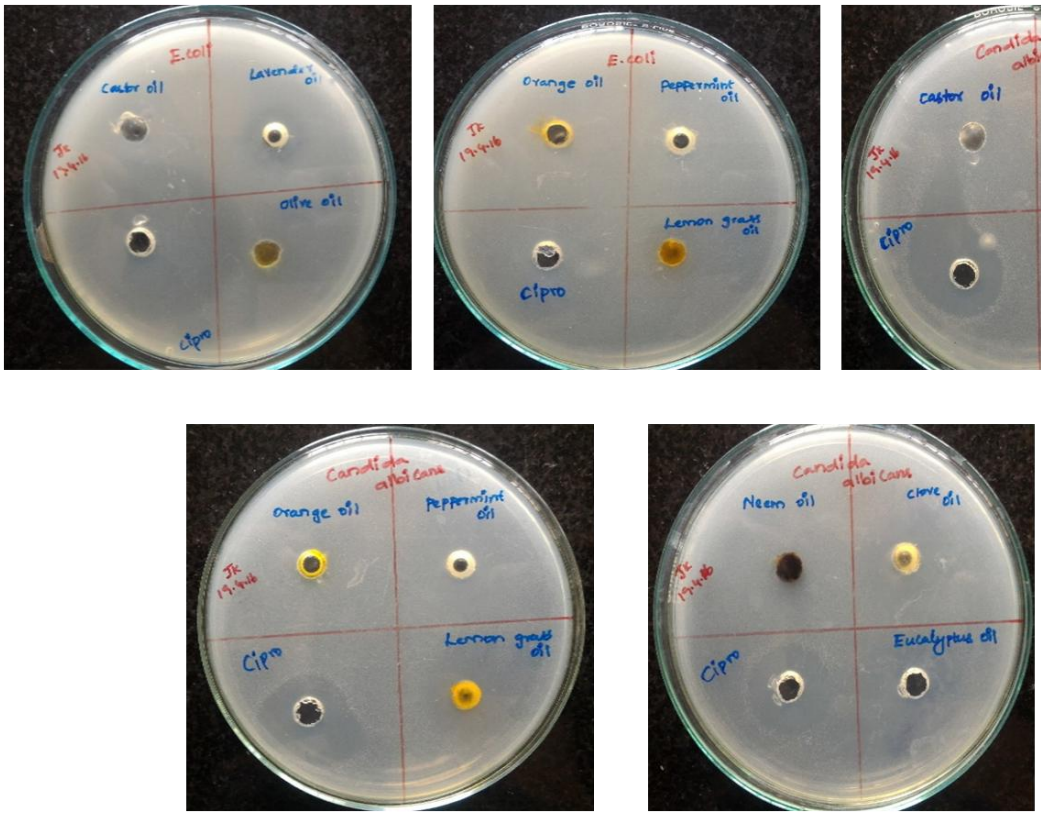


Fig 14: Images Of Zone Of Inhibition For Essential Oils

Among this clove oil, peppermint oil, lemon grass oil, lavender oil, Cinnamon oil have well effect against bacteria.Neem oil has less bacterial activity presents in the active constituent of neem bark oil other than that it has fungal activity.

7.5. Mixed proportions of essential oils were studied for antimicrobial activity for E.Coli and Klebsiella strains

Table 7: Mixed Proportion Of Essential Oils

Clove Oil + Eucalyptus Oil (1:1)	Clove Oil + Lavender Oil (1:1)
Clove Oil + Peppermint Oil (1:1)	Eucalyptus Oil + Peppermint Oil (1:1)
Peppermint Oil + Lavender Oil (1:1)	Clove Oil + Eucalyptus Oil + Peppermint Oil (1:1:1)
Clove Oil + Eucalyptus Oil + Peppermint Oil + Lavender Oil (1:1:1:1)	Clove Oil + Eucalyptus Oil + Peppermint Oil + Neem Oi l (1:1:1:1)
Clove Oil + Eucalyptus Oil + Peppermint Oil + Lavender Oil + Neem Oil (1:1:1:1)	Cinnamon Oil + Neem Oil (1:1)

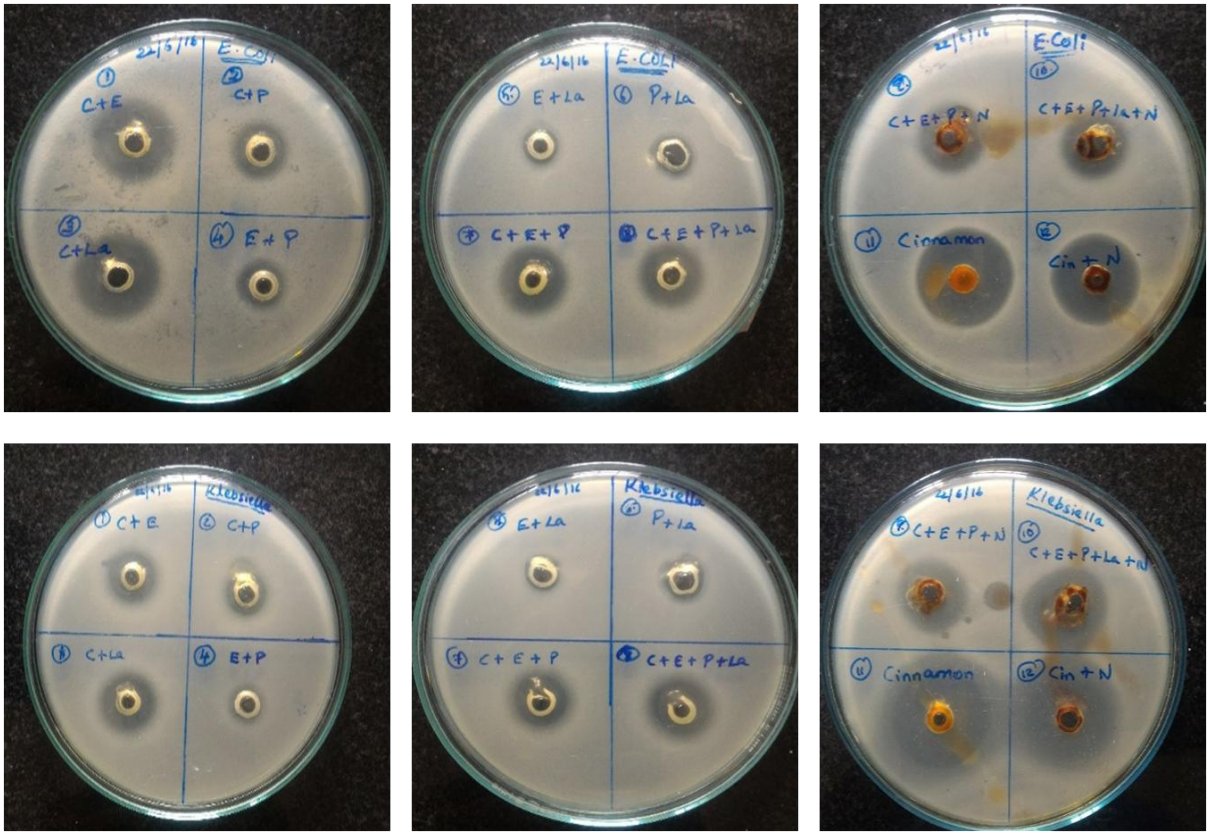


Fig 15: Images of Zone of Inhibition for Mixed Proportion

Here combination of oils shows lesser antibacterial activity than individual.

7.6. DETERMINATION OF MINIMUM INHIBITORY CONCERNTRATION

Method: 1

MIC determinations were performed in 96-wellmicroplates according to procedures described by the Clinical and Laboratory Standards Institute. The liquid media was prepared by adding 2% peptone, 0.5% Nacl, 0.3% beef extract in 100ml distilled water. Then 100µl liquid media was transferred into micro plate well. 100µl of essential oil were added in first well then serial two fold dilution was made. Add 5µl inoculum in each well. The positive control comprised of media and organism and the negative control was liquid media. Inoculated micro plate were incubated for 24hrs at 37°C. Then absorbance were measured spectrometrically at 625 nm.

Table 8: Minimum inhibitory concentration for essential oils

Control	Absorbance
Media	0.0470
Media + Organism	0.1156

Essential oil	1	2	3	4	5	6	7	8	9	10	11
Cinnamon oil	0.1956	0.4966	0.4074	0.2406	0.0921	0.0913	0.0777	0.0843	0.0751	0.0928	0.0711
Eucalyptus oil	0.0568	0.0791	0.0736	0.0768	0.0786	0.0999	0.1606	0.1432	0.1417	0.1314	0.1382
Peppermint oil	0.6197	0.1587	0.0968	0.0962	0.0902	0.1201	0.1071	0.1225	0.1244	0.1443	0.1291
Lavender oil	0.2285	0.1144	0.1425	0.0783	0.1368	0.1970	0.1752	0.2272	0.1981	0.2594	0.1268

Method :2

All microbiological assays were performed under anaerobic conditions. MIC determinations were performed in 96-wellmicroplates according to procedures described by the Clinical and Laboratory Standards Institute. Each essential oil (200mg) was dissolved in dimethyl sulfoxide (40µL) and thevolume was made to 5 mL with sterile Muller Hinton medium containing 1%Tween 80 to provide a stock solution containing 40 mg $mL^{-1}$of oil. Serial twofold dilutions of each essential oil stockwere made with Muller Hinton medium to yield final concentrations ranging from 20 to 0.625 mg $mL^{-1.}$ The diluted samples (100 µL)were transferred to microplate wells and mixed well withthe micropipette. The negative controls comprised sterile Muller Hinton medium or with dimethyl sulfoxide (at concentrations usedin the dilutions). In order to ascertain aseptic conditions, the control wells contained sterile Muller Hinton medium but withoutinoculum. The inoculated microplates were incubated at36 ± 1◦C for 48h under anaerobic conditions; and the bacterial growth was confirmed by adding 10µL of a sterile0.5% aqueous solution of triphenyltetrazolium chloride (TTC,Sigma–Aldrich) and incubating at 36◦C for 30min. Theviable bacterial cells reduced the yellow TTC to pink/red1,3,5-triphenylformazan (TPF). All assays were performed intriplicate.

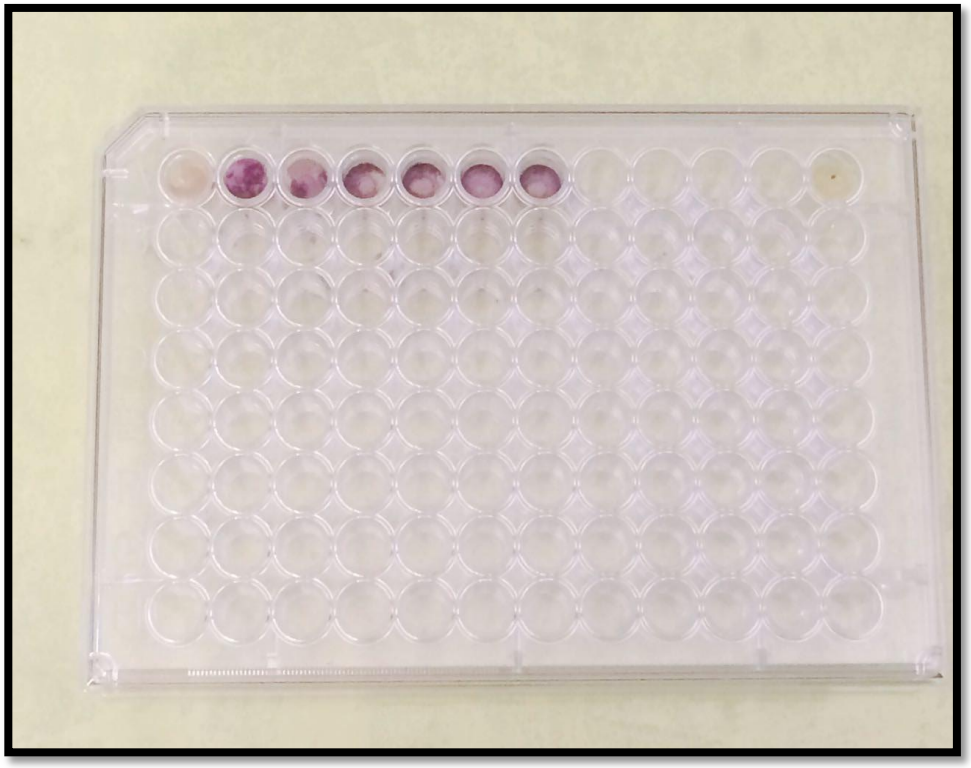


Fig 16: Minimum Inhibitory Concentration for Peppermint Oil

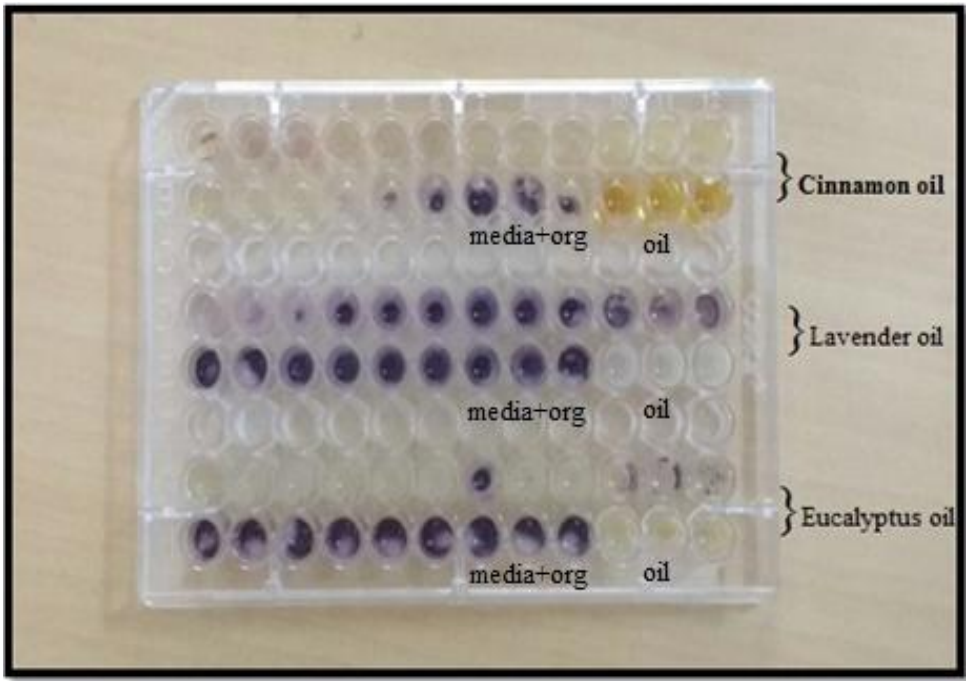


Fig 17: Minimum Inhibitory Concentration for Cinnamon Oil, Lavender Oil And Eucalyptus Oil

7.7. Determination of stability of drugs in different pH conditions

Standard solution of the Tetrahydrocurcumin(THC) were prepared by dissolving 10 mg of standard drug in methanol in 100 ml volumetric flask until it soluble and make up with distilled water(1000µg/ml). 10 ml of Prepared standard solution of THC was taken in 100 ml volumetric flask in different medium such as HCL, Acetate, Phosphate pH 6.8 and Phosphate pH 7.4 with each individual buffers (1.2, 4.0, 6.8, 7.4) respectively (volume made up with respective buffer. Determine the initial absorbance (taken at 0^{th} hour) and consider it as 100 % of assay. Place the test solutions prepared in pH buffers at room temperatures and measure the absorbance (λ_{max} = 280 nm) of the solutions at 2^{nd}, 4^{th}, 6^{th}, 24^{th} and 48^{th} hours. Determine the concentration at each time point by correlating the absorbance value from the standard graph and report the changes from the initial concentration in assay %.

Table 9: stability of drugs in different pH conditions

Time (Hours)	pH	Absorbance (nm)
0	1.2	0.579
	4	0.550
	6.8	0.544
	7.4	0.562
2	1.2	0.564
	4	0.590
	6.8	0.539
	7.4	0.547
6	1.2	0.517
	4	0.541
	6.8	0.578
	7.4	0.520

24	1.2	0.544
	4	0.533
	6.8	0.539
	7.4	0.552
48	1.2	0.540
	4	0.551
	6.8	0.544
	7.4	0.516

8. EXPERIMENTAL METHODOLOGY

Nanostructured lipid carriers (NLC) are the second Generation solid lipid nano particles (SLN) composed of solid lipid matrix which are incorporated with liquid lipids. Among the nanostructured lipid carriers that contain solid lipids together with different liquid oils .The presence of liquid lipids with different fatty acid C-chains produces NLC with less organized crystalline structure and therefore provides better loading capacity for drug accommodation. Liquid lipids are better solubilizers of drugs than solid lipids.

Approaching nano structured lipid carrier development from an emulsion perspective is faced with significant challenges. Numerous research groups subsequently commenced research efforts to improve nano structured lipid carrier development. Most researchers have approached traditional emulsion techniques.

METHODS

8.1. FORMULATION DEVELOPMENT

Preparation of Nano Structured Lipid Carriers (NLC) by High Shear Homogenization coupled with ultra probe sonication

The solid lipid(500mg) of choice andthe liquid lipid (1ml)was mixed with the drug (100mg) andwarmed to 75°C for effective melting and mixing. Simultaneously, distilled water(20ml) to which the surfactant(300mg) has been incorporated is also heated to 75°C,it is instilled into the formulation herein. Thereafter, the aqueous part is added to the lipid part maintaining the temperature at 75°C, with continous stirring followed by magnetic stirring for 3 mins. The two- phase system is then sonication using probe sonicator, at 20,000rpm for 15min followed by ultra sonication for 2 min. The prepared formulations are stored at refrigeration condition until further use.

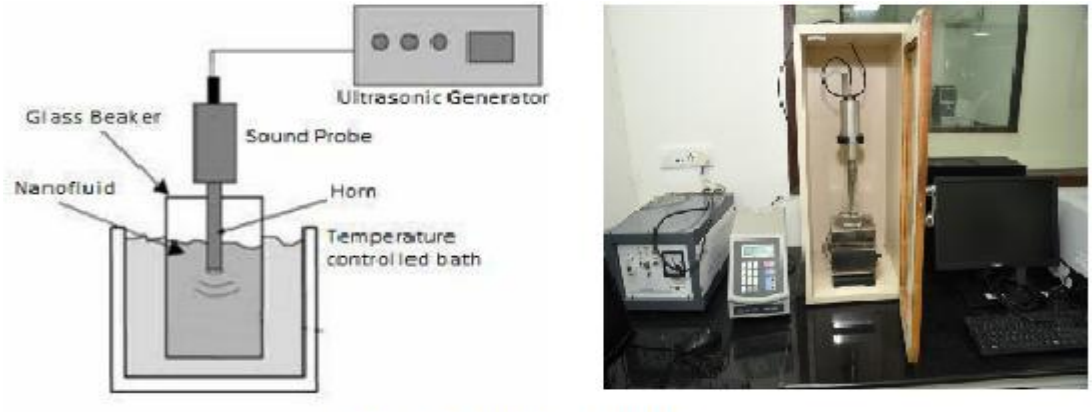


Fig 18: Schematic representation of the configuration of a Ultra Probe Sonicator

8.2. CHARACTERIZATION STUDIES

8.2.1. Particle size analysis

- ***Photon correlation spectroscopy:***

The formulated NLCs dispersions were diluted with water /suitable solvent and the sample were analyzed for particle size by photon correlation spectroscopy technique using Zeta sizer (Nano ZS 90), Malvern, UK.

- ***Zeta potential***

The prepared Nano structural Lipid particle loaded with nutraceuticals of size distribution and the charge nature was analyze using Malvern zeta seizer. The suitable dilutions of the dispersions were made using water and it was scanned under version 6.30 by using disposable sizing cuvette at the count rate of 317.5 kcps for 60 sec at the measurement position of 4.6mm at attenuator 10.

8.2.2. Entrapment efficiency:

1.5ml of the formulation was taken and subjected to centrifugation at 13000 rpm for 50 min at 4°c. The supernatant was collected and the absorbance was measured at the corresponding lamda max of 281nm.

8.2.3. Experimental Design of NLCs

In this study, a 2^3 full-factorial experimental design was used to optimize NLCs. In order to optimize, the amount of Cocoa butter, concentration of Soya Lecithin and volume of Cinnamon oil were selected as independent variables (factors). Each factor was set at a two high level and low level. Particle size, Zeta potencial, and % EE were taken as response parameters.

8.2.4. Microbial activity studies

To compare the antimicrobial activity of tetrahydrocurcumin loaded nanostructured lipid carrier was carried out by using gram negative pathogenic strains (*E.coli*) and gram positive strains (*Staphylococcus aureus*) by disc diffusion method. This both strains was prepared and swap on petriplates for growing bacterial cultures contains nutrient agar. The prepared NLCs TNC 3 and TNC 4 was added in separate sterile disc and dryed in incubate for 24 Hours. The disc containing NLCs formulation was placed in petriplate with standard as control. Then, the bacterial petriplates were incubated at 37˚C for 24 Hours. The sensitivity of test organism to each extracts were indicated by clear zone of inhibition around the disc and the diameter of the zone of inhibition was measured.

8.2.5. Phase contrast microscopy

The samples was taken on glass slide and observed under Nikon leica inverted phase contrast microscope. Images were taken on computer monitor using leica software and shape of the particles was observed.

8.2.6. Atomic Force Microscopy

A small aqueous drop of prepared THC loaded NLCs was adsorbed and dried to the surface of glass slide at room temperature. The images were examined on Multimode Scanning probe microscope (NTMDT, NTEGRA prima, Russia) in semi-contact mode with a force constant range of 0.35- 6.06 N/m and a resonating frequency range of 47- 150 KHz. The phase image and topology image were used to determine the morphology of the NLCs.

8.2.7. Scanning electron microscopy

Scanning electron microscope (SEM) is a type of electronmicroscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition. The surface characteristics of prepared NLC were examined by scanning electron microscope (SEM). The suspension was first put on clear glass stub, allowed to dry in air followed by coating with gold using Polaren E 5100 sputter coater and observed under microscope at 5.5x magnification.

8.2.8. *In vitro* drug release studies:

1 ml of NLCs formulation was taken in Franz diffusion cell apparatus. A Pig ear skin was placed in a Frans diffusion cell containing Phosphate buffer pH 7.4 solution as medium at room temperature. At various predetermined intervals i.e., 2hr, 4hr, 6hr,8hr, 24hr, 48hr. 1ml of samples was withdrawn and replaced by buffer solution, to maintain the sink conditions. Cumulative % drug release in the samples were determined by measuring the absorbance under UV visible spectrophotometry and thereby extrapolating from the calibration curves.

8.2.9. Preparation of Base Cream:

In this study, O/W emulsion was prepared by the addition of aqueous phase to the oily phase with continuous agitation. To prepare the base, an lipid phase that consisted of Cocca butter (16gm), Bees wax (1.5gm), Lanolin (2.5gm). At the same time, aqueous phase consisting of Soya Lecithin (6gm) and distilled water (50ml) was heated to the same temperature. After heating, aqueous phase was added to the lipid phase with constant stirring. Stirring was continued at 2000 rpm by the mechanical mixer for 15 min until cream was formed.

Incorporation of NLC into Base Cream

Prepared THC loaded NLC formulation (10ml) was incorporated into the 50g of Base Cream.

8.3. Texture analysis:

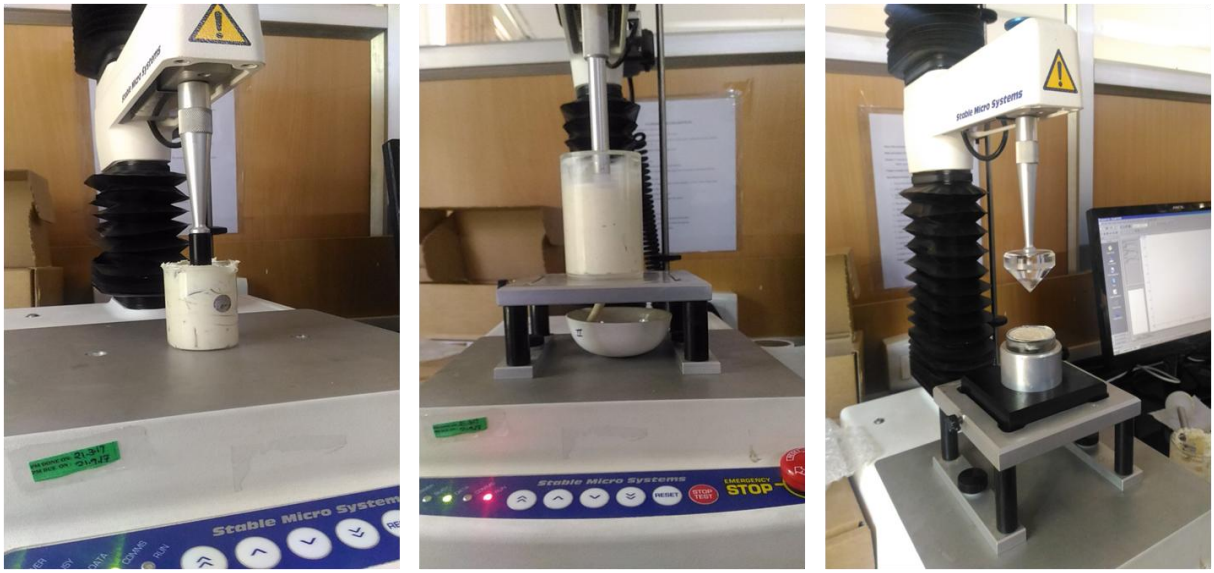


Fig 19: Texture Analysis Image for THC loaded Cream

8.3.1. Determination Of Spreadability Of Cream:

The spreadability of the cream was determined by means of Texture Analyser (TA.XT plus) equipped with 5 kg load cell using spredability rig as fixture (figure). This fixture consists of a heavy duty platform, male cone and a female cone. The Heavy duty platform was placed on the base of the machine and locked in the desiredposition by tightening the screws. An empty femalecone sample holder was placed in the base holder. The male cone probe was attached above the female cone such that the male conefits almost all the wayinto the female cone sample holder and proper care is taken to align the cones in this position. The height of the male cone was calibrated against thefemale cone so that the starting point was 25.0mm above the female cone (2 mm form the tip of the male cone and the sample). After calibration the sample was placed in the female cone sample holder and the test was run. The values of firmness (g) and work of shear (g s) were noted down by running macros.

8.3.2. Determination of Bloom Strength

The bloom strength of the cream was determined by means of Texture Analyser (TA.XT plus) equipped with 5 kg load cell using a cylindrical probe of 0.5'' diameter as fixture (figure). The sample in the container was placed centrally on the platform beneath the cylindrical probe. After calibrating the height of the probe, the test was commenced. A trigger force of 10 g was used for the study. The test results are obtaining by running the macro.

8.3.3. Extrudability of Cream:

The cream were incubated at room temperature for 2 h before measuring their extrudability using an HDP/FE forward extrusion cell of the TA-XT2 Texture Analyser equipped with a 5 kg load cell (Stable Micro Systems, UK). Prior to measurement, the cream were manually stirred and loaded (100 g) into the cell. Compression force was measured at the following conditions: pre-test speed 1 mm/s, test speed 1 mm/s, trigger force 50 g, post-test speed 10 mm/s, compression distance 20 mm, outlet diameter of extrusion cell 3 mm. The average force after reaching a plateau (at 10-15 s) was used as an indicator.

8.4. Stability studies:

Particle size analysis and entrapment efficiency studies were conducted for 3 month to evaluate the stability of the formulations. The stability studies were carried out according to ICH guidelines. The SLN formulations was stored at two different conditions i.e, 5 ± 3˚C (refrigeration conditions) and 25 ± 2˚C (room temperature).

9. RESULTS AND DISCUSSION

The profound success of lipid based formulations for highly potent, lipophilic drug molecules have gained focus in this research field from the perspective of pharmaceutical industries. To increase the success rate of these lipids based formulations, there is a need to understand the excipients role. In this line, the present study is carried out to develop nano structured lipid carrier using the selected lipid with surfactants.

9.1. FORMULATION DEVELOPMENT

- **Selection of Lipid**

Cocoa butter was selected as the lipid carrier to formulate solid lipid nanoparticles of Tetrahydrocurcumin based on the results obtained from the entrapment and stability studies of the previous study.

- **Selection of Liquid lipid**

Cinnamon oil was selected as the liquid lipid as it is of natural origin with good antibacterial activity

- **Selection of Surfactant**

Soya lecithin was selected as a surfactant as it is of natural origin.

9.2. Preparation of Nano Structured Lipid Carrier

Ultra probe sonicator were used to formulate NLC of Tetrahydrocurcumin. Totally 8 formulations were prepared using by varying the concentration of Solid lipid (Cocoa butter), one liquid lipid(Cinnamon oil), one surfactant (Soya Lecithin). The composition of the prepared NLCs was given in the table No 10.

Table 10: Formulation of Tetrahydrocurcumin loaded NLCs

S.no	Formulation Code	Drug (mg)	Lipid (mg)	Surfactant (mg)	Essential oil (ml)	Water (mg)	Remark
1	TNC 1	100	500	400	0.5	20	Good Emulsion
2	TNC 2	100	500	400	1	20	Good Emulsion
3	TNC 3	100	500	300	0.5	20	Good Emulsion
4	TNC 4	100	500	300	1	20	Good Emulsion
5	TNC 5	100	1000	400	0.5	20	Creaming on storage
6	TNC 6	100	1000	400	1	20	Creaming on storage
7	TNC 7	100	1000	300	0.5	20	Creaming on storage
8	TNC 8	100	1000	300	1	20	Creaming on storage

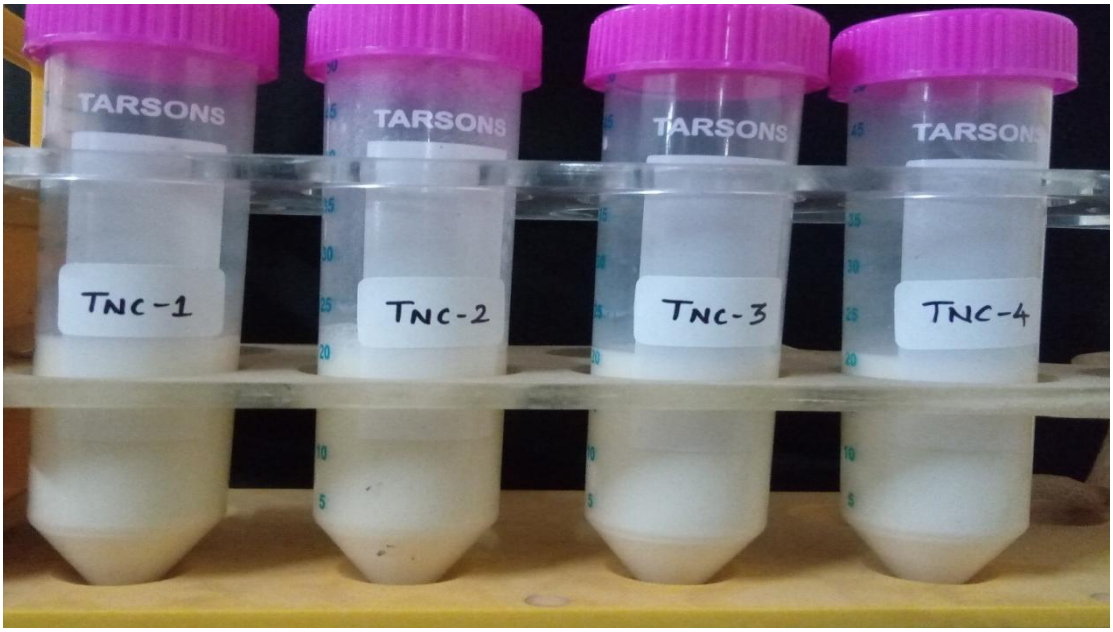


Fig 20: Formulation of THC loaded NLC (TNC 1- TNC 4)

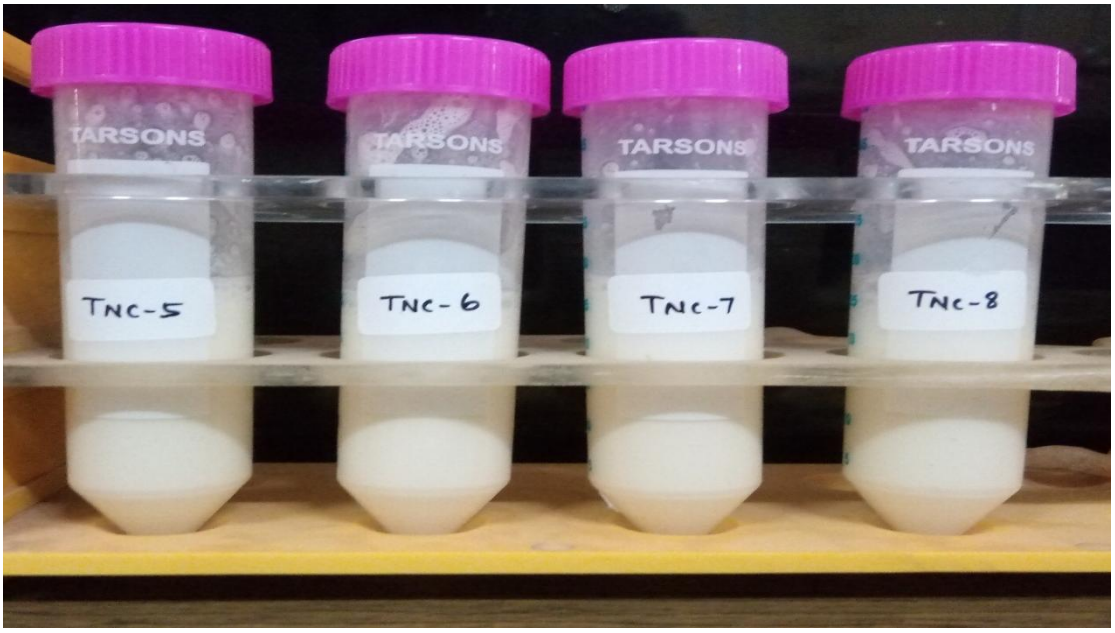


Fig 21: Formulation of THC loaded NLC (TNC 5- TNC 8)

9.3. PHYSICOCHEMICAL CHARACTERIZATION

9.3.1. Particle Size Analysis

The prepared nano structured lipid carrier was subjected to particle size analysis using Zeta sizer (nano ZS90, Malvern, UK). The formulations were sufficiently diluted with double distilled water prior to the measurement. The results showed that the particle size of prepared formulations were in the range of 50 to 190 nm with good PDI.

The results suggest that the incorporation of different surfactant showed a difference in the aggregation pattern of prepared particles. This may be due to the difference in the solubility of lipid in surfactants.

Table 11: Zeta sizer measurement results of THC loaded NLC

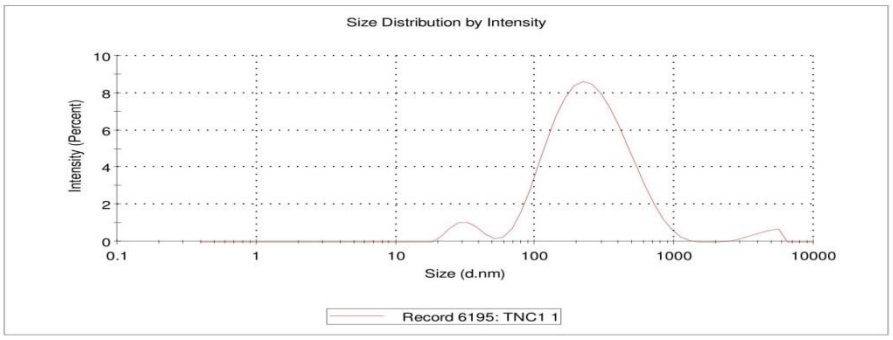
Formulation code	Particle size (nm)	Zeta potential (mV)	PDI
TNC 1	192.1	-31.6	0.325
TNC 2	136.9	-41.4	0.238
TNC 3	163.2	-31.4	0.273
TNC 4	55.9	-35.2	0.520
TNC 5	138.9	-31.6	0.305
TNC 6	83.55	-27.7	0.568
TNC 7	111.2	-33.9	0.481
TNC 8	50.85	-25.4	0.494

Fig 22: Zeta size analysis of THC loaded NLC (TNC 1)

Size distribution report by intensity

Results

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 192.1	Peak 1:	285.6	93.0	178.9
PdI: 0.325	Peak 2:	32.31	4.5	7.366
Intercept: 0.875	Peak 3:	4457	2.5	902.3
Result quality : Good				



Zeta potential report

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -31.6	Peak 1:	-31.6	100.0	7.23
Zeta Deviation (mV): 7.23	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0311	Peak 3:	0.00	0.0	0.00
Result quality : Good				

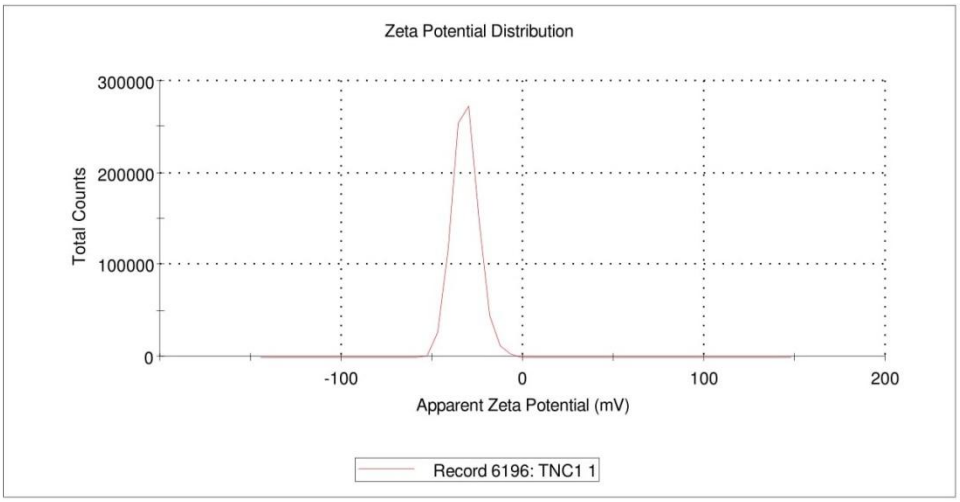
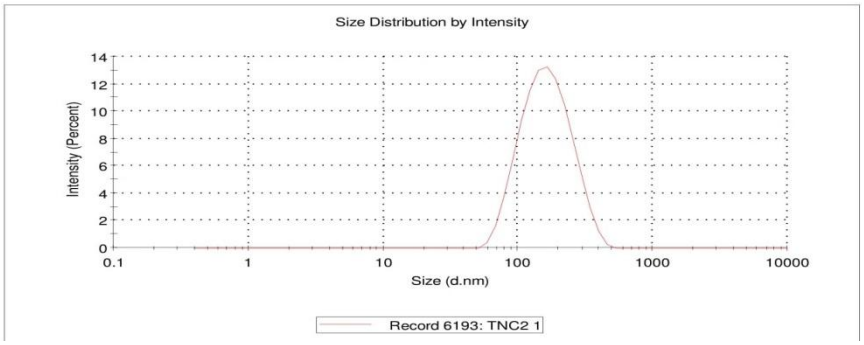


Fig 23: Zeta size analysis of THC loaded NLC (TNC 2)

Size distribution report by intensity

Results

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 136.9	Peak 1:	173.3	100.0	71.73
PdI: 0.238	Peak 2:	0.000	0.0	0.000
Intercept: 0.928	Peak 3:	0.000	0.0	0.000
Result quality : Good				



Zeta potential report

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -41.4	Peak 1:	-41.4	100.0	9.70
Zeta Deviation (mV): 9.70	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0290	Peak 3:	0.00	0.0	0.00
Result quality : Good				

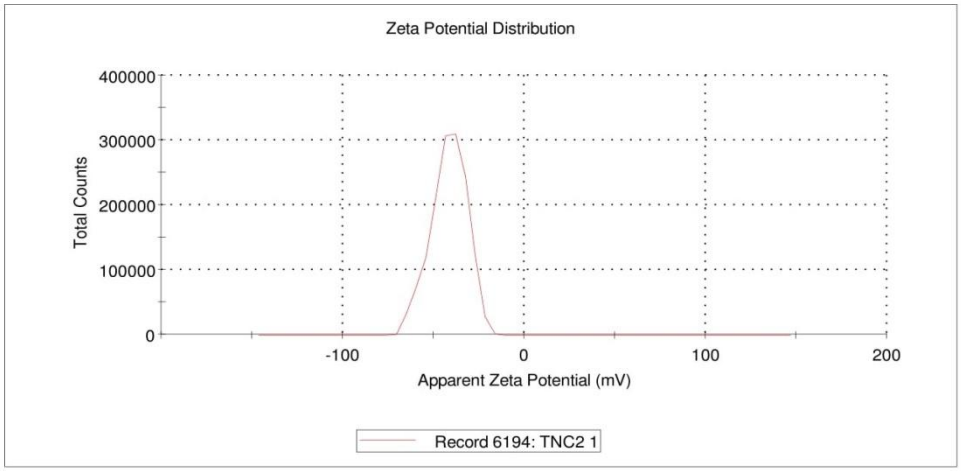
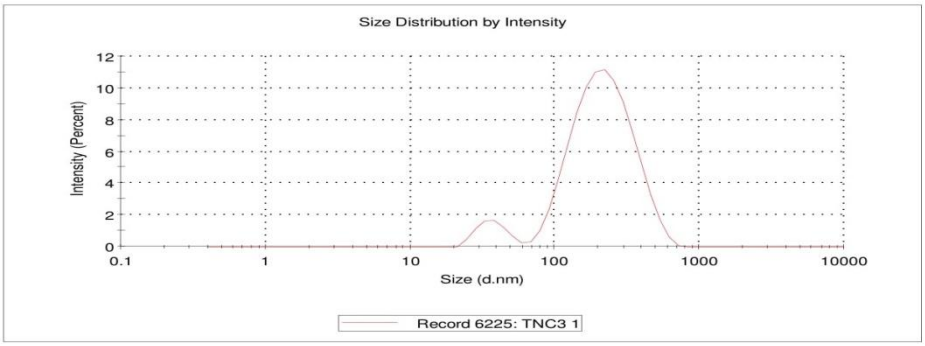


Fig 24: Zeta size analysis of THC loaded NLC (TNC 3)

Size distribution report by intensity

Results

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 163.2	Peak 1:	233.3	92.9	107.3
PdI: 0.273	Peak 2:	37.34	7.1	8.419
Intercept: 0.871	Peak 3:	0.000	0.0	0.000
Result quality : Good				



Zeta potential report

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -31.4	Peak 1:	-31.4	100.0	6.12
Zeta Deviation (mV): 6.12	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0320	Peak 3:	0.00	0.0	0.00
Result quality : Good				

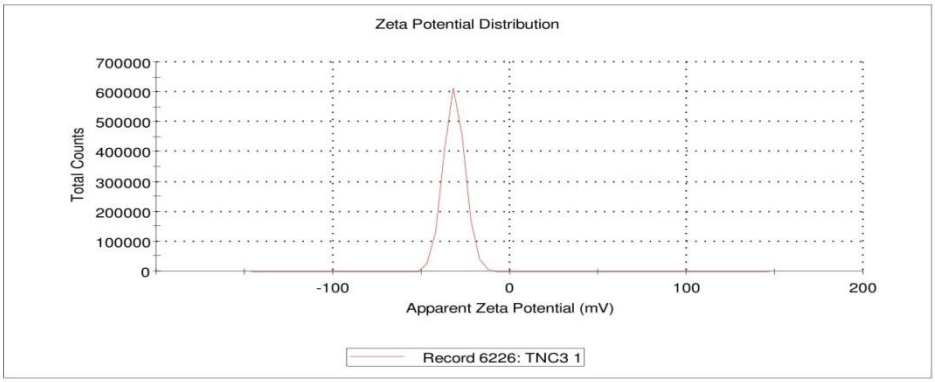
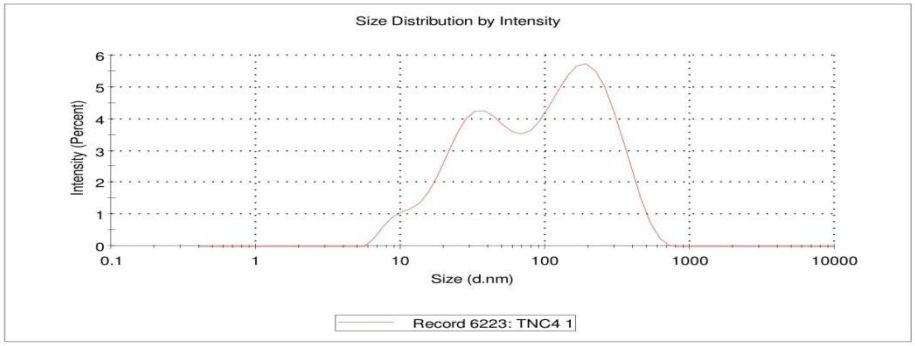


Fig 25: Zeta size analysis of THC loaded NLC (TNC 4)

Size distribution report by intensity

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	55.99	Peak 1:	196.4	58.1	108.0
PdI:	0.520	Peak 2:	34.80	41.9	17.25
Intercept:	0.720	Peak 3:	0.000	0.0	0.000
Result quality :	Good				



Zeta potential report

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-35.2	Peak 1:	-35.2	100.0	9.68
Zeta Deviation (mV):	9.68	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.0594	Peak 3:	0.00	0.0	0.00
Result quality :	Good				

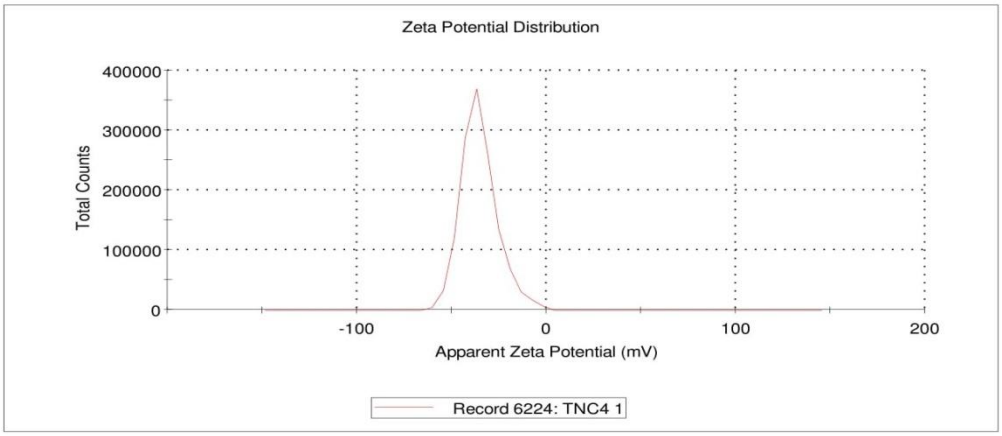
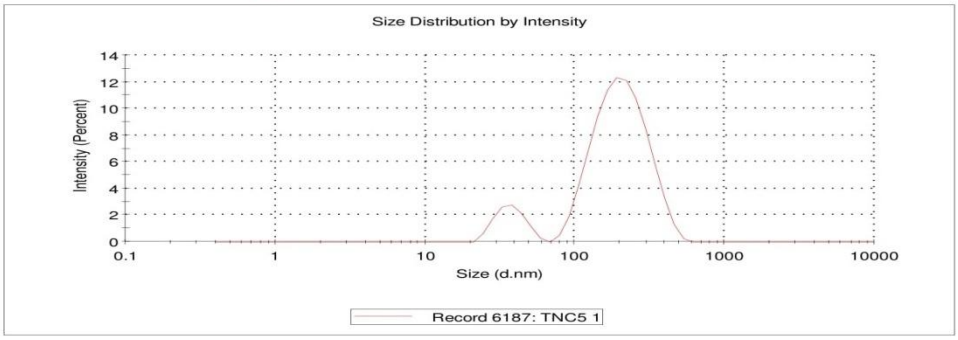


Fig 26: Zeta size analysis of THC loaded NLC (TNC 5)

Size distribution report by intensity

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	138.9	Peak 1:	214.2	88.6	83.10
PdI:	0.305	Peak 2:	37.45	11.4	8.105
Intercept:	0.857	Peak 3:	0.000	0.0	0.000
Result quality :	Good				



Zeta potential report

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-31.6	Peak 1:	-31.6	100.0	8.22
Zeta Deviation (mV):	8.22	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.0114	Peak 3:	0.00	0.0	0.00
Result quality :	Good				

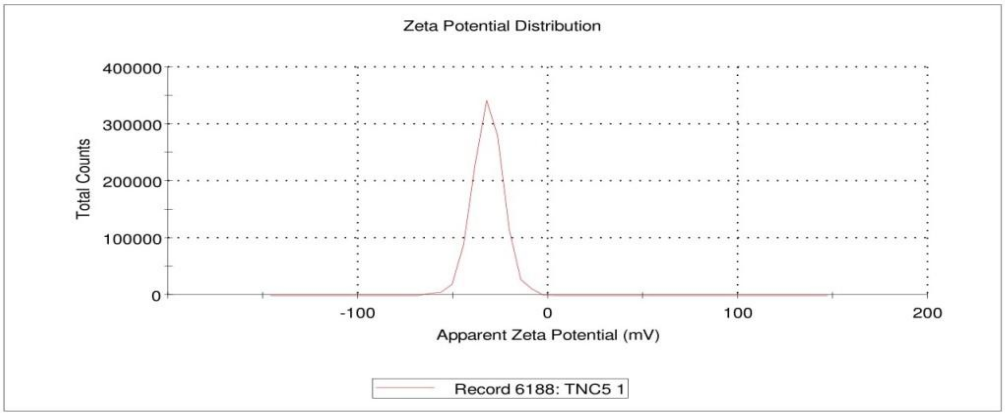
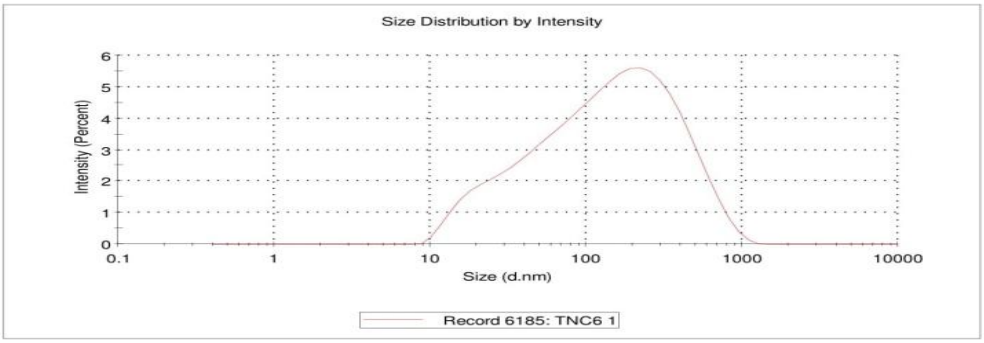


Fig 27: Zeta size analysis of THC loaded NLC (TNC 6)

Size distribution report by intensity

Results

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 83.55	Peak 1:	196.3	100.0	179.4
PdI: 0.568	Peak 2:	0.000	0.0	0.000
Intercept: 0.793	Peak 3:	0.000	0.0	0.000
Result quality : Good				



Zeta potential report

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -27.7	Peak 1:	-27.7	100.0	7.52
Zeta Deviation (mV): 7.52	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0157	Peak 3:	0.00	0.0	0.00
Result quality : Good				

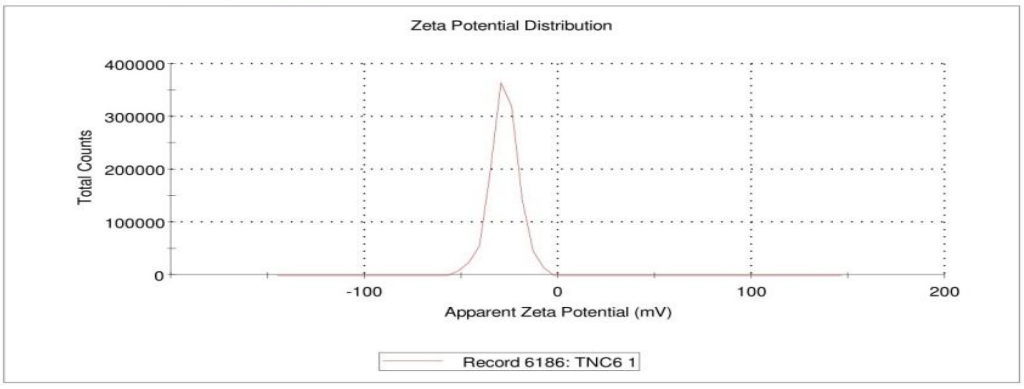
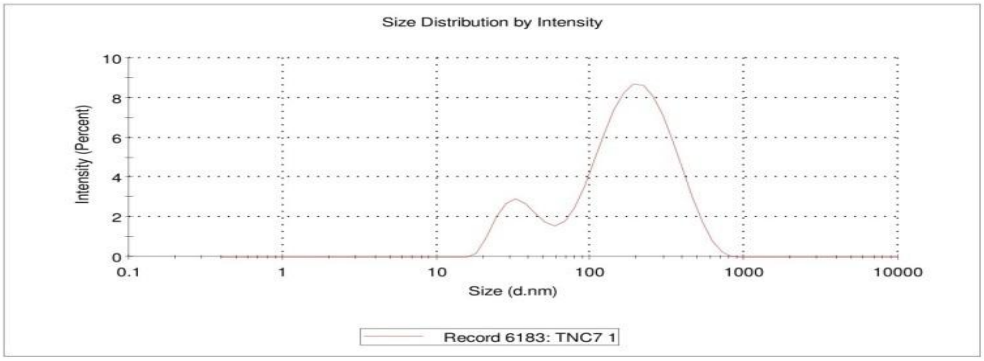


Fig 28: Zeta size analysis of THC loaded NLC (TNC 7)

Size distribution report by intensity

Results

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 111.2	Peak 1:	223.1	83.3	119.4
PdI: 0.481	Peak 2:	36.65	16.7	11.06
Intercept: 0.814	Peak 3:	0.000	0.0	0.000
Result quality : Good				



Zeta potential report

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -33.9	Peak 1:	-33.9	100.0	6.00
Zeta Deviation (mV): 6.00	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0141	Peak 3:	0.00	0.0	0.00
Result quality : Good				

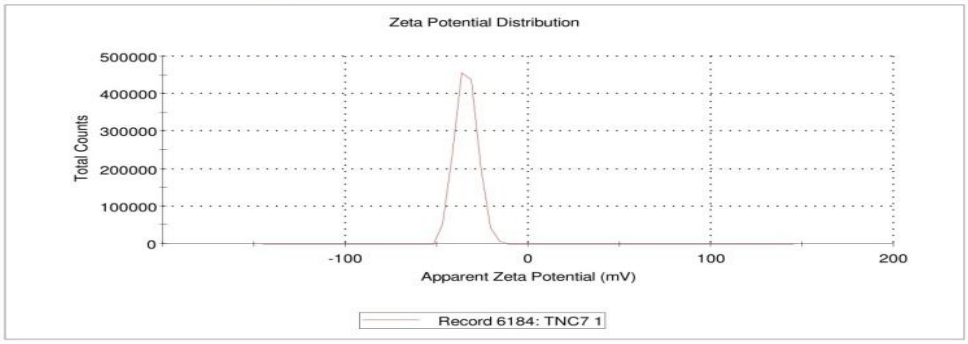
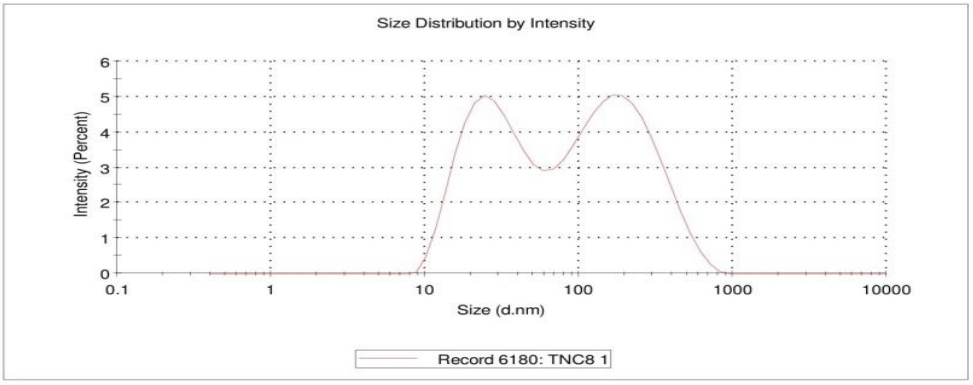


Fig 29: Zeta size analysis of THC loaded NLC (TNC 8)

Size distribution report by intensity

Results

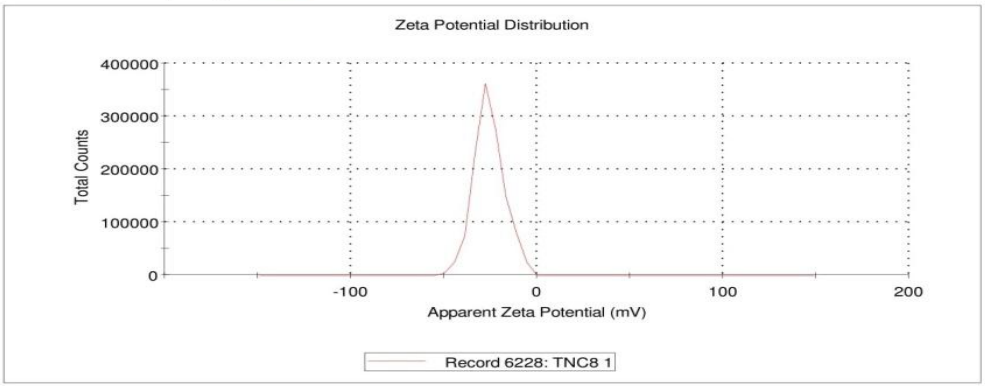
		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 50.85	Peak 1:	201.2	57.0	126.5
PdI: 0.494	Peak 2:	29.74	43.0	13.24
Intercept: 0.713	Peak 3:	0.000	0.0	0.000
Result quality : Good				



Zeta potential report

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -25.4	Peak 1:	-25.4	100.0	8.13
Zeta Deviation (mV): 8.13	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0269	Peak 3:	0.00	0.0	0.00
Result quality : Good				



9.3.2. Entrapment efficiency

The drug loading into the nanoparticles was determined by subjecting the formulations to centrifugation at 13000 rpm for 50 mins and supernatant was separated. The amount of free drug in the supernatant was measured spectrophotometrically at 281nm. A high drug loading was observed for all the formulations. Drug payload of 92.25% was observed for the formulation TNC 3. The results are depicted in table 12.

Table 12: Percentage entrapment of drug in NLC

Formulation	Entrapment Efficiency (%)
TNC 1	89.16%
TNC 2	87.79%
TNC 3	92.25%
TNC 4	90.91%
TNC 5	79.33%
TNC 6	81.11%
TNC 7	87.77%
TNC 8	88.07%

9.3.3. Experimental Design

From the results of the preliminary experiments it was predicted that the content of liquid lipid, concentration of Solid Lipid and concentration of Surfactant were the important factors affecting particle size (PS), PDI, entrapment efficiency (EE). In order to optimize the formula ingredients, a 2^3 full factorial experimental design was employed to study the effect of 3 independent variables (main factors) at 2 levels (a high level and a low level) on dependent variables (responses). A two level full factorial design contains all possible combinations between the factors and their two levels. These designs allow estimating all main factors and interaction effects between the considered factors. The details of design are as per table 1. The optimized batch was selected from among the solutions obtained for the criteria (all factors were kept in range, PS: minimum, EE: maximum) submitted in the design expert software [MiniTab software].

Table 13: Levels of independent variables

Independent variables (Factors)	Lower Level (%w/v)	Upper Level (%w/v)
Amount of Solid Lipid	500	1000
Volume of Liquid Lipid	0.5	1
Concentration of Surfactant	300	400

On the basis of defined constraints for each independent variable, the MiniTab software automatically generated the optimized formula. The experimental values were obtained by preparing 8 batches of drug loaded NLCs and simultaneously evaluating them for response parameters (PS, ZP and EE) according to the optimized formula generated by the software. Calculation of percentage prediction was done to determine the accuracy of prediction by the software and the utility of the experimental design for modifying the NLCs with desirable parameters.

Regression Equation in Uncoded Units

Size = 576.8 - 0.4733 Lipid - 903.7 Oil - 0.6910 Surfactant + 0.7530 Lipid*Oil + 0.000918 Lipid*Surfactant + 1.984 Oil*Surfactant - 0.001884 Lipid*Oil*Surfactant

Regression Equation in Uncoded Units

Zeta potential = -26.90 - 0.03620 Lipid + 12.20 Oil + 0.04700 Surfactant + 0.03240 Lipid*Oil + 0.000022 Lipid*Surfactant - 0.1480 Oil*Surfactant + 0.000056 Lipid*Oil*Surfactant

Regression Equation in Uncoded Units

Entrapment efficiency = 88.31 + 0.02892 Lipid + 3.280 Oil + 0.03800 Surfactant - 0.01156 Lipid*Oil - 0.000137 Lipid*Surfactant - 0.03080 Oil*Surfactant + 0.000060 Lipid*Oil*Surfactant

Table 14: Statistical values using 2^3 factorial design

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		88.31	*	*	*	
Lipid	0.05784	0.02892	*	*	*	500.00
Oil	6.560	3.280	*	*	*	500.00
Surfactant	0.07600	0.03800	*	*	*	100.00
Lipid*Oil	-0.02312	-0.01156	*	*	*	950.00
Lipid*Surfactant	-0.000274	-0.000137	*	*	*	590.00
Oil*Surfactant	-0.06160	-0.03080	*	*	*	590.00
Lipid*Oil*Surfactant	0.000121	0.000060	*	*	*	1031.00

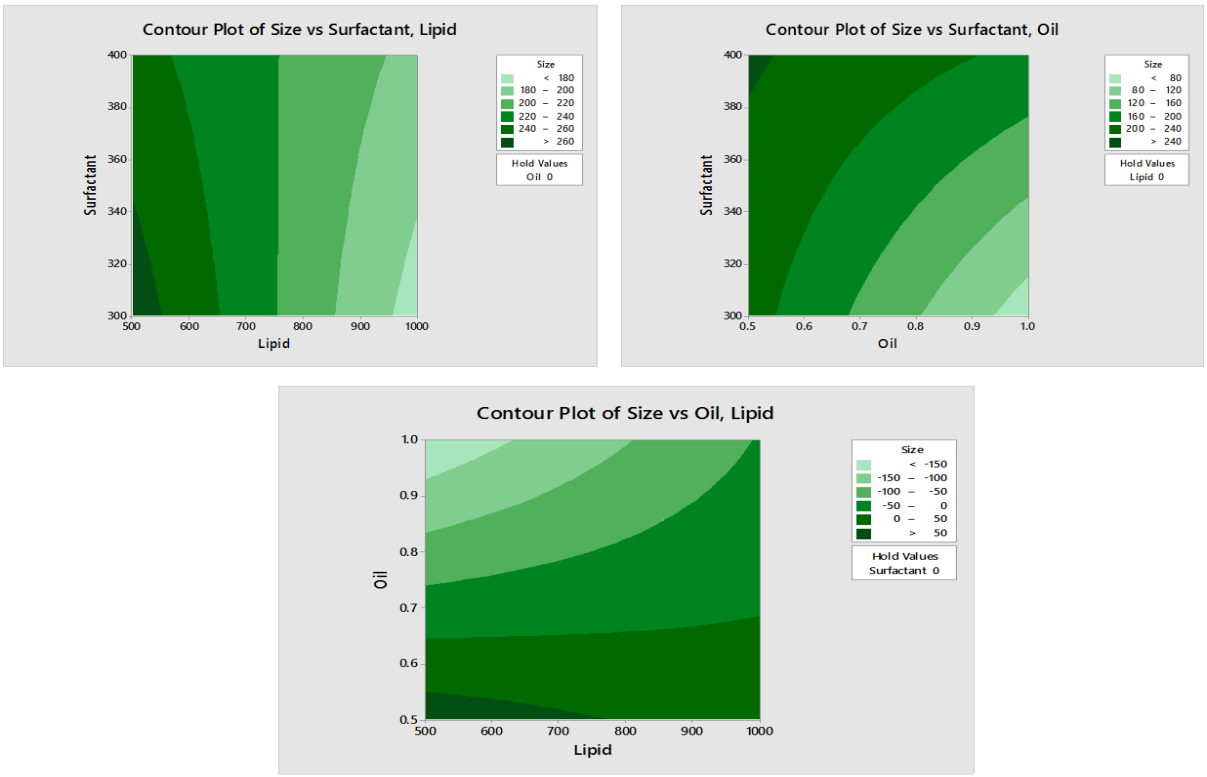


Fig 30: Influence on Experimental Design of Particle Size

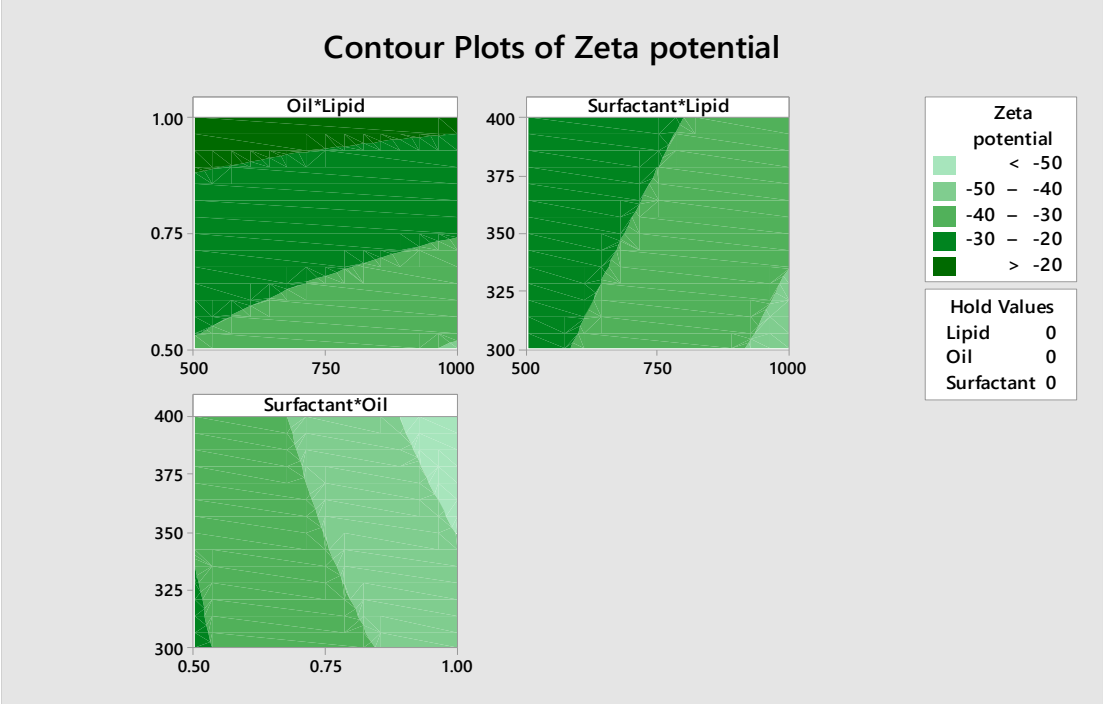


Fig 31: Influence on Experimental Design of Zeta Potencial

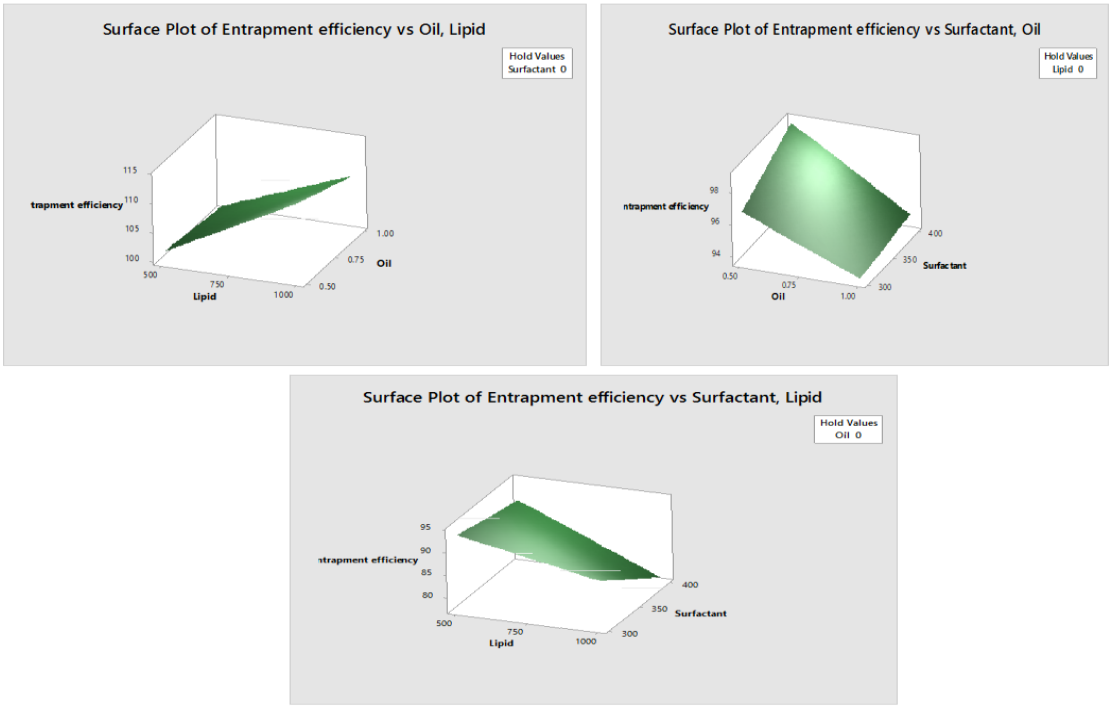


Fig 32: Influence on Experimental Design of % Entrapment Efficiency

1. Increased amount of Cocoa butter and cinnamon oil caused an decrease in particle size. The fact that the size of lipid nanoparticles is highly dependent on lipid concentration can be explained in terms of tendency of lipid to coalesce at high lipid concentration. According to Stoke's law, this behavior can be explained by difference in density between internal and external phase. Moreover, the decrease in particle size might be because increased amount of lipid provides additional space for drug molecules to entrap, thus decreasing the total surface area. As a result, least for the TNC 4 that had the minimum particle size of 59 nm.

2. On increasing the concentration of Soya lecihin, the particle size was decreased. This might be due to the surfactant-induced reduction in surface tension between aqueous phase and organic phase. In addition, surfactant helps to stabilize the newly generated surfaces and prevents particle aggregation. The % EE was decreased because of the well-known fact that the aqueous solubility of drug increases with increasing concentration of surfactant in aqueous phase. However, the percent

cumulative drug release increased because of the corresponding decrease in particle size, which in turn increased the surface area available for dissolution.

9.3.4. Microbial Activity study

The antimicrobial activity of the prepared Tetrahydrocurcumin loaded NLCs was tested against *E.coli* and *S.aureus* by disc diffusion method. The antimicrobial activity was better for TNC 4 than TNC 3 against both the bacterial strains. The antimicrobial activity of NLC was compared against Standard as control.

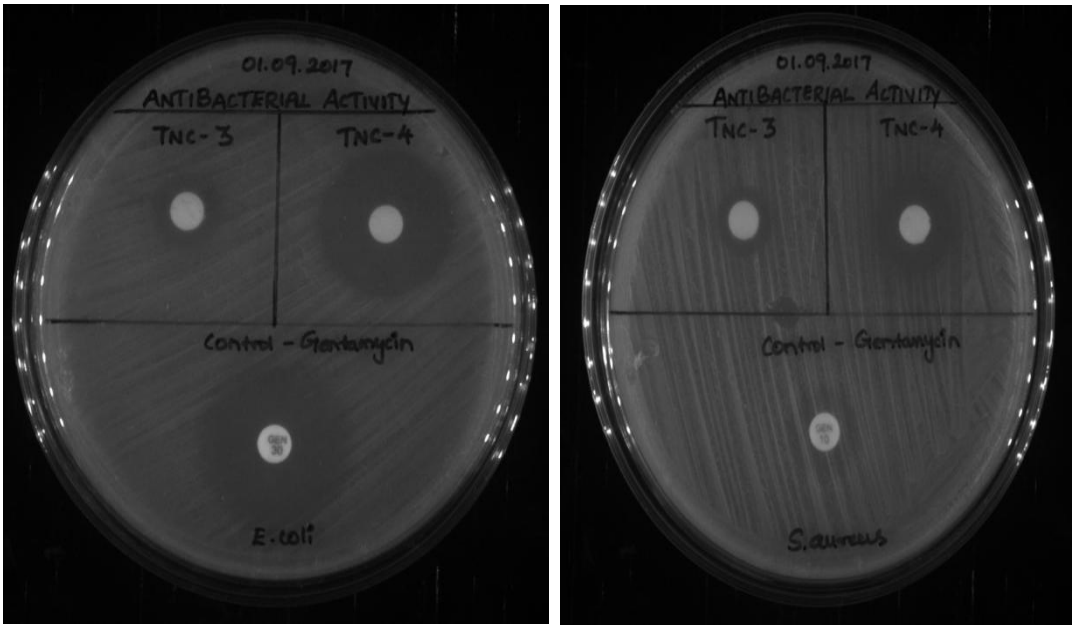


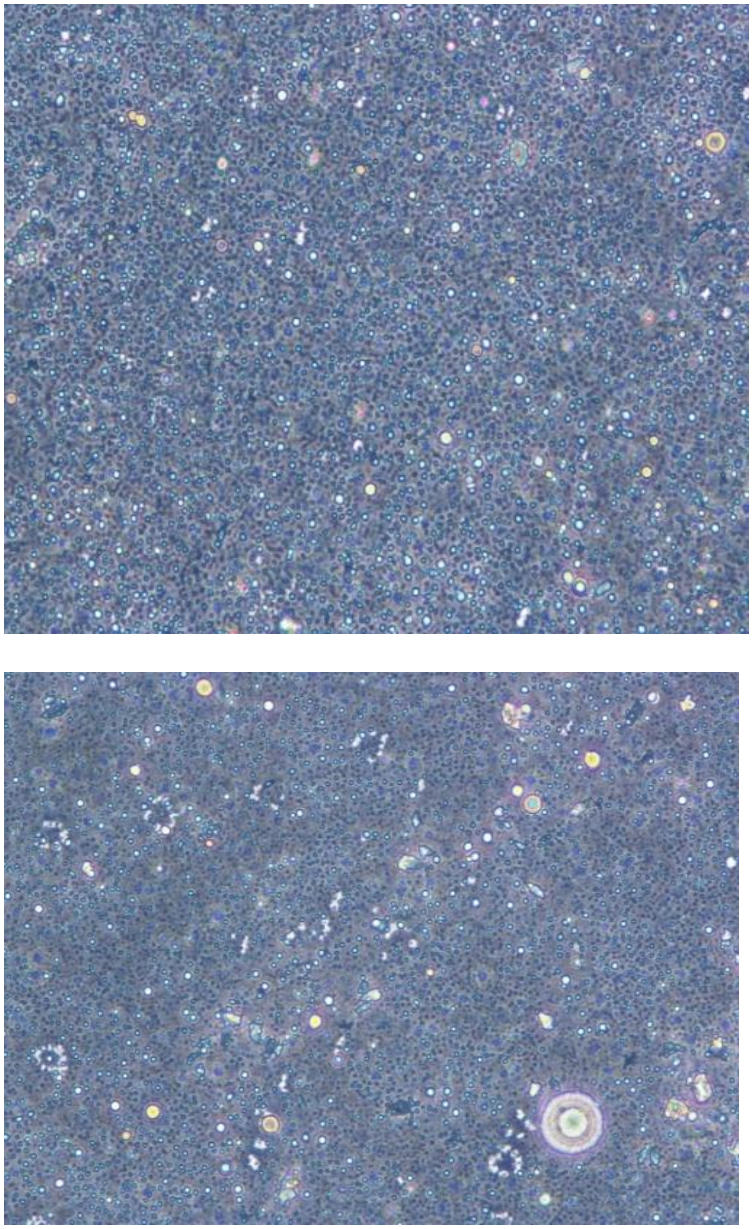
Figure 33: Anti microbial activity studies

Table 15: Measurement of zone of inhibition of anti microbial activity studies

S.no	Bacterial Strains	Zone of Inhibition(mm)		
		TNC 3	TNC 4	Control
1	E.coli	16 mm	26 mm	27 mm
2	Staphylococcus aureus	13 mm	19 mm	19 mm

9.3.5. Phase Contrast Microscopy

The Phase Contrast Microscope images for THC loaded NLC formulation, Formulation loaded cream shows particles are uniformly dispersed and it has spherical shape.



TNC 4 – 10X

Fig 34: PCM images showing the morphology of THC loaded NLC

9.3.6. SCANING ELECTRON MICROSCOPY

The surface characteristics of prepared NLC were examined by scanning electron microscope (SEM). The suspension was first put on clear glass stub, allowed to dry in air followed by coating with gold using Polaren E 5100 sputter coater and observed under microscope at different magnification.

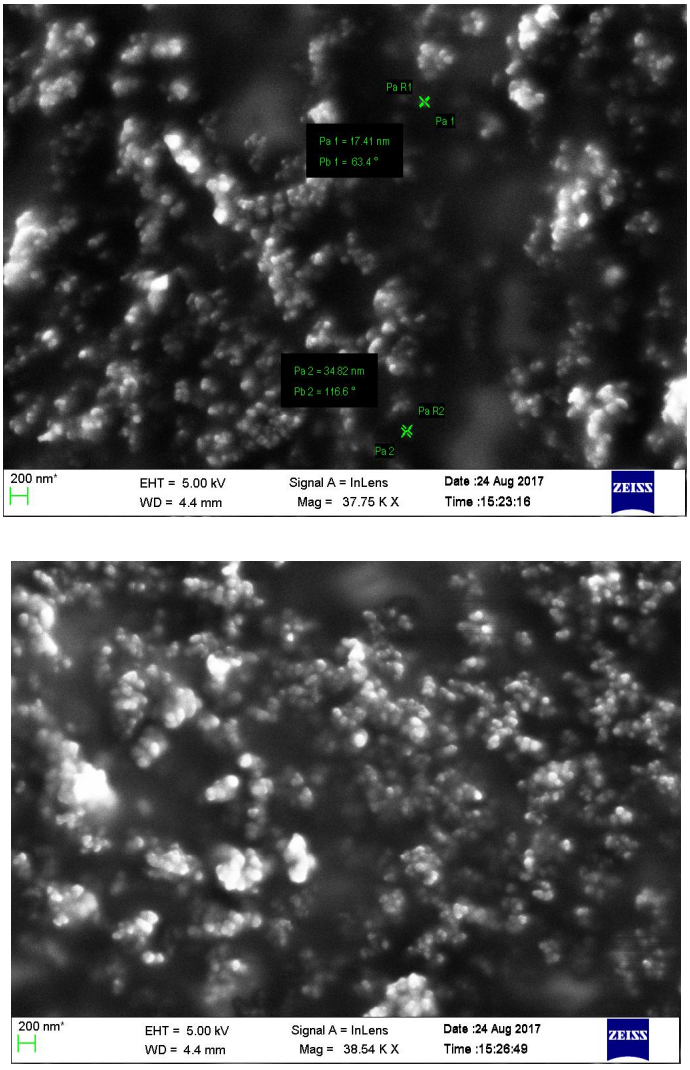


Fig 35: SEM Images of prepared NLC

Scanning electron micrographs of NLC are shown in the above Figures 35. The shape of the NLC was spherical and the size of the NLC was found within the nanometer range. Aggregation was not observed among the particles.

9.3.7. ATOMIC FORCE MICROSCOPY (AFM)

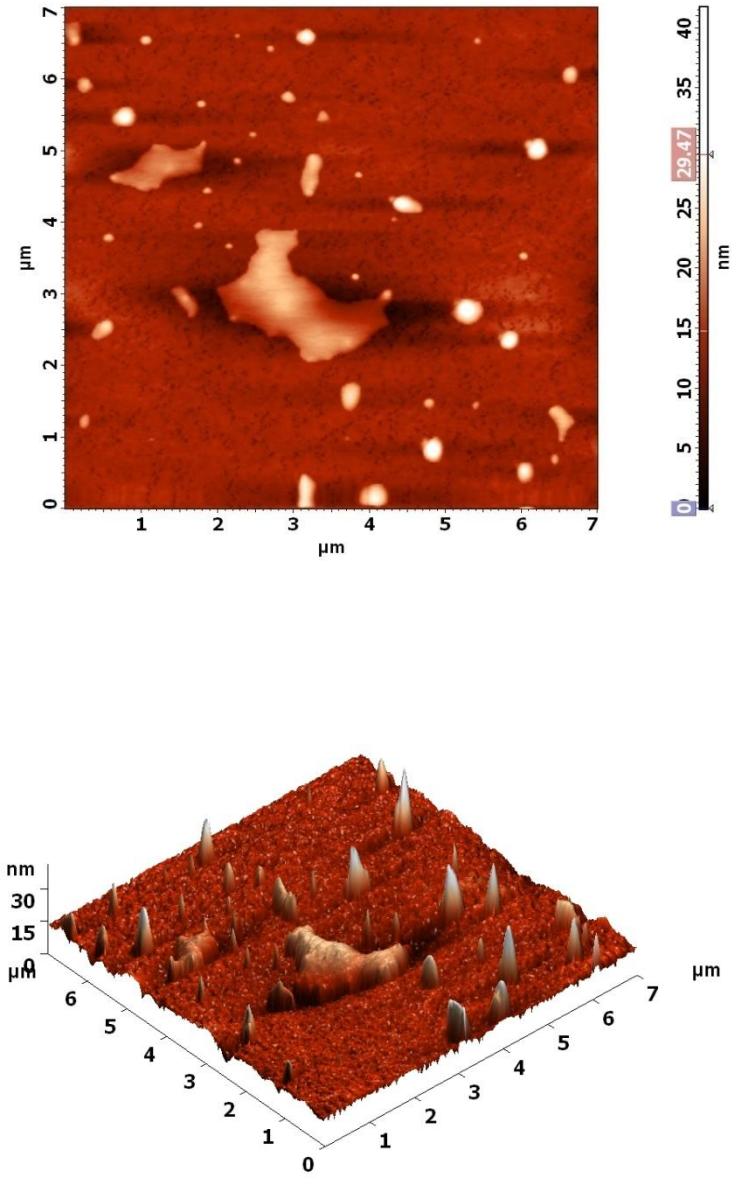


Fig 36: 2D image and 3D image of AFM analyzed particle

Atomic Force Microscopy **(**AFM) study results indicate that the prepared NLCs formulation were spherical without any aggregation.

9.3.8. *In Vitro* Drug Release:

Table 16: *In vitro* Drug Release Study

S.No	Time (hrs)	Cumulative % Drug Release
1.	2hr	0.6%
2.	4hr	4.05%
3.	6hr	8.1%
4.	8hr	11.45%
5.	24hr	57.6%
6.	48hr	96%

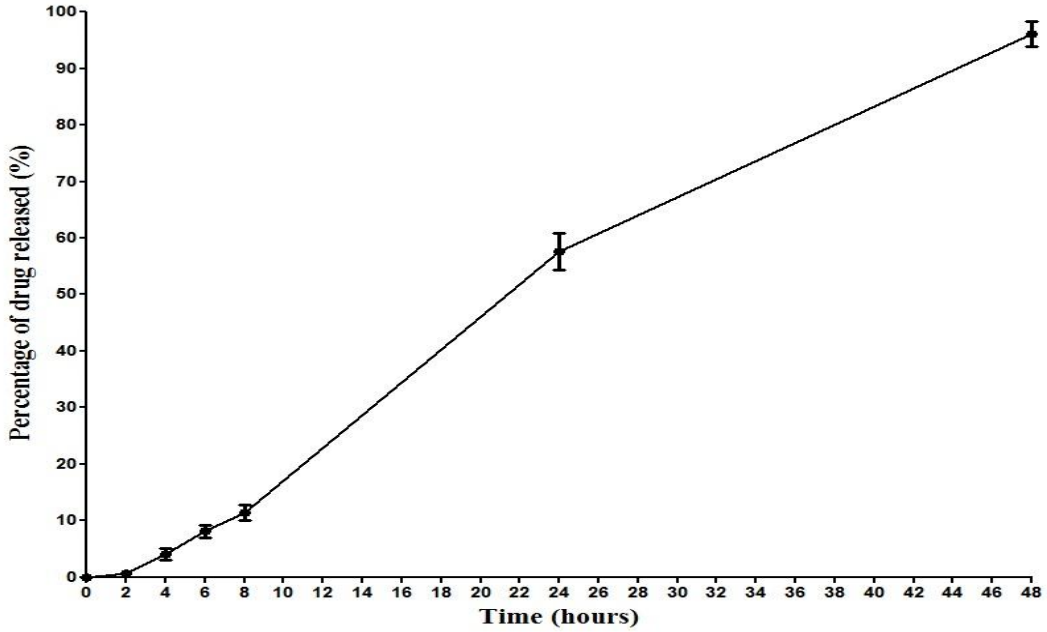


Fig 37: *In vitro* permeation study across pig ear skin

The *in vitro* release studies were carried up to 48 hours for the formulations and 96% of drug was penetrated through pig ear skin at 48hrs.

9.4. PREPARATION OF CREAM

The cream was prepared using natural excipients like Cocoa butter (lipid), Soya Lecithin (Surfactant) and emulsifying waxes/agents. The emulsifying agents like acacia, agar, tragacanth, gelatin, bees wax and lanolin has been tested. Acacia and tragacanth did not form a stable cream. Although bees wax and lanolin forms stable emulsion in the beginning, the cream showed phase separation upon storage. Cream prepared using combination of bees wax and lanolin showed good stability over 3 months.

Table 17: Preparation of cream with emulsifying agents

Lipid	Surfactant	Acacia	Tragacanth	Agar	Gelatin	Glycerine	GMS	Remarks
1gm	500mg	500mg	-	-	-	-	-	Separation
1gm	500mg	1gm	-	-	-	-	-	Separation
1gm	500mg	-	1gm	-	-	-	-	Suspension
1gm	500mg	-	1.5gm	-	-	-	-	Thicky suspension
1gm	500mg	-	-	1gm	-	-	-	Thicky solid
1gm	500mg	-	-	-	300mg	-	-	Creamy but it precipitate
1gm	500mg	-	-	-	200mg	-	-	Cream on Cooling
1gm	500mg	-	-	-	100mg	-	-	Suspension
1gm	500mg	-	-	-	100mg	5ml	-	Suspension
1gm	500mg	-	-	-	-	10ml	-	Suspension
1gm	500mg	-	-	-	-	-	500mg	Cream
3gm	1.2gm	-	-	-	-	-	1gm	Cream

Table 18: Preparation of cream with emulsifying wax as Bees wax

Lipid	**Surfactant**	**Bees wax**	**Almond Oil**	**Cinnamon oil**	**Remarks**
3gm	1.2gm	500mg	-	-	Liquid
3gm	1.2gm	700mg	-	-	Liquid
3gm	1.2gm	1gm	-	-	**Thicky Cream**
3gm	1.2gm	1gm	0.5ml	-	
3gm	1.2gm	750mg	-	-	
3gm	1.2gm	750mg	0.5ml	-	
3gm	1.2gm	750mg	1ml	-	
3gm	1.2gm	750mg	1ml	0.5ml	
3gm	1.2gm	750mg	2ml	0.5ml	
3gm	1.2gm	750mg	3ml	0.5ml	

Table 19: Preparation of cream with lanolin

Lipid	Surfactant	Lanolin	oil	Remarks
3gm	1.2gm	300mg	0.3ml	Cream on cooling
3gm	1.2gm	200mg	0.3ml	Cream on cooling
3gm	1.2gm	150mg	0.3ml	Suspension
3gm	1.2gm	300mg	-	Cream on cooling
3gm	1.2gm	500mg	-	Cream on cooling
3gm	1.2gm	1gm	-	Cream but not stable
3gm	1.2gm	1gm	0.3ml	Cream on cooling
3gm	**2gm**	1gm	-	Cream on cooling

Table 20: Preparation of cream with combination of Bees wax and lanolin

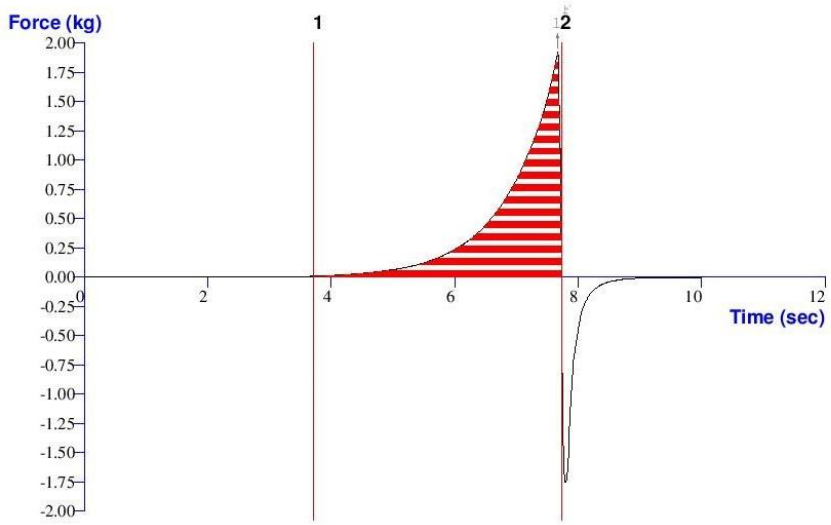
Lipid	Surfactant	Bees wax	Lanolin	Remark
3gm	1.2gm	500mg	1gm	Cream with Thicky
3gm	1.2gm	500mg	500mg	Cream with Thicky
3gm	**1.2gm**	**300mg**	**500mg**	**Cream**
3gm	1.2gm	300mg	300mg	Cream with low stabe
3gm	1.2gm	200mg	200mg	Suspension
3gm	1.2gm	250mg	250mg	Cream with low stable

The combination of bees wax with lanolin and base as lipid and surfactant have good formation of cream with no microbial growth

9.5. TEXTURE ANALYSIS

9.5.1. Spreadability:

Spreadability is the ease of which a product can be spread on skin. It is commonly a desired characteristic of ointments, gels, creams and waxes. It is related to the firmness of a product and more often than not the ease of spreading is associated with a loss in firmness. A good gel takes less time to spread and will have high spreadability. During the test the male cone approaches, penetrates and moves into the gel sample for a distance of 25mm from its start point. As the probe penetrates across the gel the force increases until a point of maximum penetration depth. This force value can be taken as the firmness at this specified depth. A firmer sample shows a correspondingly larger area that represents the total amount of force required to perform the shearing process. The probe then proceeds to withdraw from the sample. The maximum negative peak indicates the stickiness of the sample and the maximum negative area is taken as the work of adhesion. A stickier sample will require a greater force to remove the probe, yielding a larger negative area. Figure represents a typical spreadability graph of THC Cream. The firmness value obtained for THC Cream (593.330g) with force of application is (357.753g.sec).



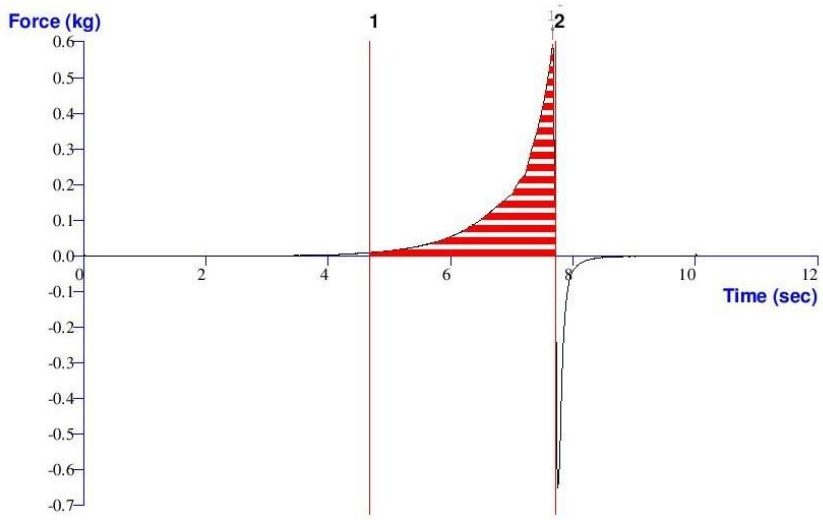


Fig 38: Spreadability plot for THC loaded Cream

9.5.2. Bloom Strength:

Bloomstrength is a measure of the ability of a colloidal dispersion to develop and retain a gel form. It is the force, expressed in grams, necessary to depress by 4 mm the surface of a gel with a standard 0.5" diameter cylinder probe. During the test when a trigger force of 10 g is attained, the probe proceeds to penetrate the cream to a depth of 4 mm. During this penetration the force drops at the point where the cream breaks. After this the resulting forces are due to continuing penetration up to the required depth. The maximum positive force (i.e. the rupture point of the gel) is taken as an indication of rupture strength. The distance that the gel penetrates before this break occurs gives an indication of the gel's elasticity, i.e. a short distance of penetration before break indicates a brittle gel whereas a large distance of penetration before rupture indicates a more elastic gel. A typical bloom strength evaluation plot of THC loaded cream is shown in figure . The rupture strength value of THC loaded cream is 51.197g.

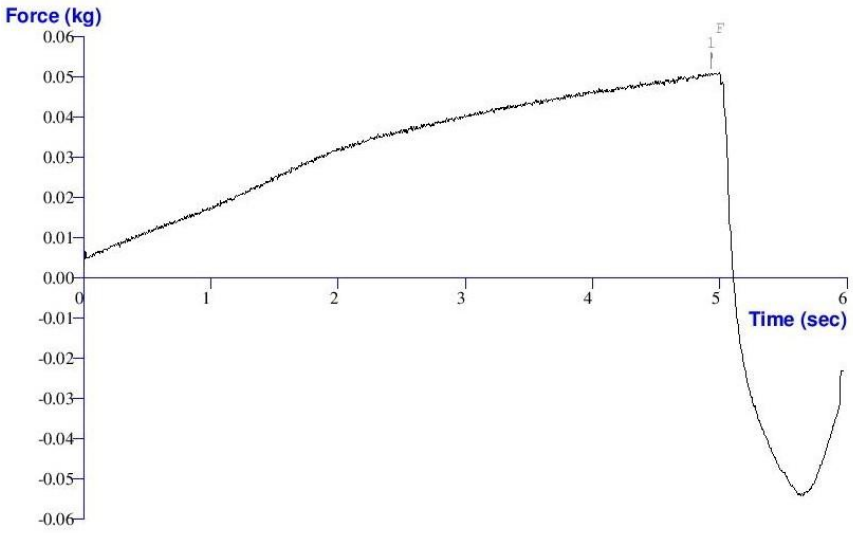


Fig 39: Bloom Strength plot for THC Loaded Cream

9.5.3. Extrudability Of Cream:

The consistency of the cream was analysed by extruding it using an HDP/FE forward extrusion cell fitted to a Texture Analyser (Stable Micro Systems, Godalming, UK). This cell measures the compression force required for a piston to extrude a product through a standard size outlet in the base of the container. The sample container can accommodate base discs with outlet diameters of 3, 5, 7 or 10 mm. The selection of the base disc depends on the consistency of the sample. The compression-extrusion test consists of applying force on a cream until it flows through an outlet that may be in the form of an annulus (hole) present in the disc at the bottom of the test cell. The tightly fitted plunger acts almost like a piston compresses the sample and causes forward flow of the cream through the annulus of the disc. The cream is compressed until the structure of the cream is disrupted and starts extruding through the outlet. The pattern of forces involved in such a test is complex. Usually the maximum force required to accomplish extrusion is measured and used as an index of texture quality.

The result in figure indicates that from 1 to 2 the sample is deformed and compressed to pack more and more tightly into the diminishing space available under the descending plunger; at this point there is little rupture of the sample. At approximately the point 2 the sample is packed solid and liquid may be expressed from it filling the interstices. At point 2 or soon afterwards the pack is solid except for small amounts of entrapped air, and the force increases steeply from 2 pressing out more liquid or air in the process. After the point 2 the sample begins to rupture and flow through the annulus (extrusion hole), and this process

continues to a point when the compressing plunger is reversed in direction and the force falls to zero. Point after 2 gives the force necessary to begin the process of extrusion, and the plateau shows the force needed to continue extrusion. It represents the increasing force being applied to an almost incompressible mixture of solids and liquid.The shape and magnitude of the compression-extrusion curve is influenced by the elasticity, viscoelasticity, viscosity, and rupture behaviour of the material; sample size, deformation rate, sample temperature, type of test cell; sample test size; and homogeneity of the sample. Preliminary tests showed that reproducible results of compression forces could be obtained using base discs with an outlet diameter of 3 mm. Lower amount of NLC in the cream gave very thick cream. The firmness of the cream in the study was 2112.834g.

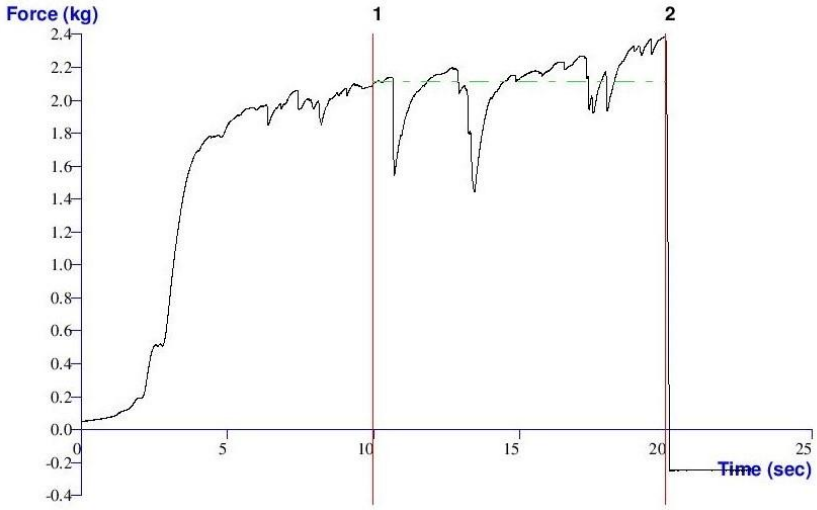


Fig 40: Extrudability of THC Loaded Cream

9.6. Stability studies

Stability studies of the prepared Tetrahydrocurcumin loaded NLCs formulations and NLCs Incorporated into cream was carried out for 3 months according to the ICH guidelines. The result showed that there were no significant changes in the cream.

Table 21: Stability studies

Formulation Code	**Particle Size (nm)**		
	1 Month	**2 Month**	**3 Month**
TNC 4 (room temperature)	79.1	83.91	96.3
TNC 4 (Refridgerator Condition)	55.1	52.2	51.9

10. SUMMARY AND CONCLUSION

Generally, lipid based formulations often show poor stability due to microbial growth. The problem of bacterial resistance to conventional preservatives has been observed in recent years. In the current study, an attempt was made to enhance the stability of THC loaded lipid based formulations for nutricosmeceutical purpose using a natural preservation system.THC loaded nanostructured lipid carriers were prepared to serve as a carrier to enhance the bioavailability of THC for nutraceutical purpose and lipid based cream loaded with NLCs of THC were prepared to serve as a carrier for the delivery of THC for cosmetic purpose.

Initially, essential oils like cinnamon oil, peppermint oil, eucalyptus oil, lavender oil, clove oil, lemon grass oil, castor oil, neem oil and their mixtures were evaluated for antimicrobial activity. The results indicated a better antimicrobial property for cinnamon oil. Hence, THC loaded nanostructured lipid carriers and the corresponding creams were prepared using cinnamon oil as liquid oil carrier. THC loaded NLC were prepared from Cocoa butter, cinnamon oil and Soya lecithin by hot homogenization technique using ultra probe sonicator. The spherical shaped nanostructure lipid particles with a particle size of 55 nm showed a sustained release pattern across pig ear skin for around 96 % for 48 hours. The antimicrobial activity was studied by disc diffusion study, TNC 4 has good activity against gram positive (*E.coli*) and gram negative (*S.aureus*) bacterial strains

These THC loaded nanostructure lipid particles were loaded in a lipid based cream (prepared from Cocoa butter, Cinnamon oil, Soya Lecithin). Evaluation of the texture properties of the lipid cream loaded with THC NLCs showed good firmness and stickiness. THC NLCs and lipid based cream loaded with THC NLCs showed good stability during the initial 3 months without any microbial contamination. Long term stability studies are in progress to evaluate the stability of the lipid based formulations for a period of 1 year.

Lipid based nutricosmeceuticals prepared using cinnamon oil as a liquid oil can be a good promising natural preservative against microbial contamination and can possibly enhance the stability of several other lipid based nutricosmeceuticals loaded with different types of drugs.

11. BIBLIOGRAPHY

- Anand P, Nair HB, Sung B, Kunnumakkara AB, Yadav VR, Tekmal RR, Aggarwal BB. Design of curcumin-loaded PLGA nanoparticles formulation with enhanced cellular uptake, and increased bioactivity in vitro and superiorbioavailability *in vivo*. Biochem Pharm 2009.
- Anna Rita Bilia, Clizia Guccione, Benedetta Isacchi, Chiara Righeschi, Fabio Firenzuoli, andMaria Camilla Bergonzi. Essential Oils Loaded in Nanosystems: A Developing Strategy for a Successful Therapeutic Approach. Volume 2014, Article ID 651593, 14 pages.
- Antonio M. Rabasco Alvarez and María Luisa González Rodríguez. Lipids in pharmaceutical and cosmetic preparations.Vol. 51. Fasc. 1-2 (2000), 74-96
- Anurag Rajvanshi1, Shalini Sharma1, Sukhbir Lal Khokra1, Ram Kumar Sahu2, Bharti Gaba, Mohammad Fazil, Saba Khan, Asgar Ali, Sanjula Baboota, Javed Ali. Nanostructured lipid carrier system for topical delivery of terbinafine hydrochloride. Cairo University (2015) 53, 147–159
- Barzegar Jalali M. Kinetic Analysis of Drug Release From Nanoparticles. J Pharm Pharm Sci 11 (1): 167-177, 2008
- Bharat B. Aggarwal, Chitra Sundaram, Nikita Malani, and Haruyo Ichikawa. Curcumin: The Indian Solid Gold 2005.
- Blasi P, Giovagnoli S, Schoubben A, Ricci M, Rossi C. Solid lipid nanoparticles for targeted brain drug delivery. Adv Drug Deliv Rev 2007; 59, 454–477.
- Bong PH. Spectral and Photophysical Behaviors of Curcumin and Curcuminoids. *Bull Korean Chem. Soc.* 2000.
- Bunjes H, Westesen K, Koch MH: Crystallization tendency and polymorphic transition in triglyceride nanoparticles. Int J Pharm 1996, 129:159–173.
- C.J.H. Porter, N. L. Trevaskis, W. N. Charman. Lipids and Lipid- based formulations: Optimizing the oral delivery of lipophilic drugs, Nature Rev. Drug Disc. 2007; 6,231-248.
- Chakraborty S, Shukla D, Mishra B, Singh S. Lipid – An emerging platform for oral delivery of drugs with poor bioavailability. Euro J Pharm and Biopharm2009; 73, 1–15.

- Cole ET, Cadé D, Benameur H. Challenges and opportunities in the encapsulationof liquid and semi-solid formulations into capsules for oral administration. Adv Drug Deliv Rev. 2008 Mar 17;60(6):747-56.
- Cream: in vitro and in vivo studies. International Journal of Nanomedicine 2011:6 611–617.
- Cui J, Yu B, Zhao Y, Zhu W, Li H, Lou H, Zhai G. Enhancement of oralabsorption of curcumin by self-microemulsifying drug delivery systems. Int J Pharm. 2009 Apr 17; 371(1-2):148-55.
- Dai WG, C P Dove, Dong LC, Li S. Advanced screening assays to rapidly identify solubility-enhancing formulations: High-throughput, miniaturization and automation. Adv Drug Deliv Rev 2008; 60, 657–672.
- Effat Sadat Farboud, Saman Ahmad Nasrollahi, Zahra Tabbakhi. Novel formulation and evaluation
- Ekta K. Kalra. Nutraceutical - Definition and Introduction. AAPS PharmSci 2003; 5 (3) Article 25 (http://www.pharmsci.org).
- Garcia-Garcia E, Andrieux K, Gil S, Couvreur P. Colloidal carriers andblood-brain barrier (BBB) translocation: a way to deliver drugs to the brain? Int J Pharm. 2005 Jul 25;298(2):274-92.
- Golovenkoand NY, Borisyuk Yu. The Biopharmaceutical Classification System-Experimental Model of Prediction of Drug Bioavailability.Biochem Supplement Series B: Biomed Chem2008; 235–244.
- Gupta NS, and Aggarwal N. Bioavailability Enhancement and Targeting of Stomach Tumors Using Gastro-Retentive Floating Drug Delivery System of Curcumin—"A Technical Note." *AAPS PharmSciTech* 2008.
- Gupta V, Aseh A,Ríos CN,Aggarwal BB, Mathur AB. Fabrication and characterization of silk fibroin-derived curcumin nanoparticles for cancer therapy. Int J Nanomed. 2009; 4, 115–122.
- Haji m. S. Khan, muhammad iqbal, akhtar rasul1 and naveed s. Bhatti. Formulation and characterization of a cream containing Extract of fenugreek seeds. Acta Poloniae Pharmaceutica ñ Drug Research, Vol. 67 No. 2 pp. 173ñ178, 2010
- Hauss DJ, Fogal SE, Ficorilli JV, Price CA, Roy T, Jayaraj AA, Keirns JJ.Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB4 inhibitor. J Pharm Sci. 1998 Feb;87(2):164-9.

- Hina Shrestha, Rajni Bala, and Sandeep Arora. Lipid-Based Drug Delivery Systems. Volume 2014, Article ID 801820, 10 pages
- Jana Pardeike, Aiman Hommoss, Rainer H. Müller. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. J. Pardeike et al. / International Journal of Pharmaceutics 366 (2009) 170–184.
- Jessie Sofia Pamudji, Rachmat Mauludin, Nasya Indriani. Development of Nanostructured Lipid Carrier Formulation Containing of Retinyl Palmitate. Int J Pharm Pharm Sci, Vol 8, Issue 2, 256-260
- Muhammad k. Waqas, naveed akhtar, mahmood ahmad, ghulam murtaza, of a Q10-loaded solid lipid nanoparticle
- Pimporn leelapornpisid*, sunee chansakaow, suvisa na-boonlong and pensak jantrawut. Development of cream containing nanostructured lipid carriers loadedmarigold (tagetes erecta linn) flowers extract for anti-wrinkles application. Int j pharm pharm sci, vol 6, issue 5, 313-314
- Rajendra Jangde3. formulation and evaluation of cyperus rotundus and cucumis sativus based herbal face cream . Pharmacologyonline 2: 1238-1244 (2011).
- S. D. Mandawgade and vandana b. Patravale. Formulation and Evaluation of Exotic Fat Based Cosmeceuticals for Skin Repair. Indian Journal of Pharmaceutical Sciences July - August 2008.
- Sanjukta duarah, kunal pujari, ramya devi durai, vedha hari b narayanan. Nanotechnology-based cosmeceuticals: a review. Int j app pharm, vol 8, issue 1, 2016, 8-12
- Shiva Golmohammadzadeh1, Mohsen Mokhtari1, Mahmoud Reza Jaafari2. Preparation, characterization and evaluation of moisturizing and UV protecting effects of topical solid lipid nanoparticles.
- Simona gherman*, daniela zavastin, adrian şpac, vasile dorneanu. Development and validation of uv spectrophotometric method for determination of enalapril maleate from commercial dosage forms. Farmacia, 2015, vol. 63, 6.
- Sin-Yeang Teow and Syed Atif Ali. Synergistic antibacterial activity of Curcumin with antibiotics against Staphylococcus aureus. Pak. J. Pharm. Sci., Vol.28 No.6, November 2015, pp.2109-2114
- Yogita R. Gonnade*, Kamlesh Niranjane, Arati Ambatkar. Lipid: an emerging platform for lipid based drug Delivery system. Volume 3, Issue 4, 572-589.