"INVESTIGATION OF ESSENTIAL OILS AS NATURAL PRESERVATION SYSTEM FOR DEVELOPMENT OF LIPID BASED NUTRICOSMETICS"

Dissertation submitted to

The Tamilnadu Dr. M.G.R. Medical University, Chennai

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MASTER OF PHARMACY

(Pharmaceutics)

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April 2017

CERTIFICATE

This is to certify that the dissertation entitled **"Investigation of Essential Oils as Natural Preservation System for Development of Lipid based Nutricosmetics"** is a bonafide work submitted by **Reg. No.261511105,** to The TamilnaduDr. M.G.R. Medical University, Chennai in partial fulfilment for **Master of Pharmacy** in **Pharmaceutics** and has been conducted under the guidance of **Dr. S.M.HabiburRahman, M.Pharm,Ph.D.,** Department of Pharmaceutics, PSG College of Pharmacy, Peelamedu, Coimbatore in the academic year of 2015-2017(April 2017)

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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
LFCS	-	Lipid Formulation Classification System
SLS	-	Sodium Lauryl Sulphate
UV	-	Ultra Violet
SEM	-	Scaning Electron Microscopy
AFM	-	Atomic Force Microscopy
PCM	-	Phase contrast Microscopy

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INTRODUCTION

LIPID BASED DRUG DELIVERY SYSTEMS

Drugs which are poorly water solubility are made well suitable for lipid-based formulation. Water insoluble and weakly basic drugs require special care in the design and development of lipid based formulation. These drugs administered in the solubilized form in the lipid vehicle may come out of the formulation due to solubilisation in the gastric fluid and may precipitate in the intestinal fluid on gastric emptying. The bioavailability of this system would depend on how rapidly the precipitates can be resolubilized by the formulation.(Sanjay Singh et al., 2009).

The percentage of new chemical entities synthesized with low aqueous solubility and high therapeutic efficacy is growing, this presents a major challenge for the drug delivery. To overcome the above challenge different methods were developed for the enhancement of bioavailability.

Lipid based formulations are more effective delivery system for oral route and improve bioavailability because of its proven safety and efficacy. Lipid Formulation Classification System was established by Pouton et al., It aims to enable *in vivo* studies for interpreting and for the identification of the most appropriate formulations for specific drugs, their physiochemical properties are taken into consideration. (Maulik Patel et al., 2011)

NANO STRUCTURED LIPID CARRIER (NLC)

A Nano structured lipid carrier (NLC) are the new generation of lipid nanoparticles, attracting major attention as novel colloidal drug carriers which is composed of physiological lipid materials suitable for topical, dermal, and transdermal administrations. To illustrate, several problems have been reported with the conventional topical preparations, e.g., low uptake due to the barrier function of the stratum corneum and unwanted absorption to the systemic circulation. Several systems which are provided in literature review can deliver active pharmaceutical ingredients across the skin presenting advantages in systemic treatment with minimal side effects, the absence of first-pass metabolism, and in topical treatment allowing targeting specific skin appendages**.** NLC has been developed to overcome the drawbacks dealing with Solid Lipid Nanoparticles (SLN). SLN is produced by replacing the oil of an

o/w emulsion by a solid lipid or a blend of solid lipid, i.e., the lipid particle matrix being solid at both room and body temperature. NLC consist mixture of solid lipid (long chain) and liquid lipid (short chain), preferably in a ratio of 70:30 up to a ratio of 99.9:0.1. The resulting matrix of the lipid particle shows a melting point depression compared to the original solid lipid, however, the matrix remains solid at body temperature. Some limitation of the SLN system regarding drug expulsion during storage, reduced particle concentration, reduced drug loading, these limitations were solved by formulating lipid particles with controlled nanostructure known as NLC. For some number of drugs, the solubility of liquid lipid is higher than that of solid lipid, which enhances drug-loading, NLC possess numerous features that are advantageous for the topical route of application. NLC are composed of physiological and biodegradable lipids that show low toxicity. The small size ensures a close contact to the stratum corneum and can increase the amount of drug penetrated into the skin. Due to the occlusive properties of lipid nanoparticles, an increased skin hydration effect is observed. Further these lipid nanoparticles are able to enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis. (shailesh patwekar et al.,2014)

ADVANTAGES OF NLCs

- Their small size and relatively narrow size distribution permits site-specific drug delivery.
- Controlled and Sustained release of active drug can be achieved.
- The incorporated drug is protected from the onslaughts of biochemical degradation.
- High drug payload.
- Incorporation of lipophilic and hydrophilic drugs feasible.
- Can be sterilized by autoclave or gamma radiation.
- Can be lyophilizes and spray dried.
- Do not generate any toxic metabolites.
- Relatively cheap and stable.
- Ease of industrial scale production by hot dispersion technique.
- Surface modification can be easily performed.

SOLID LIPID NANOPARTICLES (SLNs):

SLNs are particulate system with particle diameters ranging 50-1000nm. They are derived from oil-in-water emulsions, by replacing the liquid oil by a solid lipid. Particle size of SLN is in submicron range, ranging from 40 to 1000 nm. They have several advantages that the lipid matrix is generally made from physiologically well-tolerated lipid components, which decreases the toxicity. They have a stability of around 3 years and can easily be manufactured at industrial scales. SLNs, lipid micro particles and lipospheres have been used as alternative carriers for therapeutic peptides, proteins and antigens. Formulation as SLNs confers improved protein stability, avoids proteolysis, as well as providing sustained release of the incorporated molecules. Well-known peptides such as cyclosporine A, insulin, calcitonin and somatostatin have been incorporated into solid lipid particles. (Alka Lohani, Anurag Verma et al., 2014).

SLN PRODUCTION METHODS

There are two basic methods of SLN production: high pressure homogenization (HPH) and microemulsion technique. Also, attempts have been made to obtain those compounds using less expensive and complicated methods, such as ultrasound technique (US) and solvent cast method. Unfortunately, those methods have a number of disadvantages. (Elwira Lanson et al.,2011) The basic methods of obtaining solid lipid nanoparticles are outlined below.

High Pressure Homogenization (HPH)

HPH has proved to be an effective and reliable method of SLN production. Homogenizers of various sizes are available on the market for relatively favourable prices and HPH has been used in the production of nano-emulsions for a number of years. Contrary to other technique, HPH usually does not pose any difficulties for large-scale production. High-pressure homogenizers force the liquid through very thin orifices (a few microns in diameter) under the pressure of 100-2000 bar. On very short distances, the liquid reaches very high velocity of over 1000 km/h. High turbulence and shear disintegrate the particles to submicron sizes. The typical lipid content is 5÷10% and poses no difficulty for the homogenizer. Lipid nano-dispersion has been achieved with lipid concentration as high as 40%. HPH is further divided into hot and cold high pressure homogenization. In both methods the preparatory stage involves the introduction of active substance into the lipids through dissolution or dispersion of those substances in liquefied lipid mass.

Hot homogenization technique:

Hot homogenization is conducted at temperatures higher than lipid melting point and can thus be considered homogenization in emulsion. Pre-emulsion of the active substance, melted lipid and water phase of the emulsifier is obtained in high speed mixer. The quality of pre-emulsions largely determines the quality of the end product. The desirable particle sizes are within a few micrometres. Hot homogenization of the pre-emulsion is conducted at temperature higher than lipid melting point. In general, the higher the temperature, the smaller the particle size, caused by viscosity reduction in the internal phase. However, too high temperature may cause the active substance and the carrier to decompose. The homogenization stage can be repeated a number of times. It should be noted that homogenization under increased pressure causes the emulsion temperature to rise by approx. 10° for every 500 bar. In most cases 3 to 5 homogenization cycles under 500-1,500 bar are sufficient. The increase of homogenization pressure or the number of cycles often results in the increase in particle size due to coalescence which is the product of high kinetic energy of particles. The basis product of hot homogenization is nanoemulsion in liquid state. Solid particles are obtained by cooling the sample to room temperature or lower. Due to small particle size and the presence of emulsifiers the crystallization of lipids can take very long (up to a few months).

Cold homogenization technique:

In the cold homogenization method, the lipid microparticles are obtained by melting and subsequent cooling of drug containing lipid followed by crushing, grounding and that is diffused in cold surfactant to obtain a cold pre-suspension of micronized lipid particles. This suspension is then passes through a high pressure homogenizer at room temperature by applying 5–10 cycles at 1500 bar. This method is most suitable for hydrophilic drugs with low solubility (surfactants are added to improve solubility). This technique shortens melting process of lipid and hence it is useful for thermo-sensitive and thermo-labile drugs.

Cold homogenization has been developed in order to overcome the three fundamental problems of hot homogenization:

- Decomposition of the active substance, caused by high temperature
- Decomposition of the active substance in the water phase during homogenization
- Complexity of the crystallization stage of the nanoemulsion, leading to multiple modifications.
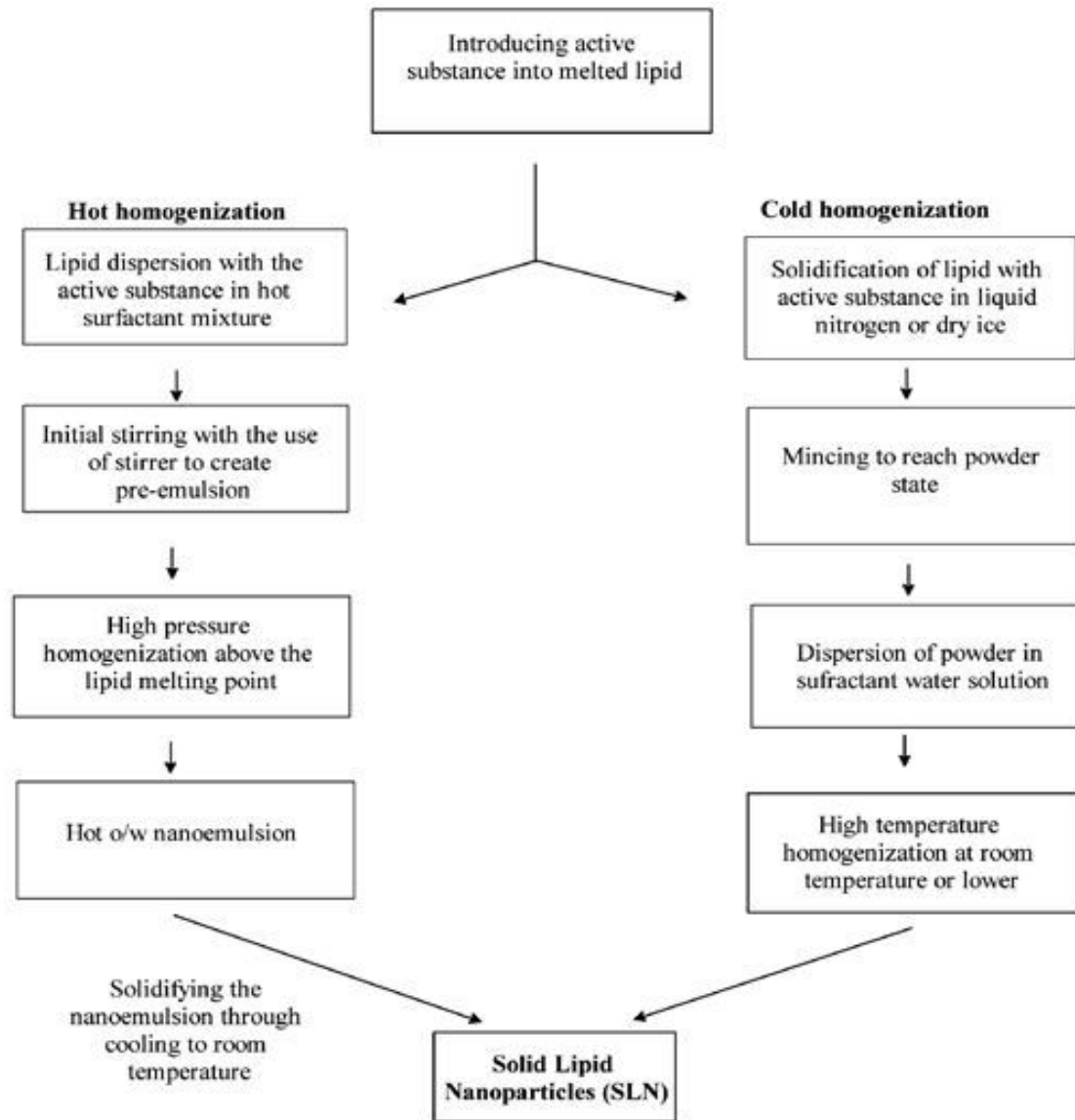
Fig 1: Schematic procedure of homogenization techniques for SLN production

Microemulsion technique

In order to obtain micro emulsion with lipids in solid state at room temperature, the process temperature must be higher than lipid melting point. Lipids (e.g. fatty acids and/or triglycerides) are melted and the mixture of water, emulsifiers and co-emulsifiers is heated to the temperature of the lipids and blended under mild conditions. If the procedure runs

correctly, we will obtain transparent, thermodynamically stable complex. The hot microemulsion is then dispersed in chilled water (2÷3°C) by smooth mechanical stirring, which ensures that the small particle size results from precipitation and not the mechanical stirring. The volume ratio of hot microemulsion to cold water should be from 1:25 to 1:50. The most popular emulsifiers are polysorbate 20, polysorbate 60 and soy lecithin. The most frequently used co-emulsifiers are usually alcohols, e.g. butanol. Technically, the precipitation of lipid particles in water is equivalent to diluting the complex, which leads to decrease in solid substance content in SLN dispersion. Due to diluting stage the achievable lipid content is lower than in formulations obtained through HPH. (Elwira Lanson et al.,2011).
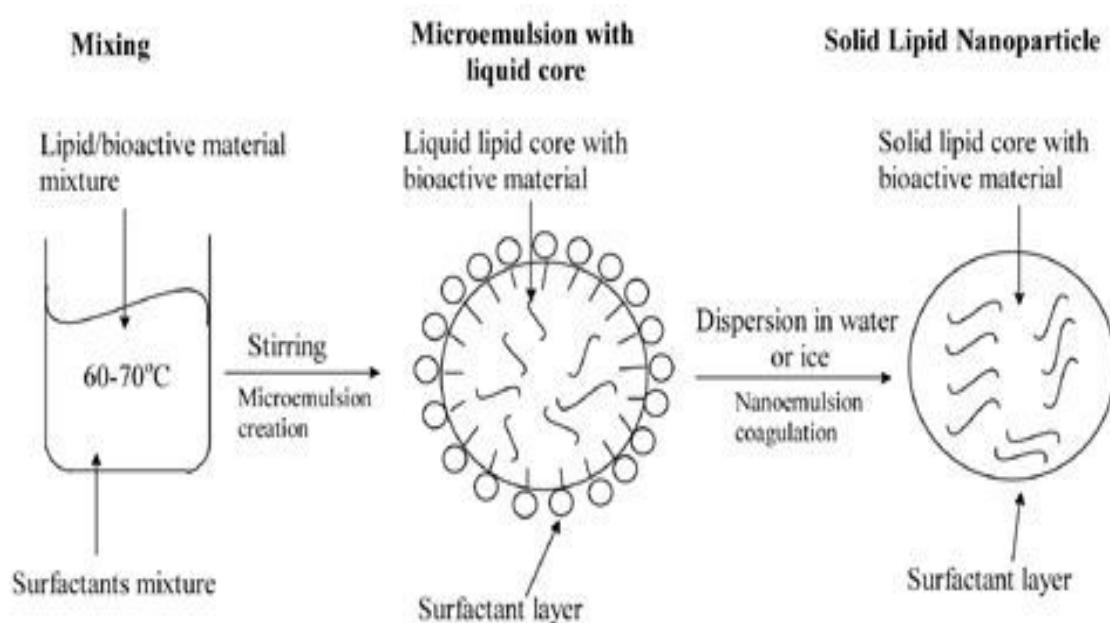
Fig 2: Schematic diagram showing the structures formed during the production of SLN by microemulsion technique

NUTRACEUTICALS

Nutraceuticals are food products that are beneficial to the health as well as helping in the prevention and treatment of disease. Few nutraceuticals are being used as pharmaceuticals and a number of others are being used by the general public as self-medication. Such products may range from dietary supplements to genetically engineered foods, herbal products and processed foods.(Swati Chaturvedi et al., 2011)

Nutraceuticals have received considerable interest because of their presumed safety and potential nutritional and therapeutic effects". The term arises from the words "Nutraceutical" "Nutrition" and "Pharmaceutical". It was coined in 1989 by Dr Stephen DeFelice, Chairman of the Foundation for Innovation in Medicine. "Nutraceutical" is a marketing term developed for nutritional supplement that is sold with the intent to treat or prevent disease and thus hasno regulatory definition. Hence a "nutraceutical" is any substance that may be considered a food or part of a food and provides medical or health benefits, encompassing, prevention and treatment of diseases. About 2000 years ago, Hippocrates correctly emphasized "Let food be your medicine and medicine your food".(A Rajasekaran et al.,2008)

ADVANTAGES OF ESSENTIAL OIL IN COSMETICEUTICAL FORMULATION

1. Enhancing the dermato-cosmetic properties and preservation, as well as the marketing image of the product.
2. At relatively high concentration in cosmetics it provide skin benefit.

NUTRICOSMETIC

Nutricosmetic means ingredients added may not only act on skin, hair, and nails but also have more systemic effect that resulting in the improved health and fitness. The important mechanism of this nutricosmetics is it mainly focusing on the carotenoid and phenolic ingredients to enhance the health and fitness by combining nutricosmetical and cosmeceutical applications, that is combined oral and topical applications and also combining nutricosmetical and physiotherapeutical interventions, that is combined oral and exercise or massage applications (Jan taeymans et al.,2014).

TETRAHYDRO CURCUMIN

Tetrahydro curcumin (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.

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. Tetrahydro curcumin is 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 (P Murugan et al., 2007).

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.

OBJECTIVE

In Recent years, Bacterial contamination changes physical and chemical properties of cosmetics usually resulting in phase separation, discoloration and release of odours etc. Rich composition of modern cosmetics in combination with aqueous formulation and direct exposure to bacterial skin flora make them an ideal environment for microbial growth. Taking into consideration the high risk of contamination and therefore a risk for consumers health, the use of preservatives is a necessity.

Preservation systems prevent and control the growth of microorganisms from contamination during manufacturing, storage or consumer use. Completely preservative-free and microbial stable cosmetics are made by sterile production and appropriate packaging. However, satisfactory results can be achieved only for some formulations and are under certain restrictions:

Preservative systems usually include various combinations of chemical biocides that operate on a broad spectrum of bacteria and fungi. They offer a high antimicrobial efficacy and therefore prolong the shelf-life of products, however, many of them can cause adverse reactions to skin.

A promising strategy to overcome these problems involves the development of suitable drug carrier systems. Nowadays, essential oils are the subject of intensive scientific research and also attract attention of cosmetic and pharmaceutical industries due to their potential therapeutic benefits as well as natural preservation effect. A new promising field of application of essential oils as natural preservatives in cosmetics or feed additives in human or animal food or as plant protection products has been studied. It is estimated that more than 3000 essential oils are of commercial importance and used in flavor and cosmetic industries. The microbial safety of cosmetics has been always of special interest for industries, as microbial spoilage can lead to product degradation and cause a risk to consumers' health.

It has become more and more evident that the development of new drugs alone is not sufficient to ensure progress in drug therapy. Exciting experimental data obtained *in vitro* are very often followed by disappointing results *in vivo* due to poor drug solubility, poor

absorption, rapid metabolism and elimination, high fluctuation of plasma levels due to unpredictable bioavailability after peroral administration.

The carriers should permit a controlled and localized release of the drug according to the specific needs of the therapy which determines the *in vivo* fate of the drug. The size of the carrier depends on the desired route of administration and ranges from few nanometres to the micrometer.

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.

Based on these facts, the aim of this work is to present current knowledge on essential oils with special focus on mechanism of antimicrobial action; assessment of their efficacy as preservatives in cosmetic formulations as well as their safety is carried out with the following objectives.

- ❖ Selection of Natural Preservatives/ Oils
- ❖ Optimization of Natural Preservatives/ Oils in various Bacterial strains.
- ❖ Determine the Minimum inhibitory Concentration of essential oils.
- ❖ The specific objective of the present work is to develop nano structured lipid carriers (NLC's) loaded with Tetrahydrocurcumin (THC) using Natural Preservatives (Natural Essential Oils)
- ❖ To optimize the developed NLC loaded with THC
- ❖ To characterize the prepared formulations

REVIEW OF LITERATURE

NANO STRUCTURED LIPID CARRIERS; POTENTIAL DRUG CARRIERS

In the pharmaceutical breakthrough today, the new technologies lead to find numerous new mighty compounds. To double-check progress in drug therapy the development of new drugs solely is not sufficient. Poor water solubility and insufficient bioavailability of the new drug substances are very widespread issues encountered. Thus, there is an expanding need to develop a pharmaceutical carrier scheme that overcomes these matters. This carrier scheme should be free of toxicity, have an adequate pharmaceutical loading capability and the possibility of pharmaceutical targeting and controlled release characteristics. The system should provide chemical and personal steadiness for the incorporated pharmaceutical. The feasibility of the production technique and as well the affordability should also be accessible.

SLN have been presented as an alternate carrier scheme to emulsions, liposomes and polymeric nanoparticles. SLN are formulated from solid lipids only. Therefore, after groundwork at smallest a part of the particles crystallizes in a higher energy modification (α or β`). Throughout storage, these modifications can transform to the low power, more organised β modification. Due to this modification high degree of alignment, the number of imperfections in the crystal lattice is small; this directs to drug expulsion .NLC have been developed to overwhelm the drawbacks affiliated with SLN. They are advised to be the second lifetime of lipid nanoparticles. Contrasted to SLN, NLC show a higher loading capability for hardworking compounds by conceiving a less organized solid lipid matrix, i.e. by blending a fluid lipid with the solid lipid, a higher element drug stacking can be achieved.

NANO TECHNOLOGY AND NANO MEDICINE

Nanotechnology is extremely valuable in the development of advanced therapeutic systems, which are termed "nanomedicines". In particular, drug delivery systems using nanoparticles area promising approach to improving the safety and bioavailability of drugs. Strongly lipophilic compounds are not generally applicable in aqueous biological systems. However, even if a drug is water insoluble, drug delivery materials permit its solubilization in the form of nanoparticle dispersions. Furthermore, targeted delivery of drugs to tissues and cells, as well as controlled release of the drug, are possible by varying the properties of these

nanoparticles. Thus, nanoparticulate drug delivery systems may lead to the development of novel therapeutic agents toward nanomedicine.

Approaches for the Development of Solid and Semi-Solid Lipid-Based Formulations

Lipid Based Drug Delivery (LBDD) has developed over the past decade fuelled by a better understanding of the multiple roles lipids may play in enhancing oral bioavailability. Moreover, the emergence of novel excipients with acceptable regulatory and safety profiles coupled with advances in formulation technologies have greatly improved the potential for successful lipid based formulations. With the growing interest in this field, there is an increasing need for guidelines in excipient selection and characterization; material handling, formulation design, and processing techniques to obtain effective and patient-compliant dosage forms. V. Jannin et al 2007 present the recent approaches in selecting the most appropriate lipid system(s); methods for characterization of their behaviour *in vitro* and *in vivo.*

Nutraceuticals

The term "Nutraceutical" was coined by combining the terms "Nutrition" and "Pharmaceutical" in 1989 by Dr Stephen DeFelice, Chairman of the Foundation for Innovation in Medicine. About 2000 years ago, Hippocrates correctly emphasized "Let food be your medicine and medicine be your food". Currently there is an increased global interest due to the recognition that "nutraceuticals" play a major role in health enhancement. Presently over 470 nutraceutical and functional food products are available with documented health benefits. "Nutraceuticals and functional foods have received considerable interest because of their presumed safety and potential nutritional and therapeutic effects" A Rajasekaran et al., (2008). Nutraceuticals have been claimed to have a physiological benefit or provide protection against the following diseases (and/or found to act as)

- Cardiovascular agents
- Antiobese agents
- Antidiabetics
- Anticancer agents
- Immune boosters
- Chronic inflammatory disorders
- Degenerative diseases

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Nano Structured lipid carriers (NLC)

Solid lipid nanoparticle (SLN) and nanostructured lipid carrier (NLC) are colloidal lipid systems, which have been proposed for several administration routes, such as parenteral, oral and topical route providing controlled release profile of many substances. Solid lipid nanoparticle (SLN) has the chance to be exploited as a delivery system in commercial products. However, there are some limitations of the solid lipid nanoparticles (SLN) system: Drug expulsion phenomenon when lipid crystallizes to the stable β-form, particle concentration in the aqueous dispersions ranging from about 1% to a maximum of only 30% and limitation of drug load by the solubility of the drug in the solid lipid(A.C. Silva, Aakanchha Jain et al.,2011). These limitations were solved by creating a lipid particle with a controlled nanostructure i.e the nano structured lipid carrier (NLC).

SLN consists of pure solid lipids and NLC contains a certain percentage of additional liquid lipids leading to imperfections in the crystal lattice. These nanoparticles are produced by one of the following techniques, namely, high pressure homogenization, microemulsion template, cold homogenization, solvent emulsification, solvent diffusion, reverse micelle-double emulsion, homogenization followed by ultrasonication, solvent injection and a very recently introduced membrane contractor techniques(Amichand Dairam et al.,2008)

The suitability of solid lipid nanoparticles (SLN) for the encapsulation of lipophilic drug was assessed for oral 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)

Nanotechnology is an enable technology that has the potential to revolutionize drug and food systems. Nanotechnology have shown enhanced oral bioavailability and biological efficacies of different phytochemicals (HuangQ et al. 2010)

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.

ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS AND POTENTIAL APPLICATIONS IN FOOD

Essential oils are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production of EOs (Helander .I M et al.,1998). The term *'essential oil'* is thought to derive from the name coined in the 16th century by the Swiss reformer of medicine. An estimated 3000 EOs are known, of which about 300 are commercially important – destined chiefly for the flavours and fragrances market. It has long been recognised that some EOs have antibacterial properties and these have been reviewed in the past that spices have antibacterial properties. Besides antibacterial properties, EOs or their components have been shown to exhibit antiviral, antimycotic, antitoxigenic, antiparasitic, and insecticidal properties.

The greatest use of EOs in the European Union (EU) is in food (as flavourings), perfumes (fragrances and aftershaves) and pharmaceuticals (for their functional properties). The well-known use of EO in aromatherapy constitutes little more than 2% of the total market. Individual components of EOs are also used as flavourings, either extracted from plant material or synthetically manufactured. The antibacterial properties of essential oils and their

components are exploited in such diverse commercial products as dental root canal sealers, antiseptics and feed supplements for lactating sows and weaned piglets. A few food preservatives containing Eos (Sara Ann Burt et al.,2007).

Recently there has preservative and antimicrobial role of spices has been an increasing interest in discovering new natural prevention of meats. Bio preservatives include a range of natural plants, animals and microorganisms which can be used to improve the keeping quality of foods. Being plant natural food stuffs, spices appear to be an alternative for the chemical antimicrobials to the consumers who tend to question their safety. Spices active compounds have been included in class of naturally occurring food preservatives and their inclusion in foods allowed by food production regulatory offices. Although, spices have been well known for their medicinal, preservative and antioxidant properties, currently they have been used with primary purpose of enhancing the flavour of foods rather than extending shelf-life. In recent years antimicrobial properties of spices have been documented and interest continued to the present. There is little information available emphasizing the preservative and antimicrobial role of spices in the prevention of meat (A. Jagadeesh Babu et al.,2011)

ESSENTIAL OILS AS NATURAL COSMETIC PRESERVATIVES

Nowadays, safety of chemical preservatives has been questioned by a big number of consumers. Traditionally used preservatives often cause skin irritation and lead to allergenic reactions. 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. Formulations containing both types of preservatives: essential oil and a synthetic one have been tested and proposed as a compromise that allows for reducing concentration of both components due to their synergistic activity. Although most essential oils are regarded as safe, some of them may cause risk of contact allergy or phototoxic reaction. (Mariola Dreger, Karolina wiergus et al.,2013).

EOs are obtained using several techniques:- water or steam distillation, solvent extraction, expression under pressure, supercritical fluid or subcritical water extractions.

Nowadays, essential oils are the subject of intensive scientific research and also attract attention of cosmetic and pharmaceutical industries due to their potential as active pharmacological compounds or natural preservatives. Enormous diversity of this group of natural compounds and wide spectrum of biological properties make them attractive for many industries and new areas of application still has not been discovered. Regardless from sensory properties of essential oils, antimicrobial and antifungal activities are the goal of research. A new promising field of application of essential oils as natural preservatives in cosmetics or feed additives in human or animal food or as plant protection products has been studied. It is estimated that more than 3000 essential oils are of commercial importance and used in flavour and cosmetic industries. Trade of the most popular oils such as eucalyptus or lemon ones was calculated at over 1000 metric tons a year and their estimated value is several hundred million Euros.

The microbial safety of cosmetics has been always of special interest for industries, as microbial spoilage can lead to product degradation and cause a risk for consumers health. Rich composition of modern cosmetics in combination with aqueous formulation and direct exposure to bacterial skin flora make them an ideal environment for growing of microorganisms. Taking into consideration the high risk of contamination and therefore a risk for consumers health, the use of preservatives is a necessity. Moreover, bacterial contamination changes physical and chemical properties of cosmetics usually resulting in phase separation, discoloration and release of odours etc. Preservation systems prevent and control the growth of microorganisms from contamination during manufacturing, storage or consumer use (Mariola Dreger, Karolina wiergus et al.,2013).

MECHANISM OF ANTIMICROBIAL ACTION OF ESSENTIAL OILS

Antimicrobial activities of EOs are well known and documented in numerous works. They are effective against both saprophytic bacteria and fungi, which are main source of cosmetic contaminations (*Bacillus* sp., *Micrococcus* sp., *Aereomonassp.*, *Acinetobacter* sp. and *Aspergillus* sp. or *Penicillium* sp.) and also against human pathogens (*Staphylococcus* sp.,

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Streptococcus sp., *Salmonella* sp. or *Candida* sp. And others). In contrast to antimicrobial activity of EOs mechanisms of their action are still not fully understood and need elucidation. In general, antimicrobial activity of essential oils is determined by their composition and concentration of components. The number of constituents in essential oil can range from several up to far more than 100. Composition and proportion of compounds varies and depends on chemotype, age of a plant, climatic and environmental conditions as well as harvest time and the distillation method. Essential oils are a complex and diverse group of natural compounds that usually consist of terpenes with terpenoids, and also aromatic and aliphatic compounds of low molecular weight. Monoterpenes are the most commonly found molecules constituting 90% of essential oils in a great variety of structures. Aromatic compounds are represented less frequently, usually in trace amounts. EOs composition is often characterized by two or three main compounds at higher concentrations (20–70%), which determine biological properties of essential oil.

EFFICACY OF ESSENTIAL OILS AS PRESERVATIVES IN COSMETIC FORMULATIONS

Efficacy of preservatives in cosmetic formulations is evaluated in a challenge test according to the European Pharmacopoeia guidelines. The challenge test is a standard procedure that involves artificial contamination of cosmetics with predetermined number of bacteria and fungi (105-106 viable cells ml-1 or g-1 of product) as well as periodic removal of samples at fixed time for counting of viable microorganisms present in the formulation during test. Microorganisms used in the challenge test include strains of bacteria: *Staphyllococcus aureus*, *Pseudomonasaeruginosa*, *Escherichia coli* and fungi: *Aspergillus niger* and *Candida albicans*. According to EP, a topical preparation is well preserved if the number of the bacteria recovered per gram is reduced by a factor of 103 (criteria A) and 102 (criteria B) within 2 days of the challenge test with no cell proliferation at 7th day up to the 28th day (Mariola Dreger, Karolina wiergus et al.,2013)..

PLAN OF WORK

- Preformulation Studies

  - Preparation of Calibration Curve for Tetrahydrocurcumin (THC) by UV Visible Spectrophotometric Analysis

  - IR Spectroscopic Analysis

  - Selection of Natural Preservatives/ Oils

  - Optimization of Natural Preservatives/ Oils in various Bacterial strains.

  - Determine the Minimum inhibitory Concentration of essential oils.

- Formulation Development

  - Formulation of nano structured lipid carrier using Ultra probe sonication technology

  - Formulation of Base Cream and Carbopol Gel

  - Incorporation of NLC into Base Cream and Carbopol Gel

- Characterization studies of the NLCs prepared

  - Particle Size Determination by Zeta sizer

  - Atomic Force Microscopy(AFM)

  - Scanning Electron Microscopy(SEM)

  - Phase Contrast Microscopy(PCM)

  - *In vitro* skin penetration

  - Texture Analysis

  - Stability Studies as per ICH guidelines

MATERIALS USED

Table 1:

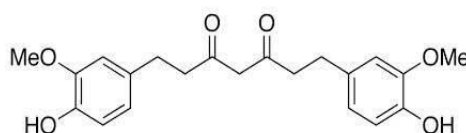| SL.NO.	MATERIALS	SOURCE
1.	Tetra Hydro Curcumin	Sami labs, Bangalore
2.	Stearic Acid	Loba Chemie Pvt. Ltd., Mumbai
3.	poloxamer	Himedia lab., mumbai
4.	Polyethylene Glycol 400	Himedia Lab., Mumbai
5.	Glyceryl mono stearate	Loba Chemie Pvt. Ltd., Mumbai
6.	Tween 80	Loba Chemie Pvt. Ltd., Mumbai
7.	Cinnamon oil pure	Sangrose Laboratories Pvt. Ltd., Mavelikara
8.	Methanol	Himedia Lab., Mumbai
9.	Sodium Lauryl Sulphate	Sangrose Laboratories Pvt. Ltd., Mavelikara
10.	Ceto stearyl alcohol	Loba Chemie Pvt. Ltd., Mumbai
11.	Cetyl alcohol	Loba Chemie Pvt. Ltd., Mumbai
12.	Myristic acid	Loba Chemie Pvt. Ltd., Mumbai
13.	Palmitic acid	Himedia Lab., Mumbai
14.	Distilled Water	Himedia Lab., Mumbai
15.	Muller Hinton Agar	Himedia Lab., Mumbai

EQUIPMENTS USED

Table 2:

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

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	:	
Molecular weight	:	372.41 Da
Physical state	:	Solid
Melting point	:	95-97°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.

EXCIPIENTS PROFILE

STEARIC ACID

Synonyms	:	Acidum stearicum; cetylacetic acid
Chemical Name	:	Octadecanoic acid
Empirical Formula	:	$C_{18}H_{36}O_2$
Structural Formula	:	

Molecular Weight	:	284.47 (pure material)

The USP32–NF27 describes stearic acid as a mixture of stearic acid ($C_{18}H_{36}O_2$) and palmitic acid ($C_{16}H_{32}O_2$).

Functional Category	:	Emulsifying agent; solubilising agent; tablet and capsule lubricant.
Applications	:	Widely used in oral and topical pharmaceutical formulations; mainly used in oral formulations as a tablet and capsule lubricant; may be used in enteric tablet coatings and as a sustained-release drug carrier; In topical formulations, stearic acid is used as an emulsifying and solubilising agent; the partially neutralized stearic acid forms a creamy base when mixed with 5–15 times its own weight of aqueous liquid; also widely used in cosmetics and food products.
Description	:	A hard, white or faintly yellow-colored, somewhat glossy, crystalline solid or a white or yellowish white powder; has a slight odour (with an odour threshold of 20 ppm) and taste suggesting tallow.
Melting point	:	69–70°C

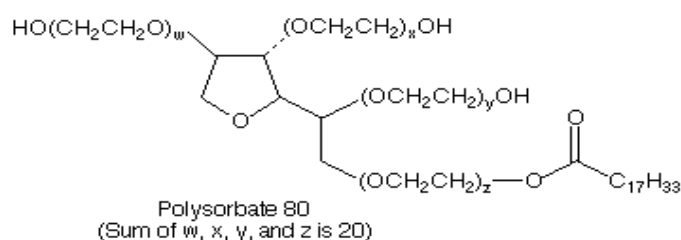|		
Solubility	:	Freely soluble in benzene, carbon tetrachloride, chloroform and ether; soluble in ethanol (95%), hexane, and propylene glycol; practically insoluble in water.

Stability and Storage

Conditions	:	A stable material; an antioxidant may also be added to it; the bulk material should be stored in a well closed container in a cool, dry place.
Incompatibilities	:	Stearic acid is incompatible with most metal hydroxides and may be incompatible with bases, reducing agents, and oxidizing agents. Ointment bases made with stearic acid may show evidence ofdrying out or lumpiness due to such a reaction when compounded with zinc or calcium salts.

TWEEN 80

Synonyms	:	Polysorbate 80
Chemical name	:	Polyoxyethylene 20 sorbitan monooleate
Empirical formula	:	$C_{64}H_{124}O_{26}$
Molecular weight	:	1310
Structural formula	:	

Polysorbate 80
(Sum of w, x, y, and z is 20)

Functional category	:	Dispersing agent; emulsifying agent; non-ionic surfactant; solubilising agent; suspending agent; wetting agent
Boiling point	:	More than $100^{o}C$
Solubility	:	Soluble in water and ethanol
Stability and storage	:	Polysorbates are stable to electrolytes and weak acids and bases, gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation. Polysorbates are hygroscopic should be examined for water content prior to use an dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides. Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry place.
Safety	:	Moderately toxic by IV route; mildly toxic by ingestion; eye irritation.

GLYCERYL MONOSTEARATE

Synonym	:	Glyceryl monostearate, Glycerin monostearate, Monostearin
Chemical name	:	2,3-Dihydroxypropyl octadecanoate
Empirical formula	:	$C_{21}H_{42}O_4$
Molecular weight	:	358.56 g·mol^{-1}
Structural formula	:	
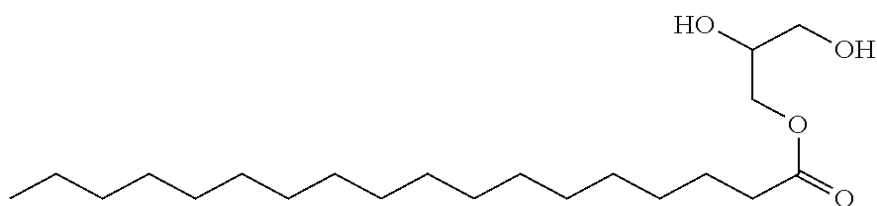
Description	:	A white or yellowish white, hard waxy mass or unctuous powder or flakes; odourless or slight, agreeable, fatty odour.
Boiling point	:	238 to 239 °C
Melting point	:	58 to 59 °C
Functional category	:	GMS is a food additive used as a thickening, emulsifying, anti-caking, and preservative agent; an emulsifying agent for oils, waxes, and solvents; a protective coating for hygroscopic powders; a solidifier and control release agent in pharmaceuticals; and a resin lubricant. It is also used in cosmetics and hair care products. It is responsible for giving ice cream and whipped cream its smooth texture. It is sometimes used as an anti-staling agent in bread.
Storage	:	Glyceryl monostearate should be kept in a tightly closed container, protected from light.

CINNAMON OIL

Synonym	:	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	:	
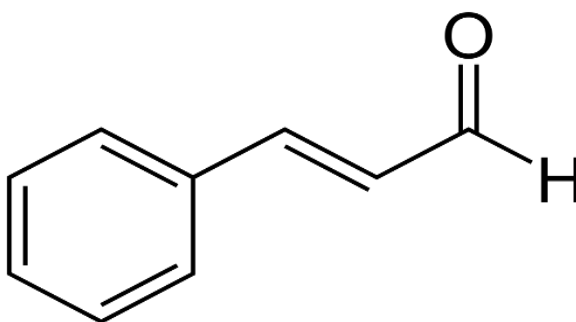
Description	:	It is of a golden-yellow colour, with the characteristic odour of cinnamon and a very hot aromatic taste. The pungent and scent come from cinnamaldehyde (about 90% of the essential oil from the bark) and, by reaction with oxygen as it ages, it darkens in colour and forms resinous compounds.
Boiling point	:	194-234 °C
Solubility	:	Insoluble in water, soluble in alcohol
Functional category	:	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 capacity.

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.

Analytical methods for Tetrahydrocurcumin (THC)

➤ UV Spectrophotometric estimation of Tetrahydrocurcumin (European Pharmacopoeia 6.0)

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.

Lambda max of Tetrahydrocurcumin:

Fig 3 : UV Spectrum of Tetrahydrocurcumin
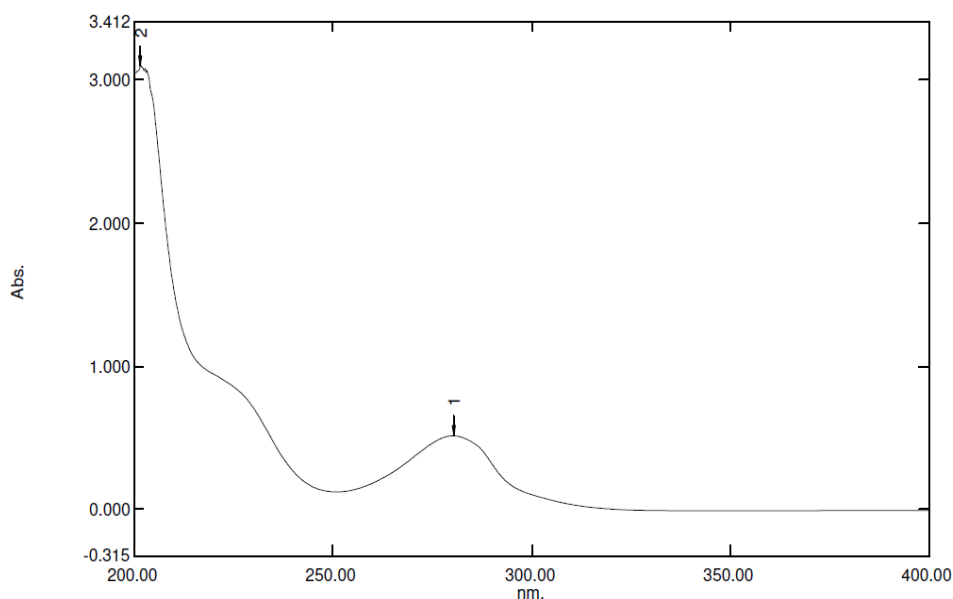
Compatibility studies

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].

IR Spectrum of Tetrahydrocurcumin

Characteristic peaks for carbonyl group C=O stretching was observed in 1603 cm-[1] and C-OH Stretching at 1156 cm-[1] which is evident that two peaks are confirming the purity of tetrahydrocurcumin molecule.

IR spectra of physical mixture of THC and stearic acid

The characteristic peaks for C=O and C-OH at 1603 and 1156 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 4 : IR Spectra of Tetrahydrocurcumin
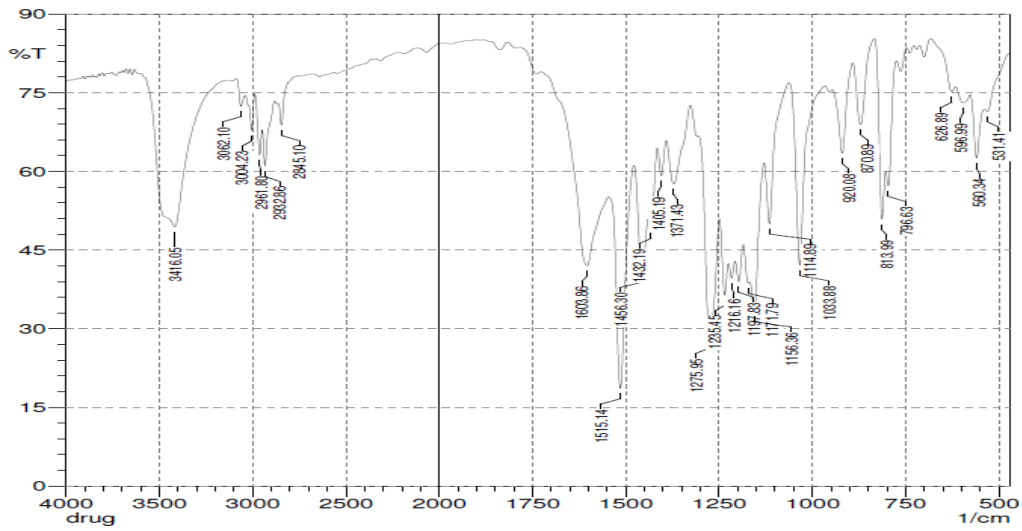
Fig 5: IR spectra of physical mixture of THC and stearic acid
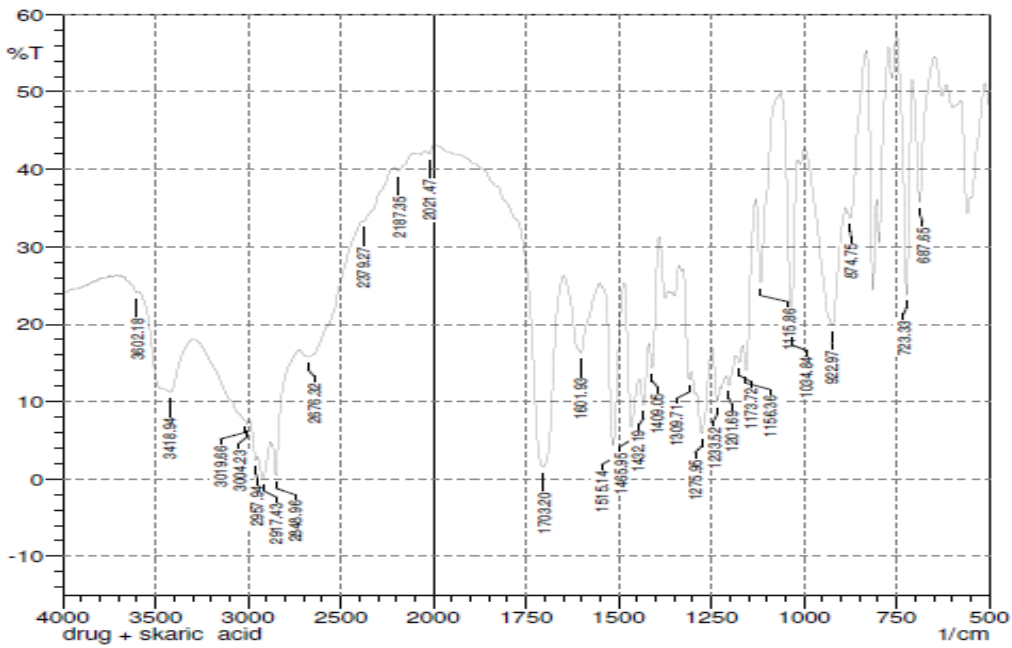
SOLUBILITY STUDIES

Apparent Solubility study

THC (5mg) was added to 1.5ml of various medium like water, 0.5-3%SLS in water, pH 7.4, 0.5-3%SLS in pH7.4, pH6.8, 0.1N HCl and kept in thermo mixer maintained at 37^0C for 1hr.Samples were then analyzed spectrophotometrically at 281nm. The results are shown in fig 6.

SPECTROPHOTOMETRIC

ANALYSIS
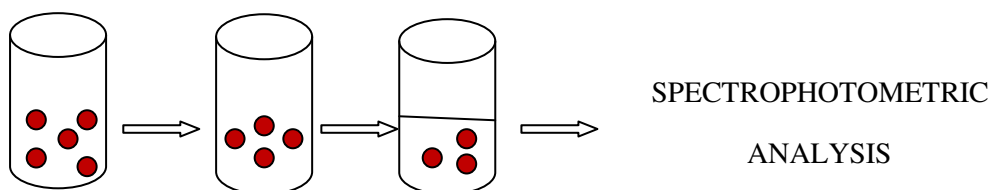
The solubility of the Tetrahydrocurcumin was found to be higher in SLS solution while compared to water .Therefore, the calibration curve was constructed using 1% SLS in water solution.

Fig 6 : Graph showing solubility of drug in different solutions
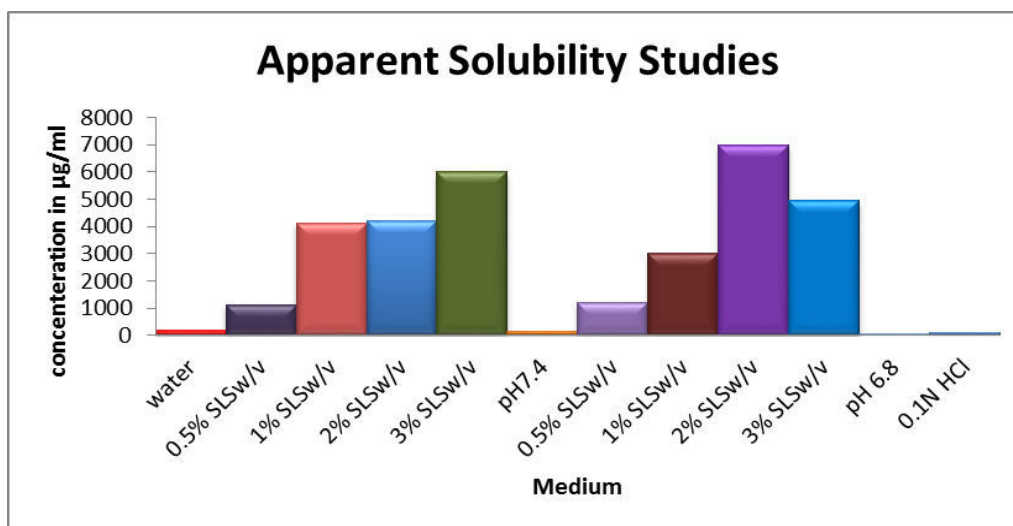
Table 3: Solubility Profile of Tetrahydrocurcumin

Drug	Solvent	Concentration(mg/ml)
THC	1% SLS	4.125

Calibration Curve

1% solution of Sodium Lauryl Sulphate (SLS) in 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 in 100ml of methanol in a volumetric flask. 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 and 10 µg/ml in 100ml volumetric flask using 1%SLS in buffer as solvent. The absorbance of THC standard solutions was measured at 281 nm (lambda max of THC) against 1% SLS 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 4: Standard Table for Tetrahydrocurcumin

| Sl.no	Concentration (µg/ml)	Absorbance at 281nm
1.	2	0.090
2.	4	0.151
3.	6	0.214
4.	8	0.279
5.	10	0.336

**Fig 7: Calibration Curve of Tetrahydrocurcumin**

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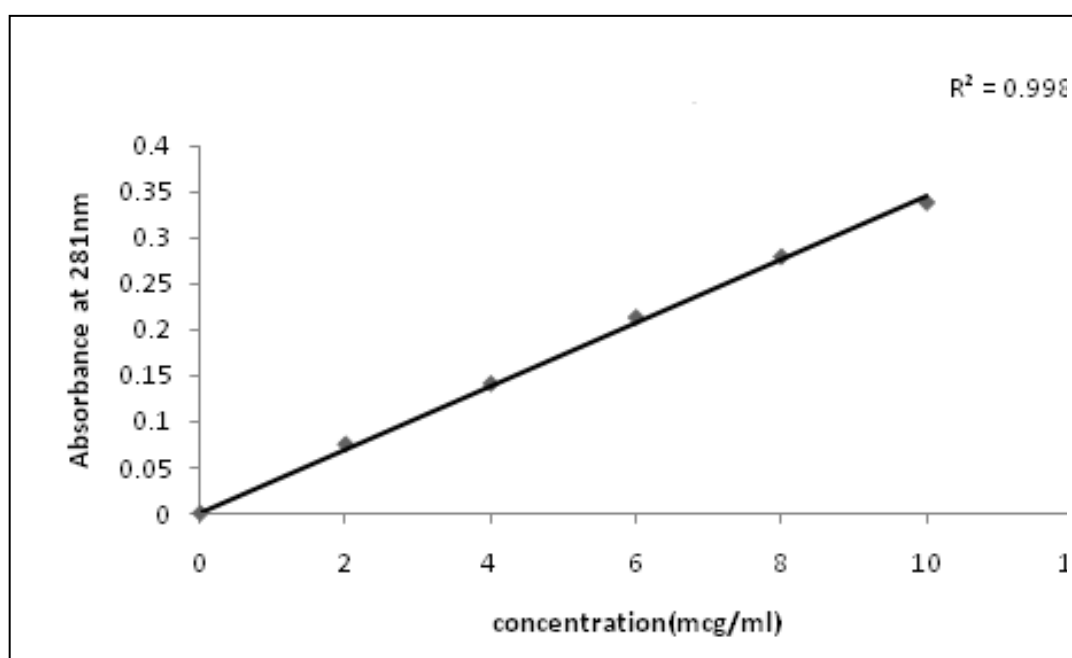
SELECTION OF OILS

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).

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	-	-	-	-	-	-	-

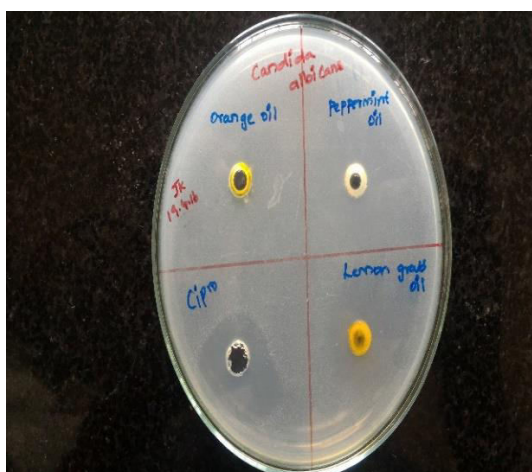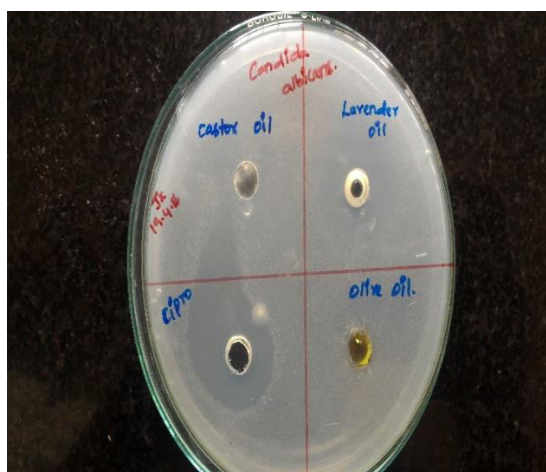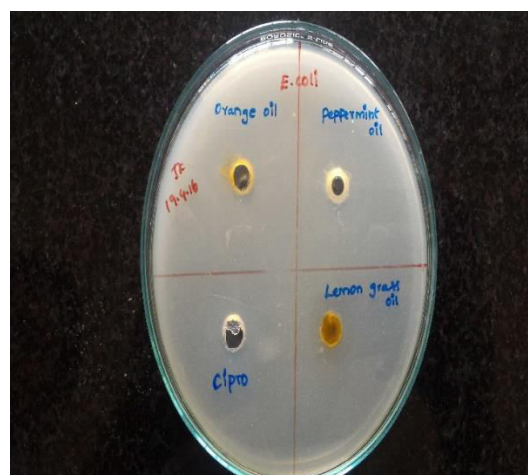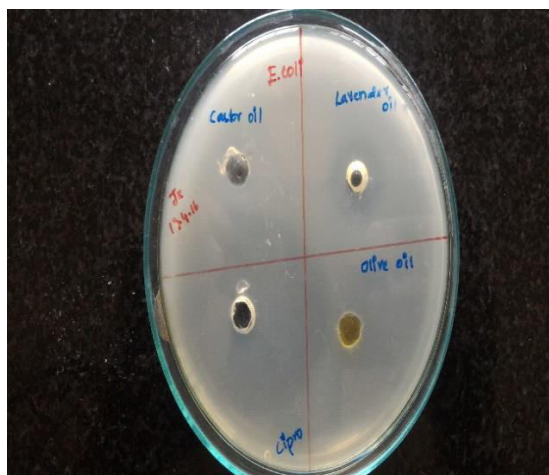**Table 5: Antibacterial activity of Essential Oils**
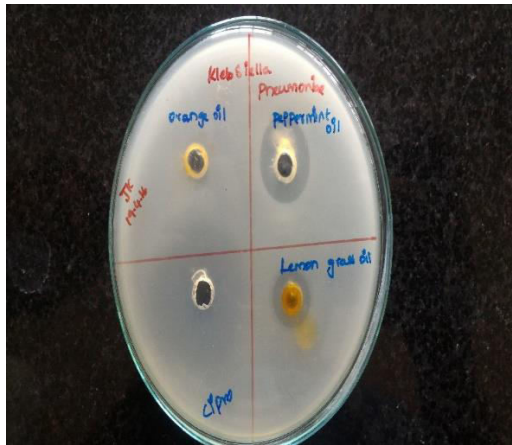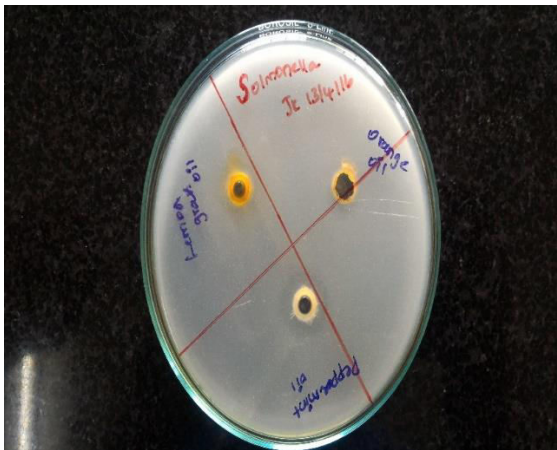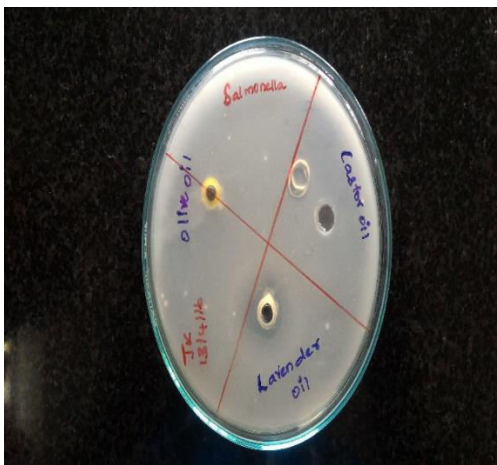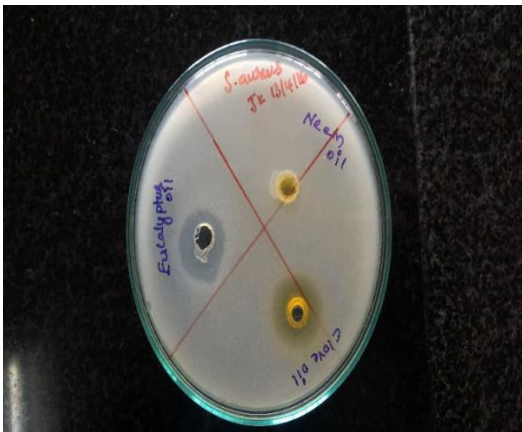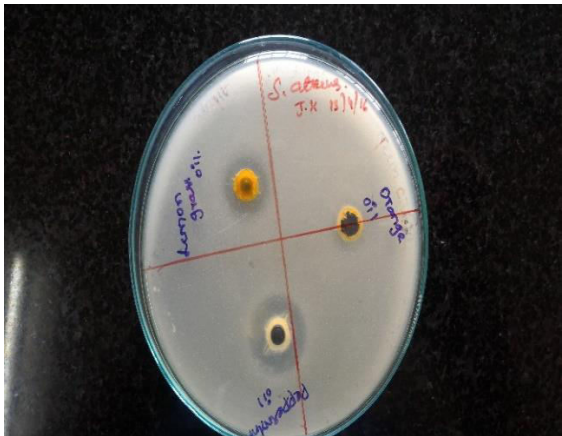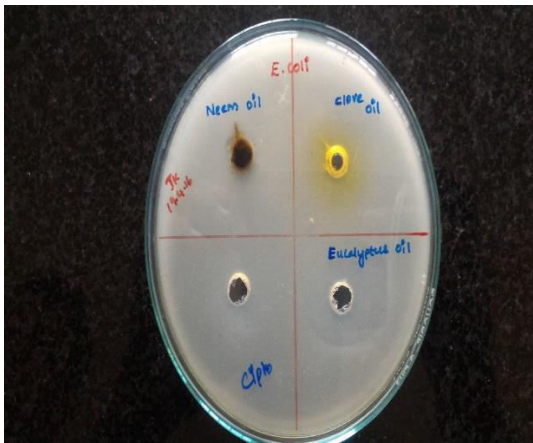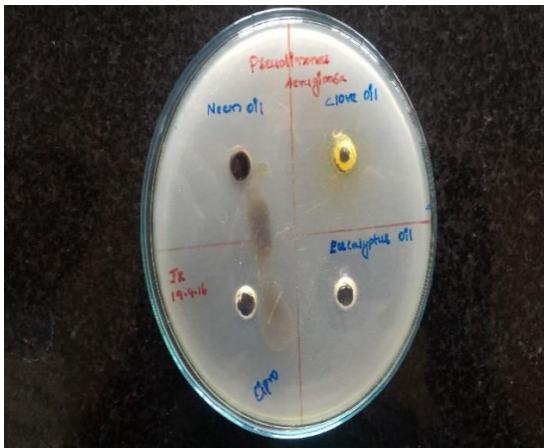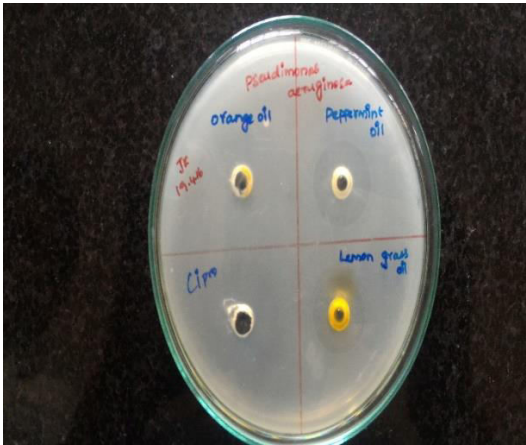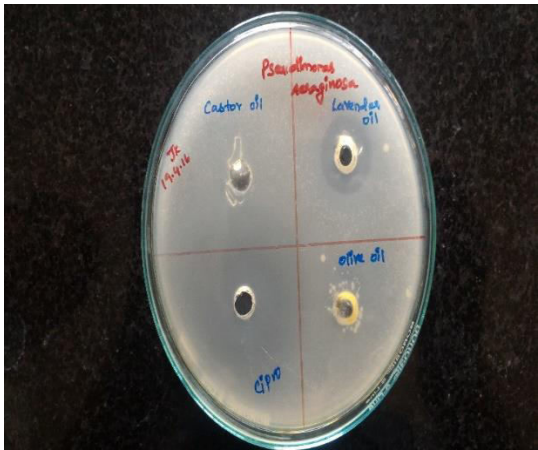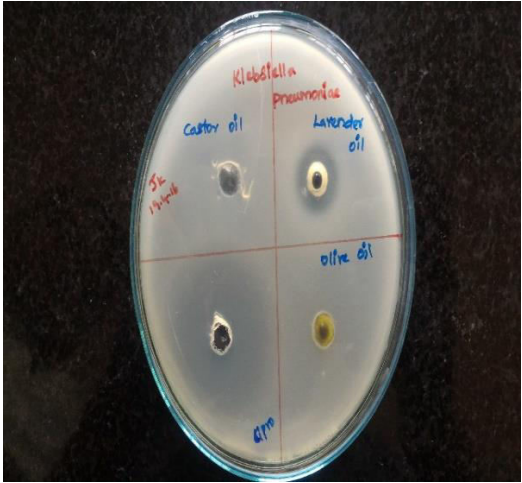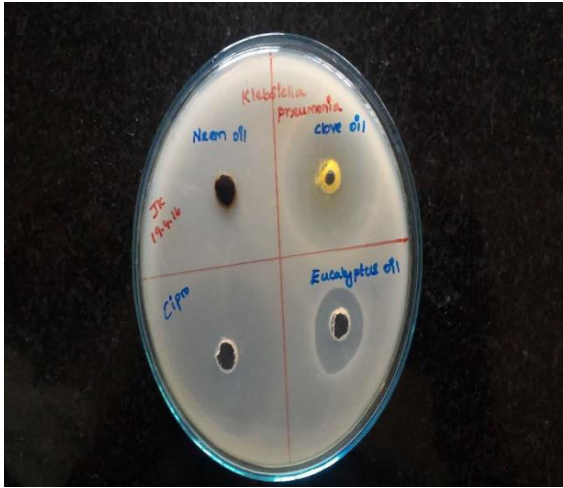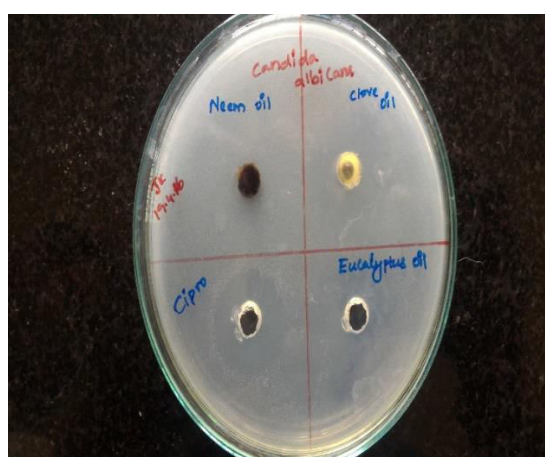
Fig 8 : Images Of Zone Of Inhibition For Essential Oils

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.

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

Clove Oil + Eucalyptus Oil	Clove Oil + Lavender Oil
Clove Oil + Peppermint Oil	Eucalyptus Oil + Peppermint Oil
Peppermint Oil + Lavender Oil	Clove Oil + Eucalyptus Oil + Peppermint Oil
Clove Oil + Eucalyptus Oil + Peppermint Oil + Lavender Oil	Clove Oil + Eucalyptus Oil + Peppermint Oil + Neem Oil
Clove Oil + Eucalyptus Oil + Peppermint Oil + Lavender Oil + Neem Oil	Cinnamon Oil + Neem Oil

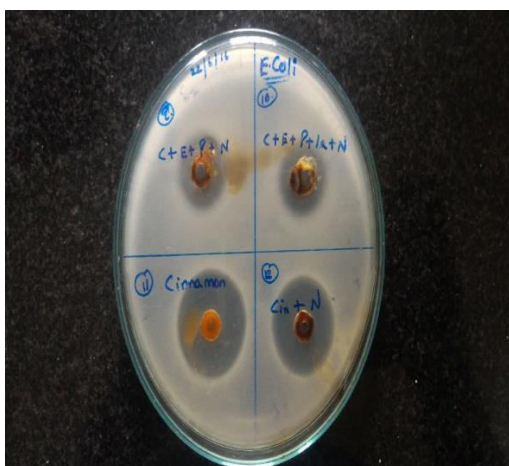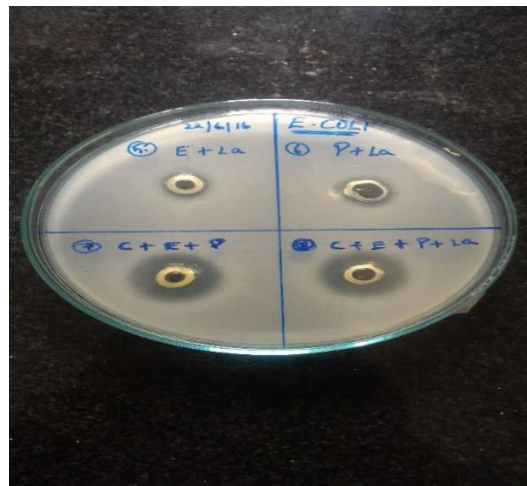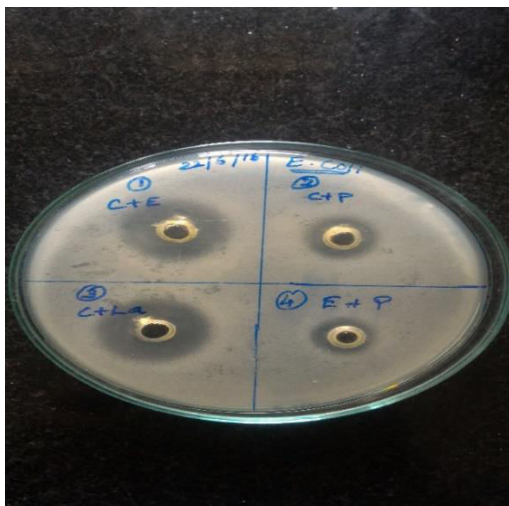**Table 6: Mixed Proportion Of Essential Oils**

Fig 9: Images of Zone of Inhibition For Mixed Proportion

Here combination of oils shows lesser antibacterial activity than individual.

DETERMINATION OF MINIMUM INHIBITORY CONCERNTRATION

Method: 1

MIC determinations were performed in 96-well micro plates 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.

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

Table 7: Minimum inhibitory concentration for essential oils

Method :2

All microbiological assays were performed under anaerobic conditions. MIC determinations were performed in 96-well micro plates according to procedures described by the Clinical and Laboratory Standards Institute. Each essential oil (200mg) was dissolved in dimethyl sulfoxide (40μL) and the volume 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 two fold dilutions of each essential oil stock were 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 micro plate wells and mixed well with the micropipette. The negative controls comprised sterile Muller Hinton medium or with dimethyl sulfoxide (at concentrations used in the dilutions). In order to ascertain aseptic conditions, the control wells contained sterile Muller Hinton medium but without inoculum. The inoculated micro plates were incubated at 36 ± 1∘C for 48h under anaerobic conditions; and the bacterial growth was confirmed by adding 10μL of a sterile 0.5% aqueous solution of triphenyl tetrazolium chloride (TTC,Sigma–Aldrich) and incubating at 36∘C for 30min. The viable bacterial cells reduced the yellow TTC to pink/red 1,3,5-triphenylformazan (TPF). All assays were performed in triplicate.

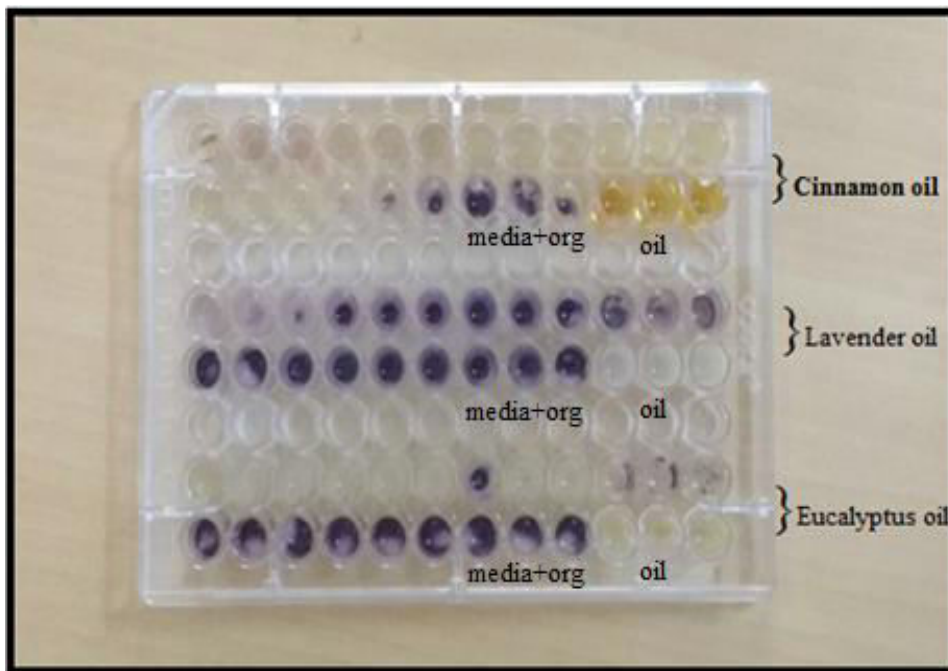**Fig 10: Minimum Inhibitory Concentration for Peppermint Oil**

**Fig 11: Minimum Inhibitory Concentration for Cinnamon Oil, Lavender Oil
And Eucalyptus Oil**

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.

MATERIALS

Stearic acid, tween 80, Glyceryl monostearate, Cinnamon oil,

FORMULATION DEVELOPMENT

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

The solid lipid (500mg) of choice and the liquid lipid (0.3ml)was mixed with the drug (100mg) and warmed to 75°C for effective melting and mixing. Simultaneously, distilled water (20ml) to which the surfactant (0.5ml) 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 20mins. The two- phase system is then sonication using probe sonicator, at 20,000 rpm for 10 min followed by ultra sonication for 2 min. The prepared formulations are stored at refrigeration condition until further use.
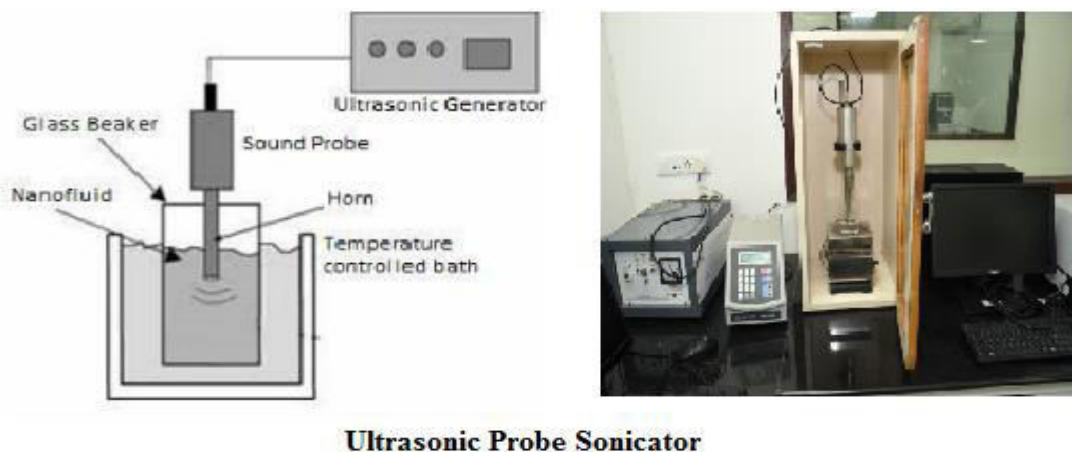
Ultrasonic Probe Sonicator

Fig 12 :Schematic representation of the configuration of a Ultra Probe Sonicator

Preparation of Base Cream:

In this study, W/O 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 cetyl alcohol (1%), ceto steryl alcohol (1%), palmitic acid (1%) and myristic acid (10%). At the same time, aqueous phase consisting of distilled water (82%), span60 (1.5%), tween80 (5%) was heated to the same temperature. After heating, aqueous phase was added to the lipid phase drop by drop. Stirring was continued at 2000 rpm by the mechanical mixer for 15 min until complete aqueous phase was added. After the complete addition of the aqueous phase, the speed of the mixer was reduced to 1000 rpm for homogenization,for a period of 5 min, and then the speed of the mixer was reduced to 500 rpm for further 5 min for complete homogenization, until the emulsion coolednto room temperature.

Incorporation of NLC into Base Cream

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

CHARACTERIZATION STUDIES

Particle size analysis

➢ *Photon correlation spectroscopy:*

The prepared SLN 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 size distribution and the charge nature of the prepared solid lipid Nano particle loaded with nutraceuticals 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.

Atomic Force Microscopy

A small aqueous drop of the THC loaded nanoparticles 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.

Scanning electron microscopy

Scanning electron microscope (SEM) is a type of electron microscope 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.

***In vitro* drug release studies:**

　　SLN formulation 2ml was taken in Franz diffusion cell apparatus. A Pig ear skin was placed in a Frans diffusion cell containing 1% SLS buffer solution as medium at room temperature. At various predetermined intervals i.e., 0.50hr, 1hr, 2hr, 4hr, 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.
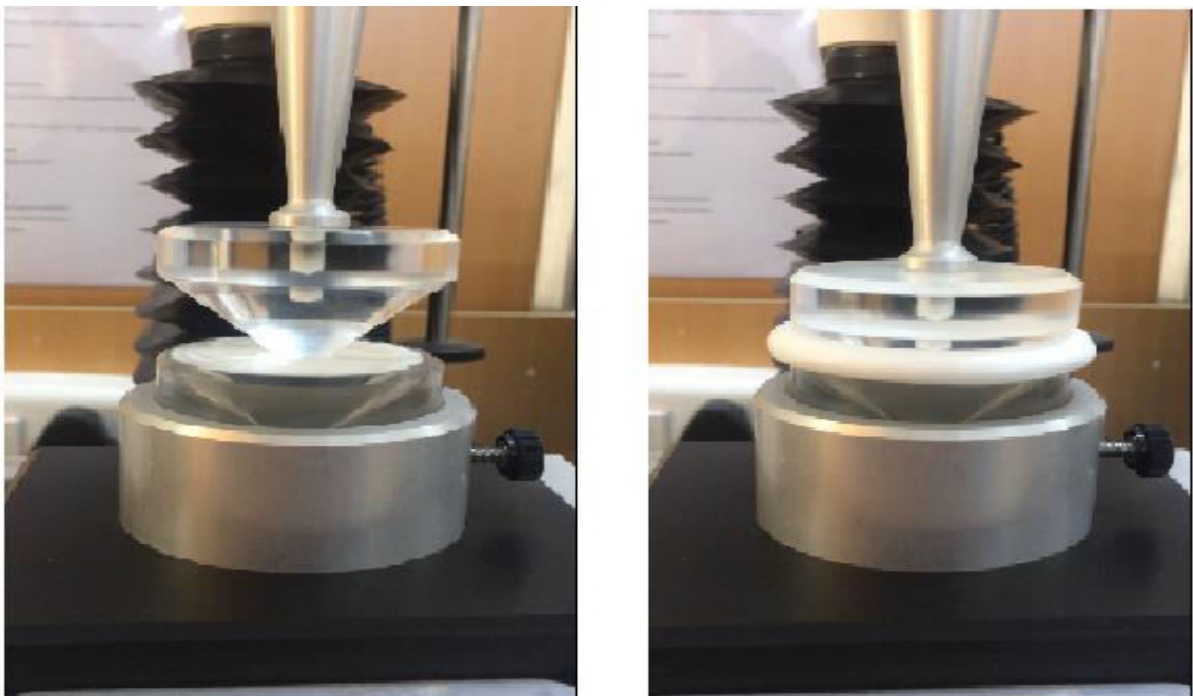
Texture analysis:

Fig 13: Texture Analysis Image for THC loaded Cream

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 desired position by tightening the screws. An empty female cone sample holder was placed in the base holder. The male cone probe was attached above the female cone such that the male cone fits almost all the way into 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 the female 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.

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.

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.

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, $5 \pm 3°C$ (refrigeration conditions) and $25 \pm 2°C$ (room temperature).

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.

FORMULATION DEVELOPMENT

> ### Selection of Lipid

The lipid Stearic acid was selected randomly based on the stability data of the previously formulated Tetrahydrocurcumin solid lipid nanoparticles. The lipid showed high drug encapsulation with good stability was selected for further studies.

Preparation of Nano Structured Lipid Carrier

Ultra probe sonicator were used to formulate NLC of Tetrahydrocurcumin. Totally, 10 formulations were prepared using one lipid (Stearic acid), one surfactant (Tween 80). The formulae for the prepared NLCs were given in the table No 5

Table 8: Batch specification of THC loaded NLC

Formulation	Drug	Lipid (Stearic acid)	Essential oil	Tween 80	PEG 4000	Polaxamer 188	GMS	Remarks
F1	-	1 gm	1ml	-	0.3gm	0.3gm	-	CREAMING ON STORAGE
F2	-	0.5gm	1ml	-	0.3gm	0.3gm	-	CREAMING ON ROOM TEMPERATURE
F3	-	1 gm	0.5ml	-	0.3gm	0.3gm	-	AGGREGATION
F4	-	0.5 gm	0.3ml	-	0.3gm	0.3gm		FLOCCULATION
F5	-	0.5 gm	0.3ml	0.3 ml	0.3gm	-	-	AGGREGATION
F6	-	1 gm	0.3ml	-	0.3gm	0.3gm	-	PHASE INVERSION
F7	-	0.5gm	0.5ml	-	0.3gm	0.3gm	-	PHASE INVERSION
F8	-	0.5gm	0.3ml	0.5ml	-	-	-	CREAMING ON STORAGE
F9		0.5gm	0.3ml	0.5 ml	-	-	0.1gm	GOOD EMULSION
F10	0.1gm	0.5gm	0.3ml	0.5 ml	-	-	0.1gm	GOOD EMULSION

Fig 14: Formulation of THC loaded NLC

**PHYSICOCHEMICAL CHARACTERIZATION**

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 73 to 828 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 9: Particle size measurement results of THC loaded NLC

Formulation	Particle Size (nm)	PDI
THC F10	246.7	0.385

Size distribution report by intensity
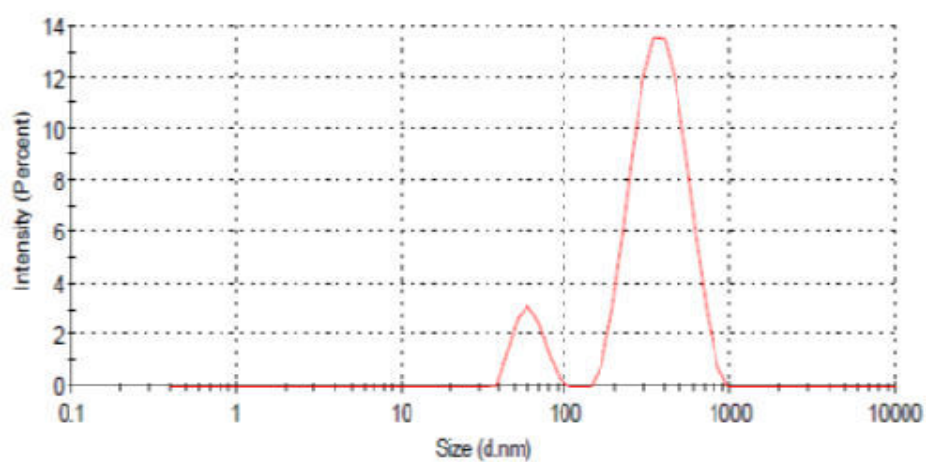
Zeta potential report
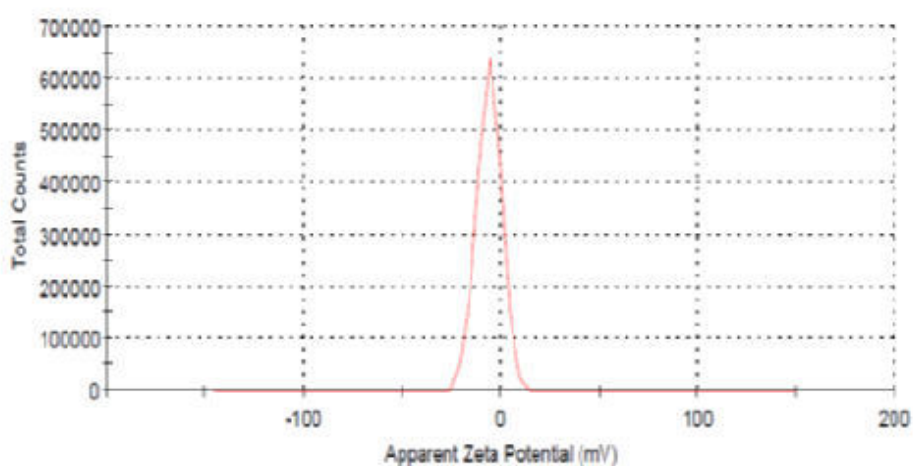
Fig 15: Zeta size analysis of THC loaded NLC prepared using Stearic acid, 0.5 ml Tween 80 and 0.3ml Cinnamon oil

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.

Fig 16: PCM images showing the morphology of THC loaded NLC
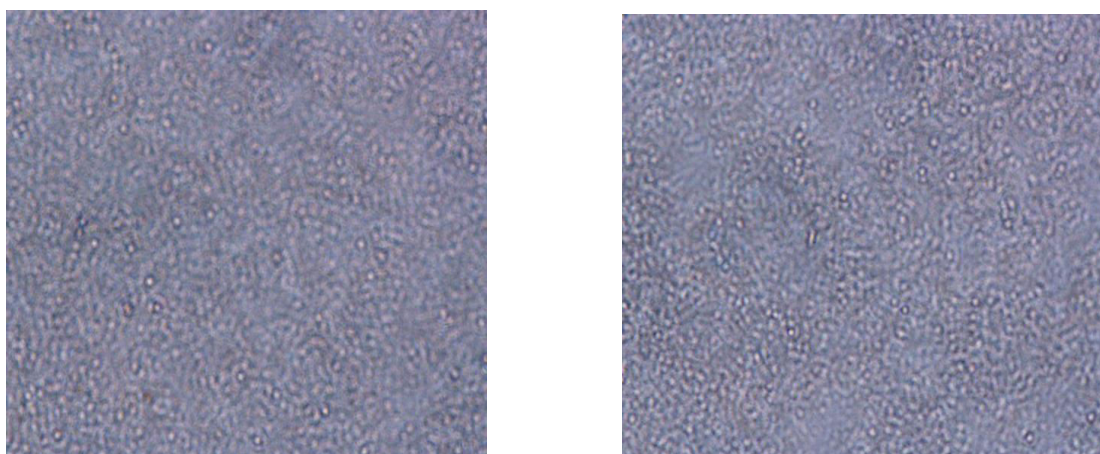
THC F10 – 40X

Fig 17: PCM images showing the morphology of NLC Loaded Base Cream
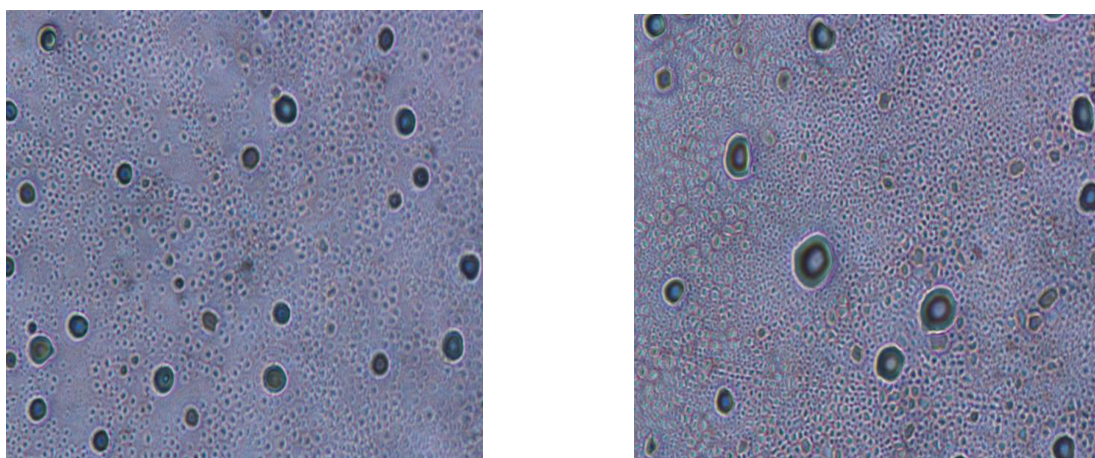
NLC Loaded Base Cream - 40x

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.
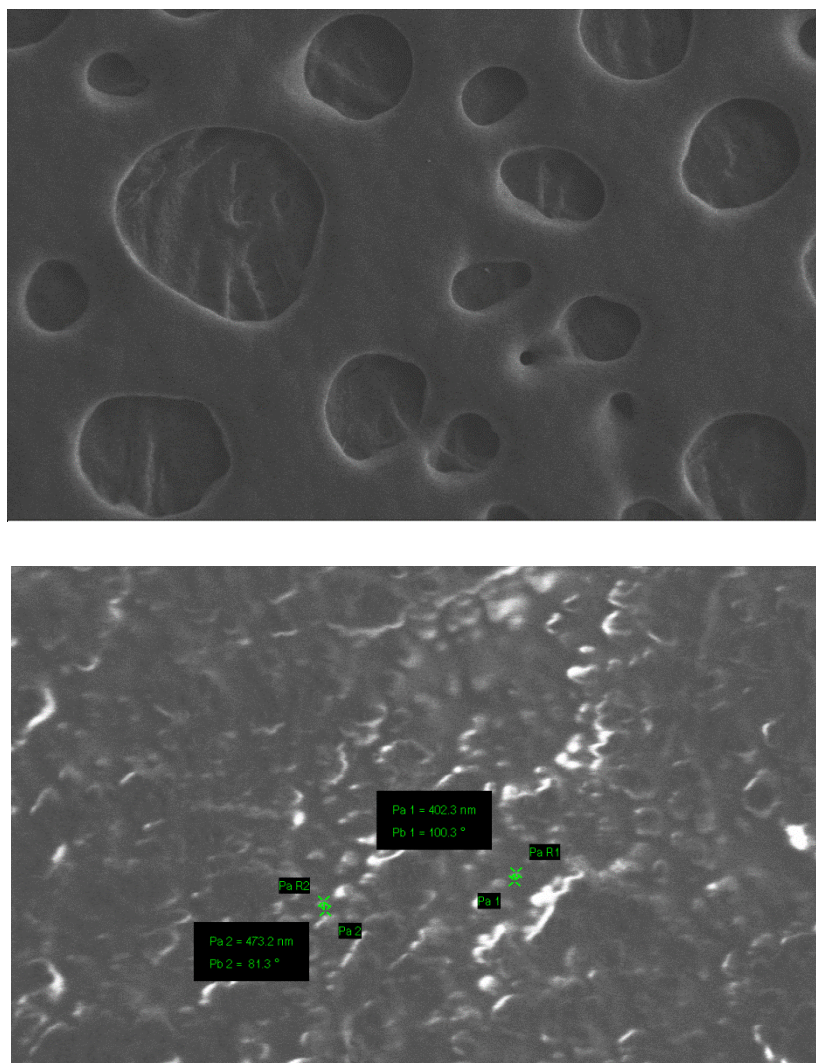

Fig 19: SEM Images of prepared NLC

Scanning electron micrographs of NLC are shown in the above Figures. The shape of the NLC was spherical and the size of the NLC was found within the nanometer range. Moreover, the micrograph also revealed the agglomeration of

nanoparticles which might be due to the lipid nature of the carrier and the drying process during sample preparation prior to SEM analysis.

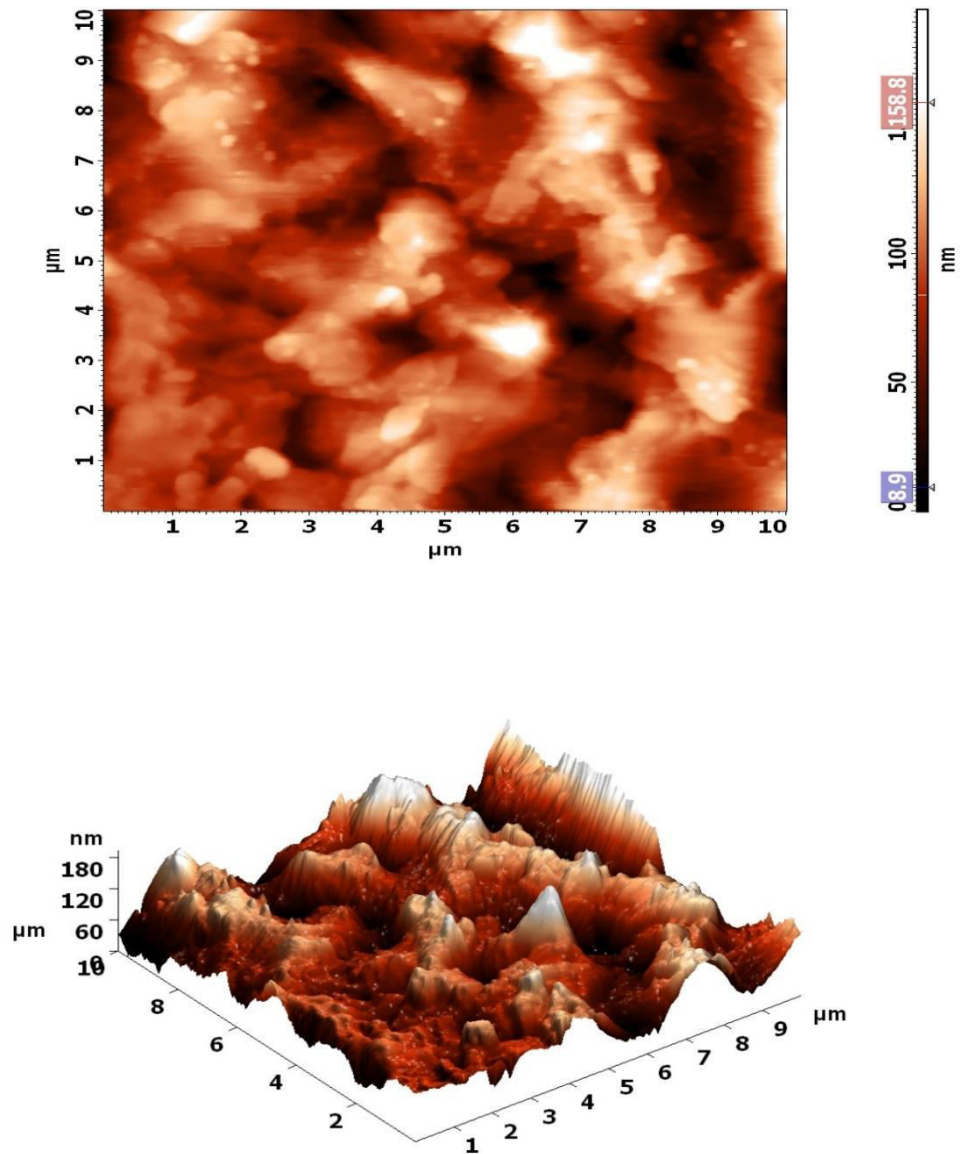**ATOMIC FORCE MICROSCOPY (AFM)**

Fig 20: 2D image and 3D image of AFM analyzed particle

Atomic Force Microscopy (AFM) study results indicate that the formulation has spherical Shape

TEXTURE ANALYSIS

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 spredability graph of THC Cream. The firmness value obtained for THC Cream (209.334g) with force of application is (178.340g.sec). Similarly the stickiness value for THC loaded cream is (280.100g).
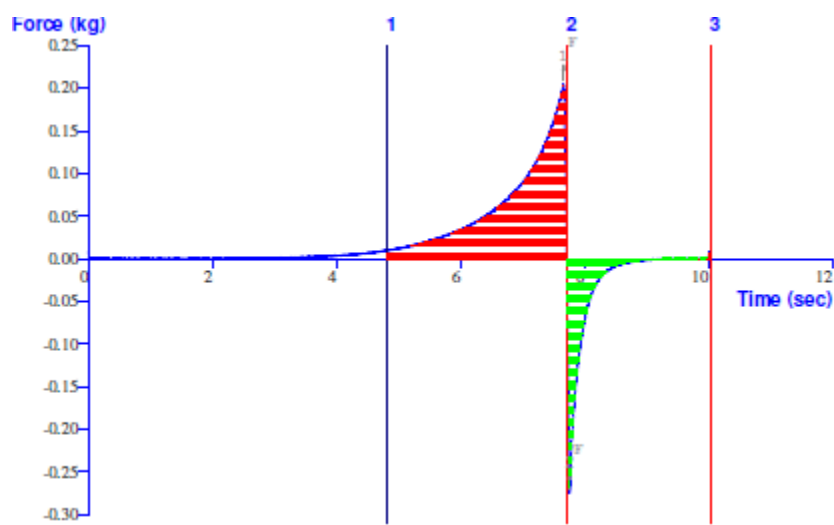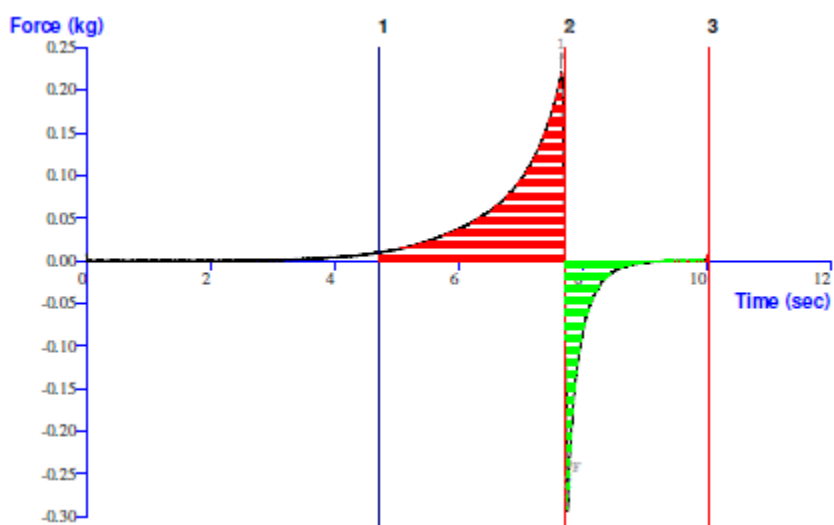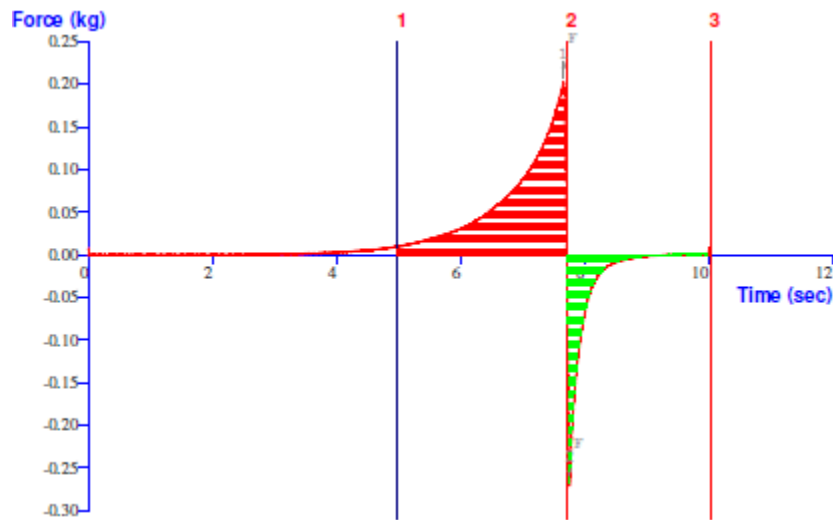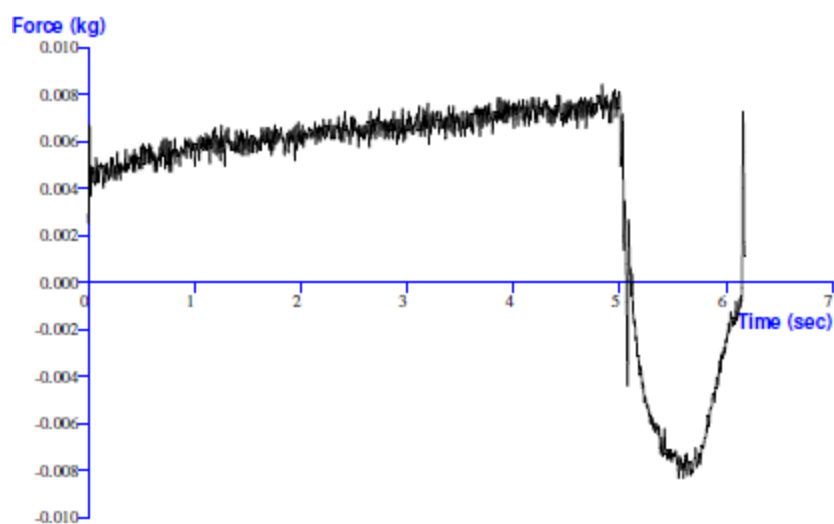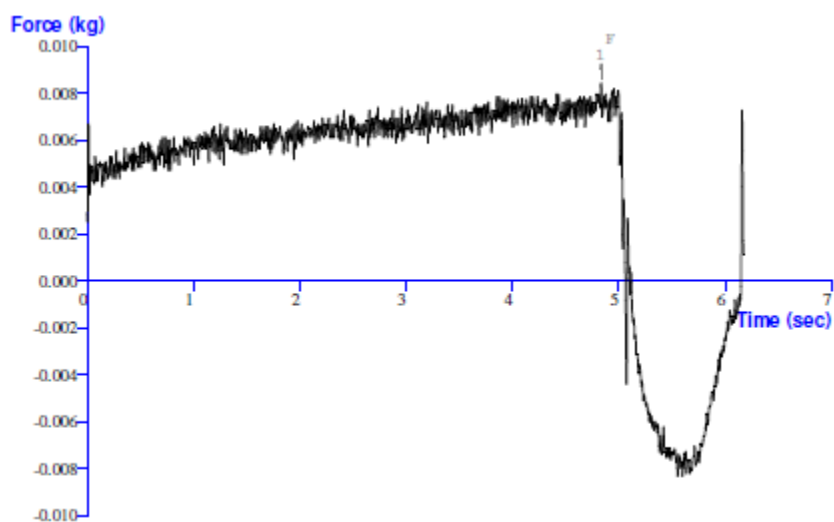
Fig 21: Spreadability plot for THC loaded Cream

Bloom Strength:

Bloom strength 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 8.552g.
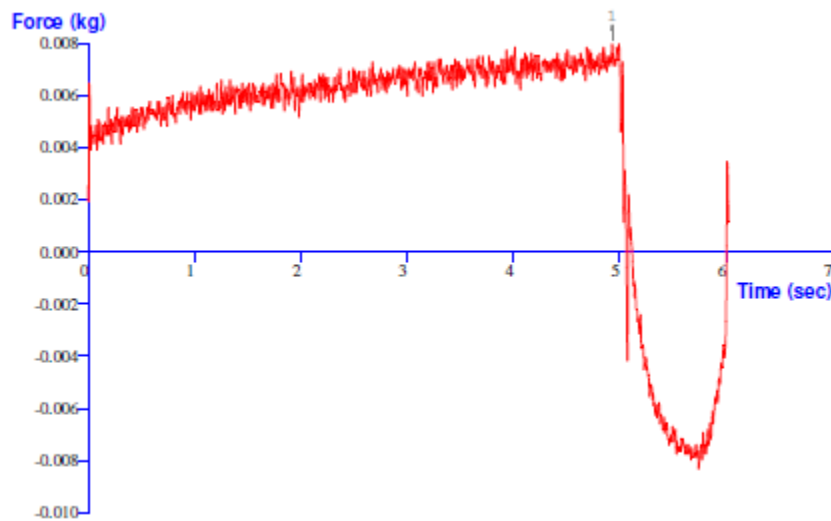
Fig 22: Bloom Strength plot for THC Loaded Cream

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 346.788g.
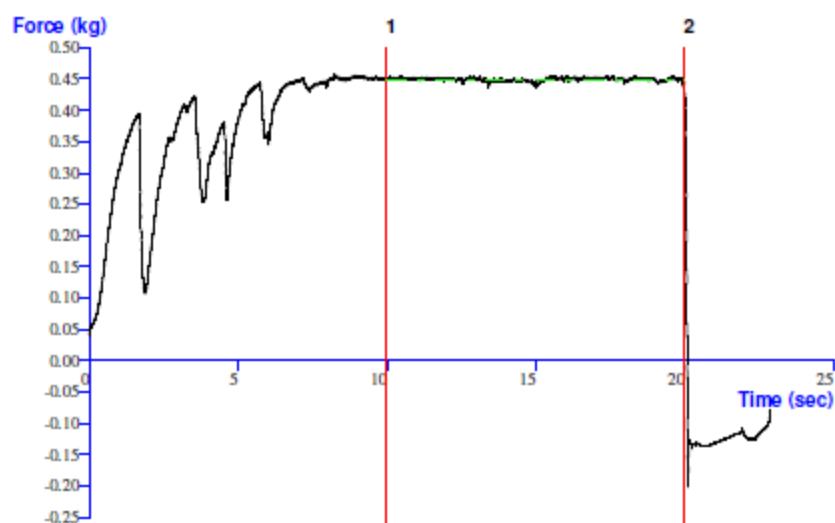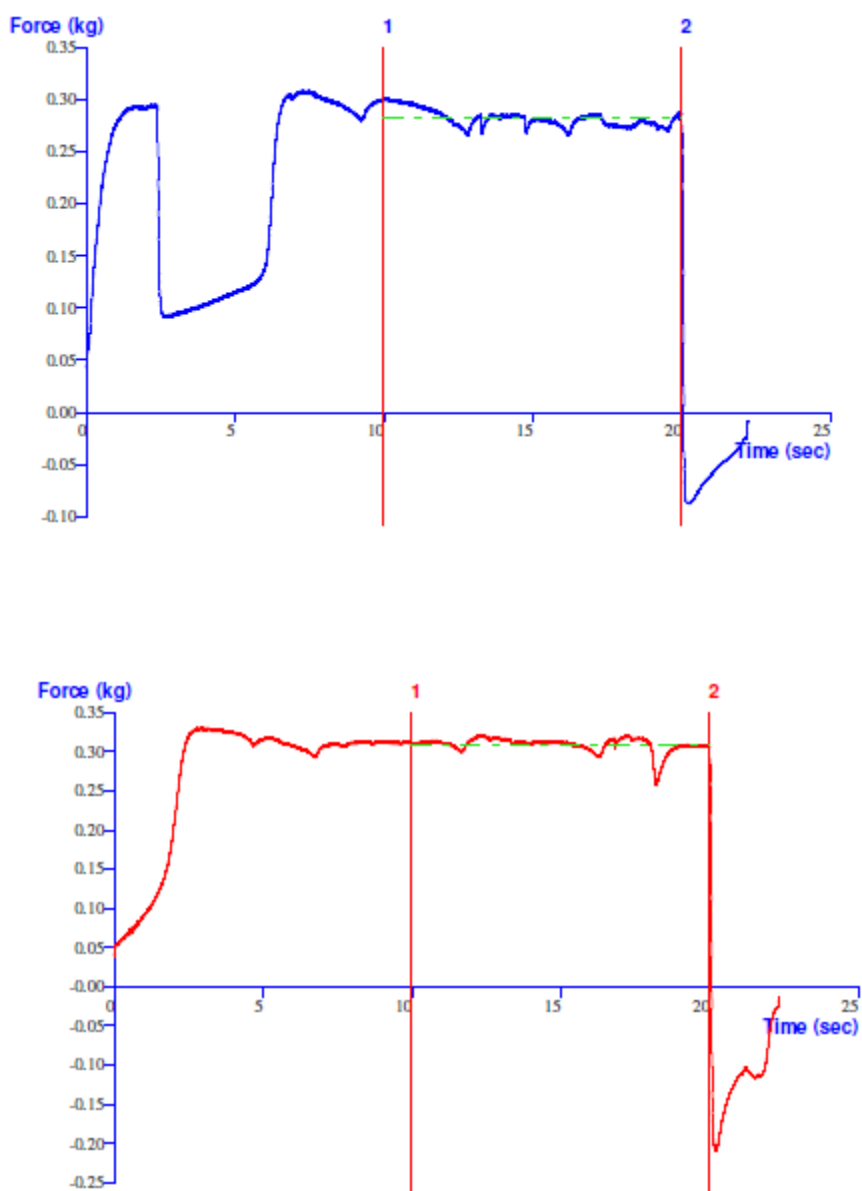
Fig 23: Extrudability of THC Loaded Cream

In Vitro **Drug Release:**

Table 10: *In vitro* Drug Release Study

S.No	Time (hrs)	Cumulative % Drug Release
1.	2hr	0.5
2.	4hr	2.5
3.	6hr	10.5
4.	8hr	15
5.	24hr	45
6.	48hr	80

Fig 24: *In vitro* permeation study across pig ear skin

The *in vitro* release studies were carried up to 48 hours for the formulations and 80% of drug was penetrated through pig ear skin at 48hrs.

Stability studies

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

SUMMARY AND CONCLUSION

In general lipid based formulations face stability problems over a longer period of time due to microbial contamination which can often change the physical and chemical properties of the drugs and excipients. 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 stearic acid, cinnamon oil and tween 80 by hot homogenization technique using ultra probe sonicator. The spherical shaped nanostructure lipid particles with a particle size of 245 nm showed a sustained release pattern across pig ear skin for around 48 hours. These THC loaded nanostructure lipid particles were loaded in a lipid based cream (prepared from cetostearyl alcohol, cetyl alcohol, myristic acid, palmitic acid and tween 80). 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.

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