EFFECT OF LIPID ON PREPARATION AND EVALUATION OF MONTELUKAST SODIUM LOADED SOLID LIPID NANOPARTICLES

Dissertation submitted to

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

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IN

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INTRODUCTION

RATIONALE OF NOVEL DRUG DELIVERY SYSTEM[1,5,7,8,9]:

Most of the drugs introduced to clinical medicine exert their effects by interactive interference with cell and cell membrane related structure and function through concentration dependent reversible interactions at specific receptor site.

Presently, conventional dosage forms (tablets, capsules, pills, injections) are primarily prescribed pharmaceutical products and are available over-the-counter.

To achieve and maintain the concentration of a administered drug within therapeutically effective range, it is often necessary to take drug dosage several times and this results in a **fluctuating drug levels** in plasma[8].

The goal in designing novel drug delivery system is to reduce the frequency of dosing of to increase effectiveness of the drug by localization at the site of action, reducing dose required or providing uniform drug delivery and the other criteria is should deliver the active entity to the site of action, thereby minimizing or eliminating side effects[5,7].

The fluctuations produced by the conventional drug delivery system can be overcome by using several approaches. They are:

1. Targeted delivery
2. Sustained release
3. Controlled release

4. Prolonged release
5. Modulated release

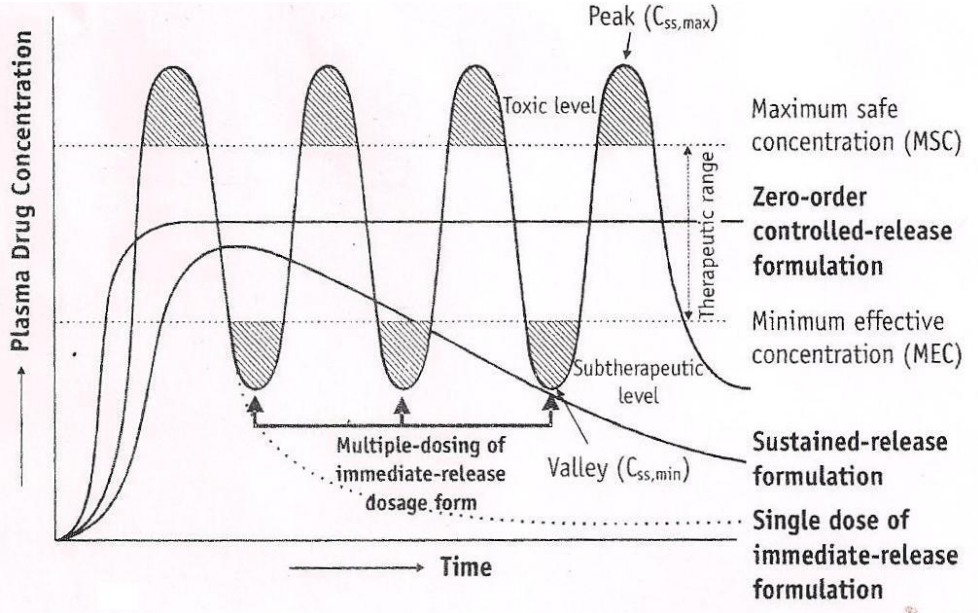


Fig.1 A hypothetical plasma drug concentration-time profile from conventional multiple dosing and an ideal controlled delivery formulation

The above figure shows the differences between conventional drug delivery system and the sustained/controlled drug delivery system. From the figure, it was concluded that fluctuations in the drug level is obtained in conventional delivery also the blood level reaches the toxic level. But as in the case of sustained drug delivery system, the sustained/controlled drug delivery system **maintains the drug level below its toxic range also reducing the multiple – dosing[1].** It should be emphasized that the plasma level of a drug should be maintained within the safe margin and effective range, for this proper and calculated dose of the drug need to be given at different time intervals by conventional dosage forms[7].

1. **Targeted drug delivery systems, TDDS:**

   It refers to the systemic administration of a drug-carrier with the goal of delivering the drug to **predetermined target** in therapeutic concentration, while restricting its access to non-target normal cellular linings, thus minimizing therapeutic index.

2. **Sustained release systems:**

   It is developed to maintain therapeutic blood or tissue levels of the drug for an **extended period of time** which is concentration dependent.

3. **Controlled release systems:**

   Zero-order release constituted drug release from the dosage form that is independent of the amount of drug in the delivery system i.e. **concentration independent and constant release time.**

4. **Prolonged release systems:**

   The release of the delivery system is attained for prolonged period of time i.e. for several weeks or even months**.**

5. **Modulated release systems:**

   It implies use of a drug delivery device that releases the drug at a variable rate controlled by environmental conditions, biofeedback, sensor input or an external control device.

Controlled and targeted delivery is one of the most enviable requirements from a carrier, which involves the multidisciplinary site- specific or targeted approach. Targeted drug delivery systems includes, liposomes, niosomes, nanoparticles, microspheres, resealed erythrocytes etc.

The advantages of novel drug delivery systems is as follows[5,7,8]:

- ✶ Controlled/sustained delivery of active agent at predetermined rate
- ✶ Maintenance of optimal and effective drug level for prolonged duration
- ✶ Reduction of untoward effects
- ✶ Increase in patient compliance
- ✶ Reduction in frequency of dosing
- ✶ Delivery of drug in the vicinity of site of action
- ✶ More efficient utilization of active agent
- ✶ Enhancement of bioavailability

Hence, by the above considerations, a novel drug delivery is needed to conquer the problems associated with conventional formulations.

TARGETED DRUG DELIVERY SYSTEMS[5]:

It implies for selective and effective localization of pharmacologically active moiety at predetermined (preselected) target in therapeutic concentration, while restricting its access to non-target normal cells.

The colloidal drug delivery systems such as liposomes, niosomes, nanoparticles enhances the bioavailability of drugs which is shown in fig.2. In general, these colloidal carrier systems have the nanometric size range, in this size range the liver cannot uptake the drug from the delivery system and are not metabolized by the liver. Hence, the drug will be circulated in the blood levels for prolonged period of time and delivers the drug at constant time which will reduces the toxicity.

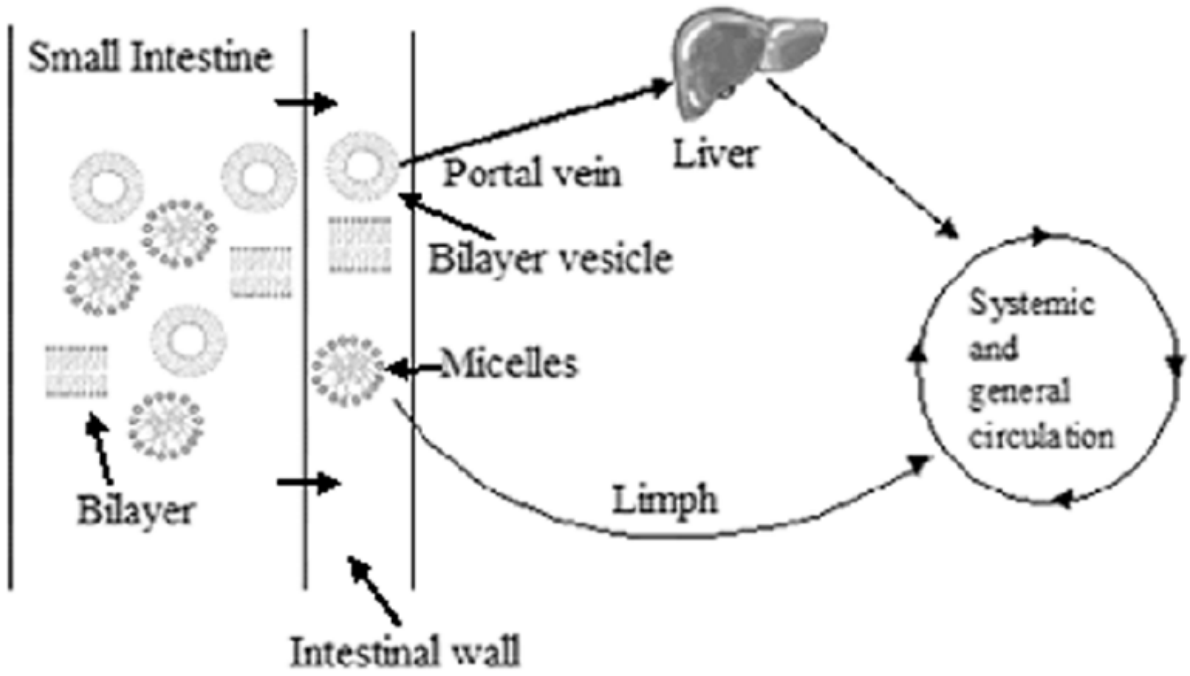


Fig.2 ***Various mechanisms of enhancement of drug bioavailability in the presence of lipids***

This may be achieved by using carrier systems, where reliance is placed on exploiting both intrinsic pathway that these carriers follow, and the bioprotection that they can offer to drugs during transit through the body.

The various carrier systems used for targeted drug delivery are as follows:

1. **Colloidal carriers:**

   A) Vesicular systems

   - Liposomes
   - Niosomes

- Pharmacosomes
- Virosomes
- Immunoliposomes

B) Microparticulate systems

- microparticles
- nanoparticles
- magnetic microspheres
- albumin microspheres
- nanocapsules
- Solid lipid nanoparticles

2. **Cellular carriers**
    - Resealed arythrocytes
    - Serum albumin
    - Antibodies
    - Platelest
    - Leukocytes
3. **Supramolecular delivery systems**
    - Micelles
    - Liquid crystals
    - Lipoproteins
4. **Polymer based systems**
    - Signal sensitive

- Micoadhesive
- Biodegradable
- Bioerodablie
- Soluble synthetic polymeric carriers

5. **Macro molecular carriers**

   Proteins; Glycoproteins; Articificial viral envelops

   Glycosylated water soluble polymers

   Monoclonal antibodies

   Toxins

   Leptons

   Polysaccharides

NANOTECHNOLOGY- INTRODUTION [20]:

- Nanotechnology is the science of that deals with the particle size in nanometer range.
- Produces new career therapies, drug delivery systems and biomaterial for implant or prosthesis.
- Small colloidal particles that are made of non-biodegradable and biodegradable polymers and the diameter is around 200 nm.
- Nano-device are some where 100 to 10000 times smaller than the human cells and are similar in size of biological molecules (enzymes and receptors)
- This offers to study and interact with normal as well as cancer cells

NANOPARTICLES[5,6,7,8,19,20,21,] :

Nanoparticles are solid polymeric, submicronic colloidal system range between 5-300nm consisting of macromolecular substances that vary in size 10nm to 1000nm. The drug of interest is dissolved, entrapped adsorbed, attached or encapsulated into the nanoparticle matrix.

Depending upon the method of preparation, nanoparticle, nanosphere or nanocapsule can be obtained with different properties and release characteristics for the encapsulated therapeutic agent.

1. Nanosphere are matrix system in which drug is physically and uniformly dispersed throughout, then particles prepared by using different polymers such as polyalkylcyanoacrylate & poly lactides or they can be solid lipid nanosphere prepared by using lipids like dipalmitoyl – phosphatidyl choline.

2. Nanocapsule are ultrafine vesicular system with a diameter less than 1 μm in which the drug is confined to a cavity surrounded by a unique polymer membrane and having aqueous or oily core containing drug substances.

Nanoparticles holds much interest, because in this range materials can have different and enhanced properties compared with the same materials of a larger size due to the following two major principle factors. The increased surfaces are of quantum effect. These factors can enhance properties such as reactivity, strength, electrical characteristics & in vivo behavior and a much greater surface area per unit mass compared with the larger particles leading to greater reactivity.

The advantages of using nanoparticles loaded with drugs, because of their small size can penetrate through small capillaries and are taken up by cells and allow the drug release at right

rate and dose at specific sites in the body for a certain time to release the accurate delivery, which enhances the therapeutic effect and reduces the toxicity and side effects. The use of biodegradable materials for nanoparticles preparation allows sustained release within the target site over a period of days or even weeks.

Types of NPS as carrier for drug & diagnostic agents

- Polymeric NPS
- Nanosuspensions and nanocrystals
- Polymeric micelles
- Ceramic NPS
- Liposome's
- Fullerenes and dendrimers
- SLN (Solid lipid nanoparticles)
- Magnetic nanoparticles
- Nanoshells coated with gold
- Nanomers and carbon nanotubes

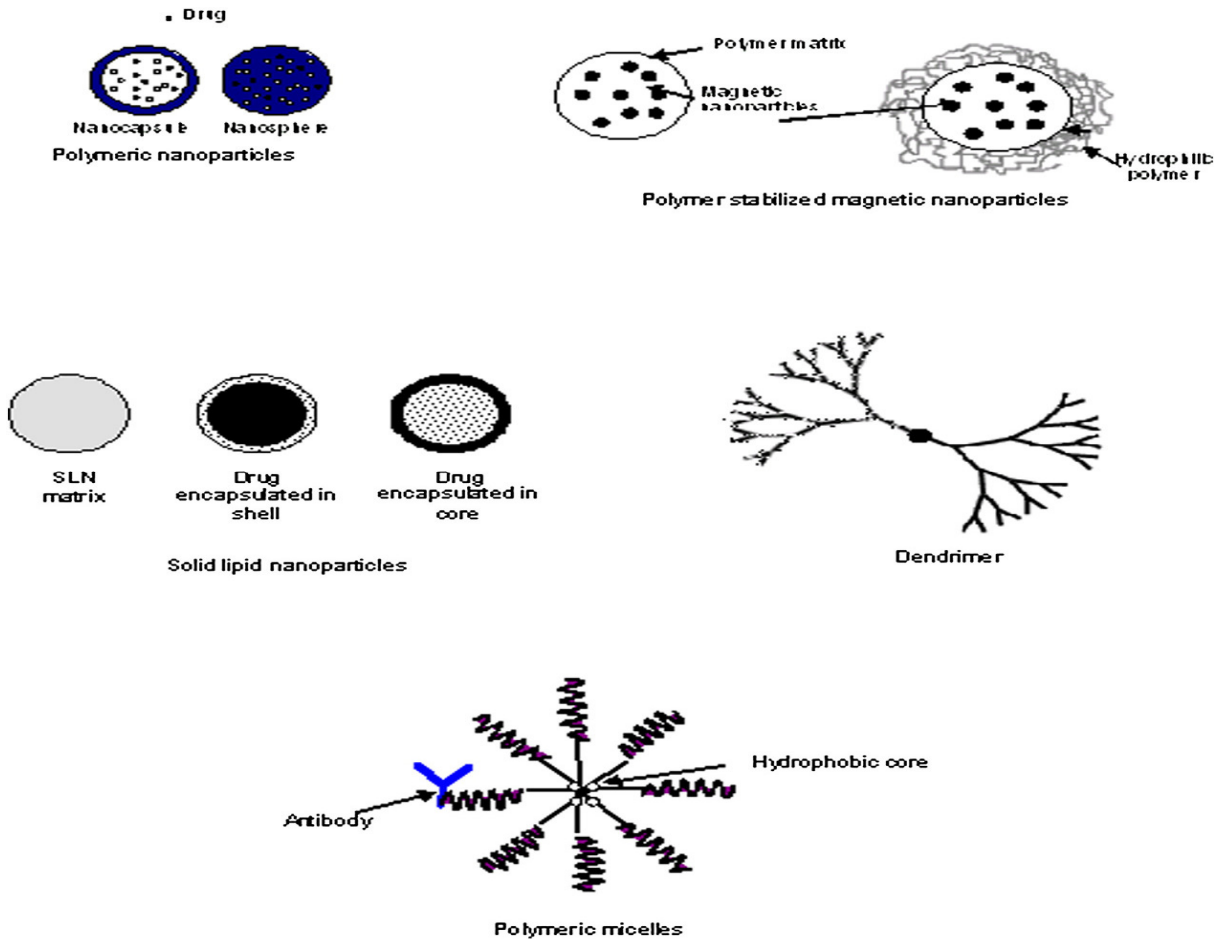


Fig. 3 represents the structure various nanoparticulate systems

Polymeric nanoparticles:

It consists of drug dispersed in an amorphous form within a polymer matrix. Examples of polymers include poly (alkyl cyanoacrylates), poly (glycolic acid) etc.

Nanosuspensions/ Nanocrystals:

Nanocrystals are crystals of poorly water-soluble drug in nanosize which when dispersed in water produce nanosuspension. Nanocrystalline drug suspensions have an advantage of higher loading capacity up to 90 % of the crystalline particle.

Polymeric micelles:

Polymeric micelles are nanosized core/shell assemblies of amphiphilic block copolymers that are suitable for the delivery of hydrophobic and amphiphilic agents.

Liposomes:

Liposomes are spherical microscopic vesicles composed of one or more concentric lipid bilayers, separated by water or aqueous buffer compartments with a diameter ranging from 25 nm to 10000 nm.

Solid lipid nanoparticles:

Melt-emulsified nanoparticles based on lipids are solid at room temperature.

CHAPTER II

SOLID LIPID NANOPARTICLES: A REVIEW

Solid lipid nanoparticles are one of the novel potential colloidal carrier systems as alternative materials to polymers which is identical to oil in water emulsion for parenteral nutrition, but the liquid lipid of the emulsion has been replaced by a solid lipid (shown on fig. 4). They have many advantages such as good biocompatibility, low toxicity and lipophillic drugs are better delivered by solid lipid nanoparticles and the system is physically stable. Solid lipid nanoparticles may be a promising sustained – release and drug targeting system for lipophilic drugs.

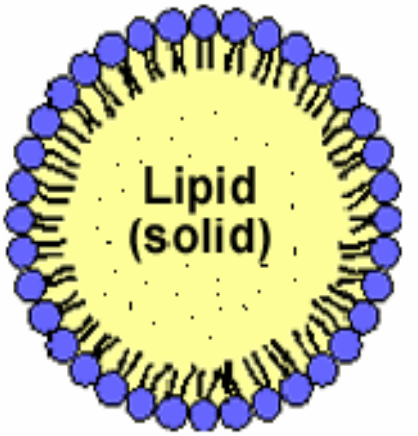


Fig.4 Structure of SLN

Solid lipid nanoparticles (SLNs) are considered to be the most effective lipid based colloidal carriers, introduced in early nineties. This is the one of the most popular approaches to improve the oral bioavailability of the poorly water soluble drugs. SLNs are in the submicron size range of 50-1000 nm and are composed of physiologically tolerated lipid components which are in solid state at room temperature. The schematic representation of different particulate drug

carriers such as emulsions and liposomes and their advantages are compared with SLNs in Fig. 5. SLNs combine all the advantages of polymeric nanoparticles, fat emulsions and liposomes.

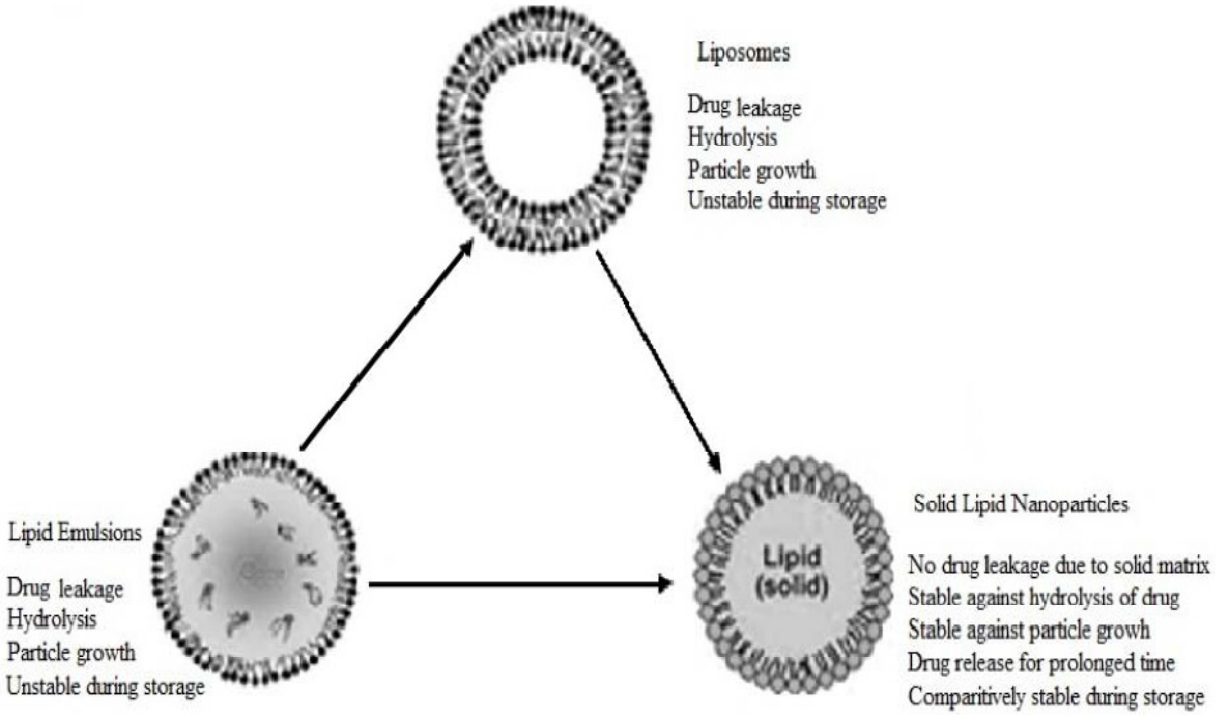


Fig. 5 A diagrammatic representation on SLN over emulsions and liposomes

ADVANTAGES OF SOLID LIPID NANOPARTICLES[5,11,14,19,21] :

- ✶ Better control over release kinetics of encapsulated compounds
- ✶ Engineering via size and lipid composition
- ✶ Melting serve as trigger
- ✶ Enhanced bioavailability of entrapped bioactive compounds
- ✶ Chemical protection of labile incorporated compounds
- ✶ Much easier to manufacture than biopolymeric nanoparticles
- ✶ No special solvent required

- ✶ Conventional emulsion manufacturing methods applicable
- ✶ Raw materials essential the same as in emulsions
- ✶ Very high long-term stability
- ✶ Application versatility
- ✶ Can be subjected to commercial sterilization procedures
- ✶ Can be freeze dried to form powdered formulation
- ✶ The use of physiological lipids
- ✶ A potential wide application spectrum (dermal, per os, intravenous)

LIPIDS AND EMULSIFIERS USED FOR THE PREPARATION OF SOLID LIPID NANOPARTICLES[5,11,]

A] LIPIDS:

Triglyceride:

- Tricaprin
- Trilaurin
- Trimyristin
- Tripalmitin
- Tristearin
- Hydrogenated coco-glycerides

Hard fat types:

- Witepsol W35

- Witepsol H35
- Witepsol H42
- Witepsol E85
- Glyceryl monostearate
- Glyceryl behenate
- Glyceryl palmitostearate
- Cetyl palmitate
- Stearic acid
- Palmitic acid
- Decanoicacid
- Behenicacid
- Acidan N12

Emulsifiers

- Soybean lecithin
- Egg lecithin
- Phosphatidylcholine
- Poloxamer (188, 182, 407)
- Poloxamine (908)
- Tyloxapol
- Span (20, 40, 60, 80)
- Tween (20,40,60,80)
- Polyvinylalcohol

Co emulsifiers

- Sodiumcholate
- Sodium glycocholate
- Taurocholic acid
- Taurodoxycholic acid
- Butyric acid

INFLUENCE OF EXCIPIENTS[11,12,16,]

Formulation variables in the product quality:

Particle size

Alteration of the size significantly affects the physical stability, biofate of the lipid particles, and release rate of the loaded drug. Hence the size of the SLNs has to be controlled within reasonable range. Well formulated systems (liposomes, nanospheres and nanoparticles) should display a narrow particle size distribution in the submicron size range (as having size below 1μm), according to the definition of colloidal particles.

Influence of the ingredients on product quality

The particle size of lipid nanoparticles is affected by various parameters such as composition of the formulation (such as surfactant/ surfactant mixture, properties of the lipid and the drug incorporated), production methods and conditions (such as time, temperature, pressure, cycle number, equipment, sterilization and lyophilization). Large particle size is obtained at lower processing temperature. The hot homogenization technique gives a smaller particle size, generally below 500 nm, and a narrow particle size distribution as compared to cold

homogenization. Mean particle size as well as polydispersity index (PI) values are reported to be reduced at increasing homogenization pressure up to 1500 bar and number of cycles (3-7 cycles).

Influence of the lipids

Using the hot homogenization, it has been found that the average particle size of SLN dispersions is increasing with higher melting lipids. However, other critical parameters for nanoparticle formation will be different for the different lipids. The examples include the velocity of lipid crystallization, the lipid hydrophilicity (influence on self-emulsifying properties and the shape of the lipid crystals (and therefore the surface area)..

Further, increasing the lipid content over 5-10% resulted in larger particles (including microparticles) and broader particle size distribution in most cases.

Influence of the emulsifiers

The concentration of the surfactant/surfactant mixture strongly affects the particle size of the lipid nanoparticles. In general, smaller particle sizes were observed when a higher surfactant/ lipid ratio was chosen. The decrease in surfactant concentration resulted in increase of particle size during storage.

Surfactants decrease the surface tension between the interface of the particles causing portioning of the particles and thereby increasing the surface area.

PREPARATION OF SOLID LIPID NANOPARTICLES[5,10,11,12,14,19,20,21] :

SLNs are prepared from lipid, emulsifier and water/solvent by using different methods and are discussed below.

Methods of Preparation of Solid Lipid Nanoparticles

1. High pressure homogenization

    Hot homogenization

    Cold homogenization

2. Ultrasonication/high speed homogenization

    Probe ultrasonication

    Bath ultrasonicatioin

3. Solvent evaporation method
4. Solvent emulsification-diffusion method
5. Supercritical fluid method
6. Microemulsion based method
7. Spray drying method
8. Double emulsion method
9. Precipitation technique

1. High pressure homogenization (HPH):

It is a reliable and powerful technique, which is used for the production of SLNs. High pressure homogenizers push a liquid with high pressure (100–2000 bar) through a narrow gap (in the range of a few microns). The fluid accelerates on a very short distance to very high velocity

(over 1000 km/h). Very high shear stress and cavitation forces disrupt the particles down to the submicron range. Generally 5-10% lipid content is used but up to 40% lipid content has also been investigated.

Two general approaches of HPH are hot homogenization and cold homogenization; work on the same concept of mixing the drug in bulk of lipid melt.

A). Hot homogenization:

Hot homogenization is carried out at temperatures above the melting point of the lipid and can therefore be regarded as the homogenization of an emulsion. A preemulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high-shear mixing device. HPH of the pre-emulsion is carried out at temperatures above the melting point of the lipid. In general, higher temperatures result in lower particle sizes due to the decreased viscosity of the inner phase. However, high temperatures increase the degradation rate of the drug and the carrier. Increasing the homogenization pressure or the number of cycles often results in an increase of the particle size due to high kinetic energy of the particles.

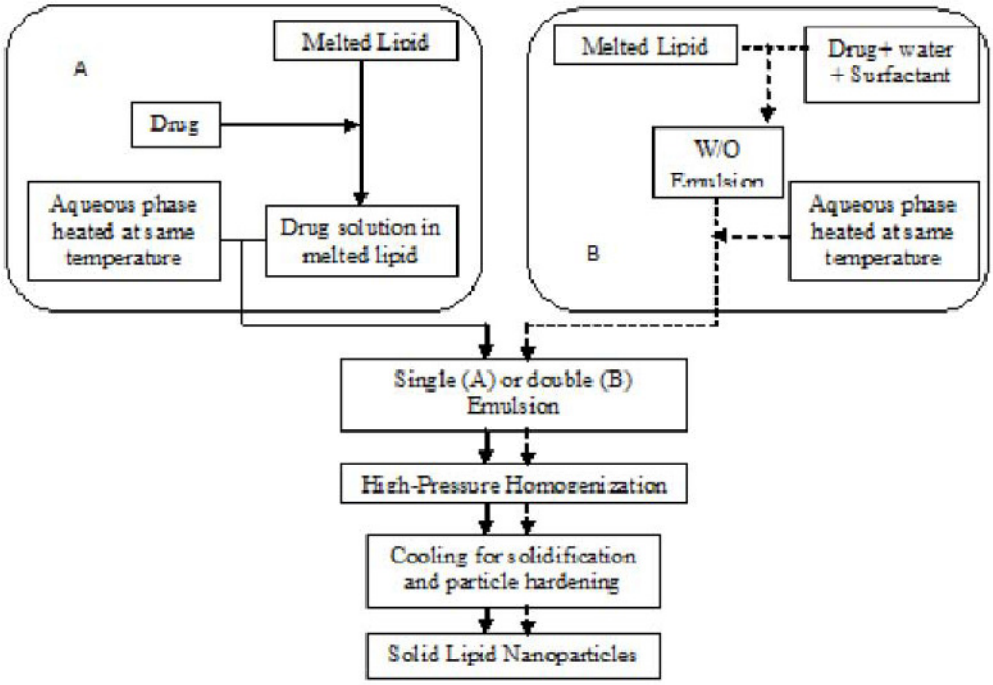


Fig. 6 Solid lipid nanoparticles preparation by hot homogenization process

B). Cold homogenization:

Cold homogenization has been developed to overcome various problems associated with hot homogenization such as: Temperature-induced drug degradation, drug distribution into the aqueous phase during homogenization, Complexity of the crystallization step of the nanoemulsion leading to several modifications and/or supercooled melts (62). In this technique the drug containing lipid melt is cooled, the solid lipid ground to lipid microparticles and these lipid microparticles are dispersed in a cold surfactant solution yielding a pre-suspension. Then this pre-suspension is homogenized at or below room temperature, the cavitation force is strong enough to break the lipid microparticles directly to solid lipid nanoparticles.

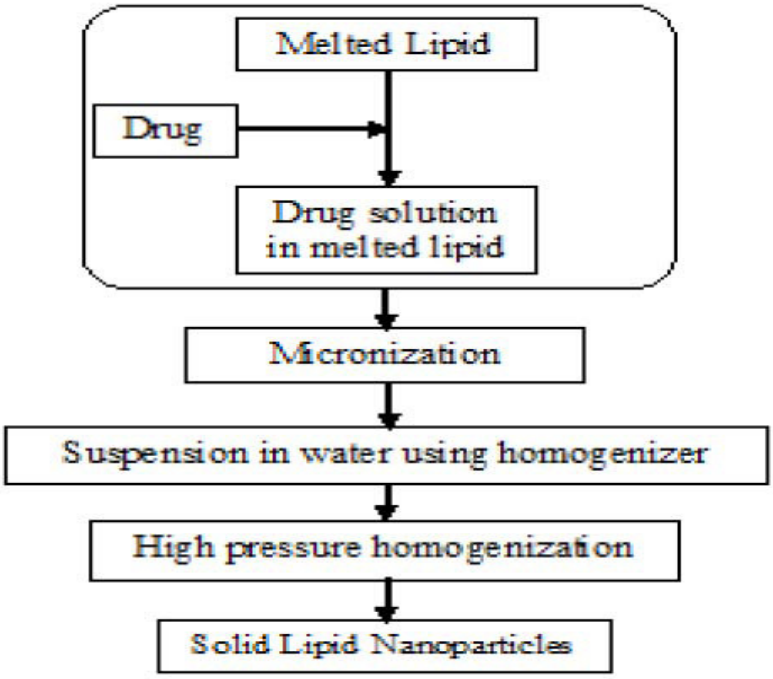


Fig. 7 Solid lipid nanoparticles preparation by cold homogenization process.

Advantages :

- ✶ Low capital cost
- ✶ Demonstrated at lab scale

Disadvantages :

- ✶ Energy intensive process
- ✶ Demonstrated at lab scale Biomolecule damage
- ✶ Polydisperse distributions
- ✶ Unproven scalability

2. Ultrasonication/high speed homogenization:

SLNs are also prepared by ultrasonication or high speed homogenization techniques. For smaller particle size combination of both ultrasonication and high speed homogenization is required.

Advantages:

- ✶ Reduced shear stress

Disadvantages:

- ✶ Potential metal contamination
- ✶ Physical instability like particle growth upon storage

3. Solvent evaporation:

SLNs are also prepared by solvent evaporation method. The lipophilic material is dissolved in a water-immiscible organic solvent (e.g. cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent, nanoparticles dispersion is formed by precipitation of the lipid in the aqueous medium by giving the nanoparticles of 25 nm mean size. The solution was emulsified in an aqueous phase by high pressure homogenization. The organic solvent was removed from the emulsion by evaporation under reduced pressure (40–60 mbar).

Advantages:

- ✶ Scalable
- ✶ Mature technology
- ✶ Continous process
- ✶ Commercially demonstrated

Disadvantages:

- ✶ Extremely energy intensive process

- ✶ Polydisperse distributions
- ✶ Biomolecule damage

4. Solvent emulsification-diffusion method:

The particles with average diameters of 30-100 nm can be obtained by this technique. Avoidance of heat during the preparation is the most important advantage of this technique.

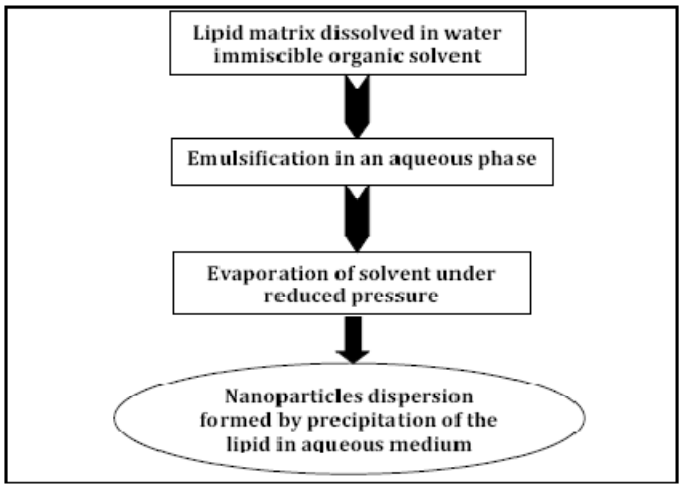


Fig. 8 Systematic representation for emulsification-diffusion method

5. Supercritical fluid method:

This is an alternative method of preparing SLNs by particles from gas saturated solutions (PGSS).

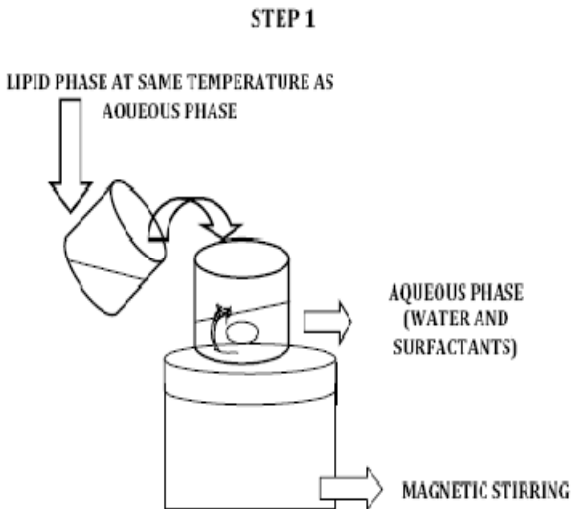
Advantages

- ✶ Avoid the use of solvents
- ✶ Particles are obtained as a dry powder, instead of suspensions
- ✶ Mild pressure and temperature conditions

✶ Carbon dioxide solution is the good choice as a solvent for this method

6. Microemulsion based method:

This method is based on the dilution of microemulsions. As microemulsions are two-phase systems composed of an inner and outer phase (e.g. o/w microemulsions). They are made by stirring an optically transparent mixture at 65-70°C which typically composed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20), co-emulsifiers (e.g. butanol,) and water. The hot microemulsion is dispersed in cold water (2-3°C) under stirring. SLN dispersion can be used as granulation fluid for transferring in to solid product (tablets, pellets) by granulation process, but in case of low particle content too much of water needs to be removed. High-temperature gradients facilitate rapid lipid crystallization and prevent aggregation. Due to the dilution step; achievable lipid contents are considerably lower compared with the HPH based formulations.



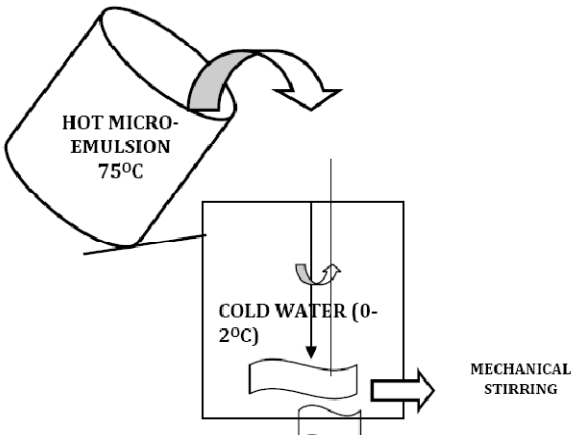


Fig. 9 Microemulsion method

Advantages:

- ✶ Low mechanical energy input
- ✶ Theoretical stability

Disadvantages:

- ✶ Extremely sensitive to change
- ✶ Labor intensive formulation work
- ✶ Low nanoparticle concentrations

7. Spray drying method:

It is an alternative technique to the lyophilization process. This recommends the use of lipid with melting point more than 70 c. The best results were obtained with SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in ethanol-water mixture.

8. Double emulsion method:

Here the drug is encapsulated with a stabilizer to prevent the partitioning of drug in to external water phase during solvent evaporation in the external water phase of w/o/w double emulsion.

9. Precipitation method:

The glycerides are dissolved in an organic solvent (e.g. chloroform) and the solution will be emulsified in an aqueous phase. After evaporation of the organic solvent the lipid will be precipitated forming nanoparticles.

SECONDARY PRODUCTION STEPS [11,14,22,23] :

Freeze drying

Lyophilization is a promising way to increase the chemical and physical stability over extended periods of time. Lyophilization had been required to achieve long term stability for a product containing hydrolysable drugs or a suitable product for per-oral administration. Transformation into the solid state would prevent the Oswald ripening and avoid hydrolytic reactions.

In case of freeze drying of the product, all the lipid matrices used, form larger solid lipid nanoparticles with a wider size distribution due to presence of aggregates between the nanoparticles. The conditions of the freeze drying process and the removal of water promote the aggregation among SLNs. An adequate amount of cryoprotectant can protect the aggregation of solid lipid nanoparticles during the freeze drying process.

Sterilization

Sterilization of the nanoparticles is desirable for parenteral administration and autoclaving which is applicable to formulations containing heat-resistant drugs. Effects of sterilization on particle size have been investigated and it was found to cause a distinct increase in particle size.

Spray drying

Spray drying might be an alternative procedure to lyophilization in order to transform an aqueous SLN dispersion into a dry product. This method has been used scarcely for SLN formulation, although spray drying is cheaper as compared to lyophilization.

The lipids with melting points at temperature >70°C had been recommended for spray drying.

DRUG INCORPORATION MODELS AND TYPES OF SLN[13]:

factors affecting loading capacity of a drug in lipid are

1. Solubility of drug in lipid melt
2. Miscibility of drug melt and lipid melt
3. Chemical and physical structure of solid matrix lipid
4. Polymorphic state of lipid material

Drug incorporation models are as follows:

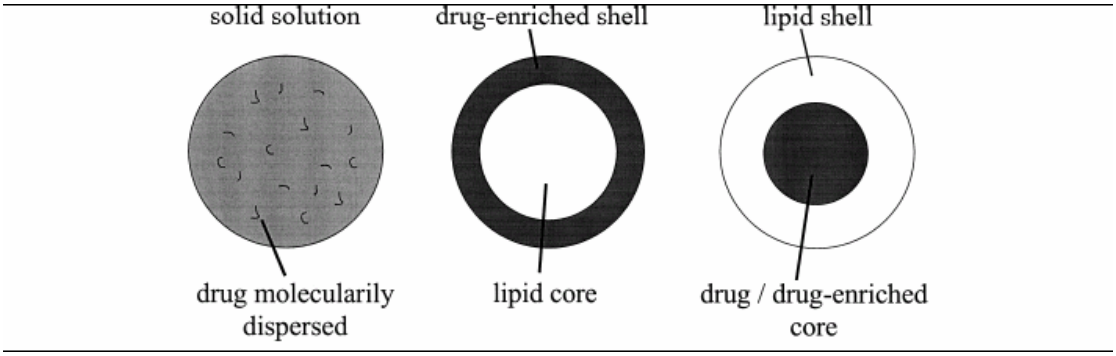


Fig. 10 Drug incorporation models

- Solid solution model:

  Drug is molecularly dispersed in lipid matrix when SLN is prepared by cold homogenization

- Drug-enriched shell model:

  A solid lipid core forms upon recrystalization temperature of the lipid is reached.

- Drug-enriched core model:

  Cooling the nanoemulsion leads to a supersaturation of the drug which is dissolved in the lipid melt leads to recrystallization of the lipid.

POSSIBLE PROBLEMS IN SLN PREPARATION AND SLN PERFORMANCE[11,13,21]:

1. High pressure-induced drug degradation:

It has been shown to decrease the molecular weight of polymers. High stress has been assumed to be the major cause and evidence of free radical formation was reported. But it's not a serious problem for majority of the drugs.

2. Lipid crystallization and drug incorporation:

The following four key aspects should be considered:

2. A) Supercooled melts:

The main reason for the formation of supercooled melts is the size dependence of crystallization processes. The tendency of the formation of supercooled melts increases with decreasing droplet size. It is therefore necessary to proof the solid state of the lipid by appropriate analytical techniques (NMR, X-ray or DSC).

2. B) Lipid modification:

The crystallized lipid may be present in several modifications of the crystal lattice. During storage, rearrangement of crystal might occur in favor of thermodynamically stable configurations and resulted in expulsion of drug. The utilization of higher drug-loading capacity in unstable configurations prevents lipid modification during storage.

2. C) Particle shape:

The shape of lipid nanoparticles may significantly differ from a sphere, lipids prefer to crystallize in the platelet form. This can be overcome by increasing the surfactant concentration.

2. D) Gelation phenomena:

The transformation of low-viscosity SLN dispersion into a viscous gel is known as gelation phenomena. It can be prevented by adding co-emulsifying surfactants with high mobility (eg. Glycocholate).

3. Coexistence of several colloidal species:

Unstable drugs will hydrolyze rapidly in contact with water and the distribution equilibrium of the drug between the different environments will be distorted. This can be overcome by increasing the matrix viscosity.

ANALYTICAL METHODS FOR CHARACTERIZATION[10,11,12,13,14,19,21,23] :

In order to develop a drug product of high quality, a precise physicochemical characterization of the SLNs is necessary. Characterization of solid lipid nanoparticles is a serious challenge due to the small size of the particles and complexity of the system. Various parameters need to be considered as:

1. Particle size measurement:

Particle size may be determined by photon correlation spectroscopy (PCS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), scanning tunneling microscopy (STM), freeze fracture electron

microscopy (FFEM) and laser diffraction (LD). PCS is a good tool for particle size measurement as it covers a size range from few nanometers to 3 μm but unable to cover larger microparticles. This can be overcome by using laser diffraction (LD).

2. Measurement of zeta potential:

Zeta potential of SLN is measured by photon correlationspectroscopy (PCS) using Malvern Zetasizer. The zetasizer measures the zeta potential based on the Smoluchowski equation.

3. Entrapment efficiency:

The amount of drug incorporated is determined after separation of the free drug and solid lipids from the aqueous medium and the separation is carried out by ultra-centrifugation, centrifugation filtration or gel permeation chromatography. Analysis is performed by UV- spectrophotometry or by HPLC.

4. Stability studies

Drug loaded SLNs are stored at 25 °C for 6 months and average size and entrapment efficiency are determined.

5. Effect of sterilization

To see the effect of sterilization on particle size, zeta potential and entrapment efficiency, blank and drug dispersions are autoclaved at 121 °C for 20 minutes.

6. Characterization by differential scanning calorimetry

Differential scanning calorimetry (DSC) (yields information on melting behavior and crystallization behavior of solid and liquid constituents of the particles) are performed.

7. Powder X-ray diffractometry (PXRD)

PXRD studies are performed in order to indentify the crystallinity behavior of the SLN.

8. In-vitro and ex-vivo methods for the assessment of drug release from SLN

8. A) *In-vitro* drug release:

I) Dialysis tubing

The SLN dispersion is placed in prewashed dialysis tubing which can be hermetically sealed. The dialysis sac is then dialyzed against a suitable dissolution medium at room temperature; the samples are withdrawn from the dissolution medium at suitable intervals, centrifuged and analyzed for drug content using a suitable analytical method.

8. B) Reverse dialysis

In this technique a number of small dialysis sacs containing 1 ml of dissolution medium are placed in SLN dispersion. The SLNs are then displaced into the dissolution medium. The direct dilution of the SLNs is possible with this method.

8. C) Franz diffusion cell

The solid lipid nanoparticle dispersion is placed in the donor chamber of a Franz diffusion cell fitted with a cellophane membrane. The dispersion is then dialyzed against a suitable dissolution medium at room temperature; the samples are withdrawn from the

dissolution medium at suitable intervals and analyzed for drug content. The maintenance of sink condition is essential.

9. Ex-vivo model for determining permeability across the gut

The jejunum 20–30 cm distal from the pyloric sphincter was used. The mucosal side was bathed with ringer buffer and the SLN were placed on the mucosal side, dispersed in ringer containing the paracellular transporter sodium fluorescein confirming for tissue integrity.

10. Pharmacokinetic analysis

Serum concentration versus time data for drug in individual rats is analyzed by non-compartmental estimations. Relative bioavailability, Cmax, Tmax, AUC and MRT can be estimated.

ADMINISTRATION ROUTES OF SLN[10,11,12,13,14,21,23]:

The various administration routes of SLN is as follows:

1. Parenteral administration:

Peptide and proteins drugs are usually available for parenteral use in the market. Since their conventional oral administration is not possible due to enzymatic degradation in GI tract. Parenteral application of SLN reduces the possible side effects of drug incorporated with the increased bioavailability. These systems are very suitable for drug targeting.

2. Oral administration:

Controlled release behavior of SLNs is reported to enable the bypass of gastric and intestinal degradation of the encapsulated drug, and their possible uptake and transport through

the intestinal mucosa. However, the assessment of the stability of colloidal carriers in GI fluids is essential in order to predict their suitability for oral administration.

3. **Rectal administration:**

When rapid pharmacological effect is required, in some circumstances, parenteral or rectal administration is preferred. This route is used for pediatric patients due to easy application.

4. Nasal administration:

Due to its fast absorption and rapid onset of drug action, avoiding degradation of labile drugs in the GIT and insufficient transport across epithelial cell layers nasal is preferred.

5. Respiratory delivery:

Nebulisation of solid lipid particles carrying anti-tubercular drugs, anti-asthmatic drugs and anti-cancer was observed to be successful in improving drug bioavailability and reducing the dosing frequency for better management of pulmonary action.

6. Ocular administration:

Biocompatibility and muco-adhesive properties of SLN improve their interaction with ocular mucosa and prolong corneal residence time of the drug, with the aim of ocular drug targeting.

7. Topical administration:

SLN are very attractive colloidal carrier systems for skin applications due to their various desirable effects on skin besides the characteristics of a colloidal carrier system. They are well suited for use on damaged or inflamed skin because they are based on non-irritant and non-toxic lipids.

APPLICATIONS[5,10,11,12,13,14,17,18,19,21] :

Solid lipid Nanoparticles possesses a better stability and ease of upgradability to production scale as compared to other colloidal drug delivery systems. This property may be very important for many modes of targeting. The several potential applications of SLNs and its pupose are given below:

1. Cancer therapy

- Targeting.
- Reduced toxicity.
- Enhanced uptake of antitumour agents.
- Improved in invitro and invivo stability.

2. Intracellular targeting

- Target reticuloendothelial systems for intracellular infections.
- Prolonged systemic circulations
- Prolonged systemic drug effect.
- Avoid uptake by RES.

3. Vaccine adjuvant

- Enhanced bioavailability.
- Protection from gastrointestinal enzymes.

4. Ocular delivery

- Improved retention of drug-reduced washout.

5. DNA delivery

- Enhanced delivery.

- Significantly higher excretion levels.

6. Oligonucleotide delivery

- Enhanced delivery of oligonucleotide.

7. Topical delivery

- Antifungals and anticancers.

8. Cosmetic application

- Preparation of sunscreens as an active carrier agent for molecular sunscreens and UV blockers.

9. Agricultural applications

- Suitable carrier for ecologically safe pesticides.

10. Stealth nanoparticles:

- Target specific cells

11. Other applications.

- Crosses blood brain barrier.
- Improved absorption and permeation.
- Enzyme immunoassay.
- Radioimaging.
- Oral delivery of peptides.

CHAPTER III

LITERATURE REVIEW

1. **Wenzhong zhou et.,** al studied formulation of enrofloxacin-loaded SLN and determined the effect of fatty acids on the characteristics and pharmacokinetics of SLN. The results showed that the encapsulation efficiency and loading capacity of SLN varied with fatty acids in the order of stearic acid > palmitic acid> tetradecanoic acid. Pharmacokinetic study revealed that the fatty acids increases the bioavailability of SLN. These results suggest that enrofloxacin-loaded SLN are promising formulations for sustained release while fatty acids had significant influences on the characteristics and performance of the SLN
2. Vinay kumar studied the effect of surface modification of solid lipid nanoparticles in brain targeting and concluded that surface modification of ropinirole loaded SLN with thiolated chitosan resulted in increased plasma levels as well as brain levels. Thiolated chitosan coated SLN were excellent carrier systems with good stability for brain targeting.
3. **Maria luisa bondi et al.,** studied SLN containing nimesulide SLNs carrying nimesulide were prepared and characterized, and the anti-proliferative effect of drug-loaded SLN verses free drug on HT-29 and SW-480 cell lines was here evaluated. All the obtained system posses colloidal size, ranging from 85 to 135 nm and negative zeta potential values. These systems show good loading capacity and drug release profile,and an invitro anti-tumour activity comparable to free drug

4. **Waree tiyaboonchai et al.,** developed curcurminoids loaded SLN using a micoemulsion technique at 75°C. It was found that variation in the amount of ingredient had profound effects on the loading capacity, mean particle size and size distribution. The results revealed that after storage in the absence of sunlight for 6months the percentage of remaining curcuminoids decreases.
5. **Mansoor A. khn et al.,** developed the non-destructive methods of characterization of risperidone SLN. Uniform distribution of risperidone and compritol ATO 888 is revealed by near infrared-chemical imaging, and good correlation was obtained. This offers the the advantage over conventional method of quantitation in that multicomponent of a formulation can be estimated instantaneously and simultaneously after the construction of model.
6. **Pallavi V pople et al.,** investigated novel particlulate carrier system [SLN] for topical application of vitamin A palmitate. SLN and its gels were evaluated for particle size, drug release, invitro penetration, invivo skin hydration and skin irritation. In conclusion, SLN represent a highly effective, nonirritant carrier for cosmetic and topical preparations, where improved skin hydration and drug penetration it desired.
7. **Nagi A.ALHaj et al.,** investigated tamoxifen loaded SLN for antitumoral activity. SLN was characterized by DSC,TEM. Zeta potential and particle size. The results of characterization studies strongly support the potential application of tamoxifen loaded SLN as a carrier system for treating breast cancer.
8. **Anna M. Fadda et al.,** studied the formulation of Artemisia arborescens L essential oil loaded SLN for agricultural use. SLN was evaluated for Zeta potential, particle size DSC

and TEM. The results suggested that SLN are good potential carriers for ecological pesticides in agriculture.

9. **Hammam A.Mowafy et al.,** developed 5-fluorouracil loaded SLN for local treatment of colon cancer. SLN were prepared by double emulsion-solvent evaporation technique. From the results, it was found that SLN has a high potential to improve the uptake of anticancer drugs inside colon tumors.
10. **R.S.R. Murphy et al.,** developed etoposide-loaded SLN with glyceride lipids and then characterized for invitro steric stability and drug release characteristics. The study demonstrate that the melt emulsification and homogenization technique followed by spray drying is a suitable method to obtain stable powder particles further invivo use of SLN prolonged the circulating carriers in blood.
11. **Mahavir chougule et al.,** studied that methotrexate-loaded SLN for topical treatment of psoriasis. Findings of the studies suggest that there is significant improvement in therapeutic index for the treatment of psoriasis.
12. **Vivek et al.,** investigated the effect of lipid matrix on olanzapine-loaded SLN by hot melt emulsification, high-pressure homogenization technique using precirol (PRE), glyceryl monostearate (GMS), glyceryl tristearate (GTS), witepsol (WE), and then characterized. SLN surface hydrophobicity was in the following order: GTS > PRE > WE > GMS.
13. **Gande suresh et al.,** studied the preparation of lovastatin-loaded SLN to improve the bioavailability by administering duodenally to rats. Results concluded that lipophilic drugs like lovastatin provide controlled release of drug, and are preferred to overcome the bioavailability problems.

14. **Helsagon et al.,** investigated the effect of surfactant surface coverage on formation and stability of tween 20 stabilized tripalmitin SLN. The results suggest that surfactant coverage at the interface may influence crystal structure and stability of SLN via surface-mediated crystal.
15. **Zaida Urban-Morlan et al.,** developed the formulation of cyclosporine-loaded SLN by emulsification-diffusion method and to study their physicochemical stability. Cyclosporine release from SLNs was relatively fast (99.60 % in 45 minutes), in association with a rapid dissolution due to the presence of many imperfections in lipid structure hence it improves the bioavailability of cyclosporine.
16. **Veerabrahma kishan at al.,** studied the nitrendipine loaded SLN for improving the oral bioavailability, using triglyceride, monoglyceride and wax. Bioavailibilty of nitrendipine SLN was increased three- to four- fold after intraduodenal administration compared to nitrendipine suspension. The obtained results are indicative of SLN as carriers for improving the bioavailability of nitrendipine.
17. **Keon wook et al.,** developed doxorubicin loaded SLN by sovent emulsification-diffusion method. In conclusion, SLN with small particle size, high entrapment efficiency, and relatively high drug loading can be obtained by this method.
18. **Doijad et al.,** developed cisplatin engineered SLN for the overall improvement in the efficacy, reduced toxicity and enhancement of therapeutic index of cisplatin. The in vitro result of formulated SLNs of cisplatin reveals that the drug is preferentially targeting to liver followed by brain and lungs.

19. **Sedef Erdal at al.,** prepared indomethacin loaded SLM and evaluated. invitro drug release is prolonged when sucroester is used as surfactant and type of surfactant was found to affect the dissolution rate of indomethacin.
20. **Qing-yu Xiang et al.,** developed a nfor the lung-targeting delivery of dexamethasone acetate by intravenous administration. The concentration of drug in the lung reached a maximum level at 0.5 hour post dexamethasone-SLN injection, 8-fold larger area under the curve was achieved compared to solution. These results indicate that SLN may be promising lung-targeting drug carrier for dexamethasone.
21. **Elisabetta et al.,** investigated the effects of processing conditions on the characteristics of solid lipid microparticles (SLM) with a potential application as carriers for pulmonary administration. The results indicate that a single intratracheal administration of SLM does not induce a significant inflammatory airway response in rats and that the SLMs might be a potential carrier for encapsulated drug via the pulmonary route.
22. **Patil et al.,** studied the preparation of miconazole nitrate loaded SLN for topical delivery. The formulations could significantly increase the accumulative uptake of miconazole in skin over marketed gel and showed a significantly enhanced skin targeting effect. These results suggests that SLN formulation with skin targeting may be a promising carrier for topical delivery of miconazole nitrate.
23. **Santa scalia at al.,** developed solid lipid budesonide microparticles for controlled release inhalation therapy. This system was prepared by oil in water wmulsification followed by spray drying and evaluated in terms of morphology, particles size distribution, crystallinity, thermal properties, aerosol performance and dissolution release. From the

studies it has shown that SLM may provide a useful approach to controlled release respiratiory therapy.

24. **Behzad et al.,** conducted the study to develop SLN of nitrofurazone using cold homogenization technique. Results show type and percentage of surfactant, type of lipid and drug/lipid ration can influence SLN character and drug release from SLN was the rate limiting step for drug permeation across rat skin and so prepared formulations can act as a drug reservoir.
25. **Guangxi Zhai et al.,** investigated the development of penciclovir-loaded SLN and evaluate the potential of SLNs as the carrier of penciclovir for topical delivery. From the results it can be concluded that SLNs provide a good skin targeting effect and may be a promising carrier for topical delivery of penciclovir.
26. **Madhushudanan et al.,** developed arthemeter-loaded lipid nanoparticles and evaluated for mean particle size, zeta potential, encapsulation efficiency and histopathological analysis. Histopathological analysis showed no significant histological changes in liver and kidney tissues in mice treated with formulations. The results highlights the suitability of lipid nanoparticles to deliver parenterally the anti-malarial drug artemether.
27. **Yingchao Li et al.,** developed the SLN loaded traditional Chinese medicine by ultrasonication method. Stability evaluation showed relatively long-term stability with only slight particle growth ($P > 0.05$) after storage at room temperature for 4 weeks. Therefore, ultrasonication is demonstrated to be a simple, available and effective method to prepare high quality SLN loaded traditional Chinese medicine.
28. **Sanjay Garg et al.,** developed the lipid nanoparticles of chlorambucil. From the results, higher AUC values of formulation as compared to CLB solution ($p < 0.01$) in tumors

suggested that the presence of DDAB on the lipid nanoparticles resulted in greater accumulation of the drug in tumors.

29. **Vobalaboina Venkateswarlu et al.,** studied the SLN delivery systems of clozapine. Homogenization followed by ultrasonication method is suitable to produce SLN of 60–380 nm size ranges. The results suggest that this system is most suitable for exploiting lymphatic transport pathway for improving oral bioavailability of clozapine.
30. **Vanna Sanna et al.,** investigated the effect of saturated fatty alcohols having different chain length (C12–C18), formulated into Precirol-based lipid nanoparticles, on the ex vivo skin permeability of Econazole nitrate. The analysis of results revealed that the effect of fatty alcohols on the Econazole nitrate permeation is structure-dependent, and associated with an increase of the permeability coefficients that can improve the interaction between alcohols and skin lipids.
31. **Marı´a J. Blanco-Prieto et al.,** developed a lipid nanoparticle system that would decrease systemic toxicity as well as to improve the therapeutic potential of the drug. It was concluded that compritol presents advantages as a matrix material for the manufacture of the nanoparticles and for the controlled release of edelfosine.
32. **Attama et al.,** described the characterization of SLN prepared using theobroma oil and fat as the main lipid matrix. And the results concluded that SLN of theobroma oil containing phospholipid could prove to be a good ocular or parenteral drug delivery system considering the low particle size, particle size stability and in vivo tolerability of the component lipids, which had higher increase in $d_{90\%}$ on storage are suitable for preparation of topical and transdermal products.

33. Chun- ching lin et el., developed quercetin-loaded nanoparticles by a nanoprecipitation technique with eudragit and polyvinyl alcohol as carriers, and to evaluate the antioxidant effects of quercetin and of its nanoparticles. This system was characterized by particle size and morphology, yield and encapsulation efficiency, DSC, X-ray diffraction, FTIR, nuclear magnetic resonance and dissolution study. Particle size of < 85 nm, encapsulation efficiency over 99%, no interaction between the drug and lipid were obtained. The release of the drug from SLN was 74-fold higher compared with the pure drug. In addition, the antioxidant activity of quercetin was more effective than pure quercetin on DPPH scavenging, anti-superoxide formation, superoxide anion scavenging and anti-lipid peroxidation.
34. Hu et al., developed clobetasol propionate by a novel diffusion method in aqueous system and physicochemical charactization were performed. The recovery lipophilic model drug clobetasol propionate was incorporated to study the recovery of nanoparticles, entrapment efficiency, zeta potential and drug delivery characterization. A novel preparation method and the optimized separation parameters in the present research for SLN were established. The results also demonstrate the principle suitability of SLN as a prolonged release formulation for lipophilic drugs.
35. **Shulka et al.,** studied the preparation and evaluation of polymethacrylic acid nanoparticles containing lamivuidine. The results suggested that the developed formulation overcome and alleviates the drawbacks and limitations of lamivudine sustained release formulations and could possibility be advantageous in terms of increased bioavailabilty of lamivudine.

36. **Mandip singh et al.,** investigated the celecoxib-loaded SLN for pulmonary targeting. Celecoxib encapsulated SLN were found to be stable and aerodynamic properties were within the respirable limits. Aerosolization of celecoxib-SLN improved the celecoxib pulmonary bioavailability compared to solution formulation which will potentially lead to better patient compliance with minimal dosing intervals.
37. **Vasheghani et al.,** developed ketoprofen-loaded SLN using beeswax and carnauba wax. The characteristics of the SLNs with various lipid and surfactant composition were investigated. It was found the SLN with more beeswax content in their core exhibited faster drug release as compared with those containing more carnauba wax in their structure.
38. **Souto et al.,** developed lopinavir SLN for intestinal lymphatic targeting. From the intestinal lymphatic transport study it became evident that SLN increased the cumulative percentage dose of lopinavir secreted into the lymph. The AUC for the lopinavir SLN was 2.13- fold higher than obtained for the conventional drug solution available in the market. From the results it was concluded that SLNs can be used both as a drug carrier for bioavailability enhancement and for targeting.
39. **Chong-kook et al.,** developed an alternative formulation of paclitaxel suitable for parenteral administration, paclitaxel-loaded sterically stabilized. SLN were prepared, characterized and examined for in vitro cytotoxicity. Treatment of the human ovarian cancer cell line and the breast cancer cell line with paclitaxel-loaded SLNs yielded cytotoxicities comparable to those of a commercially available Cremophor EL-based formulation. These results collectively suggest that our optimized SLN formulation may have a potential as alternative delivery system for pareteral administration of paclitaxe.

40. **Jia-you fang et al.,** developed lipid nanoparticles as vehicles for topical psoralen delivery. Nanostructured lipid carriers (NLC) were also prepared for comparison. SLN and NLC showed respective mean particle sizes of 300 and 200 nm respectively. Hyperproliferative or psoralen-like skin produce by repeated strippings in the dorsal skin of mouse was also used as a permeation barrier. The results showed that the nanoparticulate systems could minimize the permeation differentiation between normal and hyperproliferative skin compared to the free drug in an aqueous control.
41. **Guihua huang et al.,** studied the preparation of temozolomide SLN, evaluation of its physiochemical characteristics, and to investigate the specific drug targeting of intravenous injected SLN of temozolomide. The results showed that the average diameter of 65.9 ± 11.8 nm with a zeta potential of -37.2 ± 3.6 mV and the release behavior was in accordance with higuchi – equation. In the tested organs, the AUC/dose and the MRT of SLN were much higher and longer than those of the drug solution. These results indicated that the SLNs is a promising sustained-release and drug-targeting system for antitumor drugs.
42. **Sami nazzal et al.,** studied the preparation and in vitro antiproliferative effect of tocotrienol loaded lipid nanoparticles (NLC) against noeplastic + SA mammary epithelial cells. Sonication time and pulsar rate were initially evaluated for their effect on the size and polydispersity of the nanoparticles using a full factorial design. Tocotrienol loaded lipid nanoparticles were shown to exhibit potent antiproliferative effect against neoplatic + SA mammary epithelial cells. (NLC) had comparable IC_{50} as the reference drug solution, which signified the importance of tocotrienol encapsulation within NLCs on

their activity. Furthermore, these findings suggested that NLCs may have potential value in the treatment of breast cancer.

43. **Vandana et al.,** investigated the development of tretinoin SLN and to evaluate the viability of an SLN based gel in improving topical delivery of tretinoin. The developed SLN were characterized for particle size, polydispersibility index, entrapment efficiency and morphology. Studies were carried out to evaluate in a significant improvement in the photostability in comparison to methanolic. In vitro permeation studies through rat skin indicated that an SLN based tretinoin gel has permeation profile comparable to that of the marketed tretinoin cream.

44. **Yajiang yang et al.,** studied the characterization and release of triptolide-loaded poly (D.L- lactic acid) nanoparticles prepared by modified spontaneous emulsification solvent diffusion method (SESD). From the evaluation studies, the morphology of the nanoparticles exhibited a fine spherical shape with smooth surfaces without aggregation or adhesion. The modified SESD method was a potential and advantage method to produce an ideal polymer nanoparticles for drug delivery system.

CHAPTER IV

AIM OF WORK

Asthma is a chronic inflammatory disease in which the patient has episodes of reversible airways obstruction due to bronchial hyperresponsiveness.

Montelukast sodium is a potent, selective and orally active leukotriene receptor antagonist that acts by inhibiting physiological actions of the cysteinyl leukotrienes. It is used in the prophylaxis and treatment of asthma exercise induced bronchospasm, allergic rhinitis, urticaria and to relieve symptoms of seasonal allergies.[30,31]

Montelukast sodium is an orally active compound that binds with high affinity and selectivity to the $CysLT_1$ receptor (in preference to other pharmacologically important airway receptors, such as the prostanoid, cholinergic or β-adrenergic receptor). Montelukast inhibits physiologic actions of LTD_4 at the $CysLT_1$ receptor without any agonist activity.

The **main drawback of conventional montelukast formulation is that it undergoes hepatic first pass metabolism. Thus, it shows plasma or biological half-life of 2.5 to 5.5 hours, thereby decreasing bioavailability upto 64 %.**

.

Based on these considerations, solid lipid nanoparicles system was developed for the montelukast sodium (using various lipid such as stearic acid, glyceryl monostearate and compritol ATO 888) by hot homogenization followed by ultrasonication method) such delivery system will improve the biological half-life as well as bioavailabilty. In the proposed research work, montelukast sodium is formulated as SLN to improve the bioavailability by avoiding hepatic metabolism, greater therapeutic efficacy, and improve patient compliances.

CHAPTER V

PLAN OF WORK

PART-I

1. Determination of λ max of montelukast sodium
2. Calibration curve for montelukast sodium in 0.5 % sodiumlauryl sulphate solution

PART-II

Formulation of montelukast sodium loaded solid lipid nanoparticles using different concentrations of lipids (glyceryl behenate, glyceryl monostearate and stearic acid) by hot homogenization followed by ultrasonication.

PART-III

Evaluation of montelukast sodium loaded solid lipid nanoparticles

1. Determination of drug content of SLN formulations
2. Determination of entrapment efficiency
3. In –vitro release studies of SLN dispersion
4. Estimation of release kinetics

PART-IV

5. Measurement of particle size of SLN formulations
6. Morphological studies of SLN dispersion using scanning electron microscopy

PART-V

7. Stability studies of SLN dispersion at refrigerated and room temperature.

PART-VI

8. Differential scanning calorimetry studies of drug with lipid was conducted to determine the compatibility of drug with lipid.

9. IR studies to determine interaction between lipids and drug.

CHAPTER-VI

MATERIALS AND EQUIPMENTS

MATERIALS USED

1. Drug- Montelukast Sodium	- Microlabs, Hosur
2. Glyceryl monostearate	- Central drug house
3. Glyceryl behenate	- Orchid Pharma
4. Stearic acid	- Universal Scientific
5. Polyvinyl alcohol	- Sisco research lab
6. Sodiumlauryl sulphate	- Loba chemie
7. Dichloromethane	- Rankem
8. Methanol	- Rankem
9. Ethanol	- Central drug house
10. Dialysis membrane 50 – LA 387	- Himedia

EQUIPMENTS USED

1. Rotary Flash Evaporator - Super fit rotary flash evaporator
2. Ultra Sonicator - Vibronic's Ultrasonic processor
3. Centrifugator - Eppendorf Centrifuge 5417 R
4. Mechanical stirrer - Scientific industries
5. Electronic Balance - A&D Company, Japan
6. Magnetic Stirrer - MC Dalal & co
7. UV Visible Spectrophotometer - UV Pharma Spec 1700, Shimadzu
8. Refrigerator - Kelvinator
9. Stability chamber - Inlab equipments.

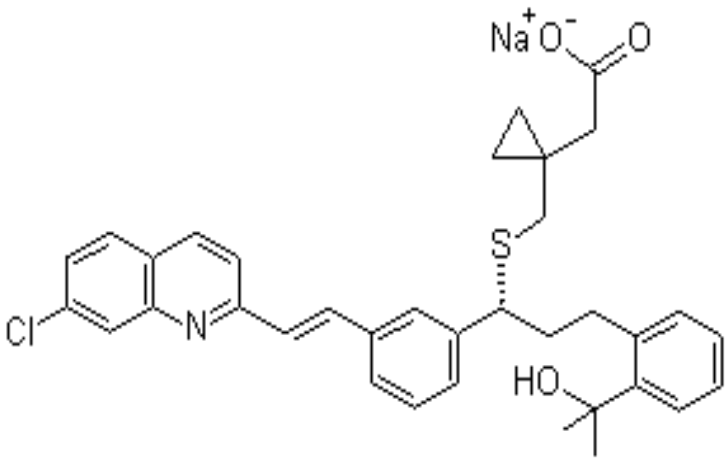
CHAPTER VII

MONTELUKAST SODIUM

DRUG PROFILE

Montelukast sodium is a potent, selective and orally active leukotriene receptor antagonist that acts by inhibiting physiological actions of the cysteinyl leukotrienes. It is used in the prophylaxis and treatment of asthma exercise induced bronchospasm, allergic rhinitis, urticaria and to relieve symptoms of seasonal allergies.[30,31]

STRUCTURAL FORMULA:[31,32]



EMPIRICAL FORMULA:[32,33,34]

$C_{35}H_{35}ClNNaO_3S^5$

CHEMICAL NAME:[1,30,32]

[R-(E)]-1-[[[1-[3-[2-(7-chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl acid] cyclopropaneacetic monosodium salt.

DESCRIPTION:[1,30,31,32]

Nature : White to off-white powder.

Solubility : Freely soluble in ethanol, methanol and water, and practically insoluble in acetonitrile.

Melting point : 135.5°C

Molecular weight : 608.2

MECHANISM OF ACTION: [34,35,36]

The cysteinyl leukotrienes { LTC_4, LTD_4, LTE_4} are products of arachidonic acid metabolism and are released from various cells, including mast cells and eosinophils. These eicosanoids bind to cysteinyl leukotriene receptors (CysLT) found in the human airway. Cysteinyl leukotrienes and leukotriene receptor occupation have been correlated with the pathophysiology of asthma, including airway edema, smooth muscle contraction and altered cellular activity associated with the inflammatory process, which contribute to the signs and symptoms of asthma.

Montelukast sodium is an orally active compound that binds with high affinity and selectivity to the $CysLT_1$ receptor (in preference to other pharmacologically important airway receptors, such as the prostanoid, cholinergic or β-adrenergic receptor). Montelukast inhibits physiologic actions of LTD_4 at the $CysLT_1$ receptor without any agonist activity.

PHARMACOKINETICS: [31,32,33,34,35,36]

Absorption:

It is rapidly absorbed after oral administration

Cmax is achieved in 2 hours

Tmax is achieved in 3 to 4 hours.

Mean oral bioavailability is 64 %

Distribution:

99 % bound to plasma proteins

Steady state volume of distribution is 8 to 11 litres

Metabolism:

Extensively metabolized by liver

Cytochrome P450 3A4 and 2C9 are involved in the metabolism of montelukast

Elimination:

The plasma clearance of montelukast averages 45 ml/min

Mean plasma half-life is 2.7 to 5.5 hours

Montelukast and its metabolites are excreted almost exclusively via the bile

Therapeutic indicatons:

Leukotriene receptor antagonist

Chronic asthma

Allergic rhinitis

Prophylaxis for exercise-induced asthma

Acute asthma attack

Dose

4 – 10 mg per day

Side effects

Oedema

Agitation

Restlessness

Allergy

Chest pain

Tremor

Dry mouth

Vertigo

Arthralgia

Sedation

Palpitations

Suicidality

Anxiousness

Drug interactions:

Phenobarbital which induces hepatic metabolism and decreased the AUC

Precautions:

Not indicated for use in the reversal of bronchospasm in acute asthma attack.

Should not be abruptly substituted for inhaled or oral corticosteroids

Should not be used as monotherapy for the treatment and management of exercise induced bronchospasm

Pateints with aspirin sensitivity should discontinue aspirin or other NSAIDs

Churg-Strauss syndrome

Contra-indications:

Hypersensitivity to any component of this product

Brand names: [24]

- Admont
- Emlukast
- Kast

- Molly
- Montal
- Montasma
- Montelast
- Monti
- Reokast
- Romilast
- Telecast

CHAPTER VIII

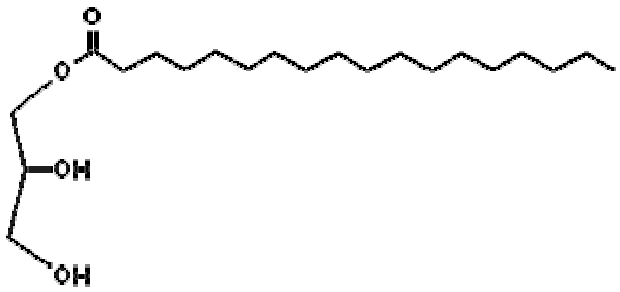
EXCIPIENTS PROFILE

GLYCERYL MONOSTEARATE

SYNONYM:[1,2,3,4,81]

Glyceryl stearate, Monostearin

STRUCTURE:



CHEMICAL NAME: [1,2,3,4,81]

3-Stearoyloxy-1,2-propanediol; Glyceryl stearate; Alpha-Monostearin; Monostearin; Octadecanoic acid, 2,3-dihydroxypropyl ester; Glycerin 1-monostearate; Glycerin 1-stearate; Glycerol alpha-monostearate; Glyceryl 1-monostearate; Stearic acid alpha-monoglyceride; Stearic acid 1-monoglyceride; 1-Glyceryl stearate; 1-Monostearin; 1-Monostearoylglycerol; 1,2,3-Propanetriol 1-octadecanoyl ester.

EMPIRICAL FORMULA:

$CH_3 (CH_2)_{16}COOCH_2CHOHCH_2OH$

MOLECULAR WEIGHT:

358.56

FUNCTIONAL CATEGORY:[81]

Emulsifying agent

DESCRIPTION :

White or cream colored waxy solid.

PROPERTIES : [1,2,3,4,81]

Physical state	:	white powder
Melting point	:	63 - 68 ^oC
Boiling point	:	> 100 ^oC
Solubility in water	:	soluble in hot water
Solvent solubility	:	soluble in methanol and chloroform mixture
HLB value	:	5.0

STABILITY AND STORAGE CONDITIONS:

It is stable under ordinary conditions, and should be stored in a well-closed container and protected from light.

SAFETY:

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

HANDLING PRECAUTIONS:[81]

Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe dust.

REGULATORY STATUS :

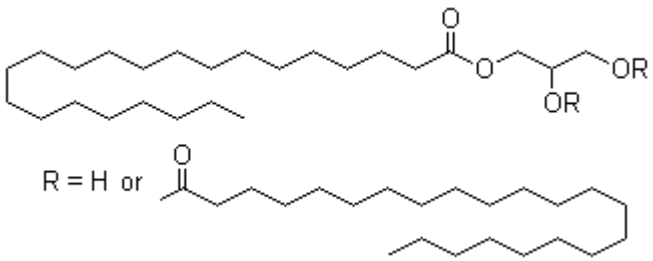
Included in the FDA inactive ingredients. Recognized by GRAS status.

GLYCERYL BEHENATE

SYNONYMS: [1,2,3,4,81]

Compritol 888 ATO; 2,3-dihydroxypropyl docosanoate; docosanoic acid, glyceryl monobehenate, 1,2,3-Propanetriol docosanoate.

STRUCTURE:



EMPIRICAL FORMULA:

$C_3H_8O_3 \cdot x(C_{22}H_{44}O_2)$

MOLECULAR WEIGHT:

414.66

FUNCTIONAL CATEGORY:

Coating agent

Tablet binder

Tablet and capsule lubricant

DESCRIPTION:

Fine white powder or hard waxy mass with a faint odor.

PROPERTIES: [1,2,3,4,81]

Physical state	:	Fine white powder
Melting point	:	65–77ºC
Boiling point	:	306 ºC
Solubility	:	Soluble, when heated, in chloroform and dichloromethane, practically insoluble in ethanol (95%), hexane, mineral oil, and water.
HLB value	:	12

STABILITY AND STORAGE CONDITIONS :

It should be stored in a tight container, at a temperature less than 358C.

SAFETY :

It is generally regarded as a relatively nonirritant and nontoxic material.

HANDLING PRECAUTIONS :

It emits acrid smoke and irritating fumes when heated to decomposition.

REGULATORY STATUS :

Included in the FDA Inactive Ingredients Guide (capsules and tablets).

STEARIC ACID

SYNONYMS: [1,2,3,4,81]

Cetylacetic acid; stereophonic acid; Tegostearic.

STRUCTURE:

CHEMICAL NAME:

Octadecanoic acid

EMPIRICAL FORMULA:

$C_{18}H_{36}O_2$

MOLECULAR WEIGHT:

284.47

FUNCTIONAL CATEGORY: [1,2,3,4,81]

Emulsifying agent

Solubilizing agent

Tablet and capsule lubricant

DESCRIPTION:

It is a hard, white or faintly yellow-colored, crystalline solid or a white or yellowish white powder.

PROPERTIES: [1,2,3,4,81]

Physical state	:	Crystalline solid/white or yellowish powder.
Melting point	:	554°C
Boiling point	:	383°C
Solubility	:	Freely soluble in benzene, carbon tetrachloride, chloroform, and ether; soluble in ethanol (95%), hexane, and propylene glycol; practically insoluble in water.
HLB value	:	15

STABILITY AND STORAGE CONDITIONS:[81]

It is 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.

SAFETY :

It is generally regarded as a nontoxic and nonirritant material. However, consumption of

excessive amounts may be harmful.

HANDLING PRECAUTIONS :

Stearic acid dust may be irritant to the skin, eyes, and mucous membranes. Eye protection, gloves, and a dust respirator are recommended. Stearic acid is combustible.

REGULATORY STATUS :

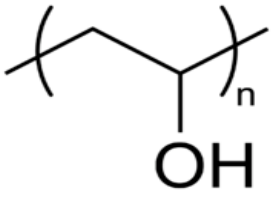
Included in the FDA Inactive Ingredients Guide (sublingual tablets; oral capsules, solutions, suspensions, and tablets; topical and vaginal preparations).

POLYVINYL ALCOHOL

SYNONYMS: [1,2,3,4,81]

Airvol; Gelvatol; Polyvinol.

STRUCTURE:



CHEMICAL NAME:

Ethenol, homopolymer

EMPIRICAL FORMULA:

(C2H4O)n

MOLECULAR WEIGHT:

20,000 – 200,000

FUNCTIONAL CATEGORY: [1,2,3,4,81]

Coating agent

Lubricant

Stabilizing agent

Viscosity-increasing agent

DESCRIPTION:

Odorless, white to cream-colored granular powder.

PROPERTIES:[4,81]

Physical state	:	White powder
Melting point	:	180–190°C
Boiling point	:	228 °C
Solubility	:	Soluble in water; slightly soluble in ethanol (95%); insoluble in organic solvents.

HLB value : 10 - 15

STABILITY AND STORAGE CONDITIONS:[81]

It is stable when stored in a tightly sealed container in a cool, dry place. It is stable on exposure to light.

SAFETY

It is generally considered a nontoxic material. It is nonirritant to the skin and eyes at concentrations up to 10%; concentrations up to 7% are used in cosmetics.

HANDLING PRECAUTIONS

Eye protection and gloves are recommended. Polyvinyl alcohol dust may be an irritant on inhalation. Handle in a well-ventilated environment.

REGULATORY STATUS

Included in the FDA Inactive Ingredients Guide (ophthalmic preparations and oral tablets).

CHAPTER IX

EXPERIMENTAL PROTOCOL

PREPARATION OF CALIBRATION MEDIUM:

0.5 % SODIUM LAURYL SULPHATE SOLUTION:

Weigh 5 grams of sodium lauryl sulphate and dissolve it in specified amount of distilled water with continuous stirring and this solution is make up to 1000 ml using distilled water to prepare 0.5 % Sodium lauryl sulphate.

PREPARATION OF CALIBRATION CURVE FOR MOTELUKAST SODIUM:[25,26,27,28]

The standard stock solution of Montelukast sodium is prepared by dissolving 100 mg of drug in 5 ml ethanol and diluted with 0.5 % SLS solution up to 100 ml. From the above stock solution, drug having different concentration of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10µg /ml concentration is prepared in using 0.5 % SLS solution with appropriate dilution.

The 10 ug/ml solution is scanned in UV spectrophotometer to find the λmax and the absorbance of the samples is measured at λmax (345nm). A graph is plotted by taking concentration in X-axis and absorbance in Y-axis to obtain the standard curve.

The standard curve prepared is used to estimate drug content, entrapment efficiency and percentage drug release.

FORMULATION OF MONTELUKAST SODIUM LOADED SOLID LIPID NANOPARTICLES:

Solid lipid nanoparticles are prepared by a **Hot homogenization** followed by **Ultrasonication** method. Montelukast sodium loaded solid lipid nanoparticles is prepared by using three different type of lipids (Compritol ATO 888, Glyceryl- monostearate and Stearic acid) of various concentrations of lipid i.e.1, 2, 5, 10, 15 and 20 %. Drug(10 mg) and Surfactant (100 mg) concentration is kept constant for all the formulations. Surfactant, Polyvinyl alcohol is used as a stabilizer to enhance the solubility of the drug in the lipid.

PROCEDURE:[37,38,42,46,49,56]

Montelukast sodium loaded SLN is prepared by a **hot homogenization** followed by **ultrasonication** method. Montelukast sodium and lipid of various concentration of lipid i.e.1, 2, 5, 10, 15 and 20 % is weighed and dissolved in ethanol. Organic solvents are completely removed using a rotary flash evaporator. The embedded lipid layer was melted by heating 5-10°C above the melting point of the lipid. An aqueous phase is prepared by dissolving polyvinyl alcohol (1% W/V) in distilled water (sufficient to produce 30 ml of preparation) and heating to the same temperature as oil phase. The hot aqueous phase is added to the oil phase and homogenization is performed (at 2000 rpm) using a mechanical stirrer for 60 minutes. The obtained coarse emulsion is allowed to cool to room temperature and stirred at 400 rpm for 30 mins. Then it is ultrasonicated using a vibronic's ultrasonic processor for 10 minutes. Montelukast sodium loaded SLN is finally stored in amber coloured containers.

CHARACTERISATION OF MONTELUKAST SODIUM LOADED SLN:

The formulated Montelukast sodium loaded solid lipid nanoparticles are evaluated for its drug content, entrapment efficiency, Particle size, SEM studies, DSC studies, FTIR studies and in vitro drug release studies.

PHYSICOCHEMICAL PROPERTIES:[40,42]

The SLN dispersions were characterized for physicochemical properties such as color, odor and stability after centrifugation. Centrifugation was performed at 2000 rpm for 30 minutes.

DETERMINATION OF DRUG CONTENT:[37,42]

1 mg equivalent of SLN dispersion is dissolved in 1 ml of ethanol and the volume is made upto 100 ml to make 10 ug / ml concentration and the absorbance is measured at 345 nm. From the absorbance drug content is measured.

DETERMINATION OF ENTRAPMENT EFFICIENCY:[37,41,44]

Entrapment efficiency of Montelukast sodium loaded SLN **is** determined by **Centrifugation** method. The nanoparticles are separated in a high speed cooling centrifuge at 14,000 rpm for 90 minutes at 4^{o}C. The sediment and supernatant liquid are separated. The supernatant solution is made up to desired volume with buffer. The amount of drug that is not incorporated in the SLNs could be obtained by the UV –spectrophotometer. The absorbance of the samples is measured at 345 nm to estimate the percentage entrapment efficiency.

The entrapment efficiency is calculated by following formula:

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount taken} - \text{Free drug}}{\text{Amount taken}} \times 100.$$

PARTICLE SIZE ANALYSIS:[47,51]

The mean diameter of SLNs in the dispersion was determined by photon correlation spectroscopy (PCS) using a laser light scattering instrument (LS230; COULTER) at a fixed angle of 90° at 25 °C. The particle size analysis data was evaluated using the volume distribution. Before measurement 1 ml of sample is diluted with distilled water.

IN VITRO DRUG RELEASE:[37,45]

In vitro release of Montelukast sodium from Montelukast sodium-loaded SLN formulations was determined by **dialysis bag method** using 0.5 % Sodium lauryl- sulphate solution as dissolution medium. Dialysis membrane having pore size 2.4 nm, molecular weight cut off 14,000 was used. Membrane was soaked in distilled water for 15 mins before using. 1 mg equivalent of drug in SLN dispersion was placed in the dialysis bag and kept in a beaker containing 250 ml of 0.5 % SLS solution as buffer. The dissolution medium was continuously stirred at 100 rpm and maintained at 37°C ± 2°C. Samples were withdrawn at predetermined time intervals, and the volume withdrawn was replaced with same volume of fresh dissolution medium. Samples were analyzed for Montelukast sodium drug content spectrophotometrically by measuring the absorbance at 345 nm against a suitable solvent blank. All experiments were repeated triplicate, and the average values were taken.

KINETIC MODELING:[69,70]

In order to understand the kinetic and mechanism of drug release, the result of in vitro drug release study of nanoparticles were fitted with various kinetic equation like zero order (cumulative % release vs. time), first order (log % drug remaining vs time), Higuchi's model (cumulative % drug release vs. square root of time). r2 and k values were calculated for the

linear curve obtained by regression analysis of the above plots. The exact mechanism by which the SLN formulations follows is determined by korse-meyer peppas model (log drug release vs log time).

SCANNING ELECTRON MICROSCOPY:[45,54,61]

Average particle size and surface morphology of the best formulation are evaluated using scanning electron microscopy. The sample was spread on an aluminium stub and allowed to dry at room temperature. The dried sample then was sputter coated with gold for 40 seconds using Hitachi Ion-Sputter E-1010. The images were captured with the Hitachi S-3400 Scanning Electron Microscope.

FT-IR STUDIES:[41,45,55]

The interaction between the drug with lipids is studied by using IR Spectrophotometer. Drug, lipid and its physical mixtures were subjected to FTIR studies. The FT-IR spectrum of pure drug and combination of drug with excipient are obtained by using Shimadzu FT-IR Spectrophotometer. The scanning range is 450-4000 cm^{-1} and the resolution is $4cm^{-1}$. Samples are prepared in KBr pellets.

DIFFERENTIAL SCANNING CALORIMETRY:[41,45,53]

Differential Scanning Calorimetry is performed using Perkin Elmer STA 6000 Thermal Analyzer. The instrument is calibrated with indium standard. Accurately weighed (it varies from 3mg-25mg) samples are placed in an open type ceramic sample pans. Thermo grams are obtained by heating the sample at a constant heating rate of 8ºC/minute. A dry purge of Argon gas (60ml/min) is used for all runs. Samples heated from 37ºC-400ºC. DSC thermograms were recorded for montelukast sodium pure drug, stearic acid, glyceryl monostearate, compritol ATO

888 and its physical mixture. For the investigation investigation of bulk material, about 1-2 mg of sample was used.

STABILITY STUDIES:[45,54,61]

According to modified ICH guidelines, all formulations of Montelukast sodium-loaded SLN were subjected to stability studies at 25 ± 2^0 C and 60 ± 5 RH for 1 month also at 4^0C and the entrapment efficiency is estimated for all the formulations at 1 week time intervals for 1 month.

CHAPTER X

RESULTS AND DISCUSSION

DETERMINATION OF λ – MAX OF MONTELUKAST SODIUM:

The λ max of montelukast sodium was estimated by scanning the 10µg/ml concentration of the drug solution in UV region (200-400 nm). It showed the λ max of **345nm**. The absorption spectra are shown in the fig. 11.

CALIBRATION CURVE OF MONTELUKAST SODIUM:

The calibration curve of montelukast sodium was prepared by using distilled water and 0.5 % SLS solution. Linear correlation coefficient was obtained for calibration of montelukast sodium in each medium. Montelukast sodium obeyed the Beer's law. Linearity was observed in the concentrations of 5 to 50 µg/ml. Calibration plot of montelukast sodium was shown in fig. 12 and the values in table 2.

DRUG CONTENT:

The drug content of all the formulations varied from 94.75 to 100 % and are shown in the table 3.

PREPARATION OF MONTELUKAST SODIUM LOADED SLN:

Homogenization followed by ultrasonication is a reliable, simple and reproducible method for preparing SLN. The composition of the formulations are shown in table 1. The prepared SLN dispersion was found to be uniform and homogenous in appearance.

PHYSICOCHEMICAL PROPERTIES:

The SLN dispersion was white in color, odorless, and fluid in nature. It was stable and did not show sedimentation even after centrifugation (2000 rpm for 30 minutes).

ENTRAPMENT EFFICIENCY:

The results of EE were shown in the table 4 and 5 and fig. 13. The influence of surfactant and lipid concentration is discussed below:

Influence of surfactant on entrapment efficiency:

Incorporation of 1 % polyvinyl alcohol, to stearic acid containing formulations (F 1 – F 6) showed EE of 23.1 – 29.3 %, to glyceryl monostearate containing formulations (F 7- F 12) showed EE of 21.7 – 42.6 % and to compritol ATO 888 containing formulations (F 13- F 18) had EE of 42.6 – 62.3 % (F 13 – F 18) shown in table 4. By Incorporating 2 % polyvinyl alcohol the EE was found to be 42.4 – 88.1 %, 40.5 – 79.3 % and 81.4 – 92.6 % respectively. From the results, it was found that an increase in surfactant concentration increases EE[33] independent of type and concentration of lipids.

Influence of lipid material on entrapment efficiency:

The EE of the formulations containing stearic acid (F 1- F 6) ranging from 42-88 %, glyceryl monostearate (F 7- F 12) ranging from 40.5- 79.3 % and compritol ATO 888 (F 13- F 18) 81.4 - 92.3 %. The results indicated that, **increasing the lipid concentration also increases the EE**. This may be due to the reason that higher concentration of lipid would provide more

space to increment of the lipid content and also reduces the escaping of drug into the external phase thus ensuring highest EE[47, 61, 63,64].

The EE of the three lipids was in the order of

COMPRITOL > GLYCERYL MONOSTEARATE > STEARIC ACID.

Among the 3 lipids used, compritol showed the highest EE when compared to glyceryl monostearate and stearic acid. This may be due to the fact, that the presence of long chain fatty alcohol could lead to the creation of a less ordered solid lipid matrix and leaves enough space to accommodate drug molecules[47, 61, 66, 79]. (Compritol molecular formula (MF): $C_{25}H_{52}O_6$, glyceryl monostearate MF: $C_{21}H_{42}O_4$ and stearic acid MF: $C_{18}H_{36}O_2$).

PARTICLE SIZE ANALYSIS:

The particle size analysis of the SLN formulations was estimated by dynamic light scattering technique. The particle size values are shown in table 6 and the distribution curves were shown in fig. 14-21.

Influence of surfactant concentration on particle size:

To optimize the surfactant concentration in order to obtain the SLN formulation in the nano-range, two concentrations of surfactant in empty SLNs were examined for mean particle size.

Empty SLN containing 1 % polyvinyl alcohol showed the mean particle size of 10.24 µm and when the concentration of polyvinyl alcohol was increased to 2 %, the SLN formulation showed the mean particle size of 50 nm. This may be due to the reason that at low surfactant

concentration, an optimal surface coverage of the surfaces may not be achieved resulting in less than optimal stabilization of the particle dispersion. Under these circumstances it would be expected that the particles would tend to aggregate[50]. Decreased particle size with increase in mobile surfactant concentration may be due to the combined effect of decreased interfacial tension and increased rate of particle disintegration during homogenization[63, 68].

These results suggested that **relatively high surfactant concentration were needed to prevent particle aggregation**[46, 47, 50]. Hence 2 % polyvinyl alcohol was optimized to get the SLN in the nano – size range.

Influence of lipid concentration on particle size:

5 % stearic acid containing formulation showed the mean particle size of 50 nm while 20 % stearic acid containing formulation showed the mean particle size of 2.30 µm. Glyceryl - monostearate containing formulations at 5% showed the mean particle size of 53 nm and at 20% concentration had 2.45 µm. For compritol containing formulations mean particle size was 80 nm and 2.60 µm for 5% and 20% respectively.

. From the results, it was noted that the drug: lipid ratio was found to have a positive effect on particle size i.e. **increase in lipid concentration resulted in larger mean particle sizes and broader size distributions**[4]. This was because of increased viscosity of inner phase that affected the shearing capacity of homogenizer.[41, 45, 46, 63]

Larger particle size with increase in lipid content could be attributed to decrease in emulsifying efficiency and increase in particle agglomeration[53].

The decreasing order of particle size for the three lipids is as follows:

Compritol < Glyceryl monostearate < Stearic acid.

Of the three lipids used stearic acid containing formulations showed lesser mean particle size than the glyceryl monostearate and compritol. This may be due to the tendency of SLN to increase in size with the increase in carbon chain length of the lipids and higher melting point lipid[37] (Compritol melting point, MP: 55°C, glyceryl monostearate MP: 60°C, and stearic acid MP: 72°C).

From the results, 5 % concentration of all three lipids was optimized to get an SLN formulation in nanometric size range.

IN VITRO RELEASE STUDIES:

SLN containing three different lipids displayed a similar **biphasic drug release pattern** with a burst release within 30 minutes followed sustained release afterwards. The reason for burst release is possibly due to the drug associated with the surface of nanoparticles. The values are shown in table 7,8,9 and 10 and graphs in fig. 22-27.

Initial burst effect:

The initial burst effect of the formulations containing stearic acid varied from 7.5-21.9 % (F 1- F 6); GMS were 9-15.4 (F 7- F12) and compritol 9.3-16.3 (F 13- F 18). From the results, it was concluded that **higher lipid ratio leads to higher initial burst release[54].**

Sustained effect:

After the burst release, SLN formulations showed sustained release. The release of drug from the formulations varied from 56.0-29.1 (F 1- F 18). Of all the formulations F 18 showed much slower release than the other formulations.

From the results, it showed that the release was chiefly dependent on the concentration of the lipids i.e. **increase in lipid concentration decreases release rate[66].**

Among the three lipids used, compritol showed more sustained release than the stearic acid and glyceryl monostearate due to its longer carbon chain length than the other two lipids. [1, 5, 6,9,20.]

The order of drug release from the three lipids as follows:

Compritol > Glyceryl monostearate > Stearic acid.

From the results it was concluded that higher lipid concentration decreases release rate[35]. The nature of fatty acids affected the release significantly and longer the carbon chain length of fatty acids, the slower the drug release[37, 41,42,45,56]

Comparison of In vitro drug release of montelukast sodium loaded SLN with montelukast sodium pure drug solution :

The release of montelukast sodium from all the formulations was varied from 59.1-29.1 at 12 hours whereas the release of montelukast sodium from pure drug solution was found to be 99.1 % at 4 hours. This confirmed that the release of drug from all SLN formulations was more

sustained than the montelukast sodium pure drug solution (1mg/ml) shown in fig 28 (pure drug with best formulation) and fig. 29.

RELEASE KINETICS:

The kinetics and mechanism of drug release were studied by release kinetics, the n, k and r^2 values are indicated in the table 11. All the formulations showed first-order release which had higher linearity than the zero-order or higuchi model.

The exact mechanism of the release kinetics was determined by korsemeyer peppas model.Results indicated that all the SLN formulations followed **non-fickian model of release kinetics.**

The kinetic modeling of the best formulation is shown in fig. 30.

SCANNING ELECTRON MICROSCOPY (SEM) STUDIES:

The morphology of montelukast sodium – loaded SLN dispersion was examined by scanning electron microscope. The best formulation, F 15 (formulation containing 5 % compritol) was chosen for SEM studies. The SEM photograph was shown in the fig. 31. It revealed that the SLN dispersion showed the particle size was found to be **less than 500 nm in size with spherical shape and almost smooth surface**.

FT - IR STUDIES:

FT-IR studies were carried out to confirm the compatibility between the drug (Montelukast sodium) and lipids used (stearic acid, glyceryl monostearate and compritol) FTIR spectra for pure drug, lipids used and its physical mixture were evaluated. The FTIR spectra are shown in fig. 32-38.

Montelukast sodium pure drug shows the bond vibrations at 3396 cm^{-1} (COOH stretching), 3057 cm^{-1} (aromatic C–H stretching), 2925 cm^{-1} (aliphatic C–H stretching), 1710 cm^{-1} (C- Ostretching), 1610 cm^{-1} (C C stretching), 1594 cm^{-1} (C N stretching), 1497 cm^{-1} (aliphatic C–H bending), 1132 cm^{-1} (C–O stretching), 1068 cm^{-1} (aromatic C–Cl stretching), 837 cm^{-1} (aromatic C–H bending) and 697 (C–S stretching) (shown in table 12).

FTIR spectrum of stearic acid shows absorption bands of C – H stretching at 2957 cm^{-1}, C – H bending at 1465 cm^{-1}, O – H bending at 1433 cm^{-1} and C – C stetching at 1099 cm^{-1}, glyceryl monostearate revealed absorption bands of C – H stretching at 3015 cm^{-1}, C – H bending at 1735 cm^{-1} and O – H bending at 1290 cm^{-1} and compritol shows the absorption bands of C – H stretching at 2815 cm^{-1} and 2849 cm^{-1} and C = O stretching at 1738 cm^{-1} (mentioned in table 12).

From the physical mixtures of drug and lipids, there are no major shifting as well as no loss of functional peaks between the spectra of drug, lipids and its physical mixtures. Hence, it was confirmed that there is **no interaction between the drug and lipids used**.

DSC STUDIES:

DSC is a highly useful means of detecting drug – excipient incompatibility. DSC thermograms of pure drug (montelukast sodium), lipids (stearic acid, glyceryl monostearate and compritol) and the physical mixtures of drug with lipids used were studied.

The drug showed the sharp melting endothermic peak at 125 °C, stearic acid showed the melting endothermic peak at 57 °C, glyceryl monostearate at 55 °C and compritol at 60 °C. The endothermic peak of physical mixtures are drug and stearic acid at 53 °C and 126 °C, drug and

glyceryl monostearate at 57°C and 125 °C and drug and compritol at 60 °C and 125 °C (shown in table 13 and fig. 39-45.

However, there was no obvious change in the endothermic peak around 125 °C of the raw material in physical mixtures. This suggested that there were no appearance of new peaks or disappearance of existing peak. Hence there was **no considerable effect on the thermal behavior of drug with the lipid matrix** under the experimental conditions.

STABILITY STUDIES OF MONTELUKAST SODIUM- LOADED SLN

2 sets of SLN formulations were examined for stability studies. One set were stored at refrigeration temperature (4 ± 2°C) and the other set were stored at 25 ± 2 °C and 60 % ± 5 %RH at the stability chamber for 1 month and the entrapment efficiency were determined at 1 week intervals.

After 1 month of the stability studies, the entrapment efficiency of all the formulations which decreased from 42.40 – 92.60 % to 41.50 – 90.30 % at 4°C and 40.10 – 89.50 % at 25 ± 2 °C. The results are shown in table 14 and 15.

From the results, lowered entrapment efficiency were observed on storage, this may be due to drug expulsion during lipid modification[13, 25, 29].

Hence it was concluded that at 4°C and 25 °C the formulations showed no significant change in entrapment efficiency[54].

CHAPTER XI

SUMMARY AND CONCLUSION

- ✶ The present research study was to estimate the effect of lipids on the preparation of montelukast sodium-loaded SLN to improve its bioavailability and to attain sustained release.
- ✶ Result suggests that the hot homogenization and ultrasonication method was a feasible method for preparing Montelukast sodium- loaded fatty acid SLN.
- ✶ SLN formulations were estimated for its particle size, morphology, entrapment efficiency, release studies, FTIR , DSC and stability studies.
- ✶ From the particle size studies, it was observed that increase in surfactant concentration from 1 to 2 % decreases the mean particle size of the formulation.
- ✶ SEM studies revealed that the SLN formulation are in the nanometric range and are spherical in shape with smooth surface.
- ✶ The entrapment efficiency were found to increase with increase in surfactant concentration and with increase in lipid concentration.
- ✶ The results obtained from the release studies revealed that all the SLN formulations have sustained release than the pure drug solution.
- ✶ The release of montelukast sodium from the SLN formulation follows first order model of release kinetics.
- ✶ From the FTIR and DSC studies, it was confirmed that there is no interaction between the drug and lipids used.

- From the stability studies it was noted that, there is a no considerable decrease in the entrapment efficiency of the SLN formulations.

CONCLUSION:

Finally, it was concluded that hot homogenization followed by ultrasonication is a feasible method. Particle sizes of SLNs revealed that the SLN prepared from higher melting point lipid showed larger particle size and with increased carbon chain length of the fatty acids. FTIR and DSC studies suggested that there is no interaction between drug and lipids. Studies showed that, increase in lipid concentration increased particle size, EE, and maintained the sustained release of drug. Among all, Compritol ATO 888 is chosen as the best lipid for formulating SLN because it had higher EE and sustained drug release profile than GMS and stearic acid. It was concluded that the SLN provide sustained release and are preferred to overcome oral bioavailability problems and suggests that montelukast sodium can formulate as SLN system.

TABLE 1

COMPOSITION OF SLN FORMULATIONS

S.NO	FORMULATIONS	SURFACTANT CONC.	LIPID	CONC.
1	F 1	2 %	STEARIC ACID	1 %
2	F 2	2 %	STEARIC ACID	2 %
3	F 3	2 %	STEARIC ACID	5 %
4	F 4	2 %	STEARIC ACID	10 %
5	F 5	2 %	STEARIC ACID	15 %
6	F 6	2 %	STEARIC ACID	20 %
7	F 7	2 %	GMS	1 %
8	F 8	2 %	GMS	2 %
9	F 9	2 %	GMS	5 %
10	F 10	2 %	GMS	10 %
11	F 11	2 %	GMS	15 %
12	F 12	2 %	GMS	20 %
13	F 13	2 %	COMPRITOL	1 %
14	F 14	2 %	COMPRITOL	2 %
15	F 15	2 %	COMPRITOL	5 %
16	F 16	2 %	COMPRITOL	10 %
17	F 17	2 %	COMPRITOL	15 %
18	F 18	2 %	COMPRITOL	20 %

TABLE 2

CALIBRATION OF MONTELUKAST SODIUM IN 0.5 % SLS SOLUTION

S.NO	*CONCENTRATION (µg/ml)*	ABSORBANCE
1	01	0.049 ± 0.002
2	02	0.098 ± 0.002
3	03	0.147 ± 0.003
4	04	0.194 ± 0.001
5	05	0.244 ± 0.001
6	06	0.294 ± 0.002
7	07	0.343 ± 0.002
8	08	0.392 ± 0.001
9	09	0.441 ± 0.001
10	10	0.492 ± 0.001

n = 3* γ - 0.9998923

TABLE 3

DRUG CONTENT OF ALL FORMULATIONS

S.NO	FORMULATIONS	DRUG CONTENT
1	F 1	94.75 %
2	F 2	97.70 %
3	F 3	97.00 %
4	F 4	98.70 %
5	F 5	100.0 %
6	F 6	99.00 %
7	F 7	99.70 %
8	F 8	100.0 %
9	F 9	99.83 %
10	F 10	97.10 %
11	F 11	98.50 %
12	F 12	98.80 %
13	F 13	98.90 %
14	F 14	100.0 %
15	F 15	97.90 %
16	F 16	99.50 %
17	F 17	98.70 %
18	F 18	98.90 %

n = 3*

TABLE 4

ENTRAPMENT EFFICIENCY OF ALL FORMULATIONS

CONTAINING 1 % POLYVINYL ALCOHOL

S.NO	FORMULATIONS	% ENTRAPMENT EFFICIENCY
1	F 1	23.10 %
2	F 2	24.60 %
3	F 3	25.50 %
4	F 4	26.90 %
5	F 5	28.10 %
6	F 6	29.30 %
7	F 7	21.70 %
8	F 8	24.80 %
9	F 9	27.90 %
10	F 10	29.20 %
11	F 11	31.10 %
12	F 12	33.60 %
13	F 13	42.60 %
14	F 14	45.10 %
15	F 15	48.90 %
16	F 16	52.10 %
17	F 17	55.50 %
18	F 18	62.30 %

n = 3*

TABLE 5

ENTRAPMENT EFFICIENCY OF ALL FORMULATIONS

CONTAINING 2 % POLYVINYL ALCOHOL

S.NO	FORMULATIONS	% ENTRAPMENT EFFICIENCY
1	F 1	42.40 %
2	F 2	45.80 %
3	F 3	62.70 %
4	F 4	66.00 %
5	F 5	75.40 %
6	F 6	88.10 %
7	F 7	40.50 %
8	F 8	45.10 %
9	F 9	48.20 %
10	F 10	53.70 %
11	F 11	76.10 %
12	F 12	79.30 %
13	F 13	81.40 %
14	F 14	83.50 %
15	F 15	84.80 %
16	F 16	86.90 %
17	F 17	89.30 %
18	F 18	92.60 %

n = 3*

TABLE 6

COMPARISON OF PARTICLE SIZE

S.NO	CONCENTRATION	MEAN PARTICLE SIZE
1	1 % Polyvinyl alcohol	10.24 µm
2	2 % Polyvinyl alcohol	50 nm
3	1 % Stearic acid	1.85 µm
4	2 % Stearic acid	895 nm
5	5 % Stearic acid	50 nm
6	10 % Stearic acid	936 nm
7	15 % Stearic acid	1.76 µm
8	20 % Stearic acid	2.30 µm
9	5 % Glyceryl monostearate	53 nm
10	5 % Compritol ATO 888	80 nm
11	20 % Glyceryl monostearate	2.45 µm
12	20 % Compritol ATO 888	2.60 µm

TABLE 7

RELEASE PROFILE OF SLN FORMULATONS

CONTAINING STEARIC ACID

Time in hours	F 1	F 2	F 3	F 4	F 5	F 6
0.25	7.50	9.0	12.0	16.2	21.0	21.00
0.5	9.40	10.7	13.4	17.1	21.2	21.90
0.75	11.5	13.2	14.8	19.0	22.2	22.90
1	13.3	13.9	15.9	20.3	23.0	23.80
1.5	14.5	16.2	16.8	21.5	23.9	24.50
2	15.9	18.8	17.6	22.7	25.7	25.50
2.5	18.1	20.6	18.4	24.4	27.7	26.60
3	19.8	23.3	19.4	24.9	28.6	27.70
3.5	22.5	25.5	20.6	25.7	29.7	28.40
4	23.4	27.0	23.3	27.8	30.7	29.20
4.5	26.4	29.4	25.9	29.1	31.5	30.10
5	30.3	31.4	26.8	30.6	32.3	30.80
5.5	31.6	32.9	27.9	31.8	33.6	32.10
6	34.8	36.6	29.3	32.7	34.1	32.80
6.5	37.2	38.4	31.3	33.9	34.8	33.20
7	39.8	39.7	33.1	35.6	35.7	33.80
7.5	41.5	41.4	34.7	36.6	36.9	34.90
8	43.2	43.5	36.5	38.3	37.7	35.30
8.5	44.7	45.7	38.6	39.0	38.8	35.80
9	46.4	47.1	41.9	40.2	39.8	36.70
9.5	47.8	48.7	44.6	41.2	41.6	37.70
10	51.5	50.6	46.9	41.6	42.4	38.30
10.5	53.9	53.0	48.3	42.1	43.2	39.10
11	56.3	53.8	49.8	43.0	43.4	39.70
11.5	57.0	54.7	51.8	44.7	44.0	40.70
12	59.1	56.0	54.0	45.6	44.5	41.60

n = 3*

TABLE 8

RELEASE PROFILE OF SLN FORMULATONS

CONTAINING GLYCERYL MONOSTEARATE

Time in hours	F 7	F 8	F 9	F 10	F 11	F 12
0.25	9.3	10.8	14.8	15.1	16.1	15.5
0.50	10.5	12.2	15.9	15.8	16.8	16.3
0.75	13.4	13.2	17.1	16.7	17.6	18.3
1.0	14.8	14.3	18.1	17.4	18.9	19.5
1.5	17.5	16.2	19.2	18.4	19.4	20.2
2.0	18.8	18.4	20.9	19.1	19.8	21.0
2.5	20.8	20.0	22.3	20.0	20.6	21.9
3.0	21.6	21.9	23.5	20.6	21.0	22.3
3.5	25.0	23.7	25.3	21.4	21.6	23.6
4.0	26.1	24.4	26.6	22.1	21.9	24.3
4.5	27.1	24.9	27.5	23.2	22.9	24.9
5.0	28.2	26.8	29.0	23.9	23.6	25.6
5.5	29.5	27.9	31.4	24.8	24.5	26.4
6.0	32.3	29.5	32.3	25.8	25.6	27.1
6.5	33.5	30.2	34.2	27.4	26.9	28.6
7.0	35.1	31.3	35.4	28.6	27.8	29.1
7.5	35.8	33.0	36.2	29.4	28.3	29.7
8.0	36.7	34.5	36.7	31.0	28.7	30.3
8.5	37.3	35.5	37.5	31.8	29.9	30.9
9.0	38.0	36.1	38.5	32.6	30.2	31.4
9.5	38.5	38.1	39.0	33.3	32.3	32.3
10.0	39.4	39.3	39.8	34.2	33.9	32.6
10.5	40.1	40.0	40.4	34.8	34.3	33.4
11.0	41.1	41.4	40.9	36.1	35.2	33.7
11.5	43.2	42.4	41.3	37.4	36.0	34.4
12.0	46.4	43.7	42.2	39.1	37.3	35.2

n = 3*

TABLE 9

RELEASE PROFILE OF SLN FORMULATONS

CONTAINING COMPRITOL ATO 888

Time in hours	F 13	F 14	F 15	F 16	F 17	F 18
0.25	9.0	10.0	11.0	14.5	15.2	15.4
0.5	11.4	11.9	11.8	15.7	16.2	16.2
0.75	12.5	12.8	13.0	16.3	17.3	17.5
1	14.1	14.2	14.5	17.2	18.5	18.6
1.5	15.1	15.2	15.3	18.2	19.3	19.5
2	16.5	16.7	16.9	19.1	20.8	20.2
2.5	17.3	18.2	18.3	20.3	20.9	20.5
3	18.9	19.5	19.9	21.2	21.6	21.0
3.5	20.3	21.0	20.8	22.3	22.9	21.7
4	22.4	22.7	21.9	22.9	23.1	22.3
4.5	23.7	23.6	23.7	23.7	23.9	23.1
5	25.1	27.5	24.6	24.8	24.6	24.3
5.5	26.7	28.6	25.7	26.1	25.3	24.3
6	30.1	30.1	27.7	27.5	26.4	24.6
6.5	30.8	31.1	28.7	28.6	27.6	25.1
7	33.8	32.0	30.2	29.4	28.2	25.3
7.5	35.5	32.6	31.4	31.7	28.9	25.8
8	37.5	33.7	32.4	32.3	29.6	26.1
8.5	39.3	34.9	33.3	33.0	30.4	26.3
9	40.4	36.1	34.1	34.1	31.0	26.4
9.5	41.7	37.9	34.8	34.3	31.5	26.8
10	43.2	38.2	35.9	35.0	31.9	26.9
10.5	44.2	40.0	37.0	35.5	32.4	27.1
11	45.9	40.7	38.2	36.1	32.8	27.4
11.5	48.3	42.3	38.6	36.8	33.1	27.8
12	52.1	44.3	39.1	37.4	33.6	28.1

n = 3*

TABLE 10

COMPARISON OF BEST FORMULATION WITH PURE DRUG

TIME in hours	F 15	PURE DRUG
0.25	15.1	16.4
0.50	15.8	27.3
0.75	16.7	37.6
1.0	17.4	54.6
1.5	18.4	69.3
2.0	19.1	81.0
2.5	20	94.3
3.0	20	99.3
3.5	21.4	99.1
4.0	22.1	99.1
4.5	23.2	-
5.0	23.9	-
5.5	24.8	-
6.0	25.8	-
6.5	27.4	-
7.0	28.6	-
7.5	29.4	-
8.0	31	-
8.5	31.8	-
9.0	32.6	-
9.5	33.3	-
10.0	34.2	-
10.5	34.8	-
11.0	36.1	-
11.5	37.4	-
12.0	39.1	-

TABLE 11
RELEASE KINETICS VALUES OF SLN FORMULATIONS

S.No	Formulation	Release Kinetics Values							
		Zero Order Release		First Order Release		Higuchi Model		Korse-Meyer Peppas Model	
		k	r^2	k (hr^{-1})	r^2	k (hr^{-1})	r^2	n	r^2
1	F 1	1.803	0.811	0.009	0.988	7.5	0.923	0.063	0.908
2	F 2	1.939	0.859	0.010	.973	7.8	0.910	0.063	0.811
3	F 3	2.169	0.900	0.011	0.992	8.6	0.940	0.072	0.811
4	F 4	2.169	0.900	0.014	0.985	10.7	0.975	0.089	0.842
5	F 5	2.873	0.951	0.016	0.997	11.4	0.989	0.091	0.779
6	F 6	3.003	0.934	0.017	0.981	12.1	0.993	0.098	0.806
7	F 7	1.239	0.655	0.005	0.925	5.4	0.827	0.047	0.982
8	F 8	1.770	0.819	0.008	0.987	7.3	0.925	0.061	0.912
9	F 9	2.208	0.895	0.011	0.992	8.9	0.958	0.072	0.837
10	F 10	2.624	0.947	0.014	0.994	10.5	0.988	0.084	0.785
11	F 11	2.724	0.955	0.015	0.990	10.8	0.976	0.084	0.759
12	F 12	3.557	0.985	0.021	0.987	13.8	0.961	0.109	0.676
13	F 13	1.971	0.771	0.010	0.996	8.3	0.888	0.075	0.911
14	F 14	2.349	0.832	0.013	0.994	9.7	0.928	0.087	0.879
15	F 15	2.693	0.903	0.015	0.992	10.9	0.969	0.097	0.849
16	F 16	3.653	0.966	0.022	0.972	14.0	0.934	0.113	0.633
17	F 17	4.173	0.980	0.027	0.998	16.4	0.984	0.138	0.700
18	F 18	4.481	0.990	0.020	0.989	17.3	0.964	0.143	0.642

TABLE 12

FOURIER TRANSFORM- INFRA RED POSITIONS OF VARIOUS BOND-VIBRATIONS IN MONTELUKAST SODIUM

S.NO	BOND	WAVENUMBER (cm^{-1})	MODE
1	C=O	3396	Stretch
2	C-H	3058	Stretch
3	C=O	2925	Stretch
4	C=O	1710	Stretch
5	C-C	1610	Stretch
6	C-N	1595	Stretch
7	C-H	1498	Bending
8	C-O	1132	Stretch
9	C-Cl	1068	Stretch
10	C-H	837	Bending
11	C-S	697	Stretch

TABLE 13

DSC STUDIES:

ENDOTHERMIC PEAKS OF DRUG, LIPIDS AND ITS PHYSICAL MIXTURES

S.NO	NAME OF THE SAMPLE	ENDOTHERMIC PEAKS
1	Montelukast sodium	125 °C
2	Stearic acid	57 °C
3	Glyceryl monostearate	55 °C
4	Compritol ATO 888	60 °C
5	Drug and Stearic acid	125 °C and 57 °C
6	Drug and Glyceryl monostearate	125 °C and 55 °C
7	Drug and Compritol ATO 888	125 °C and 60 °C

TABLE 14

STABILITY STUDIES

PERCENTAGE ENTRAPMENT EFFICIENCY OF ALL FORMULATIONS

STORED AT TEMPERATURE $4^{O}\pm2^{O}$C (WEEKS)

FORMULATION	0	1	2	3	4	5
F 1	42.40	42.10	42.00	41.80	41.60	41.50
F 2	45.80	45.30	45.10	45.00	44.80	44.50
F 3	62.70	62.60	62.50	62.40	62.20	62.10
F 4	66.00	66.00	65.80	65.70	65.50	65.40
F 5	75.40	75.10	75.10	75.00	74.80	74.50
F 6	88.10	87.80	87.70	87.70	87.50	87.10
F 8	45.10	44.80	44.70	44.50	44.40	44.10
F 9	48.20	48.00	47.90	47.40	47.30	46.90
F 10	53.70	53.30	53.20	53.20	53.10	52.90
F 11	76.10	75.70	75.60	75.40	75.30	75.20
F 12	79.30	79.00	78.80	78.70	78.60	78.50
F 13	81.40	81.20	81.10	80.00	80.00	80.00
F 14	83.50	83.50	83.40	83.20	83.10	83.00
F 15	84.80	84.70	84.40	84.10	84.10	83.90
F 16	86.90	86.70	86.60	86.40	86.10	85.50
F 17	89.30	89.20	89.10	88.70	88.10	87.90
F 18	92.60	92.50	92.10	91.40	91.10	90.30

TABLE 15

STABILITY STUDIES

PERCENTAGE ENTRAPMENT EFFICIENCY OF ALL FORMULATIONS

STORED AT TEMPERATURE $25^{O} \pm 2^{O}$C AND 60 ± 5 % RH (WEEKS)

FORMULATION	0	1	2	3	4	5
F 1	42.40	42.00	41.50	41.00	40.50	40.10
F 2	45.80	45.10	44.60	44.20	44.10	43.50
F 3	62.70	62.40	62.00	61.70	61.20	61.80
F 4	66.00	65.90	65.60	65.30	65.00	64.30
F 5	75.40	75.10	75.00	74.30	74.00	73.60
F 6	88.10	87.80	87.30	87.30	87.10	87.00
F 8	45.10	43.80	42.70	42.50	41.40	40.10
F 9	48.20	46.00	46.50	46.10	45.30	44.90
F 10	53.70	53.00	52.20	52.00	51.10	50.20
F 11	76.10	75.00	74.30	74.00	73.60	72.10
F 12	79.30	78.80	77.80	77.10	76.90	75.50
F 13	81.40	80.60	80.20	79.00	78.30	77.30
F 14	83.50	82.50	81.60	81.10	80.50	80.40
F 15	84.80	84.30	82.90	81.10	80.50	80.00
F 16	86.90	86.80	86.20	86.00	85.10	84.30
F 17	89.30	88.00	87.10	86.40	86.20	85.80
F 18	92.60	91.90	90.50	90.00	89.10	89.50

FIGURE 11

λ-MAX OF MONTELUKAST SODIUM

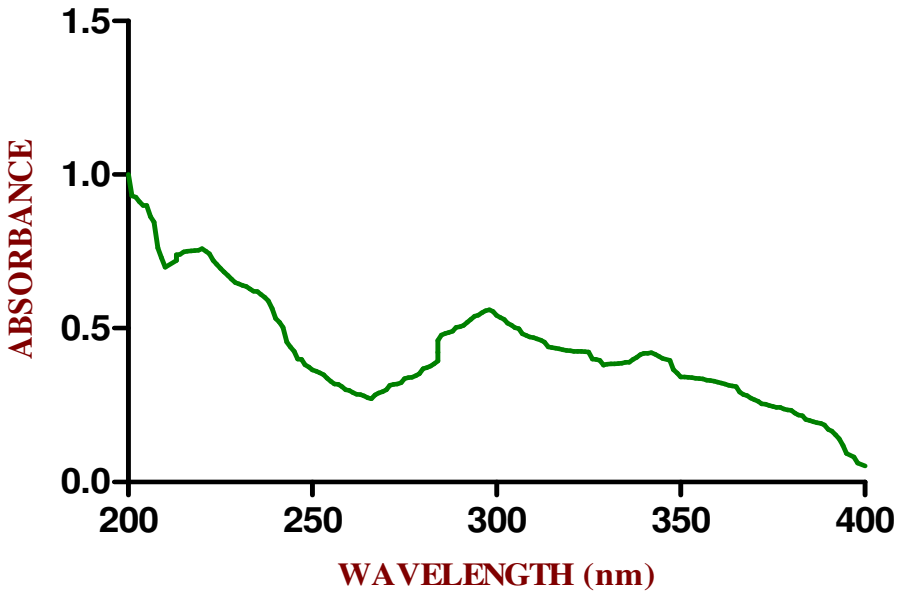


FIGURE 12

CALIBRATION CURVE OF MONTELUKAST SODIUM IN 0.5 % SLS SOLUTION

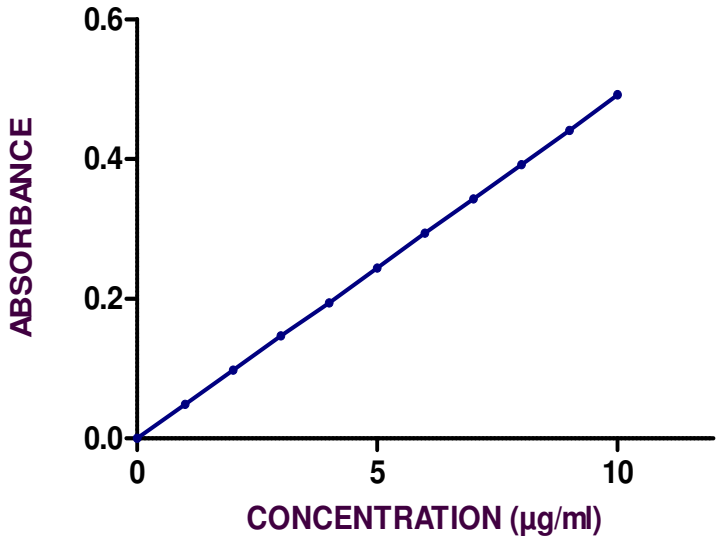


FIGURE 13

ENTRAPMENT EFFICIENCY OF ALL FORMULATIONS

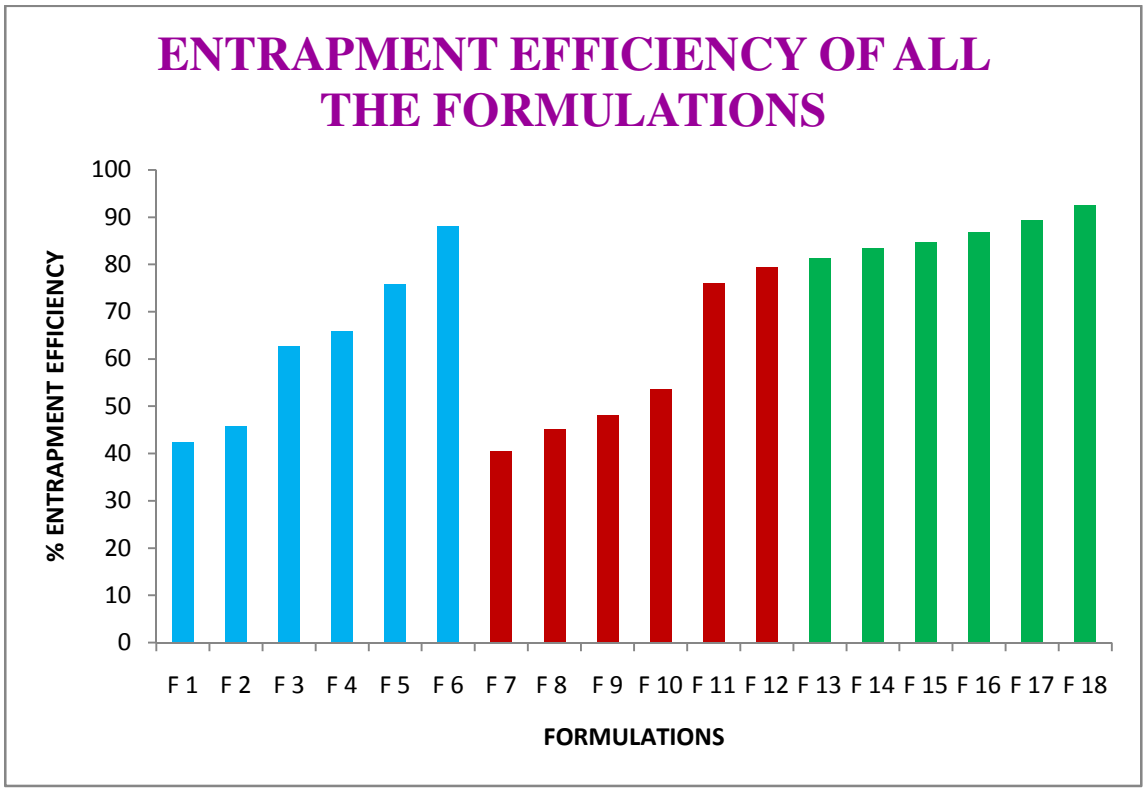


FIGURE 14

PARTICLE SIZE STUDIES:

FIGURE SHOWS PARTICLE SIZE DISTRIBUTION OF EMPTY SLN CONTAINING 1 % PVA

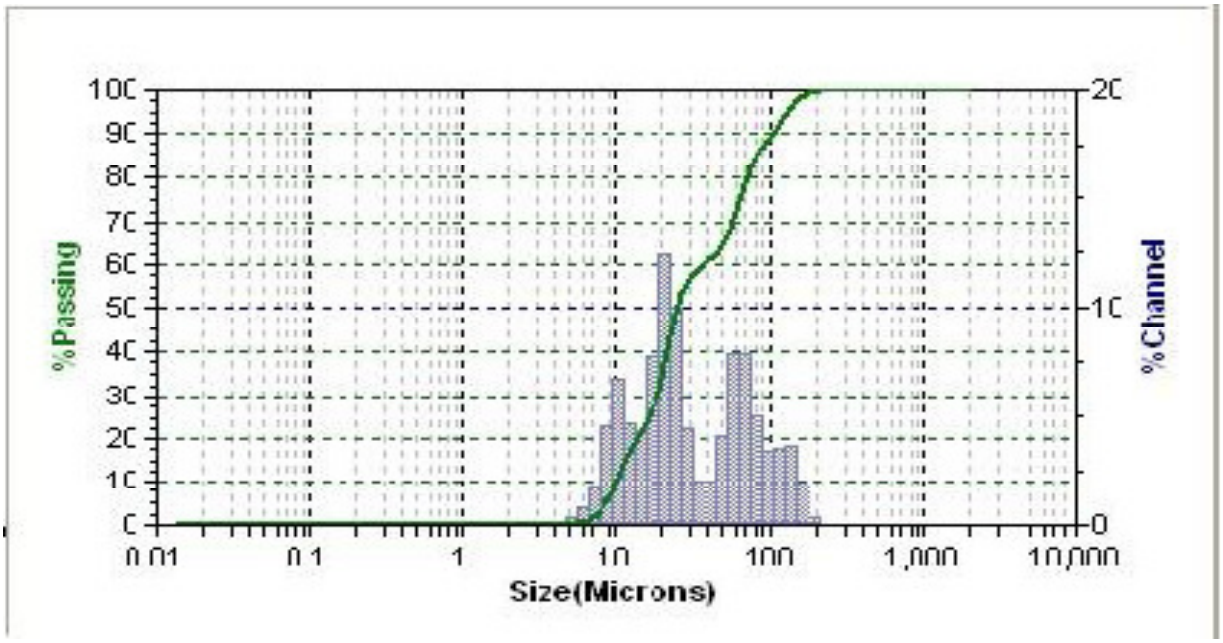


FIGURE 15

FIGURE SHOWS PARTICLE SIZE DISTRIBUTION OF EMPTY SLN CONTAINING 2 % PVA

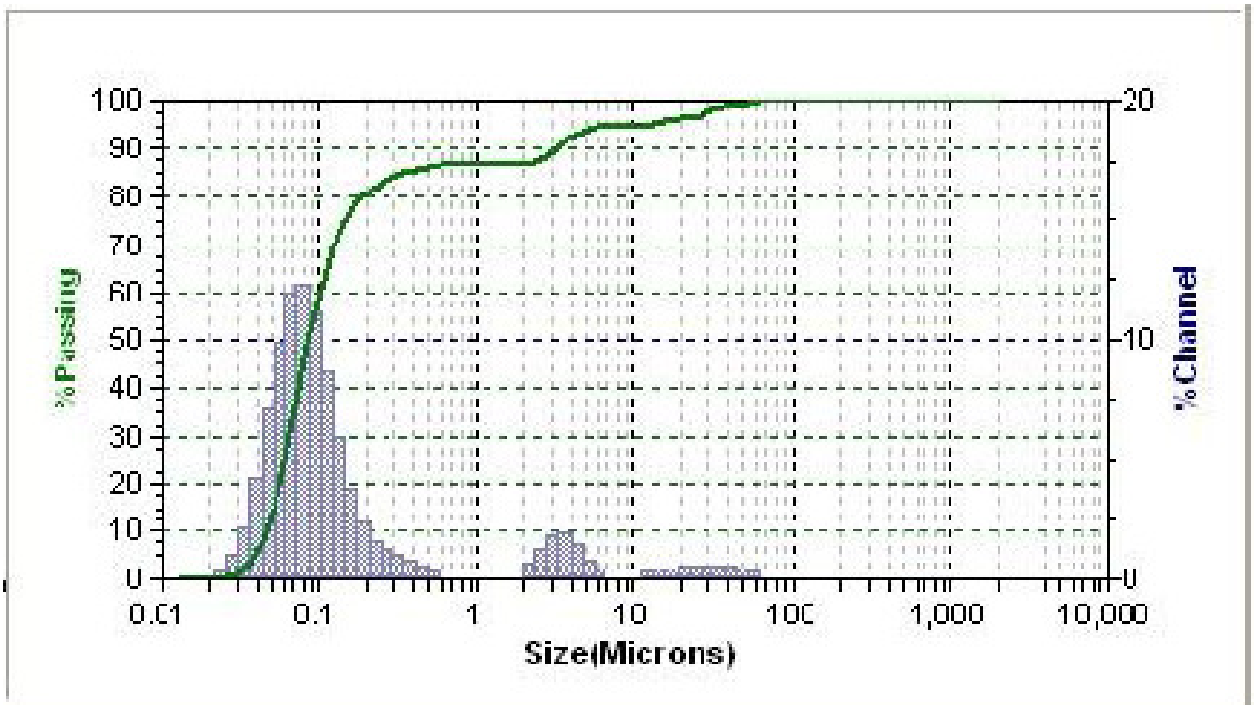


FIGURE 16

FIGURE SHOWS PARTICLE SIZE DISTRIBUTION OF F 3 (5 % STEARIC ACID)

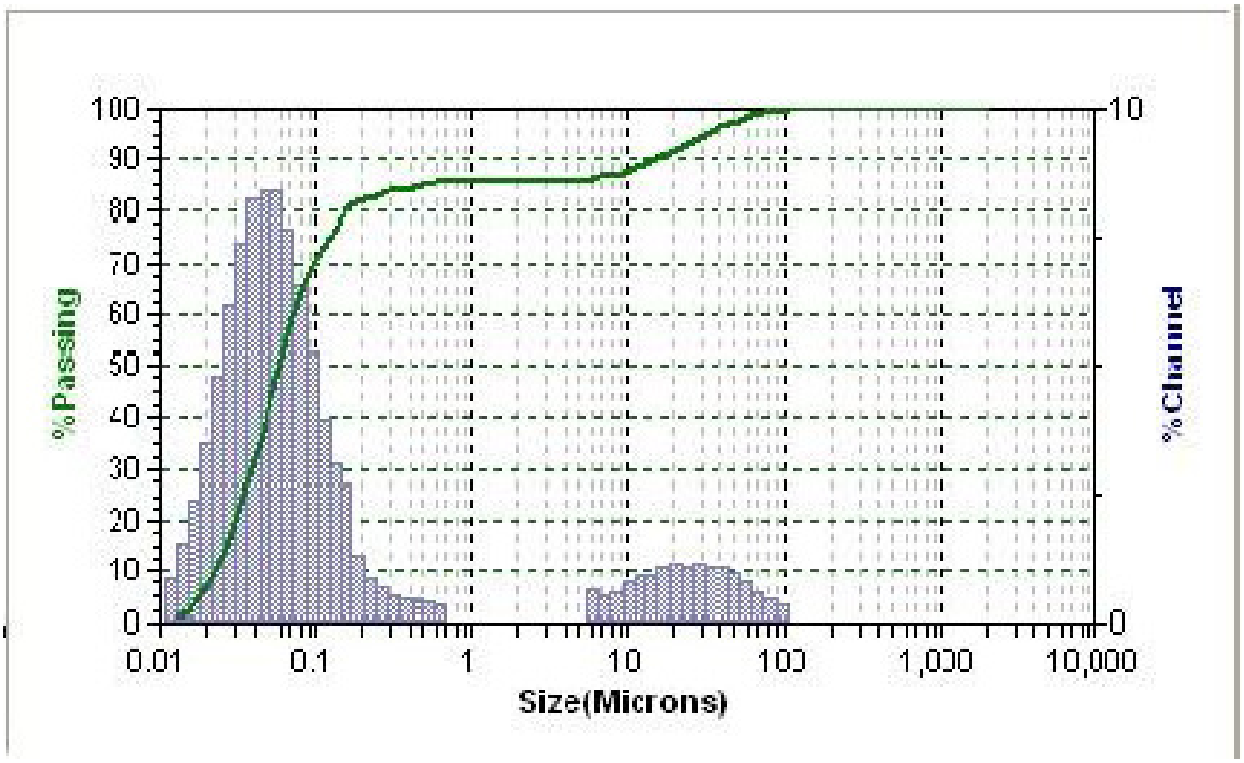


FIGURE 17

FIGURE SHOWS PARTICLE SIZE DISTRIBUTION OF F 9 (5% GLYCERYL MONOSTEARATE)

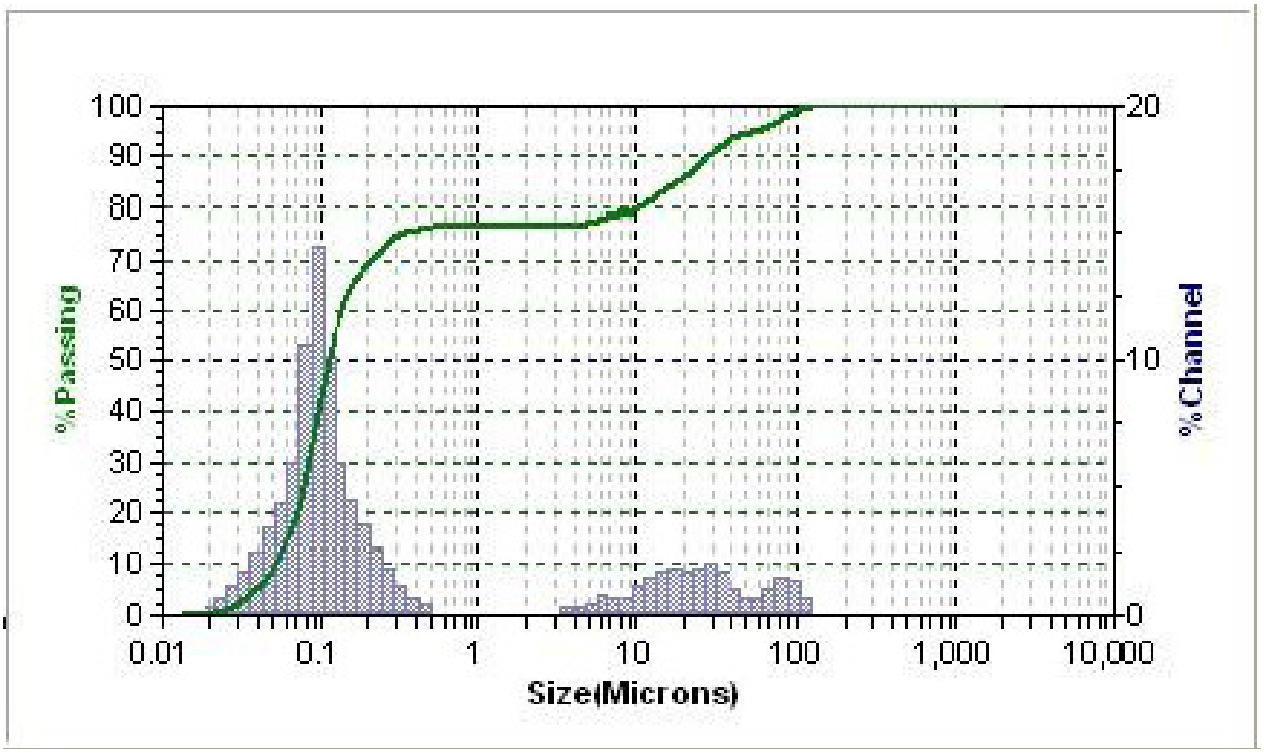


FIGURE 18

FIGURE SHOWS PARTICLE SIZE DISTRIBUTION OF

F 15 (5 % COMPRITOL ATO 888)

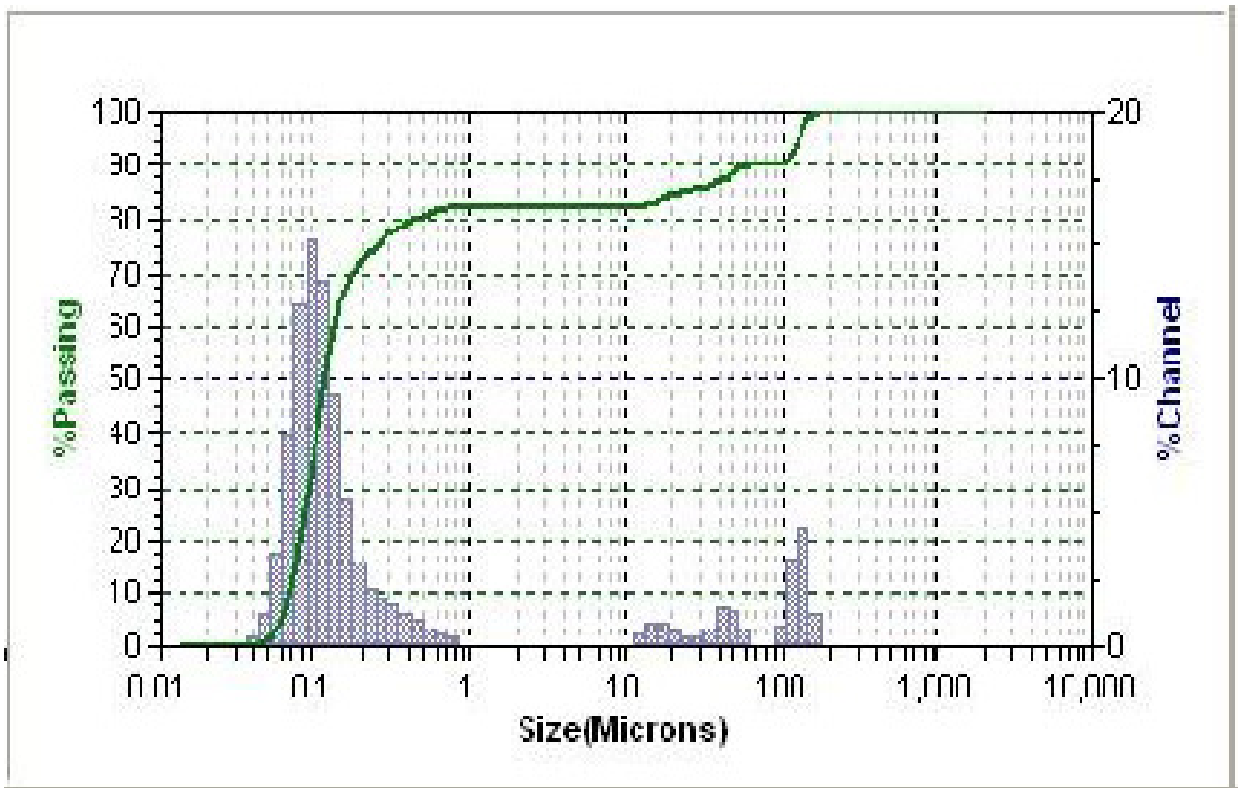


FIGURE 19

FIGURE SHOWS PARTICLE SIZE DISTRIBUTION OF F 6 (20 % STEARIC ACID)

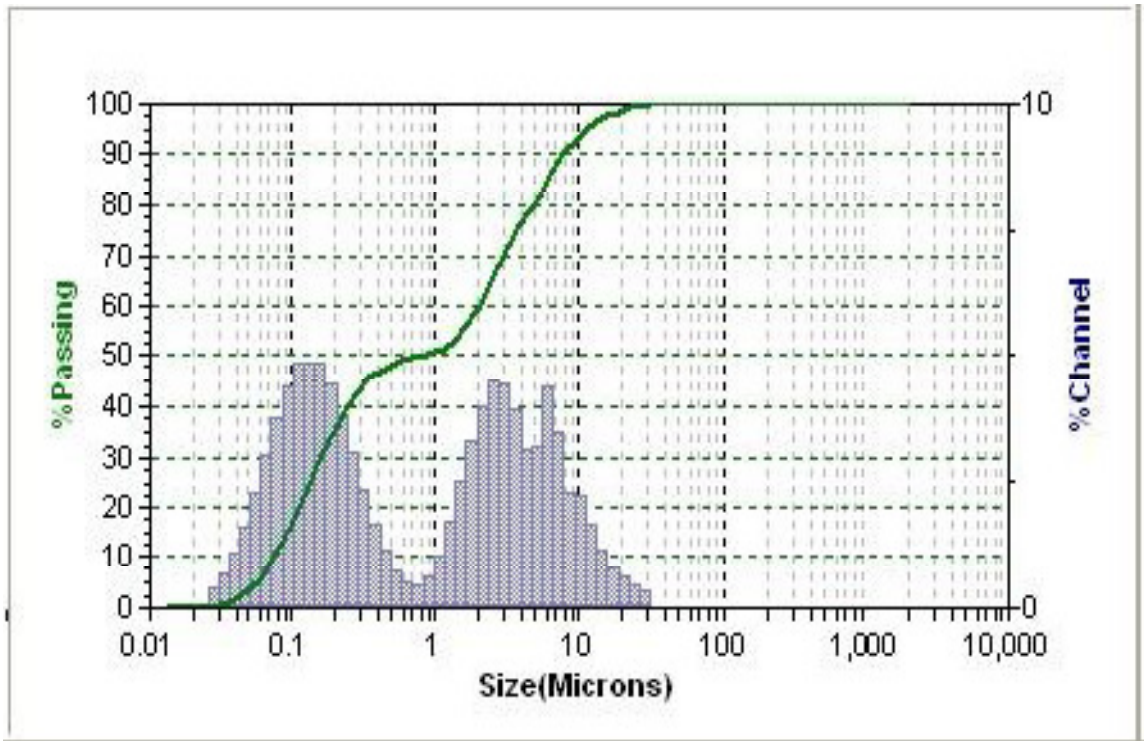


FIGURE 20

FIGURE SHOWS PARTICLE SIZE DISTRIBUTION OF F 12 (20 % GLYCERYL MONOSTEARATE

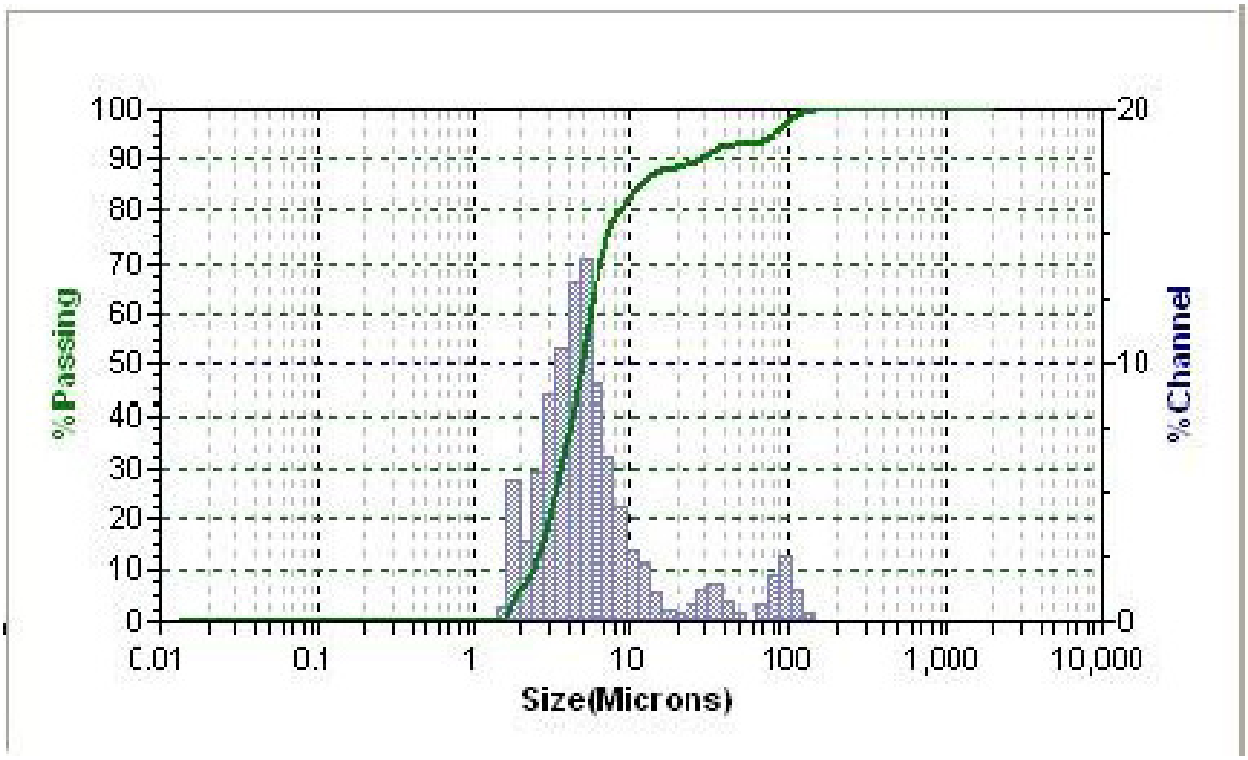


FIGURE 21

FIGURE SHOWS PARTICLE SIZE DISTRIBUTION OF F 18 (20 % COMPRITOL ATO 888)

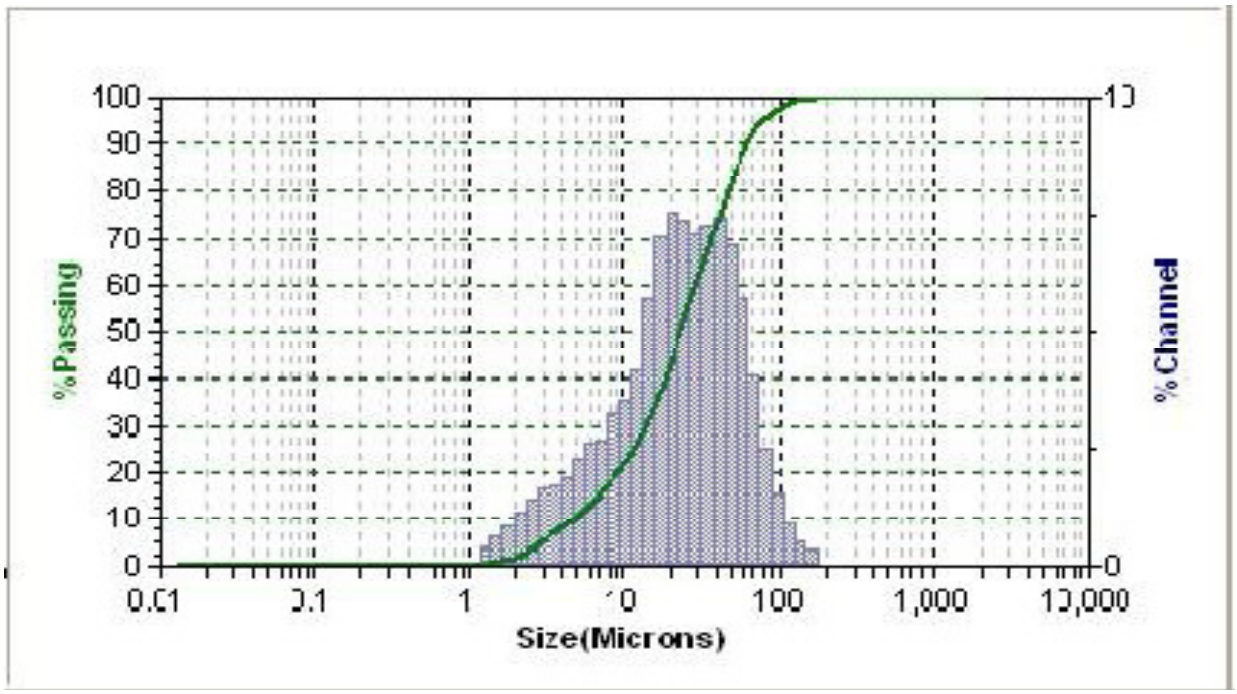
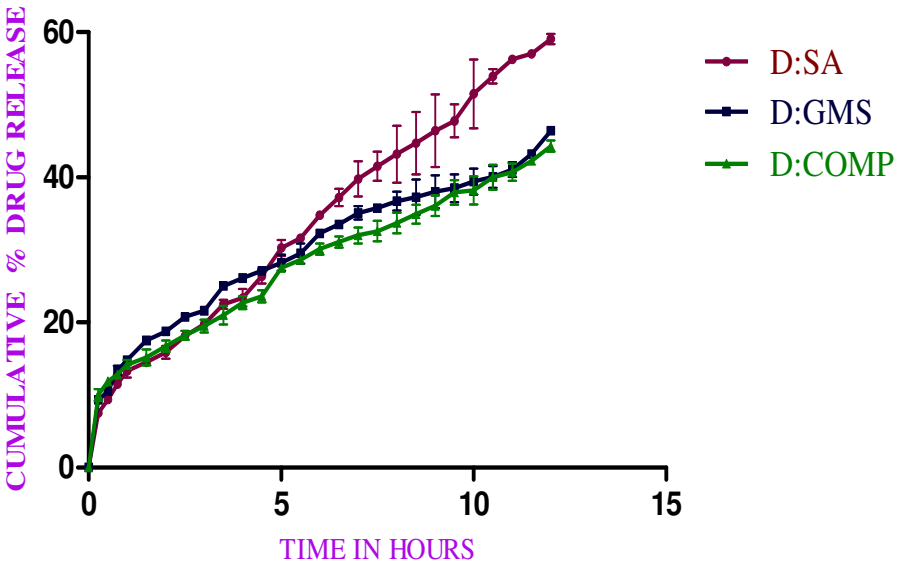


FIGURE 22

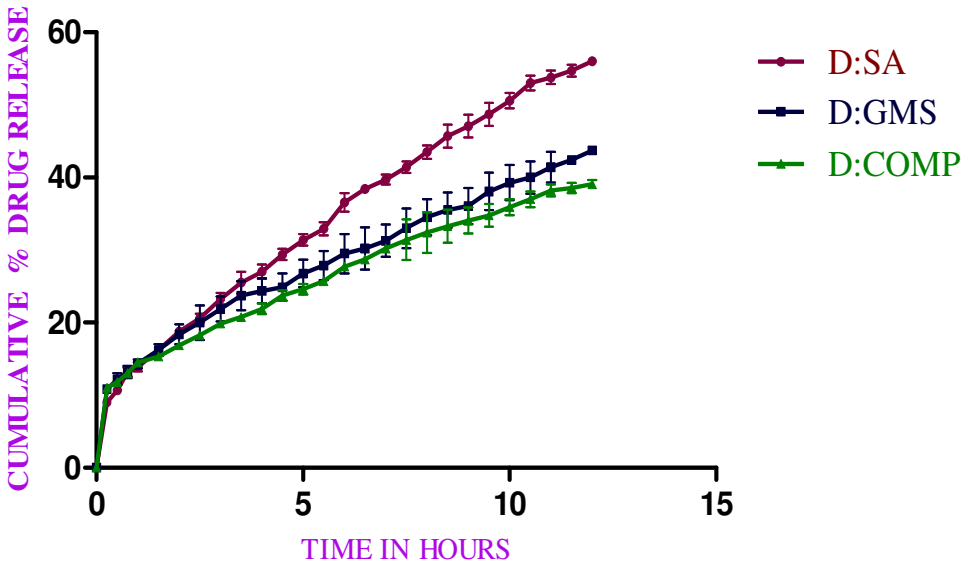
IN-VITRO RELEASE PROFILE OF SLN CONTAINING DRUG AND 1 % LIPID



Where D: SA –Drug+Stearic acid, D:GMS –Drug+ Glyceryl monostearate and D:COMP – Drug+Compritol.

FIGURE 23

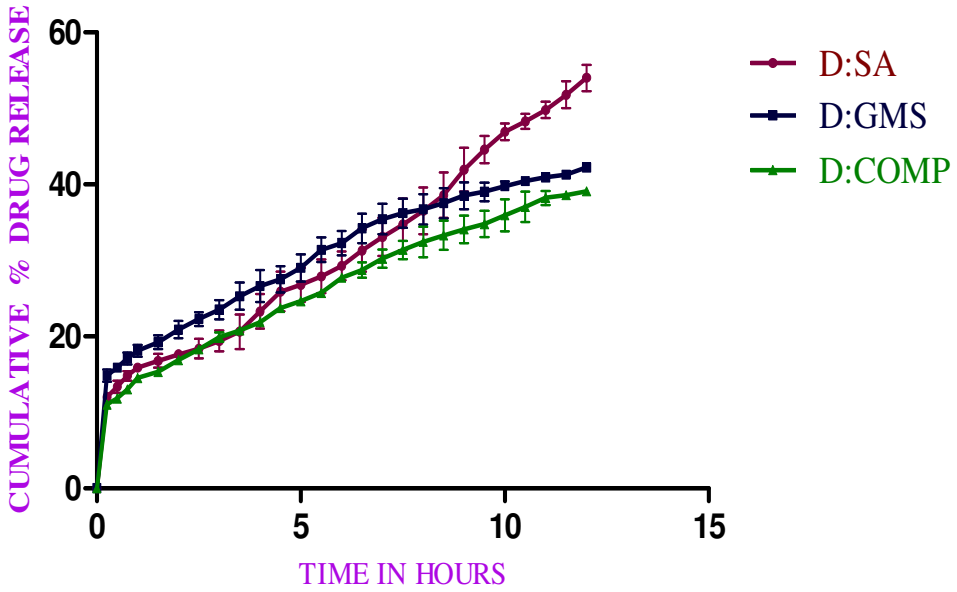
IN-VITRO RELEASE PROFILE OF SLN CONTAINING DRUG AND 2 % LIPID



Where D: SA –Drug+Stearic acid, D:GMS –Drug+ Glyceryl monostearate and D:COMP – Drug+Compritol.

FIGURE 24

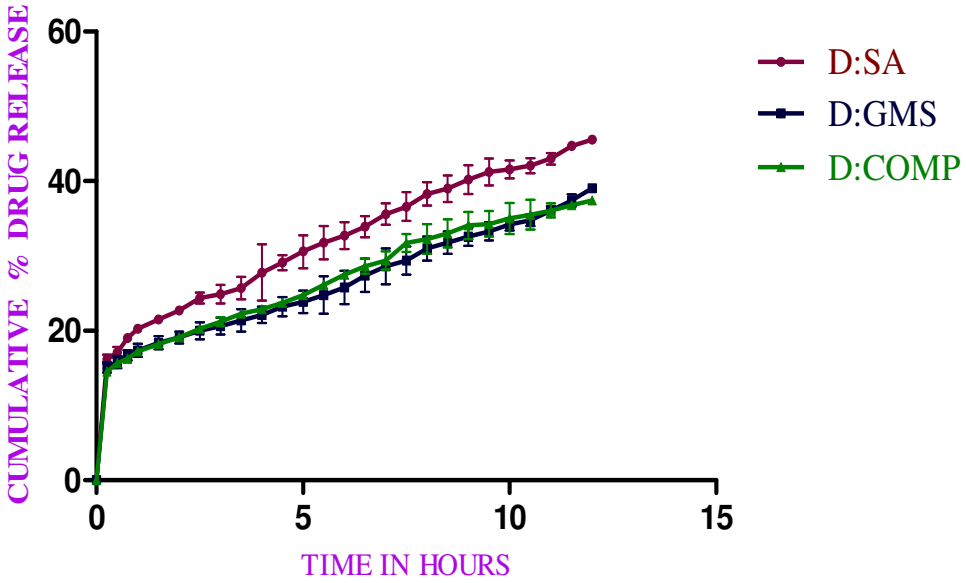
IN-VITRO RELEASE PROFILE OF SLN CONTAINING DRUG AND 5 % LIPID



Where D: SA –Drug+Stearic acid, D:GMS –Drug+ Glyceryl monostearate and D:COMP – Drug+Compritol.

FIGURE 25

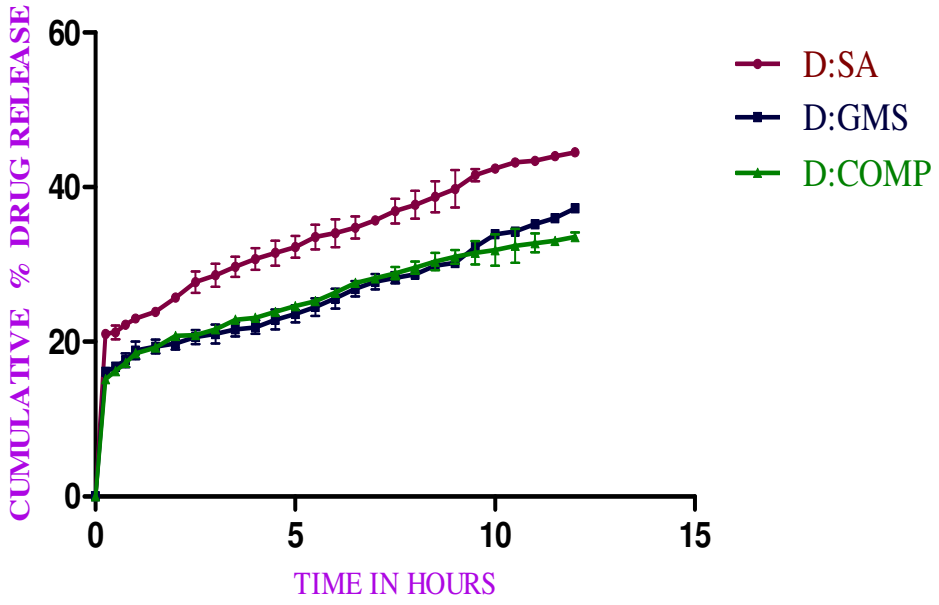
IN-VITRO RELEASE PROFILE OF SLN CONTAINING DRUG AND 10 % LIPID



Where D: SA –Drug+Stearic acid, D:GMS –Drug+ Glyceryl monostearate and D:COMP – Drug+Compritol.

FIGURE 26

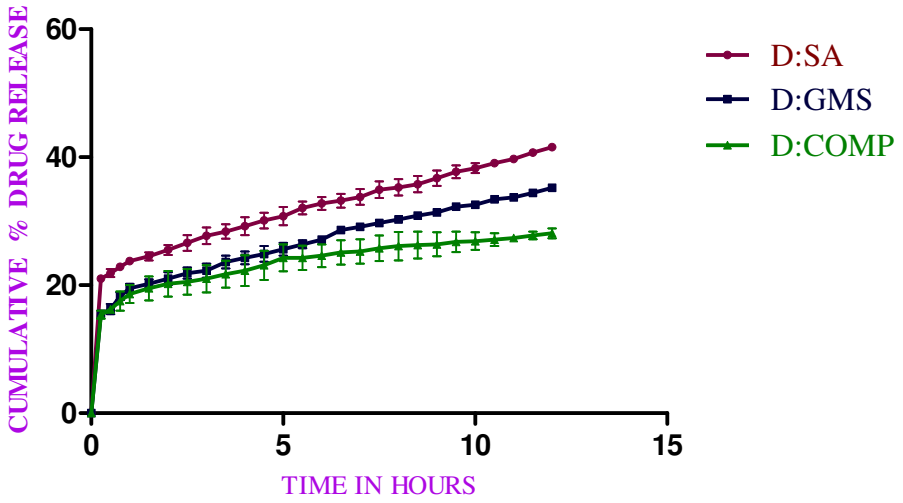
IN-VITRO RELEASE PROFILE OF SLN CONTAINING DRUG AND 15 % LIPID



Where D: SA –Drug+Stearic acid, D:GMS –Drug+ Glyceryl monostearate and D:COMP – Drug+Compritol.

FIGURE 27

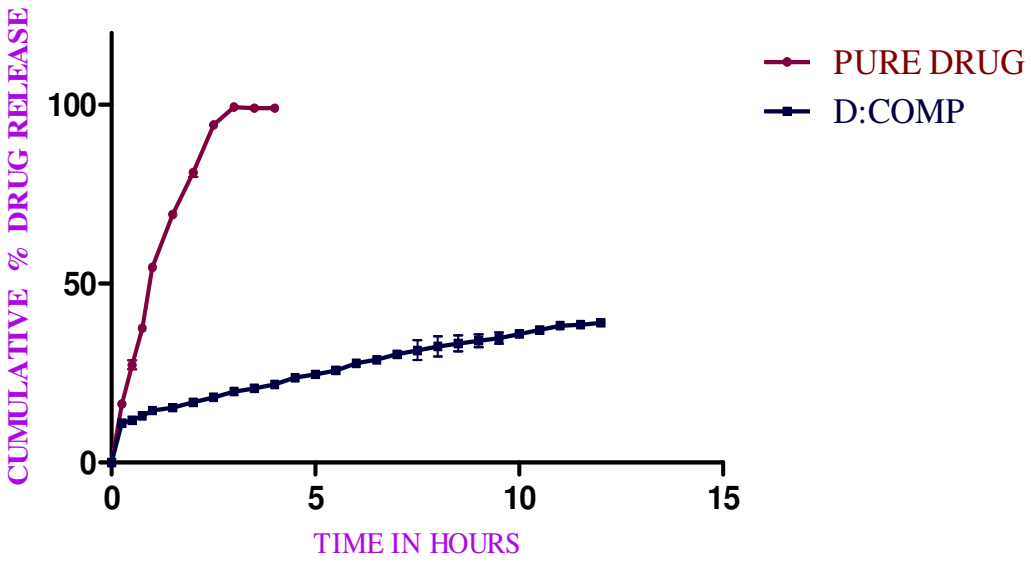
IN-VITRO RELEASE PROFILE OF SLN CONTAINING DRUG AND 20 % LIPID



Where D: SA –Drug+Stearic acid, D:GMS –Drug+ Glyceryl monostearate and D:COMP – Drug+Compritol.

FIGURE 28

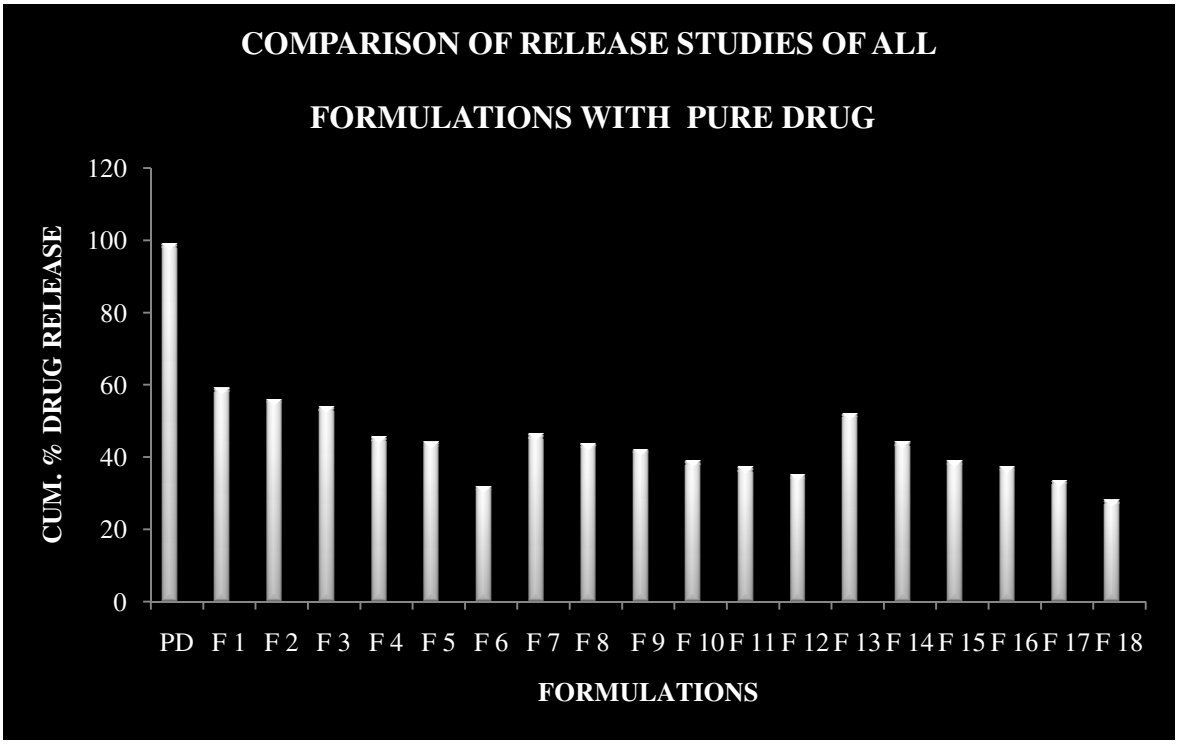
COMPARISON OF IN-VITRO RELEASE PROFILE OF BEST FORMULATION WITH PURE DRUG



Where D:COMP – Drug+Compritol.

FIGURE 29

COMPARISON OF IN-VITRO RELEASE STUDIES OFALL FORMULATIONS AND PURE DRUG

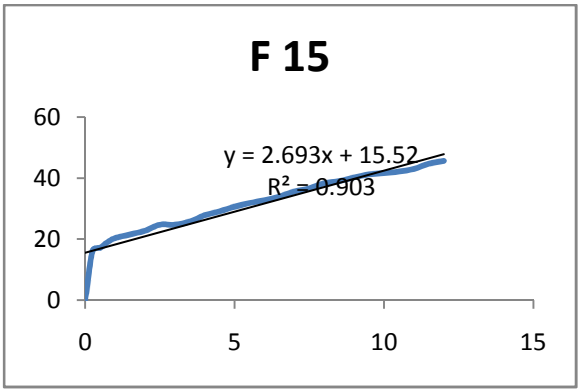


Where PD – Pure drug.

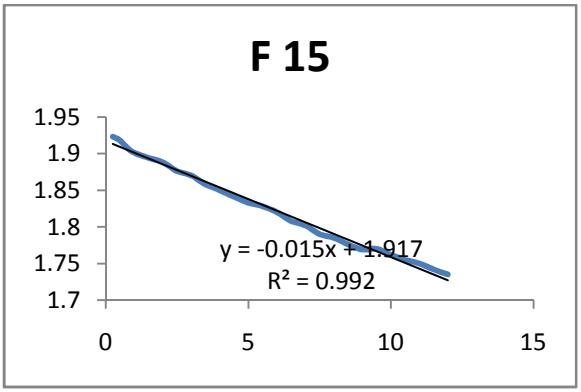
FIGURE 30

KINETIC MODELLING OF THE BEST FORMULATION (F 15)

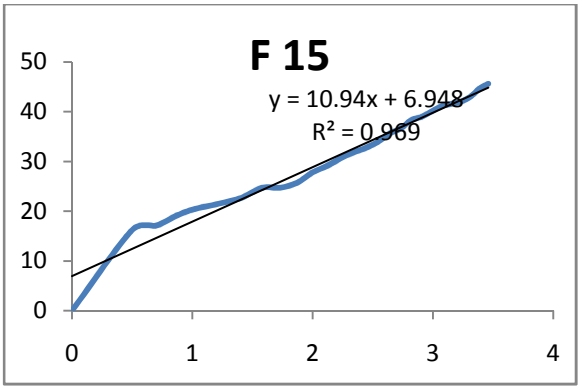
ZERO-ORDER KINTEICS MODEL



FIRST-ORDER KINTEICS MODEL



HIGUCHI KINETICS MODEL



KORSE-MEYER PEPPAS MODEL

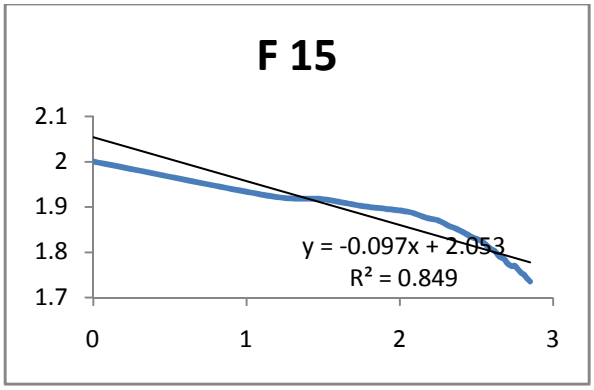


FIGURE 31

SEM PHOTOGRAPH OF THE BEST FORMULATION (F 15)

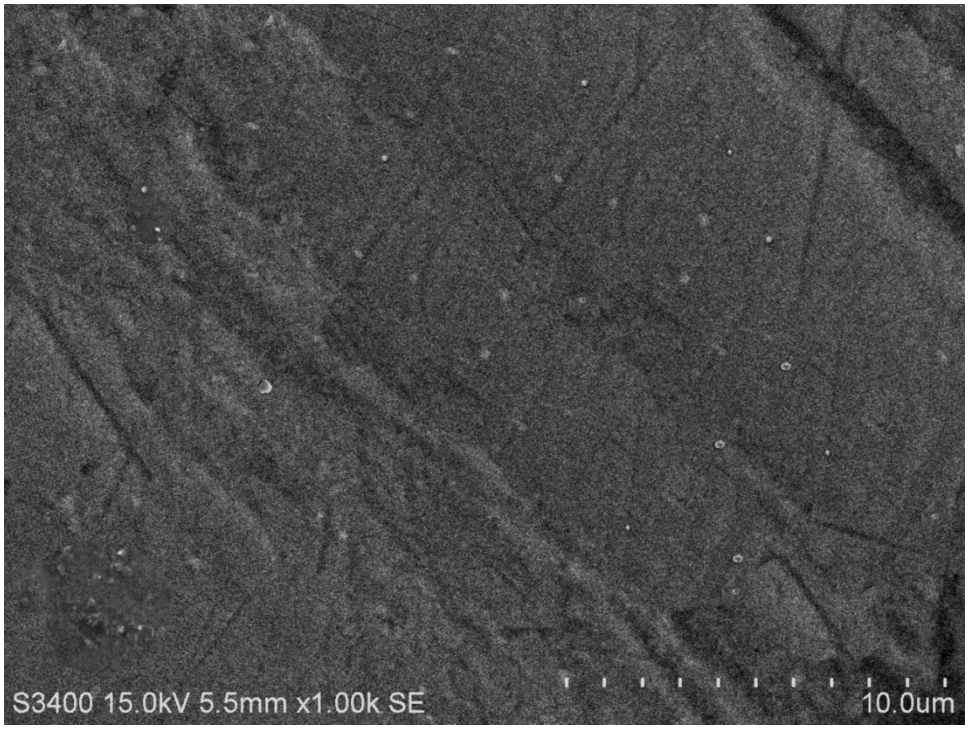


FIGURE 32

FTIR SPECTRA OF MONTELUKAST SODIUM

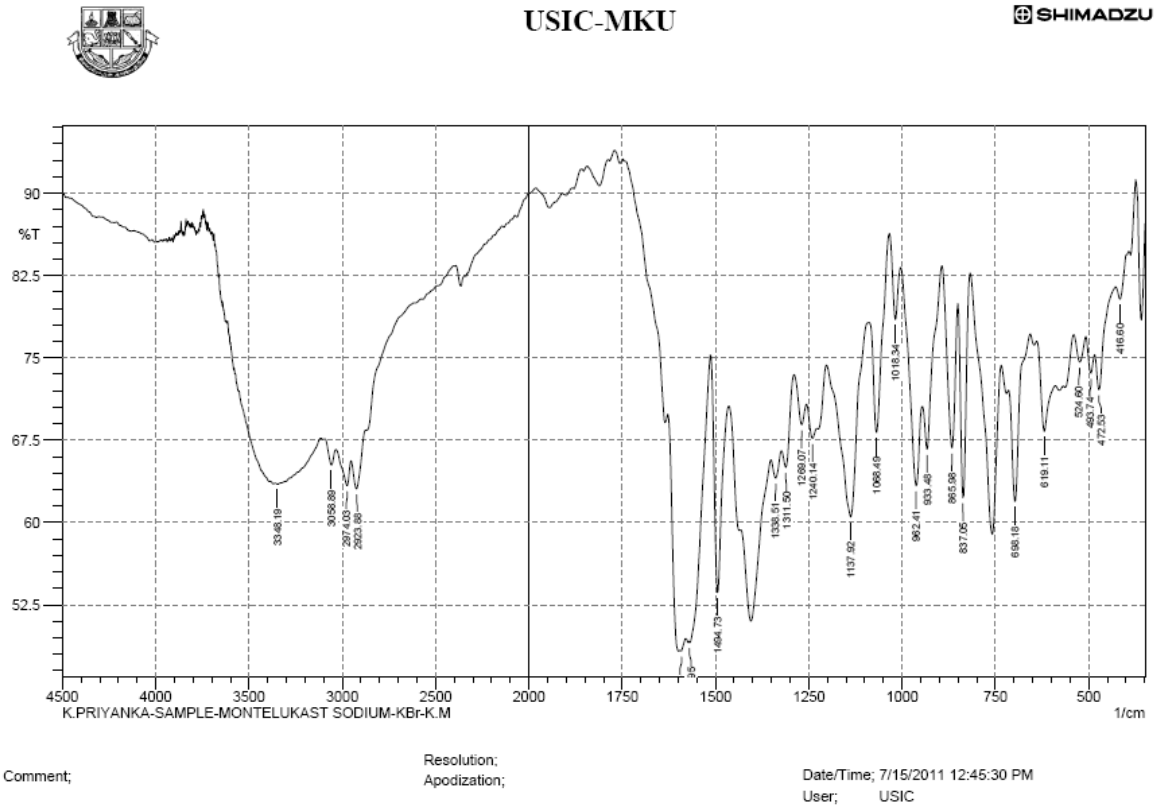


FIGURE 33

FTIR SPECTRA OF STEARIC ACID

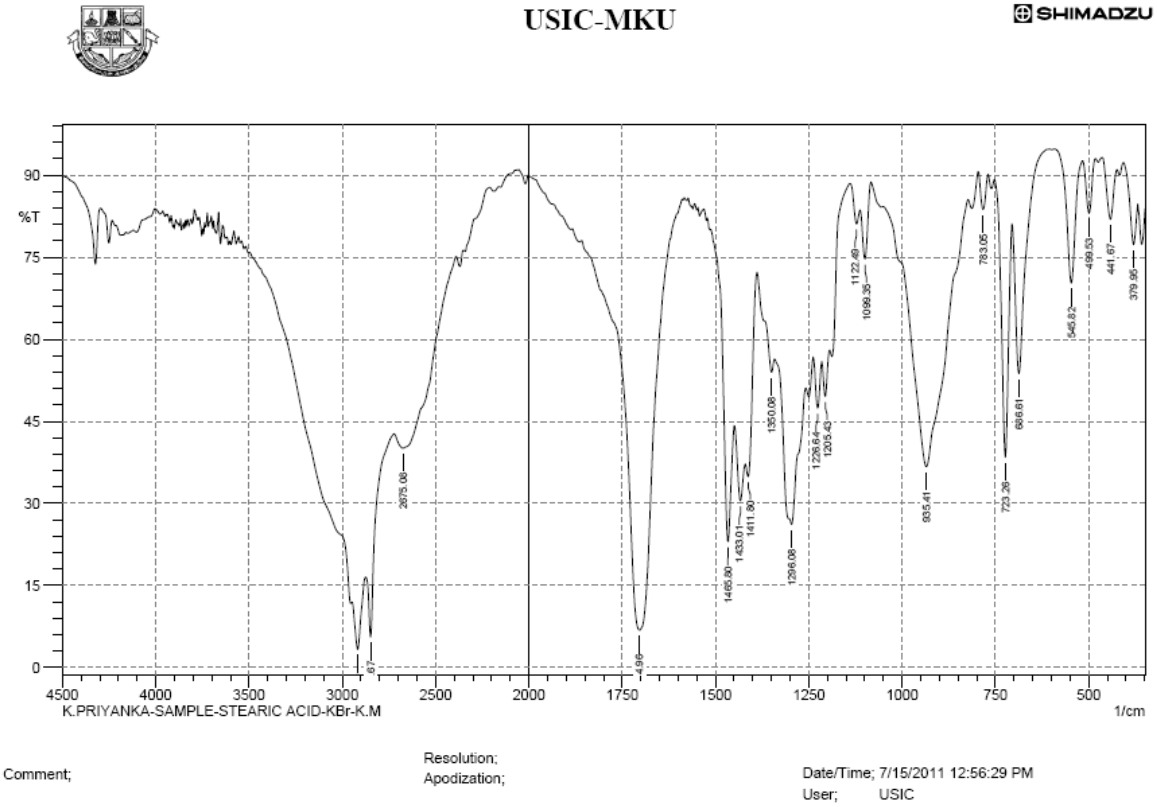


FIGURE 34

FTIR SPECTRA OF GLYCERYL MONOSTEARATE

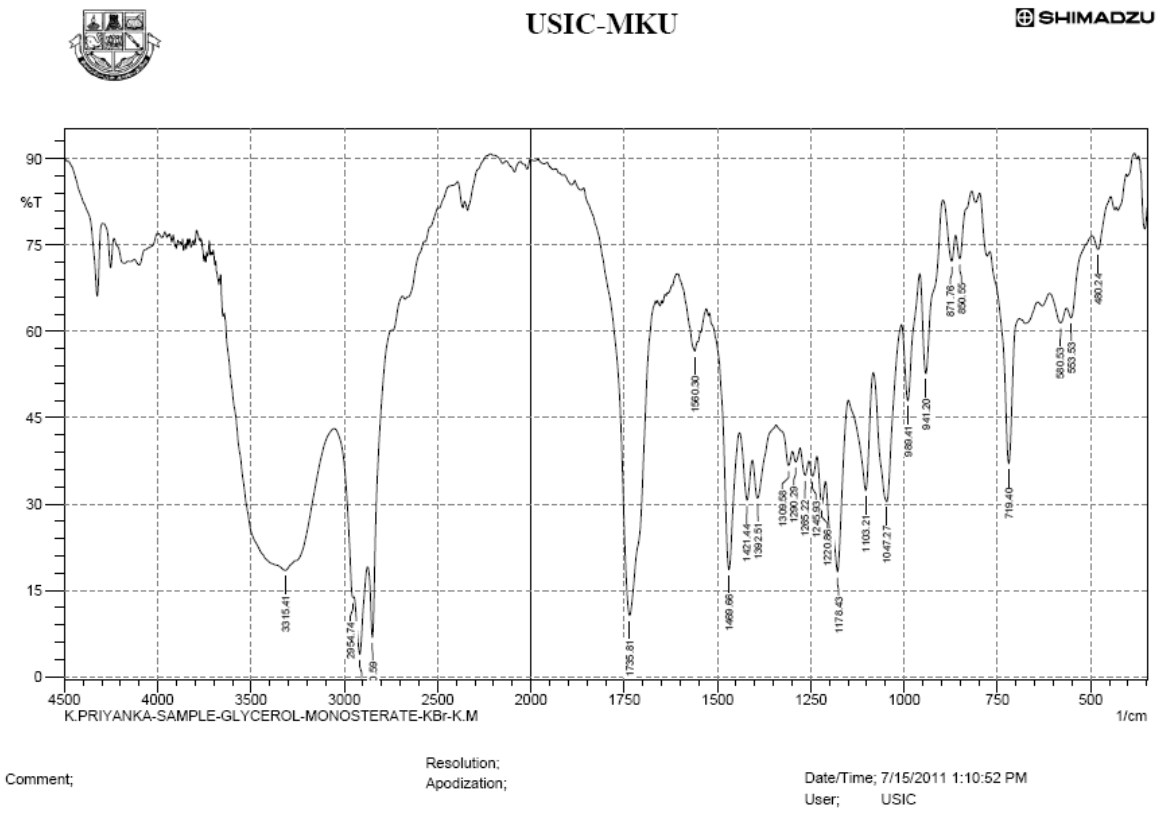


FIGURE 35

FTIR SPECTRA OF COMPRITOL ATO 888

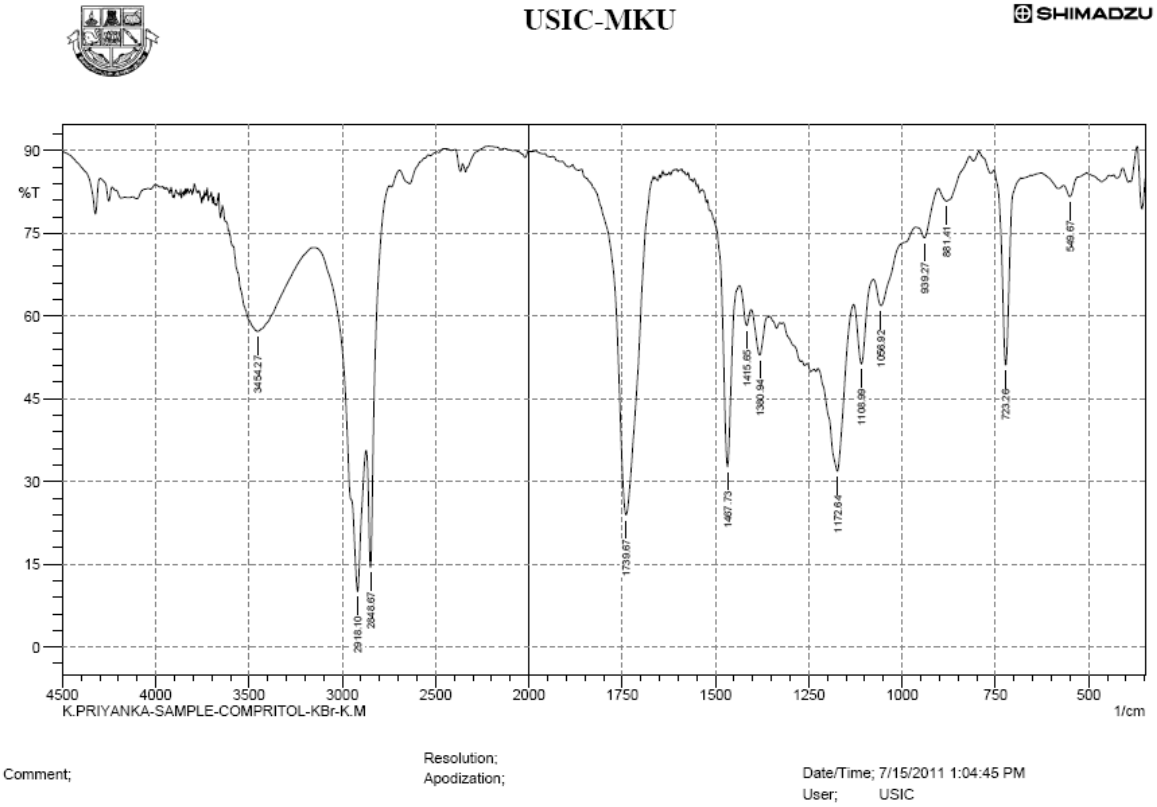
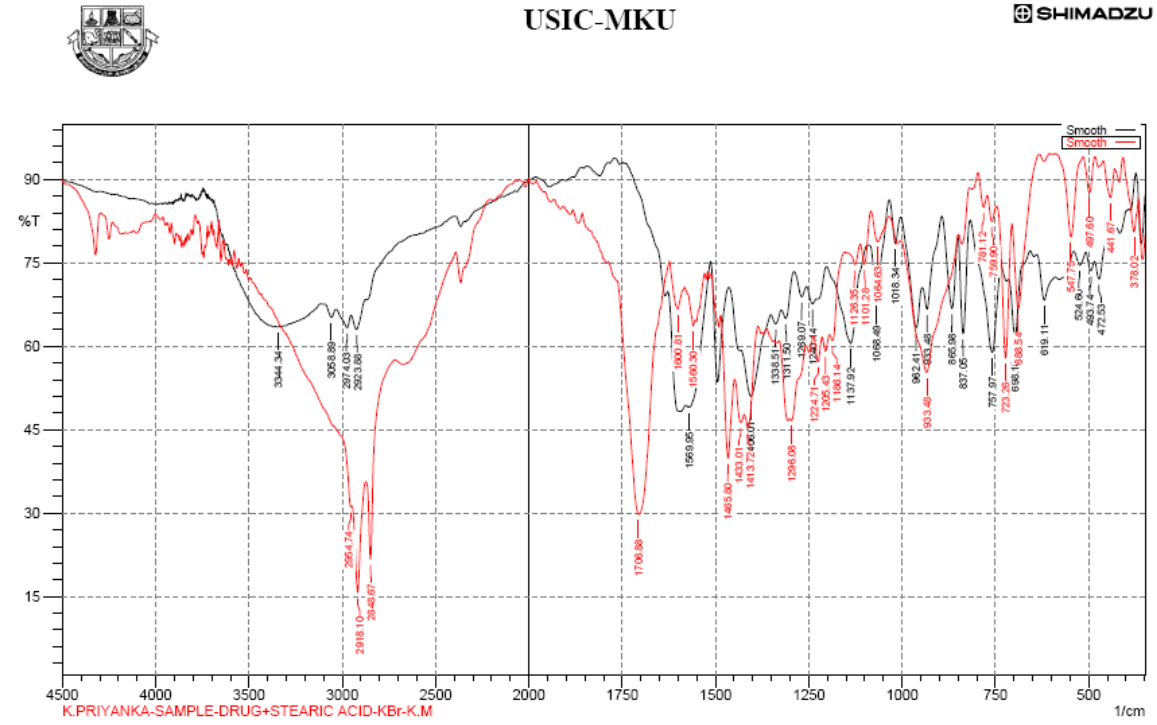


FIGURE 36

FTIR SPECTRA OF DRUG AND STEARIC ACID



Comment;

Resolution;
Apodization;

Date/Time; 7/15/2011 12:45:30 PM
User; USIC

FIGURE 37

FTIR SPECTRA OF DRUG AND GLYCERYL MONOSTEARATE

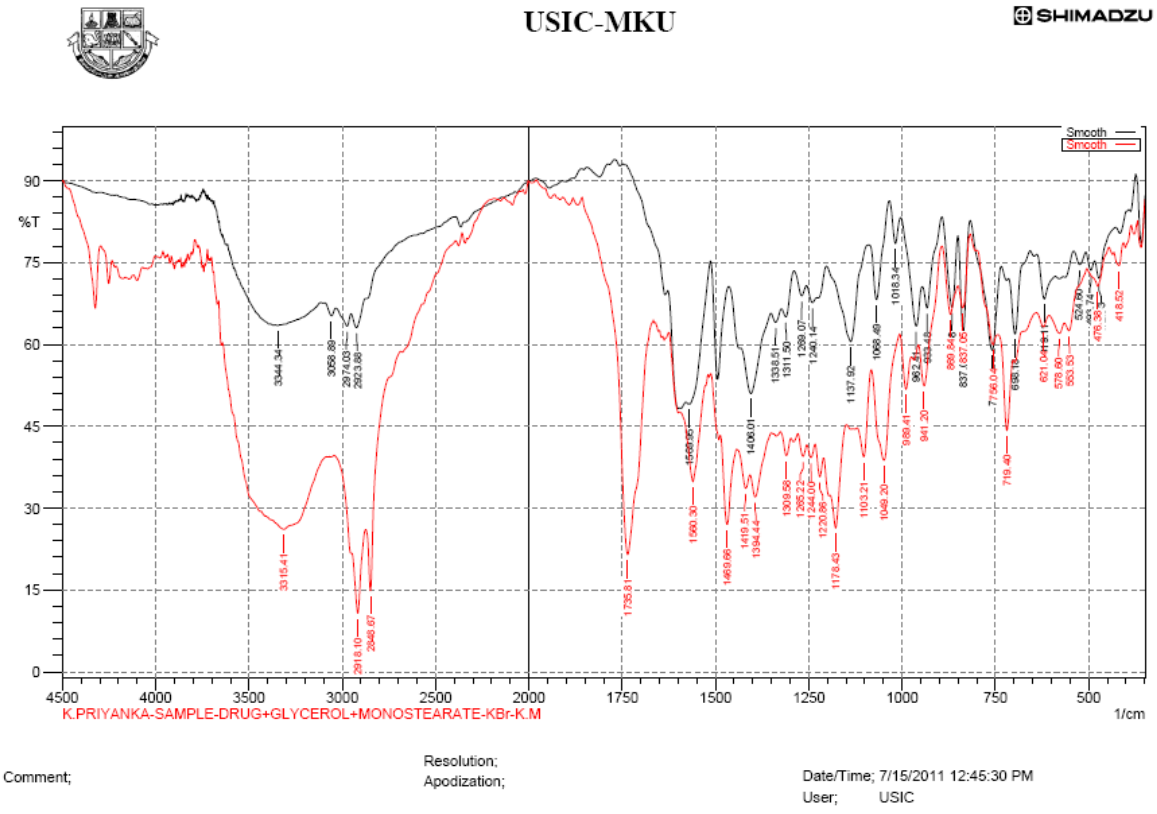


FIGURE 38

FTIR SPECTRA OF DRUG AND COMPRITOL ATO 888

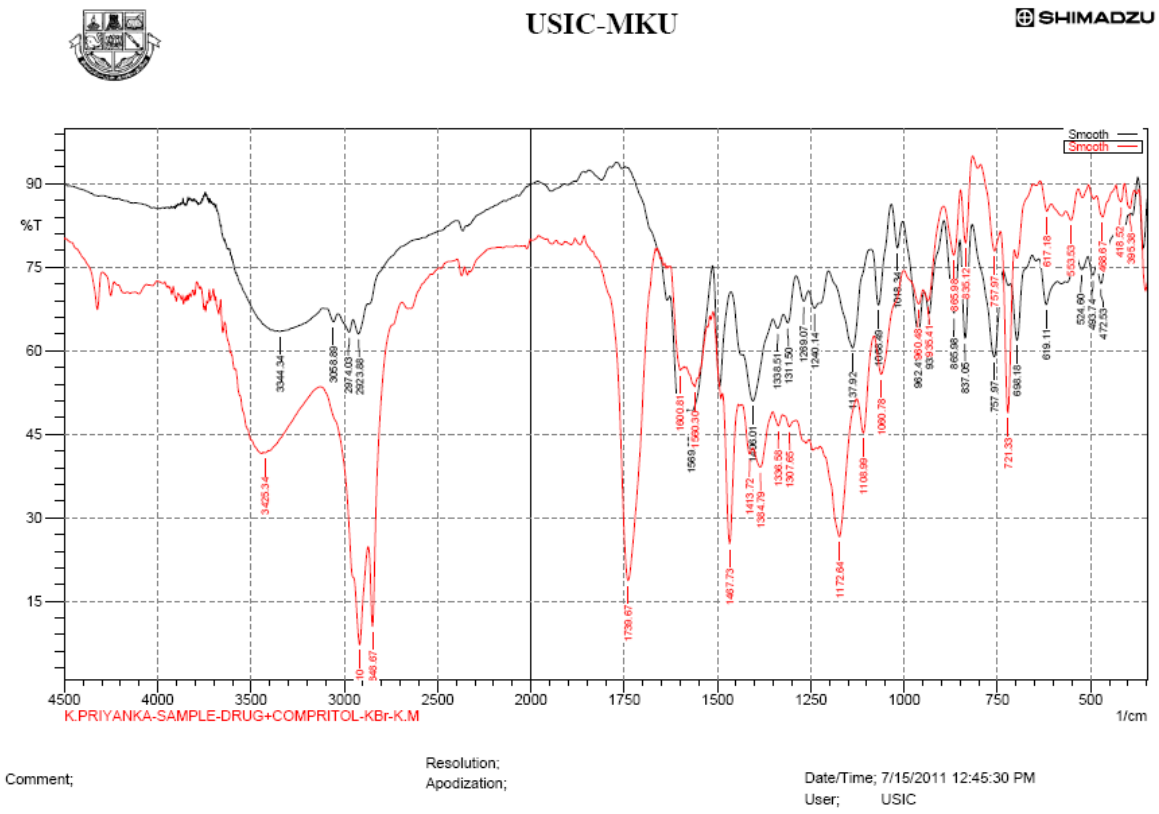


FIGURE 39

DSC THERMOGRAM OF MONTELUKAST SODIUM

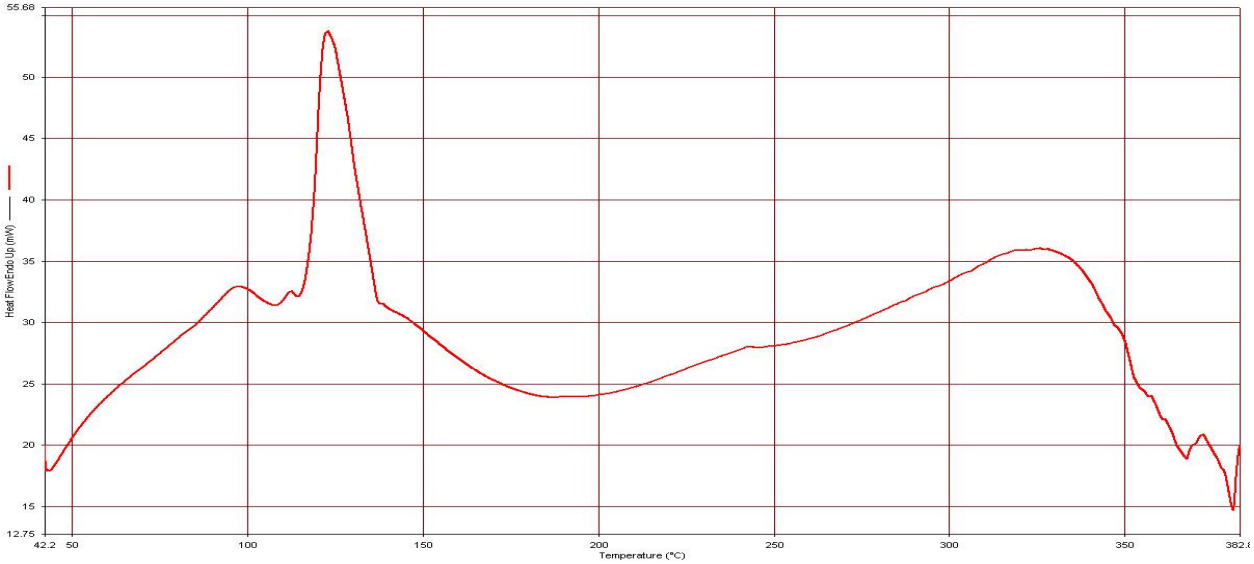


FIGURE 40

DSC THERMOGRAM OF STEARIC ACID

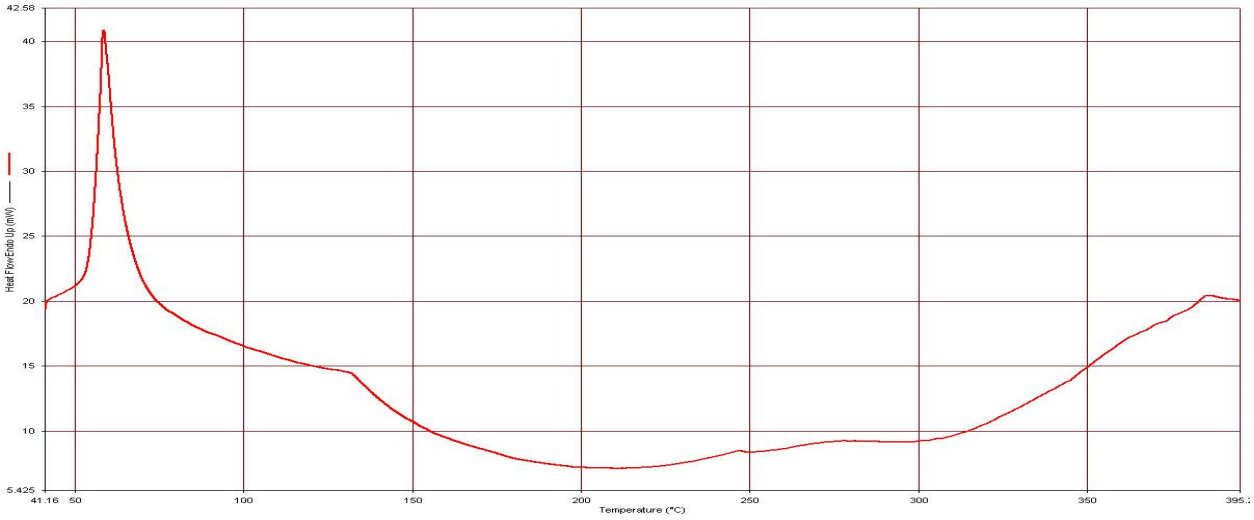


FIGURE 41

DSC THERMOGRAM OF GLYCERYL MONOSTEARATE

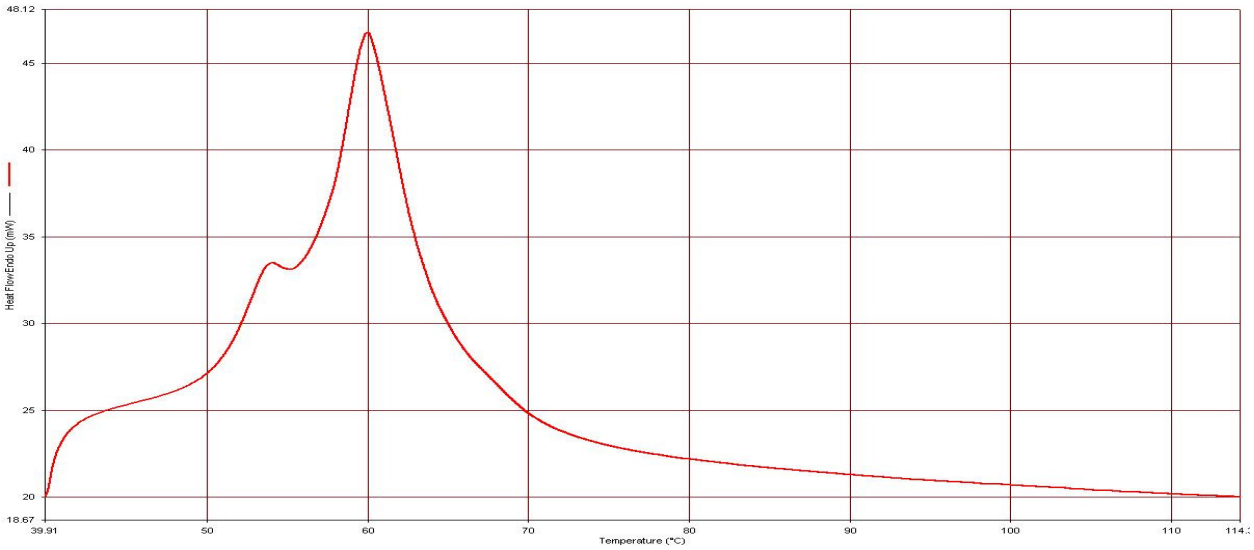


FIGURE 42

DSC THERMOGRAM OF COMPRITOL ATO 888

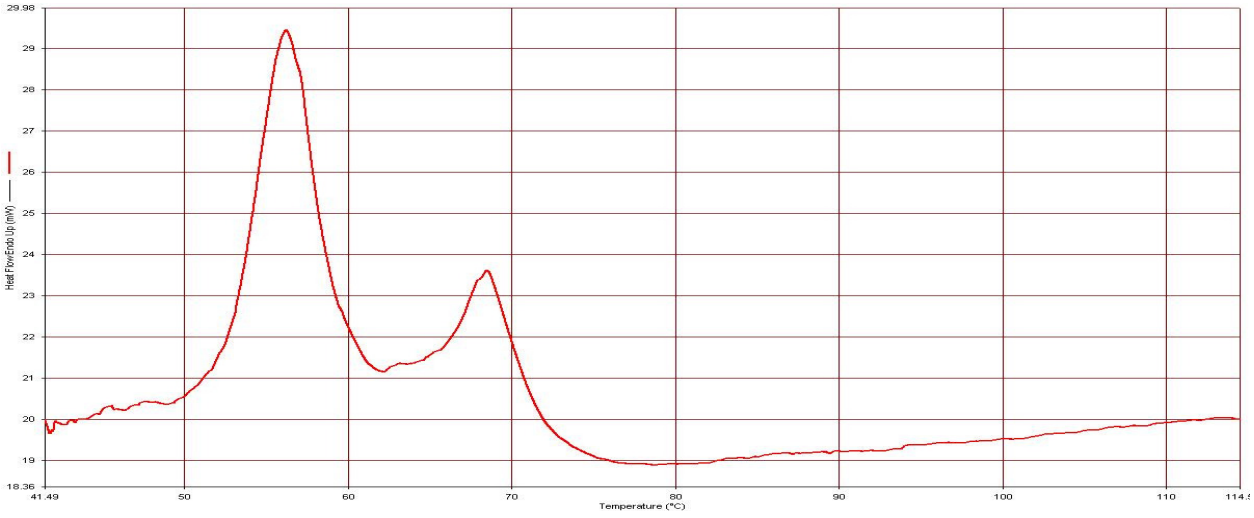


FIGURE 43

DSC THERMOGRAM OF DRUG WITH STEARIC ACID

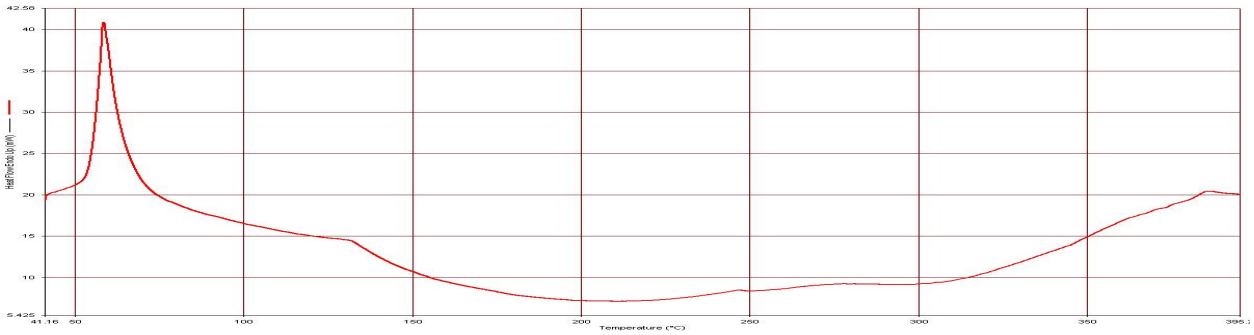


FIGURE 44

DSC THERMOGRAM OF DRUG WITH GLYCERYL MONOSTERATE

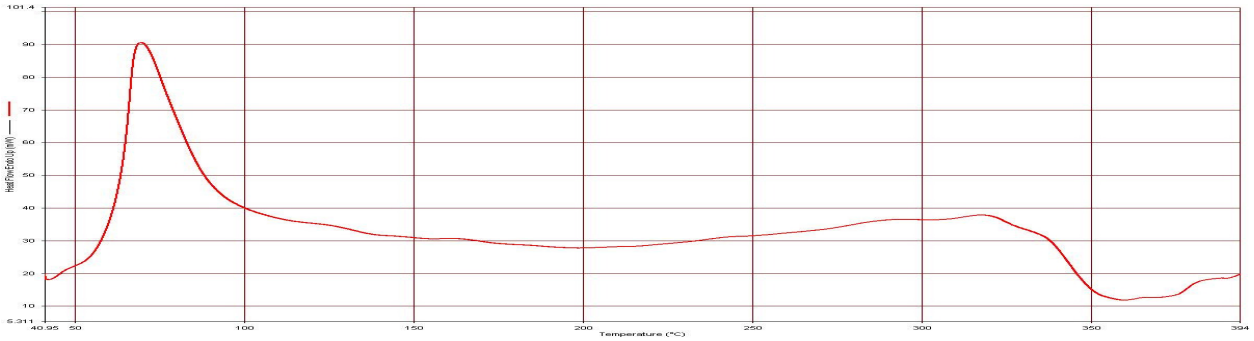
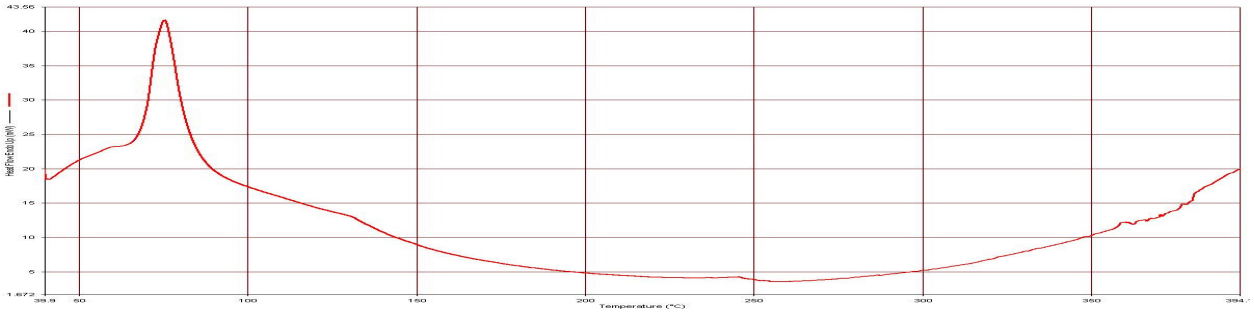


FIGURE 45

DSC THERMOGRAM OF DRUG WITH COMPRITOL ATO 888



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