FORMULATION, TISSUE DISTRIBUTION AND PHARMACOKINETIC EVALUATION OF SALBUTAMOL SULPHATE NIOSOMES

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CERTIFICATE

This is to certify that the dissertation work entitled "**FORMULATION, TISSUE DISTRIBUTION AND PHARMACOKINETIC EVALUATION OF SALBUTAMOL SULPHATE NIOSOMES "** submitted by **Mr.K.Velayutham** is a bonafide work carried out by the candidate under the guidance of **Mr. V. SANKAR M. Pharm.,** and submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** at the Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore, during the academic year 2008-2009.

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DECLARATION

I do hereby declare that the dissertation work entitled **"FORMULATION, TISSUE DISTRIBUTION AND PHARMACOKINETIC EVALUATION OF SALBUTAMOL SULPHATE NIOSOMES"** submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics,** was done by me under the guidance of **Mr. V. SANKAR, M. Pharm.,** at the Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore, during the academic year 2008-2009.

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EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"FORMULATION, TISSUE DISTRIBUTION AND PHARMACOKINETIC EVALUATION OF SALBUTAMOL SULPHATE NIOSOMES"** submitted by **Mr.K.Velayutham,** University Reg. No **26074660** to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by the candidate at the Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore and was evaluated by us during the academic year 2008-2009.

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CHAPTER - I

NOVEL DRUG DELIVERY SYSTEM (NDDS)

Introduction

[1]The basic goal of novel drug delivery systems is to achieve a steady state blood or tissue level that is therapeutically effective and non toxic for an extended period of time.

Conventional drug delivery involves the formulation of the drug into a suitable form, such as compressed tablet for oral administration or a solution for IV administration. These dosage forms have been found to have serious limitations in terms of higher doses required, lower effectiveness, toxicity& adverse effects.

NDDS are being developed rapidly, so as to overcome the limitations of conventional drug delivery.

[2]The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology.

To minimize drug degradation and loss, to prevent harmful side-effects and to

increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Among drug carriers one can name soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, and micelles. The carriers can be made slowly degradable, stimuli-reactive (e.g., pH- or temperature-sensitive), and even targeted (e.g., by conjugating them with specific antibodies against certain characteristic components of the area of interest). Targeting is the ability to direct the drug-loaded system to the site of interest. Two major mechanisms can be distinguished for addressing the desired sites for drug release: (i) passive and (ii) active targeting. An example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumors as a result of the enhanced vascular permeability of tumor tissues compared with healthy tissue. A strategy that could allow active targeting involves the surface functionalization of drug carriers with ligands that are selectively recognized by receptors on the surface of the cells of interest. Since ligand–receptor interactions can be highly selective, this could allow a more precise targeting of the site of interest.

Controlled drug release and subsequent biodegradation are important for developing successful formulations. Potential release mechanisms involve: (i) desorption of surface-bound /adsorbed drugs; (ii) diffusion through the carrier matrix; (iii) diffusion (in the case of nanocapsules) through the carrier wall; (iv) carrier matrix erosion; and (v) a combined erosion /diffusion process. The mode of delivery can be the difference between a drug's success and failure, as the choice of a drug is often influenced by the way the medicine is administered. Sustained (or continuous) release of a drug involves polymers that release the drug at a controlled rate due to diffusion

out of the polymer or by degradation of the polymer over time. Pulsatile release is often the preferred method of drug delivery, as it closely mimics the way by which the body naturally produces hormones such as insulin. It is achieved by using drug-carrying polymers that respond to specific stimuli (e.g., exposure to light, changes in pH or temperature).

For over 20 years, researchers have appreciated the potential benefits of nanotechnology in providing vast improvements in drug delivery and drug targeting. Improving delivery techniques that minimize toxicity and improve efficacy offers great potential benefits to patients, and opens up new markets for pharmaceutical and drug delivery companies. Other approaches to drug delivery are focused on crossing particular physical barriers, such as the blood brain barrier, in order to better target the drug and improve its effectiveness; or on finding alternative and acceptable routes for the delivery of protein drugs other than via the gastro-intestinal tract, where degradation can occur.

[3]Therapeutic Benefits on Novel drug delivery systems over conventional dosage forms.

- Increased efficacy of the drug.
- Site specific delivery.
- Decreased toxicity / side effects.
- Increased convenience.
- Shorter hospitalisaiton.
- Viable treatments for previously incurable diseases.
- Potential for prophylactic application.

- Lower health care costs- both short & long term.
- Better patient compliance.

[2]Drug Delivery Carriers

Colloidal drug carrier systems such as micellar solutions, vesicle and liquid crystal dispersions, as well as nanoparticle dispersions consisting of small particles of 10–400 nm diameter show great promise as drug delivery systems(fig a). When developing these formulations, the goal is to obtain systems with optimized drug loading and release properties, long shelf-life and low toxicity. The incorporated drug participates in the microstructure of the system, and may even influence it due to molecular interactions, especially if the drug possesses amphiphilic and/or mesogenic properties

Micelles formed by self-assembly of amphiphilic block copolymers (5-50 nm) in aqueous solutions are of great interest for drug delivery applications. The drugs can be physically entrapped in the core of block copolymer micelles and transported at concentrations that can exceed their intrinsic water- solubility.

Liposomes are a form of vesicles that consist either of many, few or just one phospholipid bilayers. The polar character of the liposomal core enables polar drug molecules to be encapsulated. Amphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer according to their affinity towards the phospholipids. Participation of nonionic surfactants instead of phospholipids in the bilayer formation results in niosomes.

Comparison of niosomes Vs liposomes:

a)Niosomes are now widely studied as an alternative to liposomes, which exhibit certain disadvantages such as –they are expensive, their ingredients like phospholipids are chemically unstable because of their predisposition to oxidative degradation, they require special storage and handling and purity of natural phospholipids is variable.

b) Differences in characteristics exist between liposomes and niosomes, especially since niosomes are prepared from uncharged single-chain surfactant and cholesterol whereas liposomes are prepared from double-chain phospholipids. Handjani-Vila et al were first to report the formation of vesicular system on hydration of mixture of cholesterol and a single-alkyl chain non-ionic surfactant.

c) Niosomes behave in-vivo like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability. Encapsulation of various anti neoplastic agents in these carrier vesicles has been shown to decrease drug induced toxic side effects, while maintaining, or in some instances, increasing the anti-tumor efficacy. Such vesicular drug carrier systems alter the plasma clearance kinetics, tissue distribution, metabolism and cellular interaction of the drug. They can be expected to target the drug to its desired site of action and/or to control its release.

d) As with liposomes, the properties of niosomes depends both on the composition of the bilayer and on method of their production. The intercalation of cholesterol in the bilayers decreases the entrapment volume during formulation and thus entrapment efficiency. As the concentration of cholesterol increases, entrapment efficiency decreases.

e) The entrapment efficiency increases with increase in the concentration and lipophilicity of surfactant. Chandraprakash et al made Methotrexate loaded non-ionic surfactant vesicles using lipophilic surfactants like Span 40, Span 60 and Span 80 and

found that Span 60 (HLB = 4.7) gave highest percent entrapment while Span 85 (HLB = 9.8) gave least entrapment. They also observed that as HLB value of surfactant decreased, the mean size was reduced.

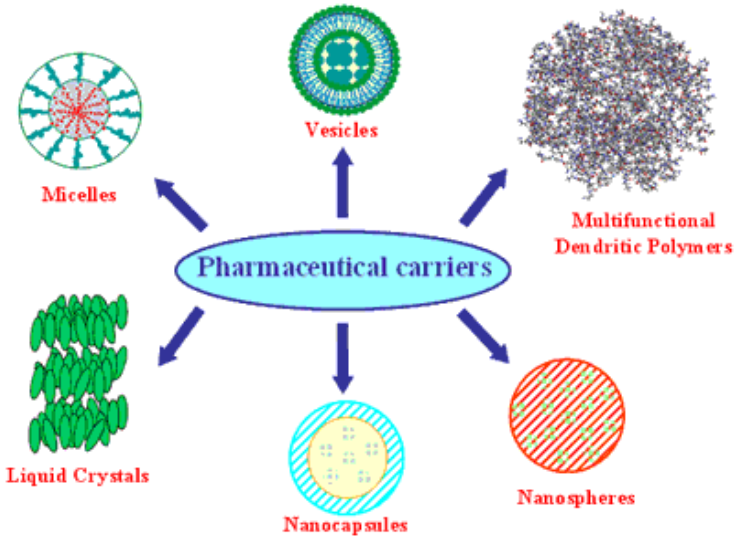


FIG a: Pharmaceutical carriers

Dendrimers are nanometer-sized, highly branched and monodisperse macromolecules with symmetrical architecture. They consist of a central core, branching units and terminal functional groups.

Nanoparticles (including nanospheres and nanocapsules of size 10-200 nm) are in the solid state and are either amorphous or crystalline. They are able to adsorb and/or encapsulate a drug, thus protecting it against chemical and enzymatic degradation.

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids. The networks are composed of homopolymers or copolymers, and are insoluble due to the presence of chemical crosslinks (tie-points, junctions), or physical crosslinks, such as entanglements or crystallites.

The ultimate goal in controlled release is the development of a microfabricated device with the ability to store and release multiple chemical substances on demand. Recent advances in microelectro-mechanical systems (MEMS) have provided a unique opportunity to fabricate miniature biomedical devices for a variety of applications ranging from implantable drug delivery systems to lab-on-a-chip devices. The controlled release microchip has the following advantages: (i) multiple chemicals in any form (e.g., solid, liquid or gel) can be stored inside and released from the microchip, (ii) the release of chemicals is initiated by the disintegration of the barrier membrane via the application of an electric potential, (iii) a variety of highly potent drugs can potentially be delivered accurately and in a safe manner, (iv) complex release patterns (e.g., simultaneous constant and pulsatile release) can be achieved, (v) the microchip can be made small enough to make local chemical delivery possible thus achieving high concentrations of drug at the site where it is needed while keeping the systemic concentration of the drug at a low level and (vi) water penetration into the reservoirs is avoided by the barrier membrane and thus the stability of protein-based drugs with limited shelf-life is enhanced.

Future Opportunities and Challenges

Nanoparticles and nanoformulations have already been applied as drug delivery systems with great success; and nanoparticulate drug delivery systems have still greater potential for many applications, including anti-tumour therapy, gene therapy, AIDS therapy, radiotherapy, in the delivery of proteins, antibiotics, virostatics, vaccines and as vesicles to pass the blood-brain barrier.

Nanoparticles provide massive advantages regarding drug targeting, delivery and release and, with their additional potential to combine diagnosis and therapy, emerge as one of the major tools in nanomedicine. The main goals are to improve their stability in the biological environment, to mediate the bio-distribution of active compounds, improve drug loading, targeting, transport, release, and interaction with biological barriers. The cytotoxicity of nanoparticles or their degradation products remains a major problem, and improvements in biocompatibility obviously are a main concern of future research.

There are many technological challenges to be met, in developing the following techniques:

- Nano-drug delivery systems that deliver large but highly localized quantities of drugs to specific areas to be released in controlled ways;
- Controllable release profiles, especially for sensitive drugs;
- Materials for nanoparticles that are biocompatible and biodegradable;
- Architectures / structures, such as biomimetic polymers, nanotubes;
- Technologies for self-assembly;

- Functions (active drug targeting, on-command delivery, intelligent drug release devices/ bioresponsive triggered systems, self-regulated delivery systems, systems interacting with the body, smart delivery);
- Virus-like systems for intracellular delivery;
- Nanoparticles to improve devices such as implantable devices/nanochips for nanoparticle release, or multi reservoir drug delivery-chips;
- Nanoparticles for tissue engineering; e.g. for the delivery of cytokines to control cellular growth and differentiation, and stimulate regeneration; or for coating implants with nanoparticles in biodegradable polymer layers for sustained release;
- Advanced polymeric carriers for the delivery of therapeutic peptide/proteins (biopharmaceutics),

And also in the development of:

- Combined therapy and medical imaging, for example, nanoparticles for diagnosis and manipulation during surgery (e.g. thermotherapy with magnetic particles);
- Universal formulation schemes that can be used as intravenous, intramuscular or peroral drugs
- Cell and gene targeting systems.

TARGETED DRUG DELIVERY SYSTEM (TDDS)

[5]Drug targeting is a phenomenon which maneunivers the distribution of drug in the body in such a manner that the major fraction of the drug interacts exclusively with the target tissue at a cellular or subcellular level. The objective of drug targeting is to achieve a desired pharmacological response at a selected site without undesirable interactions at other sites.

This is especially important in cancer chemotherapy and enzyme replacement treatment. Drug targeting is the delivery of drugs to receptors or organs or any other specific part of the body to which one wishes to deliver the drug exclusively.

The targeted or site specific delivery of drugs is indeed a very attractive goal because this provides one of the most potential ways to improve the therapeutic index of the drugs.

Earlier work done between late 1960s and the mid 1980s stressed the need for drug carrier systems primarily to alter the pharmacokinetics of the already proven drugs whose efficacy might be improved by altering the rates for metabolism in liver or clearance by the kidneys. These approaches generally were not focussed to achieve site specific or targeted delivery such as getting a cytotoxic drug to cancerous tissue while sparing other normal, though equally sensitive tissue. With the advancement in the carrier technology the issue of delivering either individual drug molecule or the entire carrier to the desired site has been addressed during the last few years.

A number of technological advances have since been made in the area of parenteral drug delivery leading to the development of sophisticated systems that

allow drug targeting and the sustained or controlled release of parenteral medicines.

[6]At present, drug targeting is achieved by one or two approaches. The first approach involves chemical modification of the parent compound to a derivative which is activated only at the target site.

The second approach utilizes carriers such as liposomes, microspheres, nano particles, antibodies, cellular carriers (erythrocytes and lymphocytes) and macromolecules to direct drug to its site of action.

Recent advancements have led to the development of several novel drug delivery system that could revolutionize the method of medication and provides a number of therapeutic benefits.

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly, and then maintain, the desired drug constant. The ideal drug delivery system delivers drug at a rate dictated by the need of the body over the period of treatment and it channels the active entity solely to the site of action. At present no available drug delivery systems can achieve all these goals. The targeted drug delivery system achieves the site specific delivery but is unable to control the release kinetics of drug in predictable manner.

Paul Ehrlich in 1906, initiated the era of development for targeted delivery when he envisaged a drug delivery mechanism that would target drugs directly to diseased cells.

[7]Number of carriers were utilised to carry drug at the target organ / tissue which include immunoglobulins, serum proteins, synthetic polymers, lipid vesicles (liposomes), microspheres, erythrocytes, reverse micells, niosomes, pharmacosomes etc. Amongst the various carriers, few drug carriers reached the stage of clinical trials

where phospholipid vesicle show strong potential for effective drug delivery to the site of action. These carriers (liposomes) are biologically inert in nature, devoid of any antigenic, pyrogenic or allergic reactions and their components can be utilised as the component of biological membrane. Drugs incorporated in liposomes are not activated under physiological conditions and do not cause unfavourable side effects as well.

[8]There are various techniques by which drug can be targeted include

1. Nanoparticles.
2. Niosomes.
3. Resealed erythrocytes.
4. Microspheres.
5. Monoclonal antibodies.
6. Liposomes.

MERITS OF TDDS

- Targeting of the drug molecule towards the tissue or organ reduces the toxicity to the normal tissues.
- Increased bioavailability.
- Improved treatment of chronic illness where symptoms break through occurs when the plasma level of the drug falls below the MEC.
- The drug is protected from first pass metabolism and GI degradation.
- Improved patient compliance can be achieved due to decrease in amount and frequency of doses administered.
- Biocompatibility can be well achieved.
- Maintenance of therapeutic action of the drug overnight.
- Systemic and local side effects are successfully reduced due to the reduction in the total amount of the drug.

- Magnetically controlled systems can be used for targeting the drug towards superficial tissues.
- Economic savings can be claimed due to reduction of total amount of drug used.

[9]LIMITATIONS OF TDDS

- TDDS such as liposomes, resealed erythrocytes and platelets suffer serious stability problems.
- Although monoclonal antibodies show very high degree of site specificity the selection and Isolation procedures are too tough.
- If the particle size of TDDS is high, they may be rapidly cleared by RES.
- Magnetically controlled TDDS sliows high specificity to superficially located ornnns and tissues but cannot be targeted to deep seated organs.
- Monoclonal antibodies may sometimes can cause unwanted antigen - antibody reaction which leads to serious consequences.
- Microspheres of particle size more than 50µg can lead to problem of thrombo-embolism in general circulation.
- Once administered the drug cannot be removed if an undesirable action is precipitated or if the drug is no longer needed.
- Most of such systems are administered by subcutaneous or Intraperitoneal route.

  The vehicles polymer employed should be sterile, hydrogen free, non irritating, biocompatible and biodegradable into non toxic compounds within an appropriate time, preferably close to duration of action.
- The products which tend to remain intact may become lodged at some sites. If this occur slow release of drug from dosage form leads to a high localised concentration of drug which causes local irritation.

- ❖ Drugs having biological half life of 1 hr or less are difficult to formulate as controlled release formulation. The high rates of elimination of such drugs from the body need an extremely large maintenance dose which provides 8 - 12hrs of continous therapy.
- ❖ As these products normally contain large amount of drug there is a possibility of unsafe over dosage if the product is improperly made.
- ❖ If it is once administered it may be difficult to stop the therapy due to toxicity or any other reasons.

APPLICATIONS OF TDDS

a. Cells as carriers

Red blood cells, leukocytes, lymphocytes and fibroblasts have all been used as potential delivery vehicles for drugs. They have an advantage of inherent biocompatibility, but they cannot cross barriers and cannot easily fuse with other cells. Erythrocytes have been, explored as possible carriers for Cytarabine and Adriamycin.

Many of the more biocompatible polymers can be used as small soluble molecular drug carriers or they can be assembled as both soluble and particulate drug vehicles. Large amount of drugs or agents can be incorporated through non-covalent forces into there assembled polymers. These particulate system are best utilized as sustained release vehicles.

Bovine albumin or bovine serum albumin and human serum albumin have been extensively investigated for target specific and sustained delivery of cancer chemotherapeutic agents.

The intra peritoneal administration of microspheres sustained the drug release over a period of time.

NIOSOMES AS NOVEL DRUG DELIVERY CARRIER[10]

Definition

Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs. Niosomes are promising vehicle for drug delivery and being non-ionic, it is less toxic and improves the therapeutic index of drug by restricting its action to target cells.

Introduction

At present no available drug delivery system achieves the site specific delivery with controlled release kinetics of drug in predictable manner.

Paul Ehrlich, in 1909, initiated the era of development for targeted delivery when he envisaged a drug delivery mechanism that would target directly to diseased cell. Since then, number of carriers were utilized to carry drug at the target organ/tissue, which include immunoglobulins, serum proteins, synthetic polymers, liposomes, microspheres, erythrocytes, niosomes etc.

Among different carriers liposomes and niosomes are well documented drug delivery.

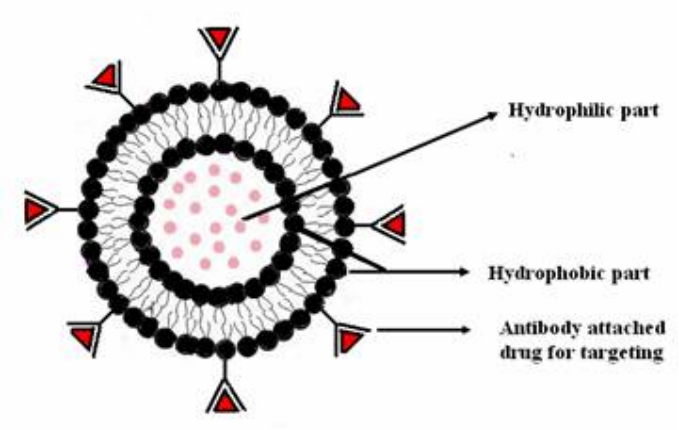
Drug targeting can be defined as the ability to direct a therapeutic agent specifically to desired site of action with little or no interaction with nontarget tissue.

Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media.

In niosomes, the vesicles forming amphiphile is a non-ionic surfactant such as Span – 60 which is usually stabilized by addition of cholesterol and small amount of anionic surfactant such as dicetyl phosphate.

Schematic representation of a drug targeting through its linkage to niosome via antibody is shown below (fig b).

Fig b: Niosome and antibody linkage



Advantages of niosomes:

The first report of non-ionic surfactant vesicles came from the cosmetic applications devised by L'Oreal.

The application of vesicular (lipid vesicles and non-ionic surfactant vesicles) systems in cosmetics and for therapeutic purpose may offer several advantages: -

- The vesicle suspension is water–based vehicle. This offers high patient compliance in comparison with oily dosage forms.
- They possess an infrastructure consisting of hydrophilic, amphiphilic and lipophilic moieties together and as a result can accommodate drug molecules with a wide range of solubilities.

- The characteristics of the vesicle formulation are variable and controllable. Altering vesicle composition, size, lamellarity, tapped volume, surface charge and concentration can control the vesicle characteristics.
- The vesicles may act as a depot, releasing the drug in a controlled manner.

Other advantages of niosomes include:

- They are osmotically active and stable, as well as they increase the stability of entrapped drug.
- Handling and storage of surfactants requires no special conditions.
- They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.
- They can be made to reach the site of action by oral, parenteral as well as topical routes.
- The surfactants are biodegradable, biocompatible and non-immunogenic.
- They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.
- Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non-aqueous phase.

Method of preparation

A. Ether injection method

This method provides a means of making niosomes by slowly introducing a solution of surfactant dissolved in diethyl ether into warm water maintained at 60°C. The surfactant mixture in ether is injected through 14-gauge needle into an aqueous

solution of material. Vaporization of ether leads to formation of single layered vesicles. Depending upon the conditions used, the diameter of the vesicle range from 50 to 1000 nm.

B. Hand shaking method (Thin film hydration technique)

The mixture of vesicles forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature (20°C) using rotary evaporator leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase at 0-60°C with gentle agitation. This process forms typical multilamellar niosomes.

Thermosensitive niosomes were prepared by Raja Naresh et al /i>[15] by evaporating the organic solvent at 60°C and leaving a thin film of lipid on the wall of rotary flash evaporator. The aqueous phase containing drug was added slowly with intermittent shaking of flask at room temperature followed by sonication.

C. Sonication

A typical method of production of the vesicles is by sonication of solution as described by Cable. In this method an aliquot of drug solution in buffer is added to the surfactant/cholesterol mixture in a 10-ml glass vial. The mixture is probe sonicated at 60°C for 3 minutes using a sonicator with a titanium probe to yield niosomes.

D. Micro fluidization

Micro fluidization is a recent technique used to prepare unilamellar vesicles of defined size distribution. This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities, in precisely defined micro channels within the interaction chamber. The impingement of thin liquid sheet along a

common front is arranged such that the energy supplied to the system remains within the area of niosomes formation. The result is a greater uniformity, smaller size and better reproducibility of niosomes formed.

E. Multiple membrane extrusion method

Mixture of surfactant, cholesterol and dicetyl phosphate in chloroform is made into thin film by evaporation. The film is hydrated with aqueous drug polycarbonate membranes, solution and the resultant suspension extruded through which are placed in series for upto 8 passages. It is a good method for controlling niosome size.

F. Reverse Phase Evaporation Technique (REV)

Cholesterol and surfactant (1:1) are dissolved in a mixture of ether and chloroform. An aqueous phase containing drug is added to this and the resulting two phases are sonicated at 4-5°C. The clear gel formed is further sonicated after the addition of a small amount of phosphate buffered saline (PBS). The organic phase is removed at 40°C under low pressure. The resulting viscous niosome suspension is diluted with PBS and heated on a water bath at 60°C for 10 min to yield niosomes.

G. Trans membrane pH gradient (inside acidic) Drug Uptake Process (remote Loading)

Surfactant and cholesterol are dissolved in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask. The film is hydrated with 300 mM citric acid (pH 4.0) by vortex mixing. The multilamellar vesicles are frozen and thawed 3 times and later sonicated. To this niosomal suspension, aqueous solution containing 10 mg/ml of drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 with 1M disodium phosphate. This mixture is later heated at 60°C for 10 minutes to give niosomes.

H. The "Bubble" Method

It is novel technique for the one step preparation of liposomes and niosomes without the use of organic solvents. The bubbling unit consists of round-bottomed flask with three necks positioned in water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck and nitrogen supply through the third neck. Cholesterol and surfactant are dispersed together in this buffer (pH 7.4) at 70°C, the dispersion mixed for 15 seconds with high shear homogenizer and immediately afterwards "bubbled" at 70°C using nitrogen gas.

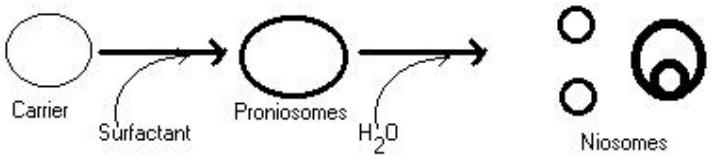
I. Formation of niosomes from proniosomes

Another method of producing niosomes is to coat a water-soluble carrier such as sorbitol with surfactant. The result of the coating process is a dry formulation. In which each water-soluble particle is covered with a thin film of dry surfactant (fig c). This preparation is termed "Proniosomes". The niosomes are recognized by the addition of aqueous phase at T > Tm and brief agitation.

T=Temperature.

Tm = mean phase transition temperature.

Fig c: Niosome from proniosome



Separation of Unentrapped Drug

The removal of unentrapped solute from the vesicles can be accomplished by various techniques, which include: -

1. Dialysis

The aqueous niosomal dispersion is dialyzed in a dialysis tubing against phosphate buffer or normal saline or glucose solution.

2. Gel Filtration

The unentrapped drug is removed by gel filtration of niosomal dispersion through a Sephadex-G-50 column and elution with phosphate buffered saline or normal saline.

3. Centrifugation

The niosomal suspension is centrifuged and the supernatant is separated. The pellet is washed and then resuspended to obtain a niosomal suspension free from unentrapped drug.

Characterization of niosomes

a) Entrapment efficiency

After preparing niosomal dispersion, unentrapped drug is separated by dialysis, centrifugation, or gel filtration as described above and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analysing the resultant solution by appropriate assay method for the drug. Where,

Entrapment efficiency (EF) = (Amount entrapped total amount) x 100

b) Vesicle diameter

Niosomes, similar to liposomes, assume spherical shape and so their diameter can be determined using light microscopy, photon correlation microscopy and freeze fracture electron microscopy.

Freeze thawing (keeping vesicles suspension at –20°C for 24 hrs and then heating to ambient temperature) of niosomes increases the vesicle diameter, which might be attributed to fusion of vesicles during the cycle.

c) In-vitro release

A method of in-vitro release rate study includes the use of dialysis tubing. A dialysis sac is washed and soaked in distilled water. The vesicle suspension is pipetted into a bag made up of the tubing and sealed. The bag containing the vesicles is placed in 200 ml of buffer solution in a 250 ml beaker with constant shaking at 25°C or 37°C. At various time intervals, the buffer is analyzed for the drug content by an appropriate assay method.

Factors affecting vesicles size, entrapment efficiency and release characteristics

a) Drug

Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayers, thereby increasing vesicle size. In polyoxyethylene glycol (PEG) coated vesicles, some drug is entrapped in the long PEG chains, thus reducing the tendency to increase the size. The hydrophilic lipophilic balance of the drug affects degree of entrapment.

b) Amount and type of surfactant

The mean size of niosomes increases proportionally with increase in the HLB of surfactants like Span 85 (HLB 1.8) to Span 20 (HLB 8.6) because the surface free energy decreases with an increase in hydrophobicity of surfactant.

The bilayers of the vesicles are either in the so-called liquid state or in gel state, depending on the temperature, the type of lipid or surfactant and the presence of other components such as cholesterol. In the gel state, alkyl chains are present in a well-ordered structure, and in the liquid state, the structure of the bilayers is more disordered. The surfactants and lipids are characterized by the gel-liquid phase transition temperature (TC). Phase transition temperature (TC) of surfactant also effects entrapment efficiency i.e. Span 60 having higher TC, provides better entrapment.

c) Cholesterol content and charge

Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency. In general, the action of cholesterol is two folds; on one hand, cholesterol increases the chain order of liquid-state bilayers and on the other, cholesterol decreases the chain order of gel state bilayers. At a high cholesterol concentration, the gel state is transformed to a liquid-ordered phase.

An increase in cholesterol content of the bilayers resulted in a decrease in the release rate of encapsulated material and therefore an increase of the rigidity of the bilayers obtained. Presence of charge tends to increase the interlamellar distance between successive bilayers in multilamellar vesicle structure and leads to greater overall entrapped volume.

d) Methods of preparation

Methods of preparation of niosomes such as hand shaking, ether injection and sonication in which Hand shaking method forms vesicles with greater diameter (0.35-13nm) compared to the ether injection method (50-1000nm).

Small sized niosomes can be produced by Reverse Phase Evaporation (REV) method .Microfluidization method gives greater uniformity and small size vesicles. Niosomes by trans membrane pH gradient (inside acidic) drug uptake process. Niosomes obtained by this method showed greater entrapment efficiency and better retention of drug.

e) Resistance to osmotic stress

Addition of a hypertonic salt solution to a suspension of niosomes brings about reduction in diameter. In hypotonic salt solution, there is initial slow release with slight swelling of vesicles probably due to inhibition of eluting fluid from vesicles, followed by faster release, which may be due to mechanical loosening of vesicles structure under osmotic stress.

Niosomes as drug carriers

Niosomes containing anti-cancer drugs, if suitably designed, will be expected to accumulate within tumors in a similar manner to liposomes. The niosomal encapsulation of Methotrexate and Doxorubicin increases drug delivery to the tumor and tumoricidal activity of the drug.

Doxorubicin niosomes possessing muramic acid and triglycerol surfaces were not taken up significantly by liver. The triglycerol niosomes accumulated in the tumor and muramic acid vesicles accumulated in the spleen. Those vesicles with polyoxyethylene surface were rapidly taken up by the liver and accumulated to a lesser extent in tumor.

Applications

Niosomal drug delivery is potentially applicable to many pharmacological agents for their action against various diseases. Some of their therapeutic applications are discussed below.

1) Targeting of bioactive agents

a) To reticulo-endothelial system (RES)

The cells of RES preferentially take up the vesicles. The uptake of niosomes by the cells is also by circulating serum factors known as opsonins, which mark them for clearance. Such localized drug accumulation has, however, been exploited in treatment of animal tumors known to metastasize to the liver and spleen and in parasitic infestation of liver.

b) To organs other than RES

It has been suggested that carrier system can be directed to specific sites in the body by use of antibodies. Immunoglobulins seem to bind quite readily to the lipid surface, thus offering a convenient means for targeting of drug carrier [37]. Many cells possess the intrinsic ability to recognize and bind particular carbohydrate determinants and this can be exploited to direct carriers system to particular cells.

2) Neoplasia

Doxorubicin, the anthracyclic antibiotic with broad spectrum anti tumor activity, shows a dose dependant irreversible cardio toxic effect. Niosomal delivery of this drug to mice bearing S-180 tumor increased their life span and decreased the rate of proliferation of sarcoma. Niosomal entrapment increased the half-life of the drug, prolonged its circulation and altered its metabolism. Intravenous administration of methotrexate entrapped in niosomes to S-180 tumor bearing mice resulted in total regression of tumor and also higher plasma level and slower elimination.

3) Leishmaniasis

Niosomes can be used for targeting of drug in the treatment of diseases in which the infecting organism resides in the organ of reticulo-endothelial system. Leishmaniasis is such a disease in which parasite invades cells of liver and spleen. The commonly prescribed drugs are antimonials, which are related to arsenic, and at high concentration they damage the heart, liver and kidney.

4) Delivery of peptide drugs

Oral delivery of 9-desglycinamide, 8-arginine vasopressin entrapped in niosomes in an in-vitro intestinal loop model and reported that stability of peptide increased significantly.

5) Immunological application of niosomes

Niosomes have been used for studying the nature of the immune response provoked by antigens. Niosomes as potent adjuvant in terms of immunological selectivity, low toxicity and stability.

6) Niosomes as carriers for Hemoglobin.

Niosomes can be used as a carrier for hemoglobin. Niosomal suspension shows a visible spectrum superimposable onto that of free hemoglobin. Vesicles are permeable to oxygen and hemoglobin dissociation curve can be modified similarly to non-encapsulated hemoglobin.

7) Transdermal delivery of drugs by niosomes

Slow penetration of drug through skin is the major drawback of transdermal route of delivery. An increase in the penetration rate has been achieved by transdermal delivery of drug incorporated in niosomes. Topical delivery of erythromycin from various formulations including niosomes or hairless mouse. From the studies, and confocal microscopy, it was seen that non-ionic vesicles could be formulated to target pilosebaceous glands.

8) Other Applications

a) Sustained Release

The role of liver as a depot for methotrexate after niosomes are taken up by the liver cells. Sustained release action of niosomes can be applied to drugs with low therapeutic index and low water solubility since those could be maintained in the circulation via niosomal encapsulation.

b) Localized Drug Action

Drug delivery through niosomes is one of the approaches to achieve localized drug action, since their size and low penetrability through epithelium and connective tissue keeps the drug localized at the site of administration.

Localized drug action results in enhancement of efficacy of potency of the drug and at the same time reduces its systemic toxic effects e.g. Antimonials encapsulated within niosomes are taken up by mononuclear cells resulting in localization of drug, increase in potency and hence decrease both in dose and toxicity.

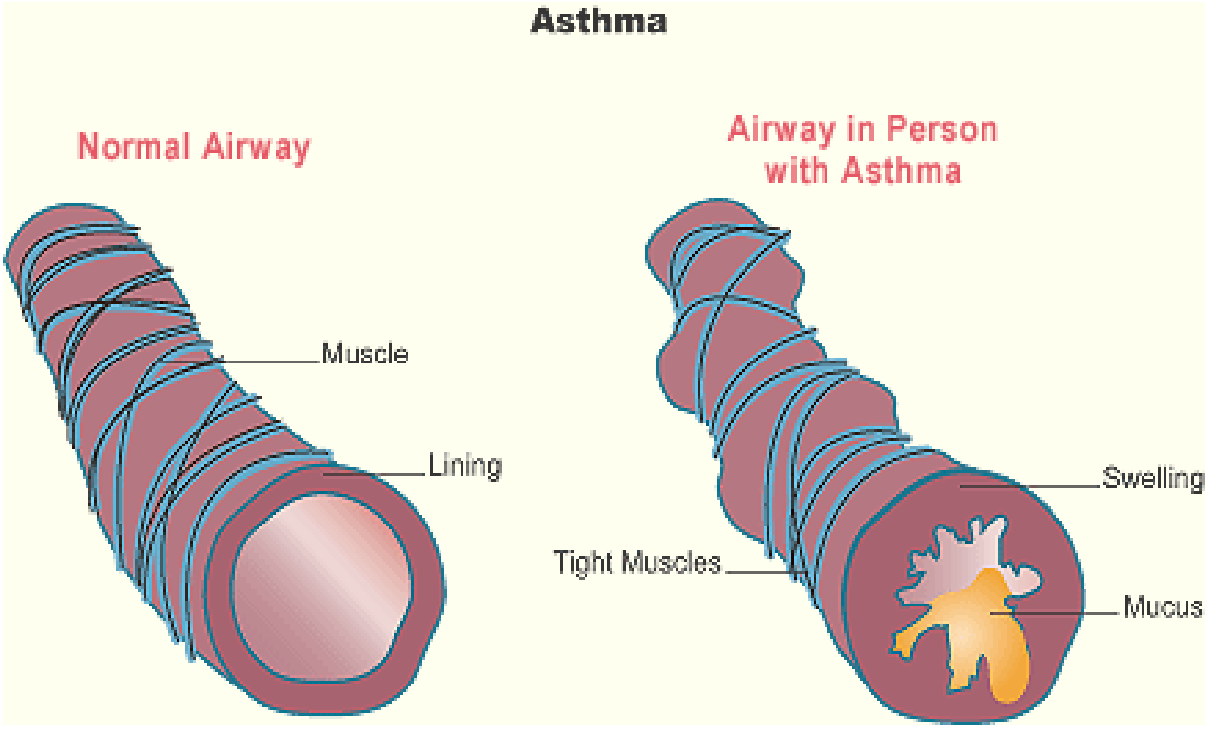
Asthma

Asthma is an inflammatory disorder of the airways, which causes attacks of wheezing, shortness of breath, chest tightness, and coughing.

Asthma is caused by inflammation in the airways. When an asthma attack occurs, the muscles surrounding the airways become tight and the lining of the air passages swell. This reduces the amount of air that can pass by, and can lead to wheezing sounds. Most people with asthma have wheezing attacks separated by symptom-free periods. Some patients have long-term shortness of breath with episodes of increased shortness of breath. Still, in others, a cough may be the main symptom. Asthma attacks can last minutes to days and can become dangerous if the airflow becomes severely restricted.

In sensitive individuals, asthma symptoms can be triggered by breathing in allergy-causing substances (called allergens or triggers). Triggers include pet dander, dust mites, cockroach allergens, molds, or pollens. Asthma symptoms can also be triggered by respiratory infections, exercise, cold air, tobacco smoke and other pollutants, stress, food, or drug allergies. Aspirin and other non-steroidal anti-inflammatory medications (NSAIDS) provoke asthma in some patients.

Asthma is thought to be increasing in prevalence in India and in other developing countries. Current estimates suggest that, although prevalence varies by age group and location, 5% of the Indian population – or approximately 50 million people – have the disease. The situation is similar in other developing countries; overall, the World Health Organization estimates that 300 million people worldwide suffer from asthma.



Exams and Tests

Allergy testing may be helpful in identifying allergens in patients with persistent asthma. Common allergens include pet dander, dust mites, cockroach allergens, molds, and pollens. Common respiratory irritants include tobacco smoke, pollution, and fumes from burning wood or gas.

The doctor will use a stethoscope to listen to the lungs. Asthma-related sounds may be heard. However, lung sounds are usually normal between asthma episodes.

Tests may include:

Lung function tests

- Peak flow measurements
- Chest x-ray
- Blood tests, including eosinophil count (a type of white blood cell)

Symptoms

- Wheezing
    - Usually begins suddenly
    - Comes in episodes
    - May be worse at night or in early morning
    - Gets worse with cold aira, exercise, and heartburn (reflux)
    - May go away on its own
    - Is relieved by bronchodilators (drugs that open the airways)
- Cough with or without sputum (phlegm) production
- Shortness of breath that gets worse with exercise or activity
- Intercostal retractions (pulling of the skin between the ribs when breathing)

Emergency symptoms:

- Extreme difficulty breathing
- Bluish color to the lips and face
- Severe anxiety due to shortness of breath
- Rapid pulse
- Sweating
- Decreased level of alertness, such as severe drowsiness or confusion, during an asthma attack

Additional symptoms that may be associated with this disease:

- Nasal flaring
- Chest pain
- Tightness in the chest

- Abnormal breathing pattern --breathing out takes more than twice as long as breathing in
- Breathing temporarily stops

Treatment

Treatment is aimed at avoiding known allergens and respiratory irritants and controlling symptoms and airway inflammation through medication.

There are two basic kinds of medication for the treatment of asthma:

Long-term control medications are used on a regular basis to prevent attacks, not for treatment during an attack. Types include:

- Inhaled steroids prevent inflammation
- Leukotriene inhibitors
- Anti-IgE therapy, a medicine given by injection to patients with more severe asthma
- Long-acting bronchodilators help open airways
- Cromolyn sodium or nedocromil sodium
- Aminophylline or theophylline (not used as frequently as in the past)

Sometimes a combination of steroids and bronchodilators are used, using either separate inhalers or a single inhaler.

Quick relief, or rescue, medications are used to relieve symptoms during an attack. These include:

- Short-acting bronchodilators (inhalers), such as Proventil, Ventolin, Xopenex, and thers.

- Corticosteroids, such as prednisone or methylprednisolone given by mouth or into a vein

Persons with mild asthma (infrequent attacks) may use quick relief medication as needed. Those with persistent asthma should take control medications on a regular basis to prevent symptoms. A severe asthma attack requires a medical evaluation and may require a hospital stay, oxygen, and intravenous medications.

A peak flow meter, a simple device to measure lung volume, can be used at home to help you "see an attack coming" and take the appropriate action, sometimes even before any symptoms appear. If you are not monitoring asthma on a regular basis, an attack can take you by surprise.

Peak flow measurements can help show when medication is needed, or other action needs to be taken. Peak flow values of 50-80% of an individual's personal best results indicate a moderate asthma attack, while values below 50% indicate a severe attack.

Prevention

Asthma symptoms can be substantially reduced by avoiding known allergens and respiratory irritants. If someone with asthma is sensitive to dust mites, exposure can be reduced by encasing mattresses and pillows in allergen-impermeable covers, removing carpets from bedrooms, and by vacuuming regularly. Exposure to dust mites and mold can be reduced by lowering indoor humidity.

If a person is allergic to an animal that cannot be removed from the home, the animal should be kept out of the patient's bedroom. Filtering material can be placed over the heating outlets to trap animal dander. Exposure to cigarette smoke, air pollution, industrial dusts, and irritating fumes should also be avoided.

CHAPTER - II

REVIEW OF LITERATURE

[12]**Majid Tabbakhian *et al*.,** investigated whether topical application of finasteride-containing vesicles (liposomes and niosomes) could enhance drug concentration at the PSU, as compared to finasteride hydroalcoholic solution (HA). Liposomes consisted of phospholipid (dimyristoyl phosphatidylcholine (DMPC) or egg lecithin): cholesterol: dicetylphosphate (8:2:1, mole ratio). Niosomes were comprising non-ionic surfactant (polyoxyethylene alkyl ethers (Brij® series) or sorbitan monopalmitate):cholesterol: dicetylphosphate (7:3:1, mole ratio). Vesicles were prepared by the film hydration technique and characterized with regard to the size, drug entrapment efficiency and gel–liquid transition temperature (*T*c). *In vitro* permeation of 3H-finasteride through hamster flank skinwas faster from hydroalcoholic solution (0.13 _g/cm2 h) compared to vesicles (0.025–0.058 _g/cm2 h). *In vivo* deposition of 3H-finasteride vesicles in hamster ear showed that liquid-state vesicle, i.e. those made of DMPC or Brij97:Brij76 (1:1), were able to deposit 2.1 or 2.3% of the applied dose to the PSU, respectively. This was significantly higher than drug deposition by gel-state vesicles (0.35–0.51%) or HA (0.76%). Both *in vitro* permeation and *in vivo* deposition studies, demonstrated the potentials of liquid-state liposomes and niosomes for successful delivery of finasteride to the PSU.

[13]**Tianqing Liu, Rong Guo** investigated the microstructure and transformation of the niosome prepared from PEG 6000/Tween 80/Span 80/H2O lamellar liquid crystal (LLC) are studied by the methods of freeze fracture-TEM, negative-staining TEM, dynamic light scattering technique and micropolarity measurements. The size of the niosome made from the LLC is little larger than that from directly mixing samples. The layer membrane of the former niosome is looser than that of the later niosome. The former niosome is held with the partial behaviors of the LLC and can tend to be transformed into the later niosome.

[14]**Abbas Pardakhty *et al*.,** studied niosomes of polyoxyethylene alkyl ethers (BrijTM) `prepared for encapsulation of insulin by film hydration method. Without cholesterol, brij 35 and brij 58 did not form niosomes, apparently because of relatively large polar head groups in comparison with their alkyl chains. The size of vesicles

depended on the cholesterol content, charge incorporation or hydrophilicity of surfactants. Entrapment of insulin in bilayer structure of niosomes protected it against proteolytic activity of α-chymotrypsin, trypsin and pepsin *in vitro*. The maximum protection activity was seen in brij 92/cholesterol (7:3 molar ratios) in which only 26.3 ± 3.98 percent of entrapped insulin was released during 24 h in simulated intestinal fluid (SIF). The kinetic of drug release for most formulations could be best described by Baker and Lonsdale equation indicating diffusion based delivery mechanism. These results indicate that niosomes could be developed as sustained release oral dosage forms for delivery of peptides and proteins such as insulin.

[15]**Tianqing Liu *et al*.,** investigated the structural behaviors of Hemoglobin (Hb) in detail by the methods of UV–vis and fluorescence spectra, circular dichroism, negative staining-TEM, FF-TEM and electrochemistry techniques in PEG 6000/Tween 80/Span 80/H2O niosome system. The obtained results show that Hb can be adsorbed and outspread on the surface of the niosome membrane. The intensities of the UV–vis and fluorescence spectra of Hb in Hb/niosome system are greater than those in Hb/H2O system. The structure behaviors of Hb are partially stabilized and protected in Hb/niosome system. With the increase of PEG 6000 content in the niosome system, the content of helix structure decreases but the contents of β-sheet and random increase for Hb. The content of β -turn structure is almost independent of PEG 6000 content. The negative zeta potential of Hb and the conductivity of the system all decrease. The zeta potential of the niosome firstly decreases and then increases.

[16]**Agnishwar Girigoswami *et al*.,** investigated that Niosomal vesicles are more stable than liposomal vesicles due to higher chemical stability of surfactants compared to phospholipids. Niosomes have been prepared from Span20, Span80, Tween20 and Tween80. Fluorescence resonance energy transfer studies have been performed in these systems to determine donor–acceptor distances. It has been found that the fluorescence resonance energy transfer efficiency is better in niosomes compared to micelles. The formation of niosomes is guided by the hydrophile–lipophile balance value of the nonionic surfactant.

[17]**CP Jain *et al*.,** prepared Niosomes (nonionic surfactant-based vesicles) containing rifampicin using various nonionic surfactants of sorbitan ester class and cholesterol in 50:50 percent mol fraction ratio. The drug-entrapped vesicles were characterized for their shape, size, drug entrapment efficiency and *in vitro* release rate. On the basis of *in vitro* characterization, the niosomes showing maximum entrapment and minimum release rate were selected for in vivo performance evaluation. Cumulative percent doses of rifampicin recovered in thoracic lymph following intravenous and intraperitoneal administrations of free rifampicin solution and niosome-encapsulated rifampicin were compared. The study revealed that effective compartmentalisation of the drug took place in the lymphatic system following intraperitoneal administration of niosome-encapsulated rifampicin. Thus rifampicin encapsulated in niosomes could successfully be used for treatment of tuberculosis along lymphatic system .

[18]**Deepika Aggarwal *et al*.,** formulated Chitosan (REVTMbio1) or Carbopol (REVTMbio2 and 3) coated niosomal timolol maleate (0.25%) formulations by reverse phase evaporation (REV) and compared to timolol solution (TMS; 0.25%) in terms of in vitro release and IOP lowering pharmacodynamic effect. The in vitro release phase of timolol (91% release in 2 h) was extended significantly by its incorporation into niosomes and further by the polymer coating (40–43% release upto 10 h). The developed formulations were evaluated for their pharmacodynamics in albino rabbits, by measuring intraocular pressure (IOP) using a non-contact pneumatonometer, and were compared to a marketed in situ gel forming solution of timolol (Timolet GFS, 0.5%; Sun Pharma). REVTMbio1 formulation showed a more sustained effect of upto 8 h (vis a vis 6 h for carbopol-coated niosomes). TMS in comparison showed effect for only 2 h though the peak effect was slightly more (14%). Lowering of IOP in the contralateral eye (20–40% as compared to 100% in case of TMS), considerably reduces with REV and REVbio formulations indicating lesser systemic side effects. Moreover, the results of REVTMbio1formulation containing 0.25% of timolol maleate compared well with the 0.5% marketed gel formulation, indicating our formulation to be significantly better considering that similar effect is obtained at half the concentration. The later becomes especially important in context to the cardiovascular side effects associated with ocular timolol maleate therapy.

[19]**Rita Muzzalupo *et al*.,** described the formation of two niosomal systems based on synthetic bolaform surfactants (4, 7, 10, 13-pentaoxa-16-aza-cyclooctadecane) - hexadecanedioc acid diamide (BD-16) and α, ω-(4,7,10,13-pentaoxa-16-aza-cyclooctadecane)-hexadecane (BC-16). Systems containing BD-16 or BC-16 and different amount of cholesterol (CH) were prepared by aqueous dispersion of films, followed by examination of methylene blue (MB) entrapment, particle size and morphology. Indeed, we also studied the hydration in the distilled water and physiological solution, in order to investigate the complexing ability on vesicle formation. The results obtained in this study show a high encapsulation capacity and this ability and the size depends on cholesterol content.

[20]**Ahmed S. Guinedi *et al*.,** investigated that Niosomes have been reported as a possible approach to improve the low corneal penetration and bioavailability characteristics shown by conventional ophthalmic vehicles. Niosomes formed from Span 40 or Span 60 and cholesterol in the molar ratios of 7:4, 7:6 and 7:7 were prepared using reverse-phase evaporation and thin film hydration methods. The prepared systems were characterized for entrapment efficiency, size, shape and in vitro drug release. Stability studies were carried out to investigate the leaching of drug from niosomes during storage. The intraocular pressure (IOP) lowering activity of acetazolamide Niosomal formulations in rabbits was measured using ShiØtz tonometer. Histological examination for the corneal tissues of rabbits receiving niosomal formulations was carried out for assessment of the ocular irritancy of niosomes. The results showed that the type of surfactant, cholesterol content and the method of preparation altered the entrapment efficiency and drug release rate from niosomes. Higher entrapment efficiencywas obtained with multilamellar niosomes prepared from Span 60 and cholesterol in a 7:6 molar ratio. Niosomal formulations have shown a fairly high retention of acetazolamide inside the vesicles (approximately 75%) at a refrigerated temperature up to a period of 3 months. Each of the tested acetazolamide niosomes prepared by either method produced a significant decrease in IOP compared to the solution of free drug and plain niosomes. Multilamellar acetazolamide niosomes formulated with Span 60 and cholesterol in a 7:4 molar ratio were found to be the most effective and showed prolonged decrease in IOP. Histological examination of corneal tissues after instillation of niosomal formulation for 40 days showed slight irritation in the substantia propria of the eye which is

reversible and no major changes in tissues were observed.

[21]**M. Manconi *et al*.,** studied the recently reported parametrization based on the differential geometry and the thermodynamics of dispersed systems to ascertain the capability of decylpolyglucoside alone or in association with cholesterol to form vesicle structures (niosomes). The theoretical calculation of the energy balance involved in the vesicles formation was carried out using values of the critical concentration of formation (ccf), surface tension and the molar surface area of the non-ionic surfactant. Furthermore, in order to confirm the theoretical results found in this work, we prepared and characterized vesicles made with decylpolyglucoside and cholesterol. The vesicles were characterized using transmission electron microscopy and dynamic light scattering.

[22]**Behrooz Nasseri** investigated the mechanical characteristics of non-ionic bilayer membranes composed of sorbitan monostearate, cholesterol and poly-24- oxyethylene cholesteryl were studied by measuring the modulus of surface elasticity (μ), a measure of membrane strength, as a function of cholesterol content and temperature. The modulus of surface elasticity increased slowly with increasing cholesterol concentration, with a sharp increase around 40 mol% cholesterol (on average an increment of 0.43×106 Nm^{-2} per molar percentage), and displayed a maximum of 6.5×106 Nm−2 around 47.5 mol% cholesterol. Further cholesterol resulted in a decrease in μ. Generally the interaction of cholesterol with the sorbitan monostearate should increase the rigidity of the membrane. However, the latter effect may be due to the formation of cholesterol clusters at high cholesterol content where excess amounts of cholesterol cannot interact with the sorbitan monostearate, and deposits on the bilayers compromising their uniformity, strength and permeability. This behaviour was evident when measurements were carried out above and below $25^{o}C$.

[23]**Suna Erdogan *et al*.,** studied about some accepted imaging techniques in clinic but most of them have several disadvantages limiting their effective use. Because of this, researchers are still performed to develop a rapid, specific means of detecting and/or imaging venous thrombi-based on the changing composition of the thrombus. Urokinase, fibrinolytic enzyme isolated form human urine, is a direct activator of plasminogen. In thrombus formation, plasminogen seems to be trapped in or absorbed

onto fibrin matrix thus leading to a localised concentration of plasminogen. This suggests that radiolabelled urokinase would be a suitable compound for the detection of thrombi. The most important disadvantage of this enzyme is short plasma half life. To overcome this problem, it was decided to encapsulate the enzyme in drug delivery systems such as liposomes, niosomes or sphingosomes. In this study, we prepared, characterized and monitored the biodistribution of three types of vesicular systems containing urokinase. All types of prepared vesicles show in vitro an acceptable encapsulation, stability and release profile. Thrombus uptake was increased by encapsulation of urokinase into vesicles.

[24]**Prem N. Gupta *et al*.,** investigated the Non-invasive vaccine delivery which is a top priority for public health agencies because conventional immunization practices are unsafe and associated with numerous limitations. Recently, the skin has emerged as a potential alternative route for non-invasive delivery of vaccine. Topical immunization (TI), introduction of antigen through topical application onto the intact skin, has many practical merits compared to injectable routes of administration. One of the possibilities for increasing the penetration of bioactives through the skin is the use of vesicular systems. Specially designed lipid vesicles are attracting intense attention and can be used for non-invasive antigen delivery. In the present study, elastic vesicle transfersomes, non-ionic surfactant vesicles (niosomes) and liposomes were used to study their relative potential in non-invasive delivery of tetanus toxoid (TT). Transfersomes, niosomes and liposomes were prepared and characterized for shape, size and entrapment efficiency. These vesicles were extruded through polycarbonate filter (50-nm pore size) to assess the elasticity of the vesicles. The immune stimulating activity of transfersomes, niosomes and liposomes were studied by measuring the serum anti-TT IgG titre following topical immunization. The immune response elicited by topical immunization was compared with that elicited by same dose of alum-adsorbed tetanus toxoid (AATT) given intramuscularly. The results indicate that optimal formulations of transfersomes, niosomes and liposomes could entrap 72.7±3.4, 42.5±2.4 and 41.3±2.2% of antigen and their elasticity values were 124.4±4.2, 29.3±2.4 and 21.7±1.9, respectively. In vivo study revealed that topically given TT containing transfersomes, after secondary immunization, could elicit immune response (anti-TT-IgG) that was equivalent to one that produced following intramuscularly alum-adsorbed TT-based immunization. In comparison to

transfersomes, niosomes and liposomes elicited weaker immune response. Thus transfersomes hold promise for effective non-invasive topical delivery of antigen(s).

[25]**Ibrahim A. Alsarra *et al*.,** investigated the Permeation of a potent nonsteroidal anti-inflammatory, ketorolac, across excised rabbit skin from various proniosome gel formulations was investigated using Franz diffusion cells. Each of the prepared proniosomes significantly improved drug permeation and reduced the lag time ($P < .05$). Proniosomes prepared with Span 60 provided a higher ketorolac flux across the skin than did those prepared with Tween 20 (7- and 4-fold the control, respectively). A change in the cholesterol content did not affect the efficiency of the proniosomes, and the reduction in the lecithin content did not significantly decrease the flux ($P <0.05$). The encapsulation efficiency and size of niosomal vesicles formed by proniosome hydration were also characterized by specific high performance liquid chromatography method and scanning electron microscopy. Each of the prepared niosomes achieved about 99% drug encapsulation. Vesicle size was markedly dependent on the composition of the proniosomal formulations. Proniosomes may be a promising carrier for ketorolac and other drugs, especially due to their simple production and facile up.

[26]**Christine Dufesa *et al*.,** evaluated the glucose-bearing niosomes as a brain targeted delivery system for the vasoactive intestinal peptide (VIP). To this end, VIP/^{125}I-VIP-loaded glucose-bearing niosomes were intravenously injected to mice. Brain uptake was determined by measuring the radioactivity of ^{125}I-labeled VIP using γ-counting, after intravenous administration of VIP in solution or encapsulated in glucose-bearing niosomes or in control niosomes. VIP integrity was assessed by reversed-phase HPLC analysis of brain extracts. Distribution of ^{125}I-VIP derived radioactivity was examined from serial brain slices. HPLC analysis confirmed the presence of intact VIP in brain after administration of VIP-loaded niosomes, but not after administration of VIP solution. Encapsulation within glucose-bearing niosomes mainly allowed a significantly higher VIP brain uptake compared to control niosomes (up to 86%, 5 min after treatment). Brain distribution of intact VIP after injection of glucose-bearing niosomes, indicated that radioactivity was preferentially located in the posterior and the anterior parts of the brain, whereas it was homogeneously distributed in the whole brain after the administration of control vesicles.

[27]**D. Gopinath *et al*.,** investigated Ascorbyl palmitate (ASP) as bilayer vesicle forming material. It formed vesicles (Aspasomes) in combination with cholesterol and a negatively charged lipid (dicetyl phosphate). Aspasomes were prepared by film hydration method followed by sonication in which aqueous azidothymidine (AZT) solution was encapsulated in aqueous regions of bilayer. Aspasomes were obtained with all compositions containing 18–72 mol% cholesterol. Differential scanning calorimetric data of aspasome dispersion and anhydrous mixtures of ascorbyl palmitate, cholesterol and dicetyl phosphate confirm the formation of bilayered vesicles with ascorbyl palmitate. Cholesterol content in aspasome did not exhibit any relation with vesicle size, zeta potential or percent entrapment.A substantial change in release rate of azidothymidine from aspasome was noticed on varying the proportion of cholesterol. Release rate and cholesterol content in Aspasomes did not exhibit any relation. A preparation with 45 mol% of cholesterol showed maximum retardation in release rate, than other compositions. The change in capture volume with time (latency) was studied for 8 h and with such a short duration study it was difficult to predict long term stability of these vesicles. But release experiments do indicate stability upto 18 h. Percent reducing activity of aspasome was estimated by measuring the absorbance of α, α diphenyl-β-picrylhydrazyl (DPPH) at 517 nm after addition of test antioxidant samples. These studies revealed that the antioxidant potency of ascorbyl moiety is retained even after converting ascorbyl palmitate into vesicles (Aspasomes). The antioxidant potency of Aspasomes was assessed by measuring the protection offered by this preparation against quinolinic acid induced lipoperoxidation of whole human blood in vitro, where in the lipoperoxidation was monitored by measuring thiobarbituric acid reactive substances (TBARS) levels. Aspasome rendered much better antioxidant activity than ascorbic acid. Transdermal permeation of aspasomal AZT, ASP-AZT aqueous dispersion and AZT-solution across excised rat skin was investigated in vitro using Franz diffusion cell. Permeation of aspasomal AZT was much higher than the other two preparations.

[28]**Y. Perrie*a* *et al*.,** compared to naked DNA immunisation, entrapment of plasmid-based DNA vaccines into liposomes by the dehydration–rehydration method has shown to enhance both humoural and cell-mediated immune responses to encoded antigens administered by a variety of routes. In this paper we have compared the

potency of lipid-based and non-ionic surfactant based vesicle carrier systems for DNA vaccines after subcutaneous immunisation. Plasmid pI.18Sfi/NP containing the nucleoprotein (NP) gene of A/Sichuan/2/87 (H3N2) influenza virus in the pI.18 expression vector was incorporated by the dehydration–rehydration method into various vesicle formulations. The DRV method, entailing mixing of small unilamellar vesicles (SUV) with DNA, followed by dehydration and rehydration, yielded high DNA vaccine incorporation values (85–97% of the DNA used) in all formulations. Studies on vesicle size revealed lipid-based systems formed cationic submicron size vesicles whilst constructs containing a non-ionic surfactant had significantly large z-average diameters (>1500 nm). Subcutaneous vesicle-mediated DNA immunisation employing two DRV(DNA) formulations as well as naked DNA revealed that humoural responses (immunoglobulin total IgG, and subclasses IgG1 and 1gG2a) engendered by the plasmid encoded nucleoprotein were substantially higher after dosing twice, 28 days apart with 10_g DRV-entrapped DNA compared to naked DNA. Comparison between the lipid and non-ionic based vesicle formulations revealed no significant difference in stimulated antibody production. These results suggest that, not only can DNA be effectively entrapped within a range of lipid and non-ionic based vesicle formulations using the DRV method but that such DRV vesicles containing DNA may be a useful system for subcutaneous delivery of DNA vaccines.

[29]**Maria Manconi *et al.*,** compared the chemical stability of tretinoin (TRA) in methanol and in vesicular suspensions exposed both to UV and artificial daylight conditions with the aim of evaluating the potential of niosomes as topical carriers capable of improving the stability of photosensitive drugs. Tretinoin-loaded niosomes were prepared from polyoxyethylene (4) lauryl ether (Brij® 30), sorbitan esters (Span® 40 and Span® 60) and a commercial mixture of octyl/decyl polyglucosides (Triton® CG110). Liposomes made from hydrogenated (P90H) and non-hydrogenated (P90) soy phosphatidylcholines were also prepared and studied. In order to evaluate the influence of vesicle structure on the photostability of tretinoin, TRA-loaded vesicles were prepared by the film hydration method, extrusion technique and sonication. After UV irradiation, TRA dissolved in methanol degraded very quickly while the incorporation in vesicles always led to a reduction of the photodegradation process. The photoprotection offered by vesicles varied depending on the vesicle

structure and composition. After fluorescent light irradiation for 21 days, not all the studied vesicular formulations improved TRA stability when compared with the free drug in methanol. Tretinoin incorporated in P90 or Span vesicles presented a half-life shorter or very close to that of the free drug. However, the inclusion of TRA in P90H liposomes and Brij® 30 or Triton® CG110 niosomes retarded the drug photodegradation.

[30]**Aranya Manosroi *et al*.,** prepared vesicles (niosomes) with hydrated mixture of various non-ionic surfactants and cholesterol was studied. The bilayer formation was characterized by X-cross formation under light polarization microscope and the ability of the vesicles to entrap water-soluble substance. Membrane rigidity was measured by means of mobility of fluorescence probe as a function of temperatures. The entrapment efficiencies of the vesicles and microviscosities of the vesicular membrane depended on alkyl chain length of non-ionic surfactants and amount of cholesterol used to prepare vesicles. The stearyl chain (C18) non-ionic surfactant vesicles showed higher entrapment efficiency than the lauryl chain (C12) non-ionic surfactant vesicles. Cholesterol was used to complete the hydrophobic moiety of single alkyl chain nonionic surfactants for vesicle formation. Niosome prepared with Tween 61 bearing a long alkyl chain and a large hydrophilic moiety in the combination with cholesterol at 1:1 molar ratio was found to have the highest entrapment efficiency of water soluble substances.

[31]**G. Redziniak** investigated various approaches to intra- and percutaneous administration of drugs, e.g. application of patches, ointments, iontophoresis, electroporation, the use of lipid vesicles like liposomes and niosomes presents numerous advantages. They are not toxic or invasive, may deliver hydrophobic and/or hydrophilic molecules, and the size of the transported molecule is not a limiting factor. Liposomes are obtained with natural amphiphilic lipids whereas niosomes are composed of synthetic amphiphilic molecules. These microscopic vesicles contain from one to several concentric lipid bi-layers with intercalated aqueous compartments. Trans-epidermal penetration of the vesicles is proportional to the “fluidity” of their lipids and their negative charge. Several drugs and cosmetics in this gallenic form are already commercially available and successfully used, presenting a better dose/effect ratio and provoking less side- effects.

[32]**S Bhaskaran *et al*.,** formulated Niosomes containing salbutamol sulphate using different non-ionic surfactants like Tween 20, 40, 60, 80, Span 20, 40, 60, 80 and Brij 35 by transmembrane pH gradient method. The drug encapsulation efficiency varied from 28 percent to 79 percent. The vesicles have been characterized by infrared spectroscopy. In vitro drug release studies were carried out using dialysis bag and phosphate buffer, pH 7.4 as a dissolution medium for 24 h. The formulation exhibited retarded release for 24 h and Span 60 was found to be the most satisfactory surfactant which released 78.4 percent of drug in 24 h. Particle size distribution studies were carried out be optical microscopy technique. Most of the niosomes were found to be spherical in shape. Thermal stability studies were carried out at 4 degree, 25 degree and 50 degree for one month. The product was lyophilized. Tissue distribution studies were carried out on rabbits. The maximum concentration was seen in lungs.

[33]**Yongmei Hao *et al*.,** prepared niosomes which have high encapsulation capacity for soluble drugs, starting from Span 60 and cholesterol, an improved method, evaporation-sonication method, was proposed. The corresponding niosomes show a good stability at least 40 days. Colchicine was chosen as a model drug for examining the capsulation capacity of these niosomes. To obtain the highest encapsulation efficiency, several factors including the structure of surfactant, level of lipid, content of drug and cholesterol were investigated and optimized. The inner cause was also discussed. The results indicate that the Span 60 is the most ideal surfactant among four kinds of Span. Furthermore, the release studies of colchicine and 5-fluorouracil (5-FU) in vitro from niosomes exhibited a prolonged release profile as studied over a period of 24 h. The results demonstrated that niosomes prepared in this way not only have high encapsulation capacity but also is expected that side effects of drugs may be reduced. It still suggests that this method may be used extensively in the field of encapsulation soluble drugs.

[34]**Tejas R. Desai *et al*.,** demonstrated the potential of encapsulating all-trans-retinoic acid (ATRA) in niosomes and delivering it as an inhaled aerosol. Niosomes may provide a means to reduce the toxicity of ATRA and alter the pharmacokinetics in a manner similar to liposomes. In addition, the low cost of the surfactants used for preparing niosomes and their greater stability compared with liposomes makes them an attractive alternative. Various nonionic surfactants were used to achieve optimum

encapsulation and nebulization efficiencies, and the best formulations were obtained with combinations of (Span 20+Tween 80) and (Span 60+Tween 80) using an ATRA concentration of 1mg/ml. The aerosol produced with the selected niosomal formulations upon nebulization in PARI LC STAR nebulizers driven by a Pulmo-Aide compressor was subsequently analyzed for the determination of size distribution and entrapment efficiencies on each stage of an Anderson cascade impactor operated in a manner that avoids spurious sizing due to droplet evaporation. Mass median aerodynamic diameters (MMADs) of 3.7±0.3 and 3.58±0.03 μm, geometric standard deviation (GSD) values of 1.59±0.17 and 1.51±0.01 and entrapment efficiencies well above 50% were obtained for the optimized formulations. The results are very encouraging and offer an alternative approach to the respiratory delivery of ATRA by aerosolization.

[35]**Maria Manconi *et al*.,** prepared Tretinoin-loaded niosomes from polyoxyethylene (**4**) lauryl ether, sorbitan esters and a commercial mixture of octyl/decyl polyglucosides, in the presence of cholesterol and dicetyl phosphate. Liposomes made of hydrogenated and non-hydrogenated phosphatidylcholine were also prepared as a comparison reference. A study was made of the influence of vesicle composition and preparation method on the vesicle structure (MLV, LUV, SUV), size distribution, entrapment efficiency and in vitro release of incorporated tretinoin. Results showed that in the presence of cholesterol all the amphiphiles used were able to form stable vesicle dispersions with or without tretinoin. Vesicle sizes were dependent on the preparation method, bilayer composition and drug load. Multilamellar (MLV) vesicles were larger than extruded (LUV) and sonicated (SUV) vesicles while drug-loaded vesicles were generally smaller than empty ones. Entrapment efficiencies of tretinoin were always very high especially for multilamellar (91–99%) and extruded (88–98%) vesicles. The in vitro release of tretinoin from the prepared vesicular formulations was studied using the vertical Franz diffusion cells. The rate of drug release through a Silastic membrane from a liposomal and niosomal tretinoin dispersion was generally faster than from a tretinoin solution. Release data showed that tretinoin delivery is mainly affected by the vesicular structure and that tretinoin delivery increased from MLVs to LUVs to SUVs.

[36]**Jia-You Fang *et al*.,** elucidated the skin permeation and partitioning of a fluorinated quinolone antibacterial agent, enoxacin, in liposomes and niosomes, after topical application. In vitro percutaneous absorption experiments were performed on nude mouse skin with Franz diffusion cells. The influence of vesicles on the physicochemical property and stability of the formulations were measured. The enhanced delivery across the skin of liposome and niosome encapsulated enoxacin had been observed after selecting the appropriate formulations. The optimized formulations could also reserve a large amount of enoxacin in the skin. A significant relationship between skin permeation and the cumulative amount of enoxacin in the skin was observed. Both permeation enhancer effect and direct vesicle fusion with stratum corneum may contribute to the permeation of enoxacin across skin. Formulation with niosomes demonstrated a higher stability after 48 h incubation compared to liposomes. The inclusion of cholesterol improved the stability of enoxacin liposomes according to the results from encapsulation and turbidity. However, adding negative charges reduced the stability of niosomes. The ability of liposomes and niosomes to modulate drug delivery without significant toxicity makes the two vesicles useful to formulate topical enoxacin.

[37]**R. Agarwal *et al*.,** studied dithranol in the topical treatment of psoriasis. However, the use of dithranol in psoriatic condition is inconvenient and troublesome, as it has irritating, burning, staining and necrotizing effect on the normal as well as the diseased skin. The entrapment of drug in vesicles is viewed to help in the localized delivery of the drug and an improved availability of the drug at the site will reduce the dose and in turn, the dose-dependent side effects like irritation and staining. The investigations deal with critical parameters controlling the formulation and stabilization of dithranol loaded liposomes and niosomes. The entrapment efficiency of dithranol in liposomes was optimized by altering the proportion of phosphatidyl choline and cholesterol, and in case of niosomes it was between Span 60 and cholesterol. Hydration and permeation mediums were also established keeping in view the poor solubility and stability of dithranol. The mean liposome and niosomes sizes were 4±1.25 and 5±1.5 μm, respectively. The drug-leakage study carried out at different temperatures of 4–8, 25±2 and 37 °C for a period of two months affirms that the drug leakage increased at a higher temperature. The in vitro permeation study using mouse abdominal skin shows significantly enhanced permeation with vesicles

as indicated by flux of dithranol from liposomes (23.13µg/cm^2/h) and niosomes (7.78µg/cm^2/h) as compared with the cream base (4.10µg/cm^2/h).

[38]**Gopi N. Devaraj *et al*.,** studied the monomers of some amphiphiles organize into bilayers to form liposomes and niosomes. Such bilayers are unstable or leaky and hence cholesterol is a common ingredient included to stabilize them. Cholesterol stabilizes bilayers, prevents leakiness, and retards permeation of solutes enclosed in the aqueous core of these vesicles. Other than cholesterol a material with good bilayer-stabilizing properties is yet to be identified. We have substituted cholesterol with fatty alcohols in niosomes containing polyglyceryl-3-diisostearate (PGDS) and polysorbate-80 (PS-80) to explore their membrane-stabilizing property via permeation studies. Niosomes of polyglyceryl-3-di-isostearate, fatty alcohol/cholesterol, and Polysorbate were prepared by ether injection method. Aqueous solution of ketorolac tromethamine (KT) was entrapped in them. The effects of alkyl chain length of fatty alcohols (C12, C14, C16, C18, and C16+18), of acyl chain length of polyoxyethylene sorbitan monoester surfactants, and of the molar ratio of lipid mixture on the release rate of ketorolac from niosomes were assessed by employing modified dissolution–dialysis method. Niosomes with cholesterol or fatty alcohols have exhibited a common release pattern. Niosomes containing fatty alcohol showed a considerably slower release rate of KT than those containing cholesterol. Based on the release rate, fatty alcohols can be ranked as stearyl<myristyl<cetyl<lauryl<cetostearyl. In niosomes containing PGDS, myristyl alcohol (MA), and polysorbate, the fatty acid chain length of polyoxyethylene sorbitan ester-type surfactants has influenced the release rate and encapsulation efficiency. Based on the release rate, polysorbates can be ranked as polysorbate-20 (C12)<polysorbate-60 (C18)<polysorbate-80 (C9=9)<polysorbate-40 (C16). In niosome preparation containing polysorbate-20 and dioctyl sodium sulfosuccinate (anionic surfactant), the release rate was slower than niosomes containing polysorbate-20. When MA concentration is kept constant at 50 mole% and the ratio of PGDS and PS-80 was altered, significant changes in entrapment efficiency and the release rate were observed. However, this ratio did not exhibit any relation with encapsulation efficiency or release rate. The release rate and entrapment exhibited an inverse correlation (r 2 =0.8774 at p <0.02 for the data of molar ratios of PGDS:MA: PS80; r 2 =0.975 at p <0.001 for the data of acyl chain length variation of polysorbates). It can be concluded that stable niosomes of

polyglyceryl-3-di-isostearate could be prepared with fatty alcohols and polysorbates instead of cholesterol and that the release of solutes from these niosomes can be optimized by altering membrane constituents and their concentrations.

[39]**P.Arunothayanun *et al*.,** investigated the effects of processing variables, particularly temperature and sonication, on the physical characteristics and phase transitional behaviour of two niosomal systems based on a hexadecyl diglycerol ether (C16G2) have been studied. Systems containing C16G2, cholesterol and poly-24-oxyethylene cholesteryl ether (Solulan C24) in the molar ratios 91:0:9 and 49:49:2 were prepared by aqueous dispersion of films, followed by examination of 5(6)-carboxyfluorescein entrapment, particle size and morphology. The thermal behaviour was examined using high sensitivity differential scanning calorimetry (HSDSC) and hot stage microscopy, while the effects of sonication were studied in terms of size and morphology, both immediately after preparation and on storing for 1 h at room temperature and 60°C. Polyhedral niosomes were formed from systems containing C16G2 and Solulan C24 alone, while cholesterol-containing systems formed spherical vesicles mixed with tubular structures; the polyhedral systems were found to have a larger particle size and higher CF entrapment efficiency. HSDSC studies showed the polyhedral systems to exhibit an endotherm at 45.4°C and a corresponding exotherm at 39.1°C on cooling which were ascribed to a membrane phase transition; no equivalent transition was observed for the cholesterol containing systems. Hot stage microscopy showed the polyhedral vesicles to convert to spherical structures at 48°C, while on cooling the spherical vesicles split into smaller structures and reverted to the polyhedral shape at 49°C. Sonication resulted in the polyhedral vesicles forming spherical structures which underwent a particle size increase on storage at room temperature but not at 60°C. The study suggests that the polyhedral vesicles undergo a reversible transition to spherical vesicles on heating or sonication and that this morphological change may be associated with a membrane phase transition.

[40]**Calum J. Drummond *et al*.,** reviewed the new impetus in surfactant self-assembly objects as agents for drug delivery that are an alternative to micellar, lamellar/liposome, niosome and transfersome or microemulsion-based vehicles. The review focus herein is on the application of hexagonal, cubic, ‘intermediate’ (viz. rhombohedral, tetragonal and monoclinic) and L_3 (‘sponge’).mesophases.

[41]**P. Arunothayanun *et al*.,** formulated formed Non-ionic surfactant vesicles (niosomes) using hexadecyl diglycerol ether (C16G2) and a series of polyoxyethylene alkyl ethers exhibit a variety of shapes dependant on their membrane composition. These surfactants form with an equimolar amount of cholesterol a mixture of largely spherical and tubular niosomes. In the absence of cholesterol, they form faceted polyhedral structures. The physicochemical and biological differences between polyhedral and spherical/tubular niosomes were studied. Polyhedral niosomes undergo a reversible shape transformation into spherical structures on heating above their phase transition temperature (Tm). The viscosity of polyhedral niosomes at room temperature is higher than their spherical counterparts due to their faceted and relatively rigid shape, and is more dependant on temperature due to shape transformation. At room temperature, polyhedral niosomes possess more rigid gel phase membranes and are less osmotically sensitive; however, they are more permeable because of a lack of or low levels of cholesterol in their membranes. Polyhedral niosomes loaded with luteinising hormone releasing hormone (LHRH), nonetheless, slow the release of drug compared to solution, albeit to a small extent.

[42]**S.P. Vyas *et al*.,** formed Nonionic surfactant vesicles using Span 60, cholesterol and dicetyl phosphate. The prepared multilamellar vesicles (MLVs) were coated by interfacial polymerization technique using *p*-phthaloyl dichloride and L-lysine. The formation of the polymeric coat was confirmed by optical microscopic and transmission electron microscopic studies. The prepared, plain and polymer-coated MLVs were studied for their size, shape, encapsulation efficiency, in vitro release profile and effect of osmotic shock on vesicle. The results observed showed that the polymer-coated MLVs were stable under various osmotic conditions. In vivo studies were carried out on albino rats. The half-life and area under curve were found to be high in the case of polymer-coated MLVs as compared to plain MLVs and plain drug solution. In vivo studies using inflamed rat model also indicated that the polymer-coated MLVs were more stable and could release the drug in a controlled fashion as compared to plain MLVs.

[43]**Gopal K. Pillai *et al*.,** prepared indomethacin incorporated in a non-ionic surfactant vesicle (niosome). The objective was to study the effect of niosomal-encapsulated indomethacin on platelet function such as inhibition of aggregation and ATP release

induced by a variety of agonists (adenosine 5%-diphosphate (ADP), epinephrine, arachidonic acid, ristocetine) and to explore the feasibility of carrier-mediated drug delivery to the platelets. Multilamellar vesicles (niosomes) were prepared from Tween-60 by the lipid hydration method. Freshly prepared human platelet rich plasma (PRP) was used for aggregation:inhibition studies, the extent of which was observed as a change in light transmission measured by the Chronolog Aggregometer. The percent inhibition of aggregation induced by the agonist ADP ranged from 28.21±0.28 at the 2.0 μmol level to 92.6±1.20 at 12.7 μmol of the encapsulated drug while the same concentrations of the drug inhibited aggregation only to the extent of 13.75±0.13 and 36.82±0.57%, respectively. A 100% inhibition of aggregation induced by arachidonic acid was achieved by niosomal indomethacin while inhibition by the free drug was 41.9% at equimolar concentrations. ATP release study showed that 100% inhibition was achieved by 8 μmol of the encapsulated drug while inhibition by the free drug was 40.00±1.82%. Therefore, at equimolar doses, the niosomal drug proved to be more efficient in inhibiting platelet aggregation than the free drug, probably due to greater quantity of the drug reaching the specific site of inhibition in the interior of the platelets and acting directly on the cyclo-oxygenase system to prevent thromboxane formation.

[44]**S.P. Vyas and N. Venkatesan** formulated Nonionic surfactant vesicles using Span 60, cholesterol and dicetyl phosphate. The prepared multilamellar vesicles (MLVs) were coated by interfacial polymerization technique using *p*-phthaloyl dichloride and L-lysine. The formation of the polymeric coat was confirmed by optical microscopic and transmission electron microscopic studies. The prepared, plain and polymer-coated MLVs were studied for their size, shape, encapsulation efficiency, in vitro release profile and effect of osmotic shock on vesicle. The results observed showed that the polymer-coated MLVs were stable under various osmotic conditions. In vivo studies were carried out on albino rats. The half-life and area under curve were found to be high in the case of polymer-coated MLVs as compared to plain MLVs and plain drug solution. In vivo studies using inflammed rat model also indicated that the polymer-coated MLVs were more stable and could release the drug in a controlled fashion as compared to plain MLVs.

[45]**C.O. Rentel *et al*.,** developed a peroral vaccine delivery system based on non-ionic surfactant vesicles (niosomes) was evaluated using BALB/c mice. Ovalbumin was encapsulated in various lyophilized niosome preparations consisting of sucrose esters, cholesterol and dicetyl phosphate. Two different formulations were compared in this study. The specific antibody titres within serum, saliva and intestinal washings were monitored by ELISA on days 7, 14, 21 and 28 after intragastric administration. Only encapsulation of ovalbumin into Wasag®7 (70% stearate sucrose ester, 30% palmitate sucrose ester (40% mono-, 60% di:tri-ester)) niosomes resulted in a significant increase in antibody titres. Administration of ovalbumin and empty niosomes did not exert a similar effect; neither did administration of any control formulation. In contrast to ovalbumin loaded Wasag®7 niosomes, application of the more hydrophilic Wasag®15 (30% stearate sucrose ester, 70% palmitate sucrose ester (70% mono-, 30% di/tri-ester)) niosome preparations did not result in an increase in antibody titres.

[46]**Sudaxshina Murdan *et al*.,** investigated multi-component organogels formed using the non-ionic surfactant sorbitan monostearate as gelator have been formulated to contain niosomes. The purpose of this study was to evaluate the potential of these vesicle-in-water-in-oil (v/w/o) gels as delivery vehicles for vaccines. Bovine serum albumin (BSA) and haemagglutinin (HA) were used as model antigens in depot and immunogenicity studies respectively. The complex gels were prepared by the addition of a hot (60^{o}C) aqueous niosome suspension (v/w) to the sol phase (*o*, an organic solution of the gelator); a vesicle-in-water-in-oil (v/w/o) emulsion was produced which cools to an opaque, semi-solid, thermoreversible v/w/o gel. Light microscopy of the organogel revealed that the microstructure consists of a tubular network of surfactant aggregates in the organic medium, the niosome suspension being dispersed in these surfactant tubules. Therefore, in such gels, the vaccine is thought to be entrapped in the niosomes, themselves located within the sorbitan monostearate tubular network in the organic medium. In vivo, a depot effect was observed following intramuscular administration of the gel containing the entrapped bovine serum albumin, cleared from the injection site over a period of days. The relatively short-lived nature of the depot was thought to arise due to interactions between the gel and the local interstitial fluid which results in gel disintegration in situ. Thus, the niosomes containing antigens are believed to be released from the organic gel. Immunogenicity studies showed that the v/w/o gel as well as one of the controls, the

water-in-oil (w/o) gel, possess immunoadjuvant properties and enhance the primary and secondary antibody titres (of total IgG, IgG_1 , IgG_{2a} and IgG_{2b}) to haemagglutinin antigen. As far as humoral immunity is concerned, the w/o gel showed stronger immunoadjuvant properties compared to the v/w/o gel, being effective at a lower antigen dose i.e 0.1µg HA.

[47]**Ijeoma F. Uchegbu *et al*.,** reviewed the self assembly of non-ionic surfactants into vesicles was first reported in the seventies by researchers in the cosmetic industry. Since then a number of groups world wide have studied non-ionic surfactant vesicles (niosomes) with a view to evaluating their potential as drug carriers. This article presents a summary of the achievements in the field to date. Niosomes may be formed form a diverse array of amphiphiles bearing sugar, polyoxyethylene, polyglycerol, crown ether and amino acid hydrophilic head groups and these amphiphiles typically possess one to two hydrophobic alkyl, perfluoroalkyl or steroidal groups. The self assembly of surfactants into niosomes is governed not only by the nature of the surfactant but by the presence of membrane additives, the nature of the drug encapsulated and the actual method of preparation. Methods of niosome preparation and the number of different morphologies that have been identified are detailed. The influence of formulation factors on niosome stability is also examined as are methods to optimise drug loading. *In vivo* these systems have been evaluated as immunological adjuvants, anti-cancer, anti-infective drug targeting agents and carriers of anti-inflammatory drugs. Niosomes have also been used in diagnostic imaging. Efforts to achieve transdermal and ophthalmic drug delivery with some formulations are also discussed.

[48]**Bhavana Vora *et al*.,** formulated proniosome based transdermal drug delivery system of levonorgestrel (LN) was developed and extensively characterized both in vitro and in vivo. The proniosomal structure was liquid crystalline-compact niosomes hybrid which could be converted into niosomes upon hydration. The system was evaluated in vitro for drug loading, rate of hydration (spontaneity), vesicle size, polydispersity, entrapment efficiency and drug diffusion across rat skin. The effect of composition of formulation, amount of drug, type of Spans, alcohols and sonication time on transdermal permeation profile was observed. The stability studies were performed at 48C and at room temperature. The biological assay for progestational

activity included endometrial assay and inhibition with the formation of corpora lutea. The study demonstrated the utility of proniosomal transdermal patch bearing levonorgestrel for effective contraception.

[49]**Ijeoma F. Uchegbu *et al*.,** investigated that PK1 is an *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-doxorubicin conjugate currently in early clinical development. Niosome encapsulation is a means to increase PK1 blood residence time, potentially promote tumour uptake and produce a slow, sustained release of active drug. Factors effecting encapsulation efficiency and size of PK1-niosome formulations were studied. Five surfactants were used to prepare PK1-niosomes; hexadecyl poly-5-oxyethylene ether ($C_{16}EO_5$); octadecyl poly-5-oxyethylene ether ($C_{18}EO_5$); hexadecyl diglycerol ether ($C_{16}G_2$); sorbitan monopalmitate (Span 40) and sorbitan monostearate (Span 60). All were mixed in equimolar ratio with cholesterol and varying amounts of Solulan C24 (a cholesteryl poly-24-oxyethylene ether) (9–39 mol%). Dicetylphosphate (DCP) was also added (2 mol%). Passive association of PK1 with preformed C16G2 and Span 60 vesicles was low (3–4%) while subsequent dehydration (freeze drying) followed by rehydration of the formulation increased the entrapment to 61% in the $C_{16}G_2$ formulation. Transmission electron microscopy revealed that these niosomes had an electron dense core, evidence of intravesicular concentration of PK1. Increasing Solulan C24 content resulted in decreased PK1 entrapment after freeze drying, and the vesicle size was also decreased. Solulan C24 (39 mol%) caused pronounced vesicle aggregation on freeze drying, whereas at lower levels (9 mol%), PK1 appeared to act as a cryoptrotectant and the mean size of C16G2 niosomes was 235 nm. A PK1:surfactant:lipid ratio of 0.3 (11.2 mg ml $^{-1}$ doxorubicin) was achieved with Span 60 niosomes. This formulation, and the C16G2 niosomes, did not induce red blood cell lysis at the proposed dose for in vivo use. Preliminary in vivo biodistribution studies showed PK1-$C_{16}G_2$ niosomes to be mainly taken up by the liver and spleen. After 24 h, 25 and 3% of dose administered was present as free doxorubicin in these organs respectively.

[50]Jain CP *et al*., investigated Niosomes (non-ionic, surfactant-based vesicles) containing rifampicin of 8-15 microns in diameter were prepared using Span-85 and cholesterol in various molar fractions. The process variables that could affect the physical characteristics of niosomes and in vitro release of the drug from the niosomes

were studied and optimized. In vivo distribution studies of the prepared niosomes found that 65% of the drug could be localized in the lungs by controlling the niosome size.

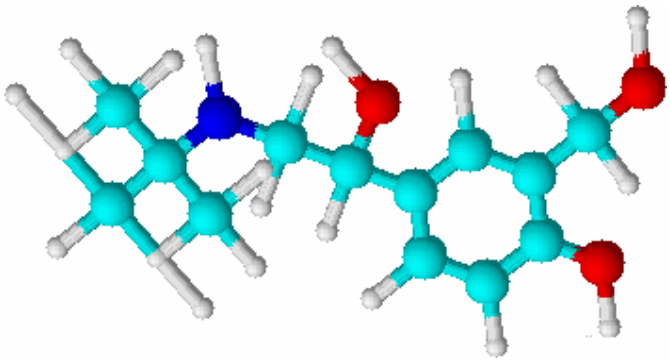
[51]**Toshimitsu Yoshioka *et al*.,** studied Formation of multilamellar vesicles (niosomes) of a series of sorbitan monoesters (span 20, 40, 60 and 80) and a sorbitan trioleate (Span 85) has been studied using a mechanical shaking technique without sonication. 5(6)-carboxyfluorescein (CF) was used as a model solute to investigate entrapment efficacy and release. For Span 80, cholesterol and dicetyl phosphate (DCP) in the molar ratio 47.5:47.5:5.0, entrapment efficacy increased linearly with increasing concentration of lipid. Entrapment efficacy per mmol lipid, however, was constant at about 34%, independent of the lipid concentration. Entrapment efficacy increased with increasing cholesterol content when vesicles were prepared by changing the molar ratio of non-ionic surfactant to cholesterol. Most efficient entrapment of CF occurred with Span 60 (HLB 4.7). Mean size of the un-sonicated niosomes showed a regular increase with increasing HLB from Span 85 (HLB 1.8). the release rate of CF from vesicles depended on the surfactant used in the preparation of the vesicles.

[52]**K.S. Chandraprakash *et al*.,** prepared Methotrexate encapsulated in niosomes. The entrapment efficacy increased in lipophilicity of surfactant. Unilamellar vesicles prepared with span 60 showed maximum entrapment and its pharmacokinetics in mice transplanted with S-180 tumor was marketedly different in comparison to unentrapped Methotrexate.

CHAPTER - III

SALBUTAMOL

Structure



Overview

1) Class

a) This drug is a member of the following class(es):

- Beta-2 Adrenergic Agonist
- Bronchodilator
- Cardiovascular Agent
- Sympathomimetic

Drug Properties

A) Synonyms

- Albuterol
- Albuterol Sulf
- Albuterol Sulfate
- Salbutamol

B) Physicochemical Properties

1) Albuterol

a) Molecular Weight

1) 239.3

b) Solubility

1) Soluble in ethanol; sparingly soluble in water; very soluble in chloroform

2) Albuterol Sulfate

a) Molecular Weight

1) 576.7

b) pH

1) Syrup: 3 to 4.5

c) Solubility

1) Soluble in water and slightly soluble in ethanol

DRUG DOSING

Adult Dosage

Inhalation, oral

Metered-Dose Inhaler

a) The recommended dose of albuterol sulfate is 2 inhalations by metered-dose inhaler every 4 to 6 hours as needed. One inhalation every 4 hours may be sufficient in some patients. Each actuation delivers 108 micrograms (mcg) of albuterol sulfate, equivalent to 90 mcg of albuterol base

b) Alternatively, 1 to 2 inhalations (0.1 milligrams (mg) to 0.2 mg) three to four times daily may be used for long-term

treatment in adults, and 1 to 2 inhalations once to treat acute symptoms, up to a maximum of 12 inhalations daily. A minimum of 3 hours is required between doses

Oral route

Tablets and Syrup

a) The recommended initial dose of albuterol is 2 or 4 milligrams (mg) orally 3 or 4 times daily. If a patient does not respond to the 4 mg dose, it may be carefully increased stepwise as tolerated, not to exceed 8 mg orally 4 times a day (32 mg total daily dose)

Extended-Release Tablets

a) The recommended dose of extended-release albuterol sulfate tablets is 8 milligrams (mg) orally every 12 hours. In some patients, including those with low body weight, a dose of 4 mg orally every 12 hours may be started and increased to 8 mg every 12 hours

Parenteral route

Injection

a) For the short-term treatment of severe bronchospasm in all types of bronchial asthma, the following dosing of albuterol is recommended for adults: one-half ampule (0.5 milliliter (mL) or 0.25 milligram (mg)) by subcutaneous or intramuscular injection stat, may repeat in 15 minutes with one-half ampule if needed. This regimen may be administered every 4 hours (Fachinfo Salbulair(R)0.5, 1997).

Infusion

a) The recommended dose for intravenous administration of albuterol in adults (depending on body weight) for the short-term treatment of severe bronchospasm in all types of bronchial asthma, is 0.2 mL to 0.4 mL (0.1 mg to 0.2 mg) albuterol infused slowly, this may be repeated in 15 minutes with up to twice the dose if necessary. The maximum recommended dose for single administration is 1 ampule (1 mL or 0.5 mg) (Fachinfo Salbulair(R)0.5, 1997).

Pharmacokinetics

Onset and Duration

A) Onset

1) Albuterol Sulfate

a) Initial Response

1) AEROSOL INHALATION: 5 to 15 minutes (Evans et al, 1971; Sillett et al, 1976); (Spector et al 1977)(Jenkinson et al, 1977; Walker et al, 1972).

2) POWDER INHALATION: 10 minutes (Pullan & Martin, 1980).

b) Peak Response

1) AEROSOL INHALATION: 30 to 120 minutes

2) NEBULIZER INHALATION: 60 TO 120 minutes

3) INTERMITTENT POSITIVE PRESSURE BREATHING: 30 to

120 minutes

4) ORAL: 15 to 180 minutes.

a) After 4 to 8 mg doses, or 0.15mg/kg

5) SUBLINGUAL: 2 to 3 hours

B) Duration

1) Albuterol Sulfate

a) Single Dose

1) AEROSOL INHALATION: 4 to 6 hours

2) ORAL IMMEDIATE RELEASE: 6 to 8 hours

Drug Concentration Levels

A) Albuterol Sulfate

1) Therapeutic Drug Concentration

a) Premature labor, 8 to 33 ng/mL

2) Time to Peak Concentration

a) Aerosol inhalation: 3 to 4 h

b) Nebulized inhalation: 0.5 h

c) Oral, extended-release tablet: 6 h

d) Oral, inhalation: 0.42 h

e) Oral, syrup: within 2 h

f) Oral, tablet: 2 h to 2.6 h

g) ORAL: 1 to 4 hours

h) SUBLINGUAL: Regular release, 2 to 3 hours

i) IPPB INHALATION: 2 to 4 hours

j) AEROSOL INHALATION: 3 to 4 hours

ADME

Absorption

A) Albuterol Sulfate

1) Bioavailability

a) Nebulized inhalation: less than 20%

b) Oral, extended-release tablet: approximately 100% relative to immediate-release tablet

c) Oral: 50% to 85%

d) BUCCAL: Less than 20%

e) IPPB INHALATION: 21.4% to 30.2%

2) Effects of Food

a) Extended-release tablet: food decreases rate of absorption without altering extent of bioavailability

Distribution

A) Distribution Sites

1) Albuterol Sulfate

a) Protein Binding

1) 10%

B) Distribution Kinetics

1) Albuterol Sulfate

a) Volume of Distribution

1) 156 +/- 38 liters

Metabolism

A) Metabolites

1) Albuterol Sulfate

a) Active metabolite: 4-O-sulfate ester

Excretion

A) Kidney

1) Albuterol Sulfate

a) Renal Clearance (rate)

1) 238 mL/min after intravenous administration (Hutchings et al, 1987).

b) Renal Excretion (%)

1) Nebulized inhalation: most of absorbed dose

2) Oral: 76% over 3 days

3) 64% to 98%

a)In pregnant patients, 18.7% of the drug was excreted unchanged after oral administration

B) Feces

1) Albuterol Sulfate

a) 1.2% to 7% eliminated in feces from oral dose

b) Oral: 4%

C) Total Body Clearance

1) Albuterol Sulfate

a) Clearance: 480 +/- 123 mL/min

b) Clearance: 501 +/- 185 mL/min pregnant patients

Elimination Half-life

A) Parent Compound

1) Albuterol Sulfate

a) Oral, extended-release tablet: 9.3 h

b) Oral, inhalation: approximately 4.6 h to 5 h

c) Oral, tablet and syrup: about 5 h to 7.2 h

d) 3 to 6.5 hours

Precautions

A) Albuterol sulphate

1) cardiovascular disorders, especially coronary insufficiency, cardiac arrhythmias, and hypertension; may produce clinically significant changes on pulse rate, blood pressure, symptoms, and/or electrocardiogram

2) convulsive disorders

3) diabetes mellitus

4) hyperthyroidism

5) hypokalemia

Adverse Reactions

- Nervousness
- shakiness
- dizziness
- headache
- uncontrollable shaking of a part of the body
- muscle cramps
- excessive motion or activity sudden changes in mood
- nosebleed
- nausea
- increased or decreased appetite
- difficulty falling asleep or staying asleep
- pale skin

Symptoms of overdose may include:

- seizures
- chest pain
- fast, irregular or pounding heartbeat
- nervousness
- headache
- uncontrollable shaking of a part of the body
- dry mouth
- nausea
- dizziness
- excessive tiredness
- lack of energy
- difficulty falling asleep or staying asleep

Marketed formulations

- Ventmax SR
- Volmax
- Ventolin injection
- Ventolin solution for Intravenous infusion

CHAPTER - IV

POLYOXYETHYLENE SORBITAN FATTY ACID ESTERS[11]

Functional Category

Emulsifying agent; nonionic surfactant; solubilizing agent; wetting, dispersing/ suspending agent.

Applications in Pharmaceutical Formulation or Technology

Polyoxyethylene sorbitan fatty acid esters (polysorbates) are a series of partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20, 5, or 4 moles of ethylene oxide for each mole of sorbitol and its anhydrides. The resulting product is therefore a mixture of molecules of varying sizes rather than a single uniform compound. Polysorbates containing 20 units of oxyethylene are hydrophilic nonionic surfactants that are used widely as emulsifying agents in the preparation of stable oil-in-water pharmaceutical emulsions. They may also be used as solubilizing agents for a variety of substances including essential oils and oil-soluble vitamins, and as wetting agents in the formulation of oral and parenteral suspensions. They have been found to be useful in improving the oral bioavailability of drug molecules that are substrates for p-glycoprotein. Polysorbates are also widely used in cosmetics and food products

Stability and Storage Conditions

Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides.

Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry place.

Uses of polysorbates

Uses	Concentration (%)
Emulsifying agent	-
Used alone in oil-in-water emulsions	1–15
Used in combination with hydrophilic emulsifiers in oil-in-water emulsions	1–10
Used to increase the water-holding properties of ointments	1–10
Solubilizing agent	-
For poorly soluble active constituents in lipophilic bases	1–10
Wetting agent	-
For insoluble active constituents in lipophilic bases	0.1–3

Incompatibilities

Discoloration and/or precipitation occur with various substances, especially phenols, tannins, tars, and tar like materials. The antimicrobial activity of paraben preservatives is reduced in the presence of polysorbates

Regulatory Status

Polysorbates 60, 65, and 80 are GRAS listed. Polysorbates 20, 40, 60, 65, and 80 are accepted as food additives in Europe. Polysorbates 20, 40, 60, and 80 are included in the FDA Inactive Ingredients Guide (IM, IV, oral, rectal, topical, and vaginal preparations). Polysorbates are included in parenteral and nonparenteral medicines licensed in the UK. Polysorbates 20, 21, 40, 60, 61, 65, 80, 81, 85, and 120 are included in the Canadian List of Acceptable Non-medicinal Ingredients

Tween 20

Nonproprietary Names

Polysorbate 20

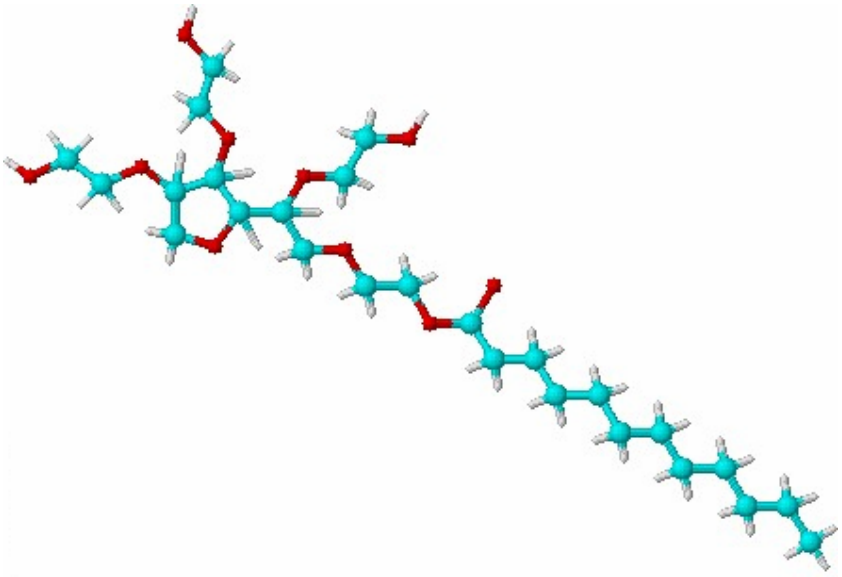
Synonym

Armotan PML 20; Capmul POE-L; Campul POE-L Low PV; Crillet 1; Drewmulse; E432; Durfax 20; E432; Eumulgin SML; Glycosperse L-20; Hodag PSML-20; Lamesorb SML-20; Liposorb L-20; Liposorb L-20K; Montanox 20; Nissan Nonion LT-221; Norfox Sorbo T-20; POE-SML; Ritabate 20; Sorbax PML-20; sorbitan monododecanoate; Sorgen TW-20; T-Maz 20; TMaz 20K; poly(oxy-1,2-ethanediyl) derivatives; polyoxyethylene 20 laurate; Protasorb L-20; Tego SML 20; Tween 20.

Chemical name

Polyoxyethylene 20 sorbitan monolaurate

Structure



Emprical formula and molecular weight

$C_{58}H_{114}O_{26}$

M.W: 1128

Colour and form

Yellow oily liquid

Solubility

Soluble in water and ethanol. Insoluble in mineral oils

Safety

Moderate toxicity by IP and IV routes. Moderately toxic by ingestion. Human skin irritant.

· LD50 (hamster, oral): 18 g/kg

· LD50 (mouse, IV): 1.42 g/kg

· LD50 (rat, oral): 37 g/kg

Tween 40

Nonproprietary Names

Polysorbate 40

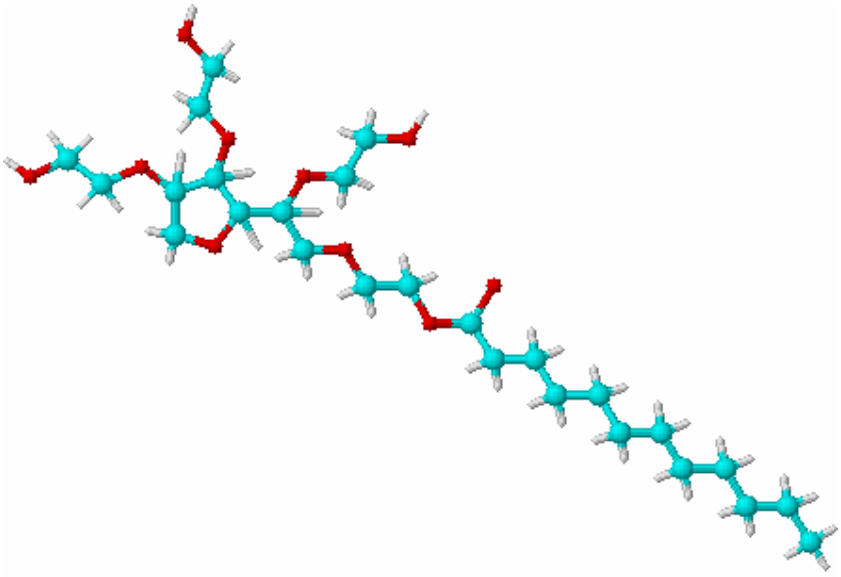
Synonym

Crillet 2; E434; Eumulgin SMP; Glycosperse S-20; Hodag PSMP-20; Lamesorb SMP-20; Liposorb P-20; Lonzest SMP-20; Montanox 40; poly(oxy- 1,2-ethanediyl) derivatives; Protasorb P-20; Ritabate 40; sorbitan monohexadecanoate; Sorbax PMP-20; Tween 40.

Chemical name

Polyoxyethylene 20 sorbitan monopalmitate

Structure



Emprical formula and molecular weight

C62H122O26

M.W: 1284

Colour and form

Yellow oily liquid

Solubility

Soluble in water and ethanol. Insoluble in mineral oils

Safety

LD50 (rat, IV): 1.58 g/kg.

Moderately toxic by IV route.

Tween 60

Nonproprietary Names

Polysorbate 60

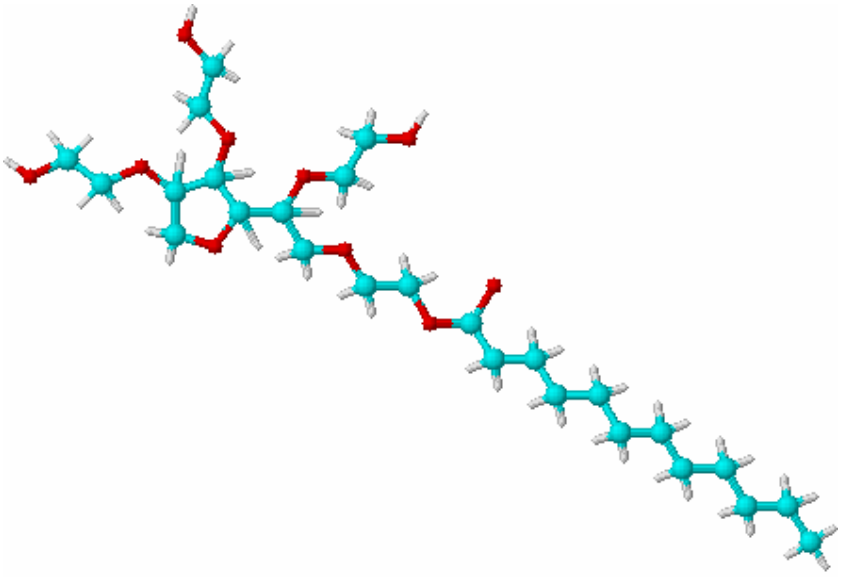
Synonym

Atlas 70K; Atlas Armotan PMS 20; Capmul POE-S; Cremophor PS 60; Crillet3; Drewpone 60K; Durfax 60; Durfax 60K; E435; Emrite 6125; Eumulgin SMS; Glycosperse S-20; Glycosperse S-20FG; Glycosperse S-20FKG; Hodag PSMS-20; Hodag SVS-18; Lamsorb SMS-20; Liposorb S-20; Liposorb S-20K; Lonzest SMS-20; Nikkol TS-10; Norfox SorboT-60 Montanox 60; Polycon T 60 K; polyoxyethylene 20 stearate; Ritabate 60; Protasorb S-20; Sorbax PMS-20; sorbitan monooctadecanoate poly(oxy-1,2-ethanediyl) derivatives; T-Maz 60; T-Max 60KHS; Tween 60; Tween 60K; Tween 60 VS.

Chemical name

Polyoxyethylene 20 sorbitan monostearate

Structure



Emprical formula and molecular weight

$C_{64}H_{126}O_{26}$

M.W: 1312

Colour and form

Yellow oily liquid

Solubility

Soluble in water and ethanol. Insoluble in mineral oils

Safety

LD50 (rat, IV): 1.22 g/kg.

Moderately toxic by IV route. Experimental tumorigen; reproductive effects.

Tween 80

Nonproprietary Names

Polysorbate 80

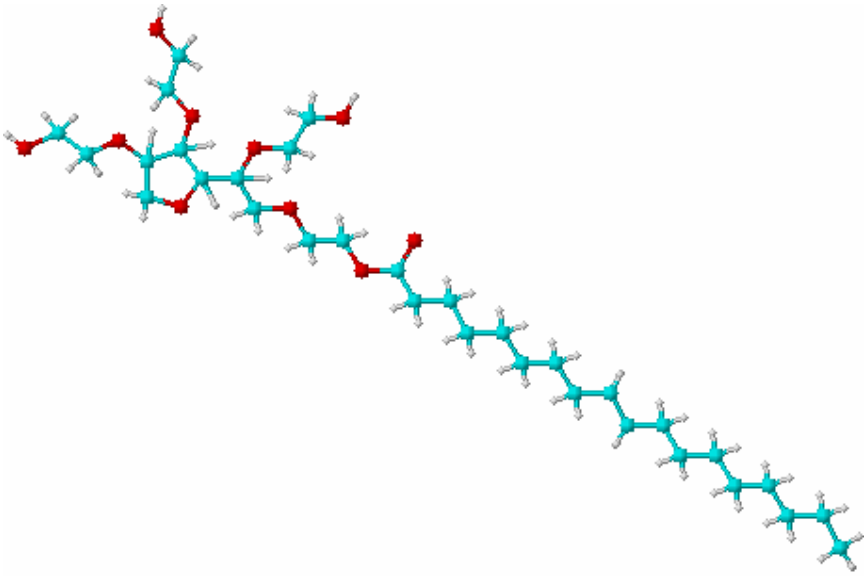
Synonym

Atlas E; Armotan PMO 20; Capmul POE-O; Cremophor PS 80; Crillet 4; Crillet 50; Drewmulse POE-SMO; Drewpone 80K; Durfax 80; Durfax 80K; E433; Emrite 6120; Eumulgin SMO; Glycosperse O-20; Hodag PSMO-20; Liposorb O-20; Liposorb O-20K; Montanox 80; polyoxyethylene 20 oleate; Protasorb O-20; Ritabate 80; (Z)-sorbitan mono-9-octadecenoate poly(oxy1,2- ethanediyl) derivatives; Tego SMO 80; Tego SMO 80V; Tween 80.

Chemical name

Polyoxyethylene 20 sorbitan monooleate

Structure



Emprical formula and molecular weight

$C_{64}H_{124}O_{26}$

M.W: 1310

Colour and form

Yellow oily liquid

Solubility

Soluble in water and ethanol. Insoluble in mineral oils

Safety

Moderately toxic by IV route. Mildly toxic by ingestion. Eye irritation. Experimental tumorigen, reproductive effects. Mutogenic data.

· LD50 (mouse, IP): 7.6 g/kg

· LD50 (mouse, IV): 4.5 g/kg

· LD50 (mouse, oral): 25 g/kg

· LD50 (rat, IP): 6.8 g/kg

· LD50 (rat, IV): 1.8 g/kg

Sorbitan Esters (Sorbitan Fatty Acid Esters) [11]

Functional Category

Emulsifying agent; nonionic surfactant; solubilizing agent; wetting and dispersing/suspending agent.

Applications in Pharmaceutical Formulation or Technology

Sorbitan monoesters are a series of mixtures of partial esters of sorbitol and its mono- and dianhydrides with fatty acids. Sorbitan diesters are a series of mixtures of partial esters of sorbitol and its monoanhydride with fatty acids.

Sorbitan esters are widely used in cosmetics, food products, and pharmaceutical formulations as lipophilic nonionic surfactants. They are mainly used in pharmaceutical formulations as emulsifying agents in the preparation of creams, emulsions, and ointments for topical application. When used alone, sorbitan esters produce stable water-in-oil emulsions and microemulsions but are frequently used in combination with varying proportions of a polysorbate to produce water-in-oil or oil-in-water emulsions or creams of varying consistencies.

Sorbitan monolaurate, sorbitan monopalmitate and sorbitan trioleate have also been used at concentrations of 0.01–0.05% w/v in the preparation of an emulsion for intramuscular administration.

Stability and Storage Conditions

Gradual soap formation occurs with strong acids or bases; sorbitan esters are stable in weak acids or bases. Sorbitan esters should be stored in a well-closed container in a cool, dry place.

Solubility

sorbitan esters are generally soluble or dispersible in oils; they are also soluble in most organic solvents. In water, although insoluble, they are generally dispersible.

Safety

Sorbitan esters are widely used in cosmetics, food products, and oral and topical

pharmaceutical formulations and are generally regarded as nontoxic and nonirritant materials. However, there have been occasional reports of hypersensitive skin reactions following the topical application of products containing sorbitan esters. When heated to decomposition, the sorbitan esters emit acrid smoke and irritating fumes. The WHO has set an estimated acceptable daily intake of sorbitan monopalmitate, monostearate, and tristearate, and of sorbitan monolaurate and monooleate at up to 25 mg/kg body-weight calculated as total sorbitan esters.

Regulatory Status

Certain sorbitan esters are accepted as food additives in the UK. Sorbitan esters are included in the FDA Inactive Ingredients Guide (inhalations; IM injections; ophthalmic, oral, topical, and vaginal preparations). Sorbitan esters are used in nonparenteral medicines licensed in the UK. Sorbitan esters are included in the Canadian List of Acceptable Non-medicinal Ingredients

Uses of Sorbitan Esters

Uses	Concentration (%)
Emulsifying agent	-
Used alone in oil-in-water emulsions	1–15
Used in combination with hydrophilic emulsifiers in oil-in-water emulsions	1–10
Used to increase the water-holding properties of ointments	1–10
Solubilizing agent	-
For poorly soluble active constituents in lipophilic bases	1–10
Wetting agent	-
For insoluble active constituents in lipophilic bases	0.1–3

Span 20

Nonproprietary Names

Sorbitan monolaurate

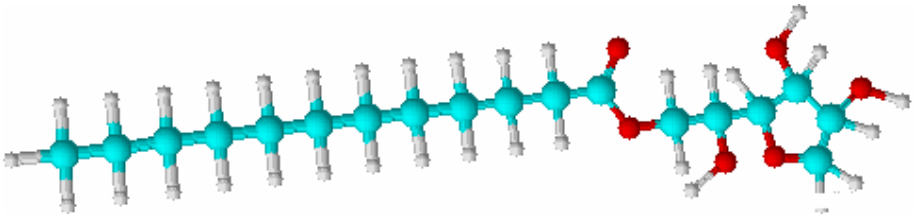
Synonym

Arlacel 20; Armotan ML; Crill 1; Dehymuls SML; E493; Glycomul L; Hodag SML; Liposorb L; Montane 20; Protachem SML; Sorbester P12; Sorbirol L; sorbitan laurate; Span 20; Tego SML.

Chemical name

Sorbitan monododecanoate

Structure



Emprical formula and molecular weight

$C_{18}H_{34}O_6$

M.W: 346

Colour and form

Yellow viscous liquid

Safety

LD50 (rat, oral): 33.6 g/kg.

Experimental neoplastigen.

Span 40

Nonproprietary Names

Sorbitan monopalmitate

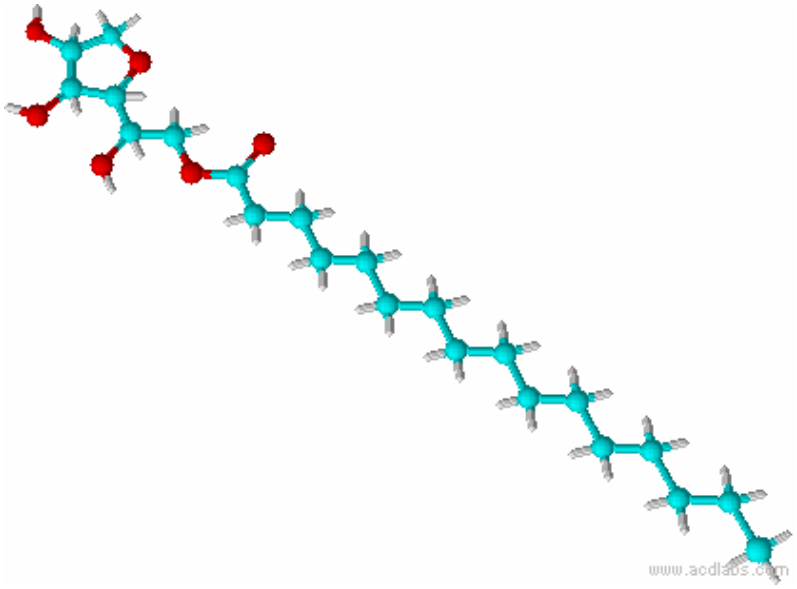
Synonym

1,4-Anhydro-D-glucitol, 6-hexadecanoate; Ablunol S-40; Arlacel 40; Armotan MP; Crill 2; Dehymuls SMP; E495; Glycomul P; Hodag SMP; Lamesorb SMP; Liposorb P; Montane 40; Nikkol SP-10; Nissan Nonion PP-40R; Protachem SMP; Proto-sorb SMP; Sorbester P16; Sorbirol P; sorbitan palmitate; Span 40.

Chemical name

Sorbitan monohexadecanoate

Structure



Emprical formula and molecular weight

$C_{22}H_{42}O_6$; M.W: 403

Colour and form

Cream solid

Span 60

Nonproprietary Names

Sorbitan monostearate

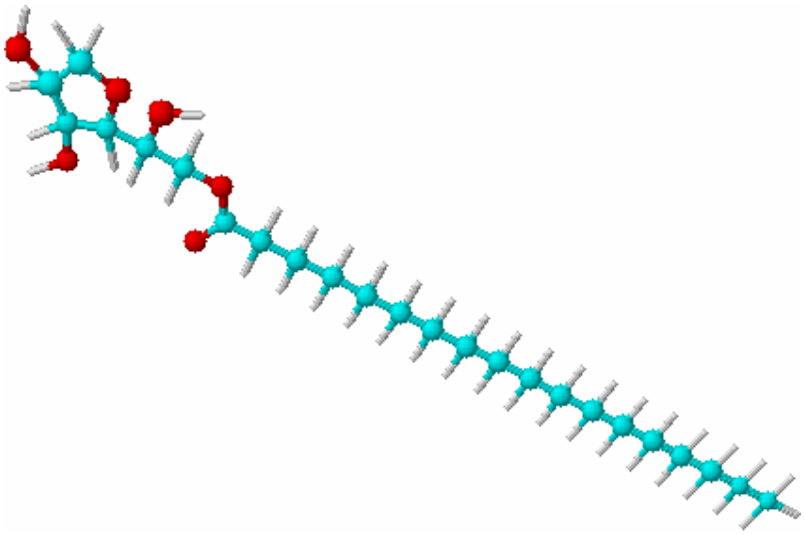
Synonym

Ablunol S-60; Alkamuls SMS; 1,4-Anhydro-D-glucitol, 6-octadecanoate; anhydrosorbitol monostearate; Arlacel 60; Armotan MS; Atlas 110K; Capmul S; Crill 3; Dehymuls SMS; Drewmulse SMS; Drewsorb 60K; Durtan 6O; Durtan 60K; E491; Famodan MS Kosher; Glycomul S FG; Glycomul S KFG; Hodag SMS; Lamesorb SMS; Liposorb S; Liposorb SC; Liposorb S-K; Montane 60; Nissan Nonion SP-60R; Norfox Sorbo S- 60FG; Polycon S60K; Protachem SMS; Prote-sorb SMS; S-Maz 60K; SMaz 60KHS; Sorbester P18; Sorbirol S; sorbitan stearate; Sorgen 50; Span 60; Span 60K; Span 60 VS; Tego SMS.

Chemical name

Sorbitan mono-octadecanoate

Structure



Emprical formula and molecular weight

$C_{24}H_{46}O_6$

M.W: 431

Colour and form

Cream solid

Safety

LD_{50} (rat, oral): 31 g/kg.

Very mildly toxic by ingestion. Experimental reproductive effects.

Span 80

Nonproprietary Names

Sorbitan monooleate

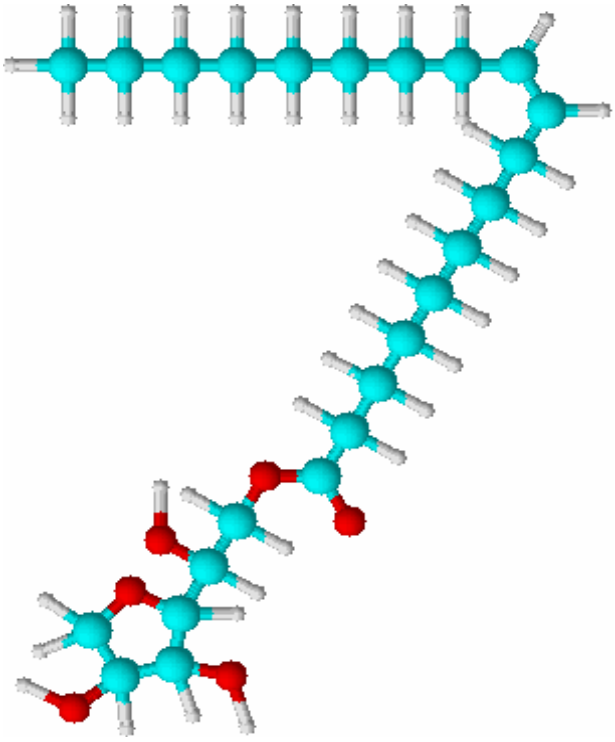
Synonym

Ablunol S-80; Arlacel 80; Armotan MO; Capmul O; Crill 4; Crill 50; Dehymuls SMO; Drewmulse SMO; Drewsorb 80K; E494; Glycomul O; Hodag SMO; Lamesorb SMO; Liposorb O; Montane 80; Nikkol SO-10; Nissan Nonion OP-80R; Norfox Sorbo S-80; Polycon S80 K; Proto-sorb SMO; Protachem SMO; S-Maz 80K; Sorbester P17; Sorbirol O; sorbitan oleate; Sorgen 40; Sorgon S-40-H; Span 80; Tego SMO.

Chemical name

(*Z*)-Sorbitan mono-9-octadecenoate

Structure



Emprical formula and molecular weight

$C_{24}H_{44}O_6$; M.W: 429

Colour and form : Yellow viscous liquid

Dicetyl phosphate

Synonym : Dihexadecyl phosphate

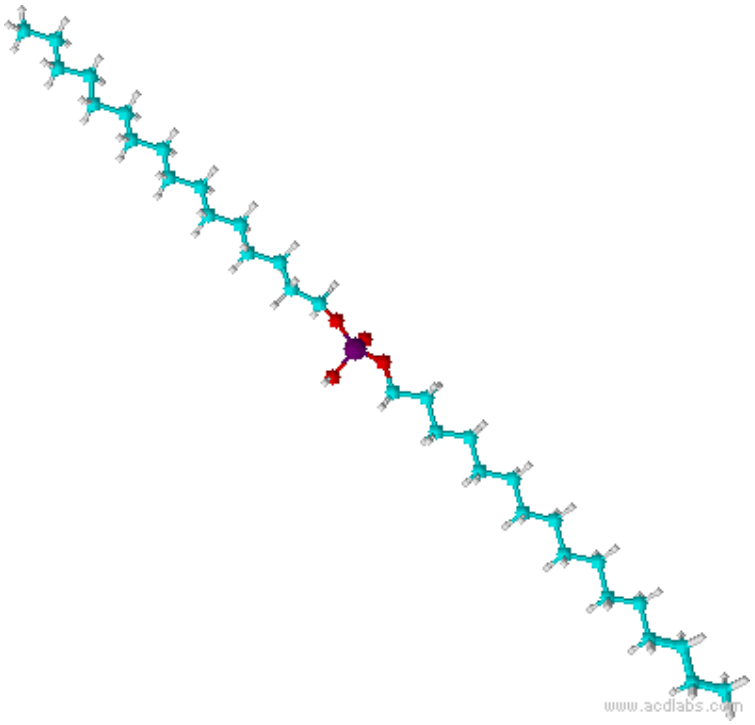
Formula : $C_{32}H_{67}O_4P$

Molecular Weight : 546,85 g/mol

Appearance

Form solid

Structure



Toxicological Information

Chronic exposure

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as

probable, possible or confirmed human carcinogen by IARC.

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation.

Skin May be harmful if absorbed through skin. May cause skin irritation.

Eyes May cause eye irritation.

Ingestion May be harmful if swallowed

Cholesterol

Nonproprietary Names

Cholesterol

Synonyms

Cholesterin; cholesterolum.

Chemical Name

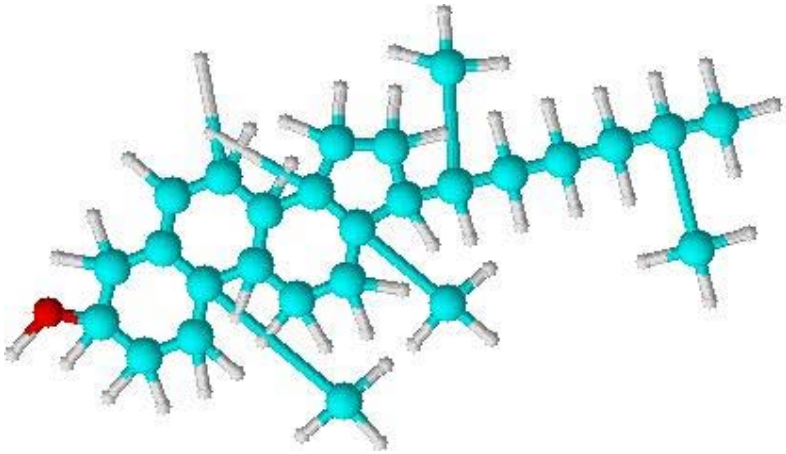
Cholest-5-en-β-ol [57-88-5]

Empirical Formula and Molecular Weight

C27H46O

386.67

Structure



Functional Category

Emollient; emulsifying agent.

Applications in Pharmaceutical Formulation or Technology

Cholesterol is used in cosmetics and topical pharmaceutical formulations at concentrations of 0.3–5.0% w/w as an emulsifying agent. It imparts water-absorbing power to an ointment and has emollient activity. Cholesterol also has a physiological

role. It is the major sterol of the higher animals, and it is found in all body tissues, especially in the brain and spinal cord. It is also the main constituent of gallstones.

Description

Cholesterol occurs as white or faintly yellow, almost odorless, pearly leaflets, needles, powder, or granules. On prolonged exposure to light and air, cholesterol acquires a yellow to tan color.

Stability and Storage Conditions

Cholesterol is stable and should be stored in a well-closed container, protected from light.

Solubility

Solvent	Solubility
Acetone	Soluble
Benzene	1 in 7
Chloroform	1 in 4.5
Ethanol	1 in 147 at 0°C
	1 in 78 at 20°C
	1 in 29 at 40°C
	1 in 19 at 50°C
	1 in 13 at 60°C
Ethanol (95%)	1 in 78 (slowly)
	1 in 3.6 at 80°C
Ether	1 in 2.8
Isopropyl myristate	1 in 19
Methanol	1 in 294 at 0°C
Vegetable oils	Soluble
Water	Practically insoluble

Safety

Cholesterol is generally regarded as an essentially nontoxic and nonirritant material at the levels employed as an excipient. It has, however, exhibited experimental teratogenic and reproductive effects, and mutation data have been reported.Cholesterol is often derived from animal sources and this must be done in accordance with the regulations for human consumption. The risk of bovine spongiform encephalopathy (BSE) contamination has caused some concern over the use of animal-derived cholesterol in pharmaceutical products. However, synthetic methods of cholesterol manufacture have been developed.

Regulatory Status

Included in the FDA Inactive Ingredients Guide (injections, ophthalmic, topical, and vaginal preparations). Included in nonparenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

CHAPTER - V

OBJECTIVES OF THE STUDY

Salbutamol is a relatively selective beta-2-adrenoreceptor stimulant. After parenteral administration, stimulation of the beta receptors in the body, both beta-1 and beta-2, occurs because (a) beta-2 selectivity is not absolute, and (b) higher concentrations of salbutamol occur in the regions of these receptors with parentral mode of administration. This results in the beta-1 effect of cardiac stimulation, and beta-2 effects of peripheral vasodilatation and hypotension, skeletal muscle tremor and uterine muscle relaxation.

In order to reduce the uptake of salbutamol by beta-1 receptor and to increase the drug concentration in lungs with minimum dose through niosomes was tried in this study.

- To formulate and optimize Salbutamol Sulphate niosomes for targeting lungs.
- To reduce the dose level and dosing frequency of Salbutamol Sulphate by sustained release niosome formulation.
- To determine the stability of Salbutamol Sulphate niosome formulation with and without stabilizing agent on storage.
- To determine the stability of salbutamol sulphate niosomes as per ICH guidelines.
- To compare the bio-distribution nature of niosomal formulation and drug in solution to different organs in mice when administered via intravenous route.
- To evaluate the altered pharmacokinetics and metabolism of Salbutamol Sulphate niosomal formulation in rabbits.

PLAN OF WORK

1. Calibration of standard curve.
2. Preformulation study and optimization of formulation with different ratios of non-ionic surfactants.
3. Formulation of Salbutamol Sulphate niosomes with different non-ionic surfactants like Tween 20, 40, 60, 80 and Span 20, 40, 60, 80 by thin film hydration technique using rotary flash evaporator.
4. Characterization of Niosomes.
    a) Vesicle size determination of both empty and drug loaded formulations by optical microscopy.
    b) Photomicrographs using optical microscope to confirm lamellarity of vesicles before and after sonication of formulations.
    c) Surface morphology of vesicles by Scanning Electron Microscopy.
    d) Evaluation of osmotic shock of vesicles when incubated with three different medium.
    e) Viscosity determination using Ostwald viscometer.
    f) Determination of entrapment efficacy by ultra centrifugation technique.
    g) Determination of SPAN index.
5. *In vitro* release of different formulations and drug in solution.
6. Stability of Salbutamol Sulphate niosomes as per ICH guidelines.
7. Tissue distribution nature of two best niosomal formulations and drug in solution using mice.
8. Pharmacokinetic evaluation of Tween and Span niosomal formulations using rabbits.

CHAPTER - VII

Calibration of standard curve

Preparation of Standard Stock Solution

Accurately, about 100 mg of Salbutamol Sulphate was weighed and transferred to a 100 ml volumetric flask. The drug was dissolved in 100 ml of PBS pH 7.4 with shaking and then the volume made up to the mark with PBS pH 7.4 to obtain a standard stock solution of a drug concentration, 1000 µg/ml.

Selection of Analytical Wavelength

With appropriate dilution of the standard stock solution with PBS pH 7.4, the solution was scanned using the double beam UV visible spectrophotometer (Model: UV- 1650 PC, SHIMADZU) in the spectrum mode between the wavelength range of 400 nm to 200 nm. The λ_{max} of Salbutamol Sulphate was found to 276 nm, which was selected as the analytical wavelength for further analysis. (Fig 1)

Standard Plot

Stock solution was further diluted to get the different concentrations to determine the linearity range. Linearity was obtained in the concentration between 20 - 200 µg/ml. The standard samples were analyzed at 276 nm using UV Spectrophotometer. (Table1 & Fig 2)

Table 1: Calibration of standard Curve

S.No	Concentration (µg/ml)	Absorbance at 276 nm
1	20	0.13049
2	40	0.22803
3	60	0.34167
4	80	0.45667
5	100	0.55981
6	120	0.65942
7	140	0.77563
8	160	0.87244
9	180	0.98254
10	200	1.10425

Fig 1: Spectrum report

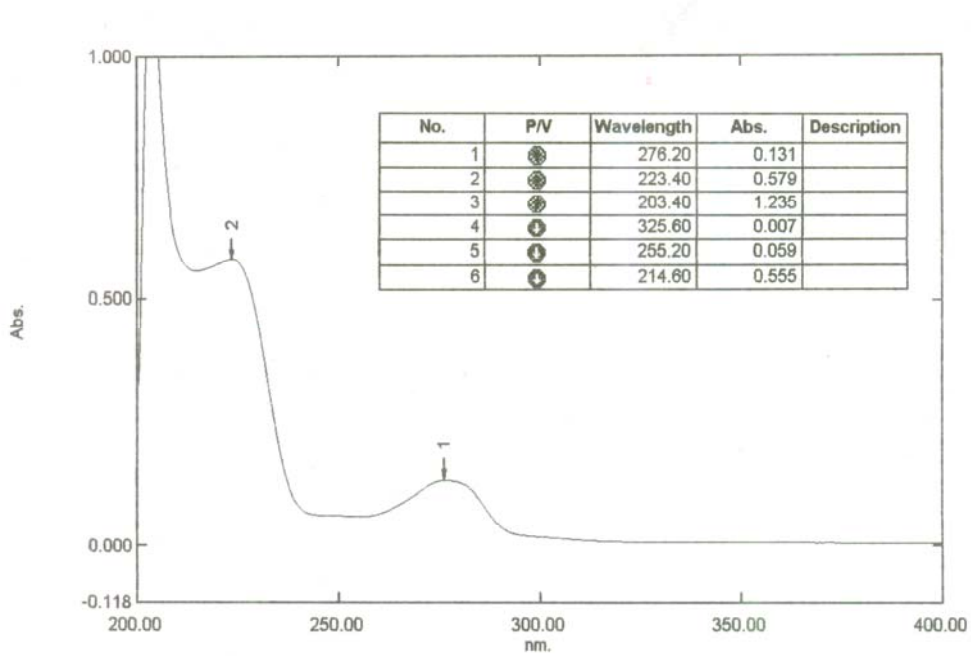
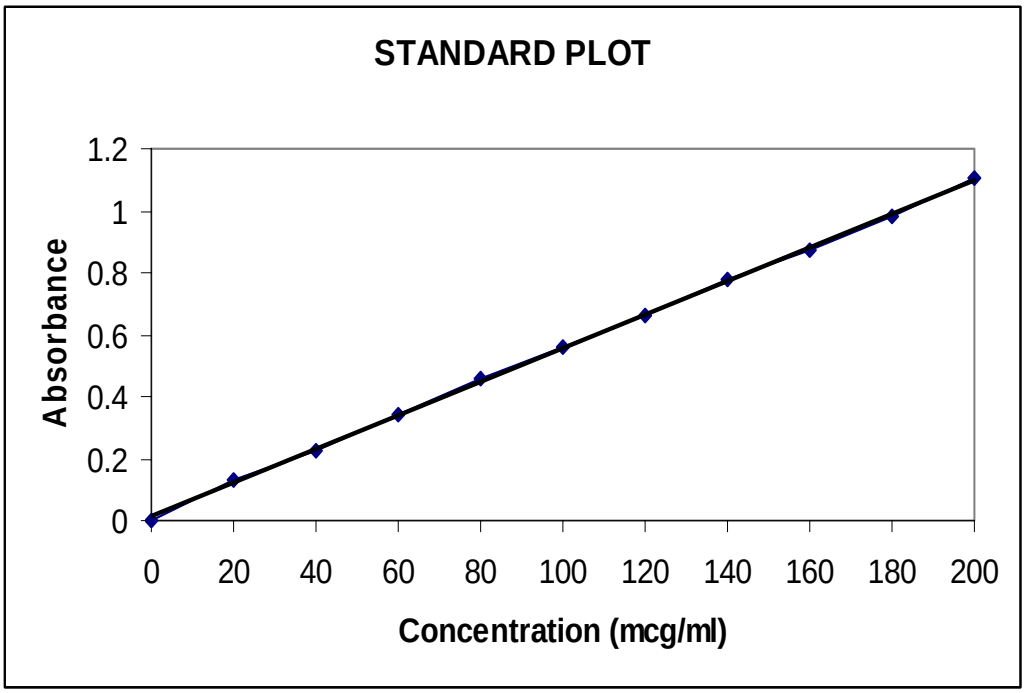


Fig 2: Calibration of standard curve



Straight line equation: $y = 0.00542x + 0.01312$

Correlation co-efficient: $r^2 = 0.99957$

Preformulation study and optimization of Formulation

Formulations were optimized by taking different micro molar ratios of Tween 80 and keeping cholesterol in a constant ratio i.e. 20 µM (Table 2). 20:90 µM (cholesterol: Tween 80) ratio gives the spherical vesicle of niosomes. With this ratio formulations were tried with different non-ionic surfactants such as Tween (20, 40 & 60) and Span (20, 40, 60 & 80) by thin film hydration technique. Vesicles were found to be spherical in all the formulations. Thus 20:90 µM ratio of cholesterol and non-ionic surfactant respectively was taken for the further development of formulations.

Table 2: Optimization of formulation

S.No	Cholesterol : Surfactant (Tween 80)		Result
	Ratio (µM)	Weight (mg)	
1	20 : 30	7.6:39.3	Spherical vesicles not seen
2	20 : 50	7.6:65.5	Spherical vesicles not seen
3	20 : 60	7.6:78.6	Spherical vesicles not seen
4	20 : 70	7.6:91.7	*Spherical vesicles with crystals*
5	**20: 90**	**7.6:117.9**	***Spherical vesicles***
6	20 : 120	7.6:157.2	*Spherical vesicles with crystals*

Preparation of Niosomes

Thin film hydration technique

20 µM of cholesterol, 90 µM of Non Ionic surfactant were dissolved in 800 ml of round bottom flask with 10 ml of chloroform and it was allowed to rotate in a flash evaporator at 60^{o}C. Vacuum was applied at 620 mmHg from vacuum pump for one hour**.** The flask was allowed to rotate 125 rpm for 1 hour to get a dry film. Film was hydrated with 5 ml of Phosphate Buffer Saline pH 7.4 containing 8 mg of Salbutamol Sulphate and it was allowed to rotate for 60 min. The same procedure was carried out with different non ionic surfactants. All the formulations were sonicated for 2 minutes using Bath sonicator.

Formulations were also tried without the addition of stabilizing agent cholesterol and also with the inclusion of charge inducing agent dicetyl phosphate (DCP). Niosome vesicles were also formulated without the addition of drug in order to determine any changes in vesicle size after the addition of drug. Formulation identity was shown in table 3.

Quantities of surfactant, cholesterol and DCP used in the formulations were given in table 4.

Table 3: Formulation identity

S.No	Code	Surfactant used
1	F1	Tween 20
2	F2	Tween 40
3	F3	Tween 60
4	F4	Tween 80
5	F5	Span 20
6	F6	Span 40
7	F7	Span 60
8	F8	Span 80
9	F9	Tween 40 without cholesterol
10	F10	Tween 60 without cholesterol
11	F11	Span 40 without cholesterol
12	F12	Span 60 without cholesterol
13	F13	Span 60 +cholesterol + DCP

Table 4: Quantities of different variables used in formulation

S.No	Code	Amount of surfactant (mg)	Amount of cholesterol (mg)	Amount of DCP (mg)
1	F1	101.5	7.7	-
2	F2	115.5	7.7	-
3	F3	118.1	7.7	-
4	F4	117.9	7.7	-
5	F5	31.4	7.7	-
6	F6	36.3	7.7	-
7	F7	38.8	7.7	-
8	F8	38.6	7.7	-
9	F9	115.5	7.7	-
10	F10	118.1	7.7	-
11	F11	36.3	7.7	-
12	F12	38.8	7.7	-
13	F13	38.8	7.7	2.7

Characterization of niosomes

a. Vesicle size determination

Vesicle size of unsonicated formulation was determined by optical microscopy using a pre calibrated eye piece. Eye piece was calibrated using stage micrometer at 40 X magnification. Size of each division of eye piece micrometer was determined using the formula

$$\text{Size of each division} = \frac{\text{Number of divisions of stage micrometer}}{\text{Number of divisions of eye piece micrometer}} \times 10$$

Each division of eye piece micrometer was found to be 2 μm at 40 X magnification.

The average size of 50 vesicles was counted for 3 times at different time intervals after 4 hours of the formulation (Table 5).

b. Photomicrographs[37]

A drop niosomal suspension was placed on the microscopic glass slide. Photographs of sonicated and unsonicated formulations were taken at 40 X magnification using the digital camera (Olympus 8 Mega pixel) attached to the eye piece of the microscope. Shape and lamellar nature of the unsonicated vesicles was confirmed with the photographs. (Fig 3 to Fig 18)

Table 5: Vesicle size determination

S.No	Code	Empty formulation (µm)	Formulation with drug (µm)
1	F1(Tween 20 + CHOL)	< 2	3.04
2	F2(Tween 40 + CHOL)	< 2	3.62
3	F3(Tween 60 + CHOL)	< 2	2.75
4	F4(Tween 80 + CHOL)	< 2	2.37
5	F5(Span 20 + CHOL)	2.44	4.12
6	F6(Span 40 + CHOL)	2.36	3.58
7	F7(Span 60 + CHOL)	2.16	3.12
8	F8(Span 80 + CHOL)	2.04	2.50
9	F9(Tween 40)	< 2	3.91
10	F10(Tween 60)	< 2	2.91
11	F11(Span 40)	4.32	5.25
12	F12(Span 60)	4.18	4.75
13	F13(Span 60 + CHOL+DCP)	2.84	2.95

Fig 3: F1 unsonicated (Tween 20 +CHOL)

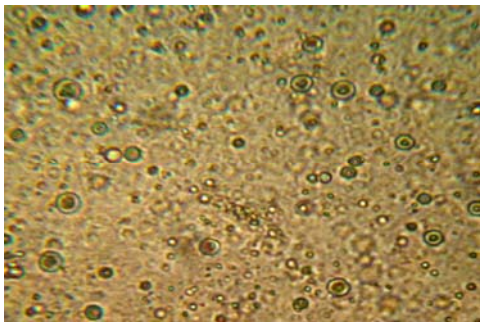


Fig 4: F1 sonicated (Tween 20 +CHOL)

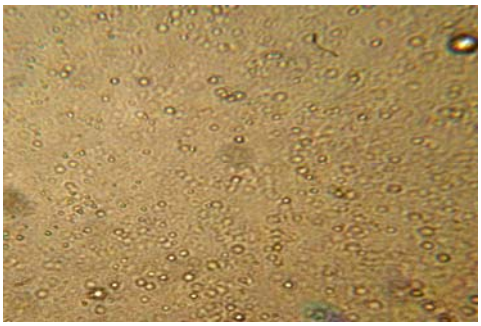


Fig 5: F2 unsonicated (Tween 40 +CHOL)

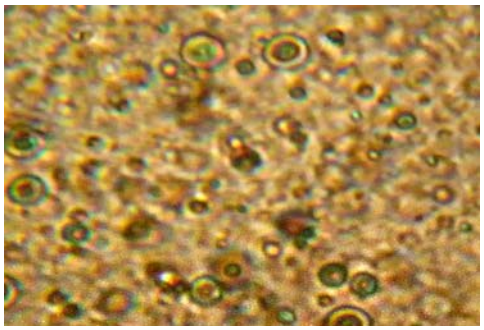


Fig 6: F2 sonicated (Tween 40 +CHOL)

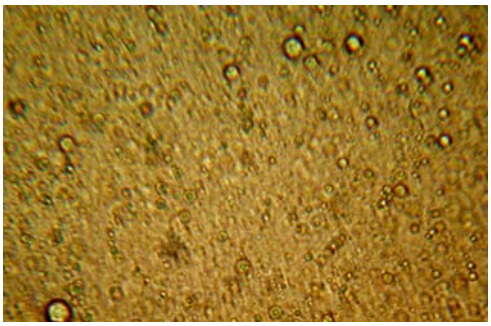


Fig 7: F3 unsonicated (Tween 60 +CHOL)

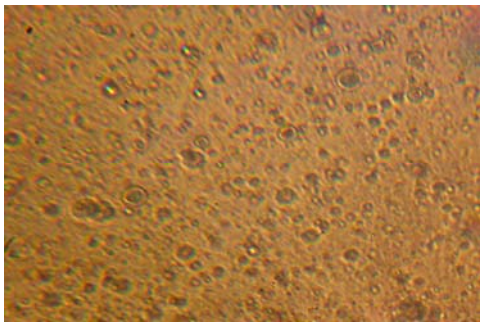


Fig 8: F3 sonicated (Tween 60 +CHOL)

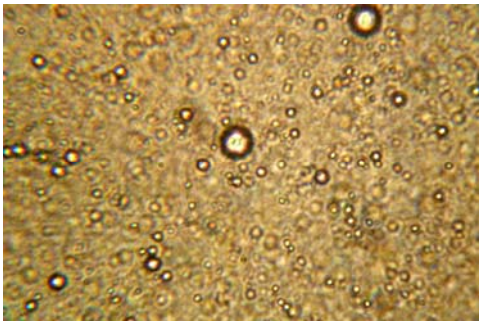


Fig 9: F4 unsonicated (Tween 80 +CHOL)

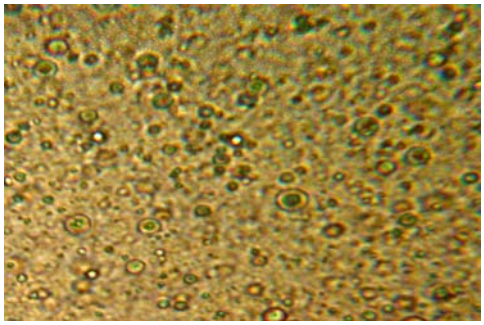


Fig 10: F4 sonicated (Tween 80 +CHOL)

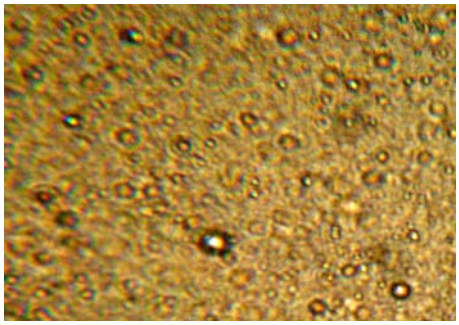


Fig 11: F5 unsonicated (Span 20 +CHOL)

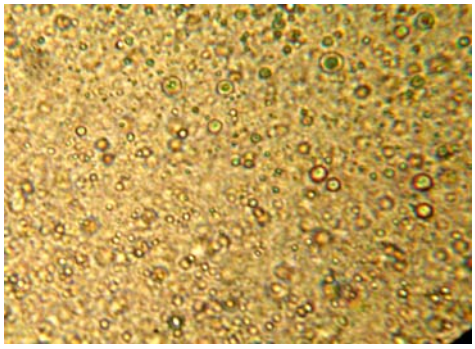


Fig 12: F5 sonicated (Span 20 +CHOL)

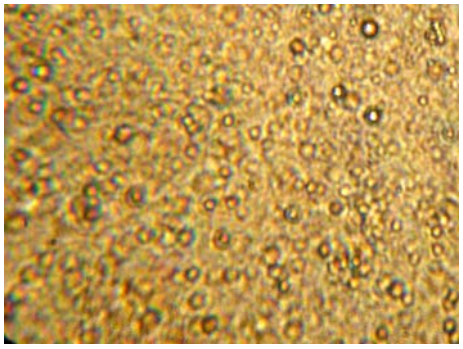


Fig 13: F6 unsonicated (Span 40 +CHOL)

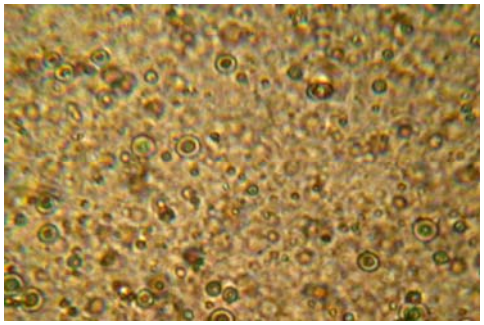


Fig 14: F6 sonicated (Span 40 +CHOL)

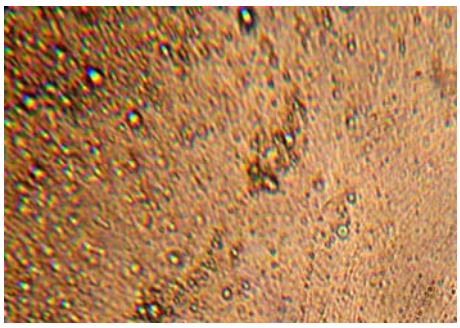


Fig 15: F7 unsonicated (Span 60 +CHOL)

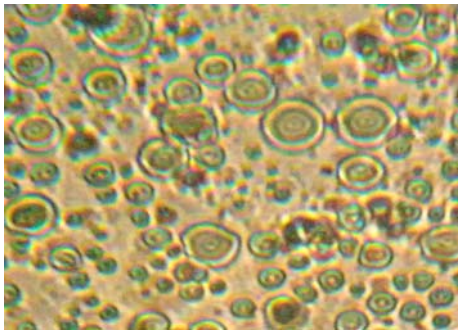


Fig 16: F7 sonicated (Span 60 +CHOL)

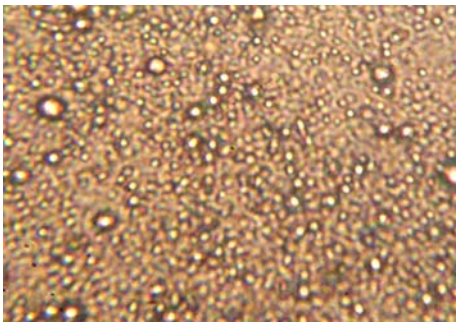


Fig 17: F8 unsonicated (Span 80 +CHOL)

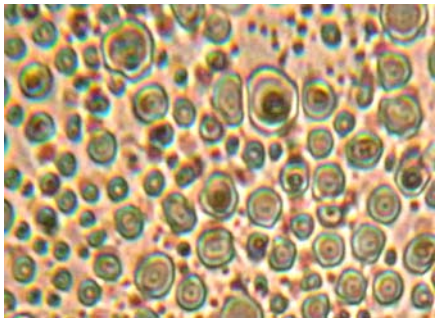
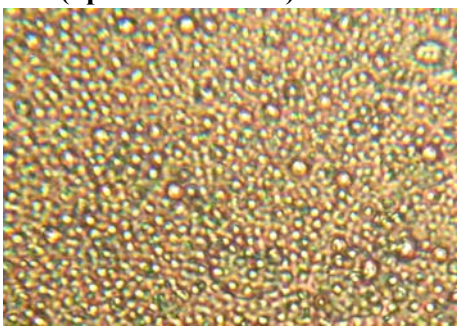


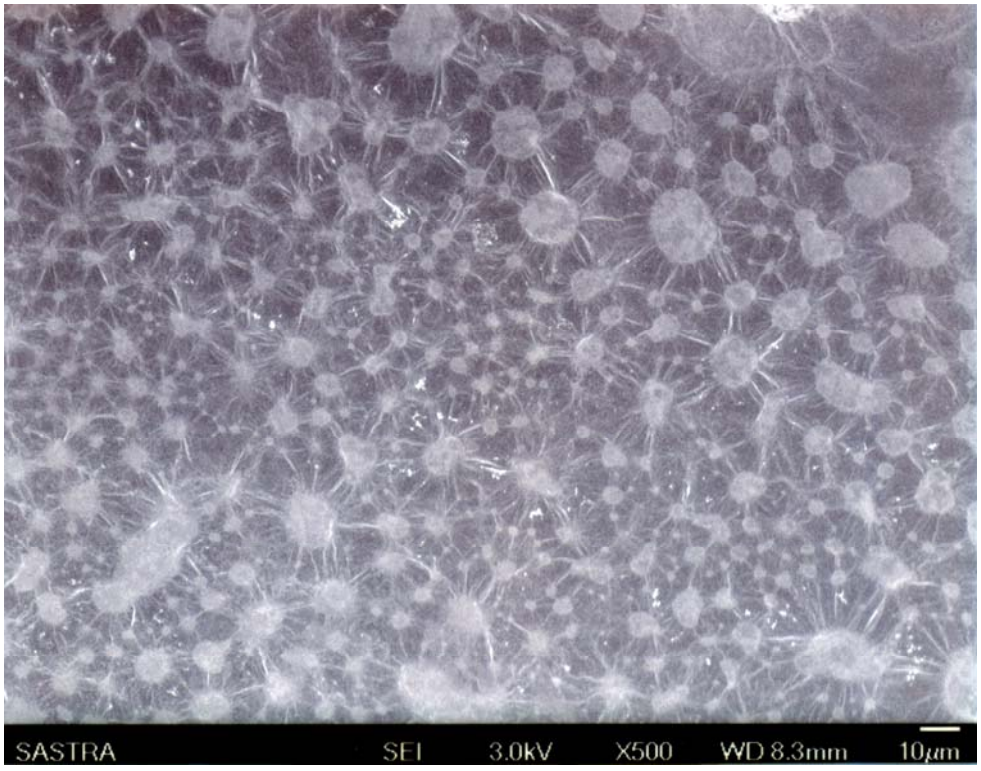
Fig 18: F8 sonicated (Span 80 +CHOL)



c. Scanning Electron Microscopy

Vesicles were viewed and photographed by Scanning electron microscopy in order to determine the surface characteristics of the formulated vesicles. 100 µ litres of the samples were mounted on the metal stub and allowed to dry at 20 ^{o} C. The dried sample was coated with platinum for 30 seconds with the thickness of 20 mA(10^{-3}) using JEOL JF1600 Auto fine coater. Then the sample was viewed and captured under 500X resolution by secondary electron detector using JEOL JSM –F, Field emission scanning electron microscopy at a voltage of 3.0 kV. (Fig 19)

Fig 19: SEM Image



d. Osmotic Shock[27,44]

The effect of osmotic sensitivity and aggregation tendency was studied on niosomal formulations with and without cholesterol. The effect of osmotic change was investigated by monitoring the change in vesicle diameter following the incubation of the vesicular suspension in medium of different tonicity. A hypertonic media was simulated using 1 M NaI solution, normal saline with 0.9% NaCl and hypotonic media with 0.5% NaCl. The vesicular suspension was incubated in these media for 3 h following which the change in vesicle size was measured. This helped in determining the effect of osmotic shock on the prepared MLVs with and without cholesterol. (Table 6)

Table 6: Effect of osmotic shock on niosomal formulation

S.No	Code	Average size of formulation (µm)	Average size in hypotonic solution (µm)	Average size in saline solution (µm)	Average size in hypertonic solution (µm)
1	F1(Tween 20 + CHOL)	3.0	3.6	3.4	Shrinked
2	F2(Tween 40 + CHOL)	3.6	4.0	3.8	Shrinked
3	F3(Tween 60 + CHOL)	2.7	3.0	2.8	Shrinked
4	F4(Tween 80 + CHOL)	2.3	2.6	2.4	Shrinked
5	F5(Span 20 + CHOL)	4.1	4.6	4.2	Shrinked
6	F6(Span 40 + CHOL)	3.5	4.2	3.6	Shrinked
7	F7(Span 60 + CHOL)	3.1	3.8	3.4	Shrinked
8	F8(Span 80 + CHOL)	2.5	3.6	3.2	Shrinked
9	F9(Tween 40)	3.9	4.8	4.2	Shrinked
10	F10(Tween60)	2.9	4.9	4.4	Shrinked
11	F11(Span 40)	5.2	6.9	5.7	Shrinked
12	F12(Span 60)	4.7	6.5	5.2	Shrinked
13	F13(Span 60 + CHOL+DCP)	2.9	3.9	3.18	Shrinked

* Hypertonic solution – 1 M Sodium Iodide (NaI)

* Hypotonic solution – 0.5 % Sodium Chloride

* Saline solution - 0.9% sodium Chloride

e. Determination of viscosity[41]

Density was determined using specific gravity bottle. The weight of water and formulations were substituted in the formula and the density was calculated.

$$\text{Density of sample } (d_2) = \frac{w_2}{w_1} \text{ X } d_1$$

Where,

w_1 – weight of water

w_2 – weight of sample

d_1 – density of water (0.998)

d_2 – density of sample

Viscosity of the formulations was determined using Ostwald viscometer. The time taken for water and formulations to flow from point A to B was calculated and substituted in the formula and the viscosity was calculated (Table 7).

$$\text{Viscosity of sample } (\eta_1) = \frac{\rho_1 \text{ x } t_1}{\rho_2 \text{ x } t_2} \text{ X } \eta_2$$

Where,

ρ_1 – density of sample

ρ_2 – density of water

η_1 – viscosity of sample

η_2 – viscosity of water

t_1 – time taken to sample to flow from point A to B

t_2 – time taken to water to flow from point A to B

Table 7: Determination of viscosity

S.No	Code	Viscosity (centipoise)
1	F1(Tween 20 + CHOL)	2.052
2	F2(Tween 40 + CHOL)	2.072
3	F3(Tween 60 + CHOL)	2.121
4	F4(Tween 80 + CHOL)	1.80
5	F5(Span 20 + CHOL)	3.269
6	F6(Span 40 + CHOL)	2.56
7	F7(Span 60 + CHOL)	1.839
8	F8(Span 80 + CHOL)	2.936
9	F9(Tween 40)	2.311
10	F10(Tween 60)	2.065
11	F11(Span 40)	4.826
12	F12(Span 60)	4.059
13	F13(Span 60 + CHOL+DCP)	1.928
14	Drug in solution	0.9349

f. Determination of entrapment efficacy[25]

One ml of the formulation was taken and ultra centrifuged at 13, 000 rpm (15700 X g) at 4^{o} C for 90 minutes using eppendorf centrifuge (model 5415 R, Fig-20). The supernatant was recovered using micro pipette and analyzed by UV method for Salbutamol sulphate content. The percentage of drug encapsulation (EP (%)) was calculated by the following equation. (Table 8)

$$\mathbf{EP\ (\%) = [(C_t - C_r)/C_t] \times 100}$$

Where,

C_t – Concentration of total Salbutamol Sulphate.

C_r – Concentration of free drug in supernatant solution.

Fig 20: Eppendorf centrifuge

Table 8: Percentage entrapment efficacy

S.No	Code	Entrapment efficacy (%)
1	F1(Tween 20 + CHOL)	81.4
2	F2(Tween 40 + CHOL)	86.9
3	F3(Tween 60 + CHOL)	89.6
4	F4(Tween 80 + CHOL)	79.2
5	F5(Span 20 + CHOL)	87.7
6	F6(Span 40 + CHOL)	90.0
7	F7(Span 60 + CHOL)	90.5
8	F8(Span 80 + CHOL)	77.7
9	F9(Tween 40)	64.8
10	F10(Tween 60)	69.2
11	F11(Span 40)	76.1
12	F12(Span 60)	78.4
13	F13(Span 60 + CHOL+DCP)	89.6

g. Determination of polydispersity (SPAN) index[12]

Polydispersity or SPAN index is used to determine the narrow size distribution of the vesicle size. Standard deviation of the vesicle size was determined and the width of the vesicle size distribution was given by a SPAN index which was calculated by the following equation. The cumulative percentage size distribution of different formulations is depicted in fig 21 and fig 22 and the SPAN index is shown in table 9.

$$\textbf{SPAN index} = (D_{0.9} - D_{0.1})/D_{0.5}$$

Where,

$D_{0.9}$ – particle diameter determined at 90^{th} percentile of particle undersized

$D_{0.1}$ - particle diameter determined at 10^{th} percentile of particle undersized

$D_{0.5}$ - particle diameter determined at 50^{th} percentile of particle undersized

Table 9: SPAN Index

S.No	Code	SPAN index
1	F1	1.18
2	F2	1.11
3	F3	1.62
4	F4	2.16
5	F5	1.45
6	F6	1.50
7	F7	1.84
8	F8	2.14
9	F9	0.758
10	F10	1.77
11	F11	1.56
12	F12	0.97
13	F13	2.0

Fig 21: Cumulative % size distribution (Tween formulations)

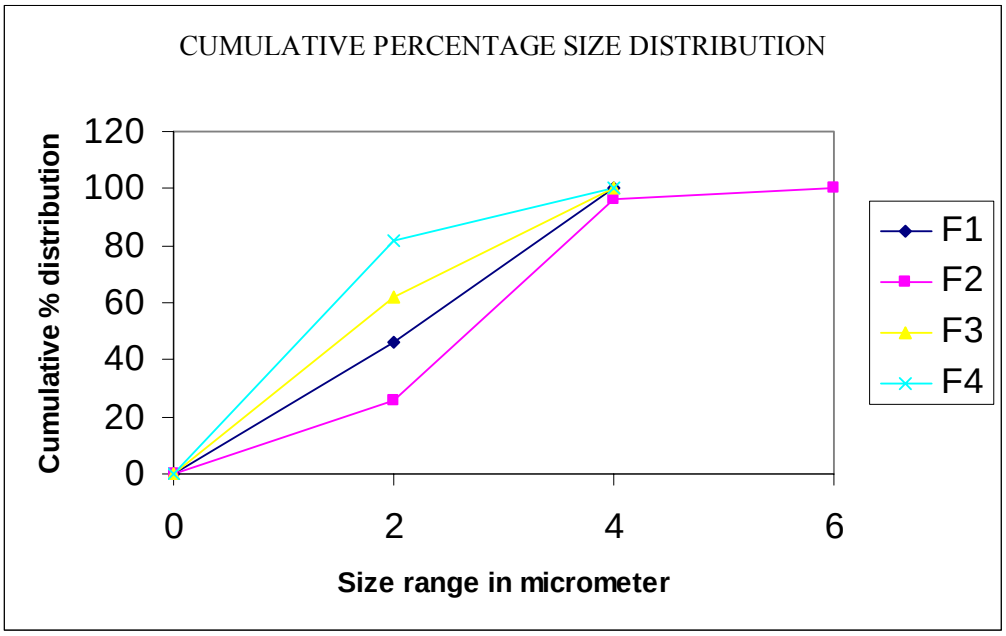
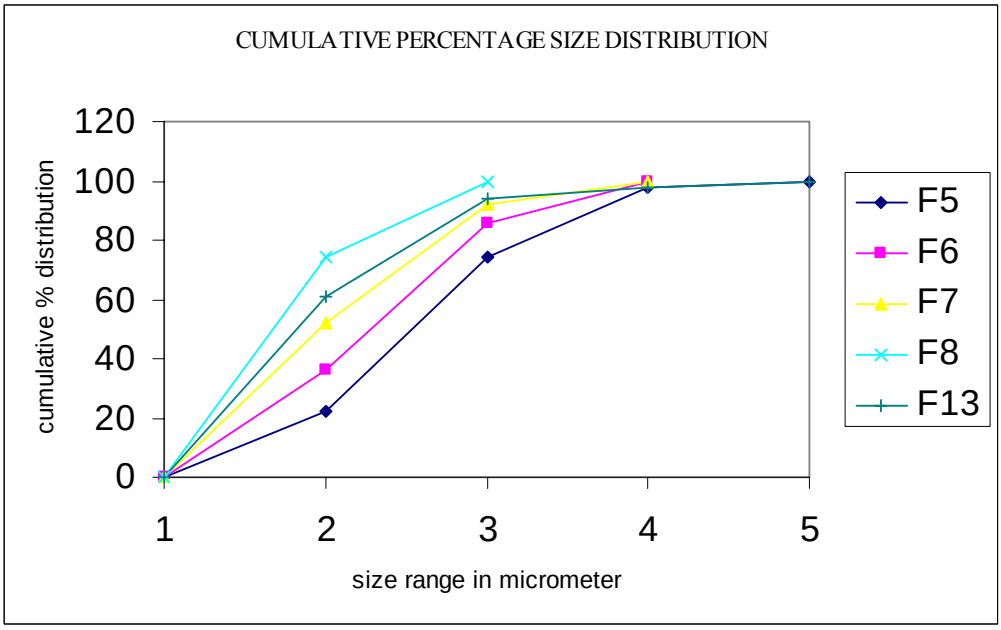


Fig 22: Cumulative % size distribution (Span formulations)



5. *In vitro* release studies[55]

In vitro release was carried out using Himedia dialysis membrane 50 with the molecular weight cut-off ranges from 12000 – 14000 daltons which has the capacity of holding 1.61 ml/cm. Dialysis bag which acts as a donor compartment was soaked in warm water for 10 minutes and Himedia closure clips are used to close dialysis bag on both the sides to prevent the leakage of formulation during the release study (Fig 23, Fig 24 and Fig 25).

The niosomal formulations were centrifuged at 13, 000 rpm for 90 minutes at 4^{o} C. The sediment which has the entrapped drug was taken for the release study after reconstituted with 1 ml of PBS pH 7.4. The dispersed entrapped drug was taken in the dialysis bag and it closed using closure clips. Dialysis bag was placed in 100 ml of PBS pH 7.4 which acts as a receptor compartment. The medium was stirred by using the magnetic stirrer at 100 rpm in room temperature. At each one hour interval 5 ml of samples were withdrawn and after each withdrawn same volume of medium was replaced. Then the samples were assayed spectrophotometrically, at 276 nm using PBS pH 7.4 as blank. Release data of Tween 20, 40, 60 & 80 formulations were shown in table 10 - table 15 and fig 26, Span 20, 40, 60 & 80 in table 16 – 22 and fig 27. The releases of all formulations were compared with pure Salbutamol sulphate solution. Drug in solution data was shown in table 23.

Fig 23: Dialysis membrane

Fig 24: Closure clips

Fig 25: *In vitro* release set-up

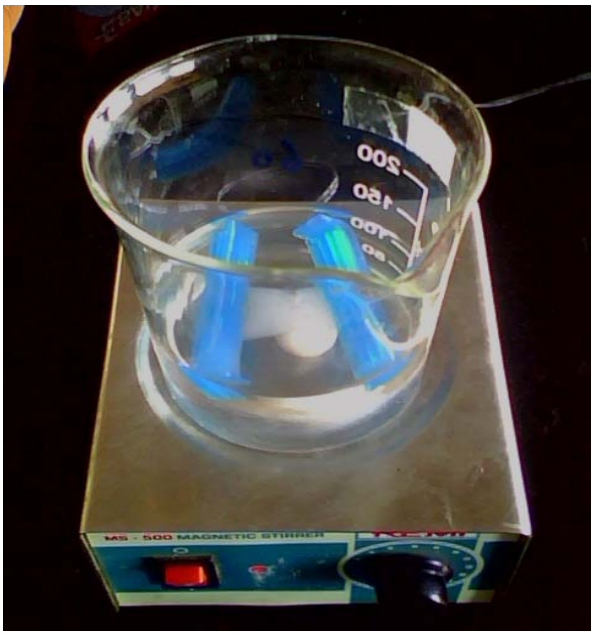


Table 10: Release data for formulation F1 (Tween 20 + CHOL)

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.02601	2.378229	0.002378	0.237823	13.43632
2	0.03345	3.750923	0.003751	0.375092	21.19165
3	0.04016	4.98893	0.004989	0.498893	28.18604
4	0.05103	6.994465	0.006994	0.699446	39.51675
5	0.05969	8.592251	0.008592	0.859225	48.54379
6	0.0625	9.110701	0.009111	0.91107	51.47289
7	0.06335	9.267528	0.009268	0.926753	52.35891
8	0.06653	9.854244	0.009854	0.985424	55.67369
9	0.07092	10.66421	0.010664	1.066421	60.24976
12	0.09253	14.65129	0.014651	1.465129	82.77566

Table 11: Release data for formulation F2 (Tween 40 + CHOL)

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.02637	2.444649	0.002445	0.244465	12.93465
2	0.02686	2.535055	0.002535	0.253506	13.41299
3	0.03589	4.201107	0.004201	0.420111	22.22808
4	0.03833	4.651292	0.004651	0.465129	24.61001
5	0.05493	7.714022	0.007714	0.771402	40.81493
6	0.05774	8.232472	0.008232	0.823247	43.55805
7	0.07629	11.65498	0.011655	1.165498	61.66657
8	0.08911	14.0203	0.01402	1.40203	74.18146
9	0.09192	14.53875	0.014539	1.453875	76.92458
12	0.09643	15.37085	0.015371	1.537085	81.32724

Table 12: Release data for formulation F3 (Tween 60 + CHOL)

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.01831	0.957565	0.000958	0.095756	4.910588
2	0.01941	1.160517	0.001161	0.116052	5.951367
3	0.02076	1.409594	0.00141	0.140959	7.228688
4	0.02173	1.588561	0.001589	0.158856	8.146466
5	0.02612	2.398524	0.002399	0.239852	12.30012
6	0.02948	3.01845	0.003018	0.301845	15.47923
7	0.03216	3.512915	0.003513	0.351292	18.01495
8	0.03357	3.773063	0.003773	0.377306	19.34904
9	0.0376	4.516605	0.004517	0.451661	23.16208
12	0.07628	11.65314	0.011653	1.165314	59.75967

Table 13: Release data for formulation F4 (Tween 80 + CHOL) [n=2]

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.02808	2.760148	0.00276	0.276015	16.04±0.82
2	0.02502	2.195572	0.002196	0.219557	12.76±2.36
3	0.03149	3.389299	0.003389	0.33893	19.70±1.34
4	0.03268	3.608856	0.003609	0.360886	20.98±1.02
5	0.03479	3.998155	0.003998	0.399815	23.24±0.98
6	0.04211	5.348708	0.005349	0.534871	31.09±1.32
7	0.05396	7.535055	0.007535	0.753506	43.80±1.28
8	0.06478	9.531365	0.009531	0.953137	55.41±1.24
9	0.07316	11.07749	0.011077	1.107749	64.40±0.86
12	0.09342	14.8155	0.014815	1.48155	86.13±0.32

Table 14: Release data for formulation F9 (Tween 40)

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.0193	1.140221	0.00114	0.114022	8.086677
2	0.01611	0.551661	0.000552	0.055166	3.912486
3	0.01892	1.070111	0.00107	0.107011	7.589438
4	0.02087	1.429889	0.00143	0.142989	10.14106
5	0.03162	3.413284	0.003413	0.341328	24.20769
6	0.0321	3.501845	0.003502	0.350185	24.83578
7	0.0564	7.98524	0.007985	0.798524	56.63291
8	0.05713	8.119926	0.00812	0.811993	57.58813
9	0.05811	8.300738	0.008301	0.830074	58.87048
12	0.06616	9.785978	0.009786	0.978598	69.4041

Table 15: Release data for formulation F10 (Tween 60)

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.01976	1.225092	0.001225	0.122509	8.167282
2	0.02016	1.298893	0.001299	0.129889	8.659287
3	0.02563	2.308118	0.002308	0.230812	15.38745
4	0.03162	3.413284	0.003413	0.341328	22.75523
5	0.04089	5.123616	0.005124	0.512362	34.15744
6	0.04504	5.889299	0.005889	0.58893	39.26199
7	0.06494	9.560886	0.009561	0.956089	63.73924

Fig 26: Comparative *In vitro* release profile of Tween formulations

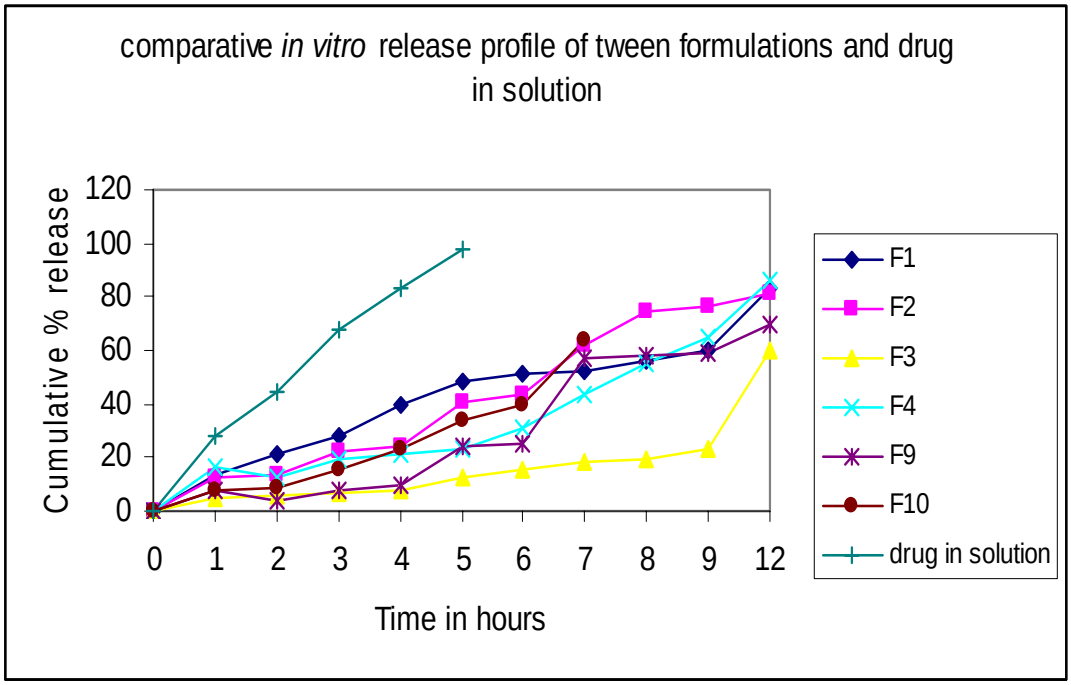


Table 16: Release data for formulation F5 (Span 20 + CHOL)

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.03003	3.119926	0.00312	0.311993	16.33469
2	0.03105	3.308118	0.003308	0.330812	17.31999
3	0.03918	4.808118	0.004808	0.480812	25.17339
4	0.04316	5.542435	0.005542	0.554244	29.01799
5	0.04792	6.420664	0.006421	0.642066	33.61604
6	0.05326	7.405904	0.007406	0.74059	38.77437
7	0.05796	8.273063	0.008273	0.827306	43.31446
8	0.06327	9.252768	0.009253	0.925277	48.44381
9	0.06247	9.105166	0.009105	0.910517	47.67103
12	0.08327	12.9428	0.012943	1.29428	67.76337

Table 17: Release data for formulation F6 (Span 40 + CHOL)

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.03088	3.276753	0.003277	0.327675	16.71813
2	0.03254	3.583026	0.003583	0.358303	18.28074
3	0.03293	3.654982	0.003655	0.365498	18.64787
4	0.04749	6.341328	0.006341	0.634133	32.35372
5	0.05216	7.202952	0.007203	0.720295	36.74976
6	0.05432	7.601476	0.007601	0.760148	38.78304
7	0.05976	8.605166	0.008605	0.860517	43.90391
8	0.06794	10.11439	0.010114	1.011439	51.60404
9	0.06462	9.501845	0.009502	0.950185	48.4788
12	0.08674	13.58303	0.013583	1.358303	69.30115

Table 18: Release data for formulation F7 (Span 60 + CHOL)

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.01354	0.077491	0.0000775	0.00775	0.393401
2	0.01536	0.413284	0.000413	0.041328	2.097889
3	0.02384	1.97786	0.001978	0.197786	10.0399
4	0.02936	2.99631	0.002996	0.299631	15.2097
5	0.03369	3.795203	0.003795	0.37952	19.26499
6	0.03906	4.785978	0.004786	0.478598	24.2943
7	0.04218	5.361624	0.005362	0.536162	27.21636
8	0.05429	7.595941	0.007596	0.759594	38.55808
9	0.06392	9.372694	0.009373	0.937269	47.57713
12	0.07324	11.09225	0.011092	1.109225	56.30584

Table 19: Release data for formulation F8 (Span 80 + CHOL)

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.01929	1.138376	0.001138	0.113838	6.735955
2	0.02466	2.129151	0.002129	0.212915	12.59853
3	0.02612	2.398524	0.002399	0.239852	14.19245
4	0.02661	2.48893	0.002489	0.248893	14.7274
5	0.0271	2.579336	0.002579	0.257934	15.26234
6	0.03857	4.695572	0.004696	0.469557	27.78445
7	0.04384	5.667897	0.005668	0.56679	33.53785
8	0.05469	7.669742	0.00767	0.766974	45.38309
9	0.06125	8.880074	0.00888	0.888007	52.54482
12	0.07218	10.89668	0.010897	1.089668	64.47739

Table 20: Release data for formulation F11 (Span 40)

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.01526	0.394834	0.000395	0.039483	2.378518
2	0.0199	1.250923	0.001251	0.125092	7.535678
3	0.0304	3.188192	0.003188	0.318819	19.20598
4	0.03052	3.210332	0.00321	0.321033	19.33935
5	0.03198	3.479705	0.00348	0.34797	20.96208
6	0.03772	4.538745	0.004539	0.453875	27.34184
7	0.04226	5.376384	0.005376	0.537638	32.38785
8	0.05957	8.570111	0.00857	0.857011	51.62717

Table 21: Release data for formulation F12 (Span 60)

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.04016	4.98893	0.004989	0.498893	29.17503
2	0.0542	7.579336	0.007579	0.757934	44.3236
3	0.05457	7.647601	0.007648	0.76476	44.72282
4	0.06042	8.726937	0.008727	0.872694	51.03472
5	0.06299	9.201107	0.009201	0.920111	53.80764
6	0.06873	10.26015	0.01026	1.026015	60.00086
7	0.07764	11.90406	0.011904	1.190406	69.61438
8	0.08643	13.52583	0.013526	1.352583	79.09842

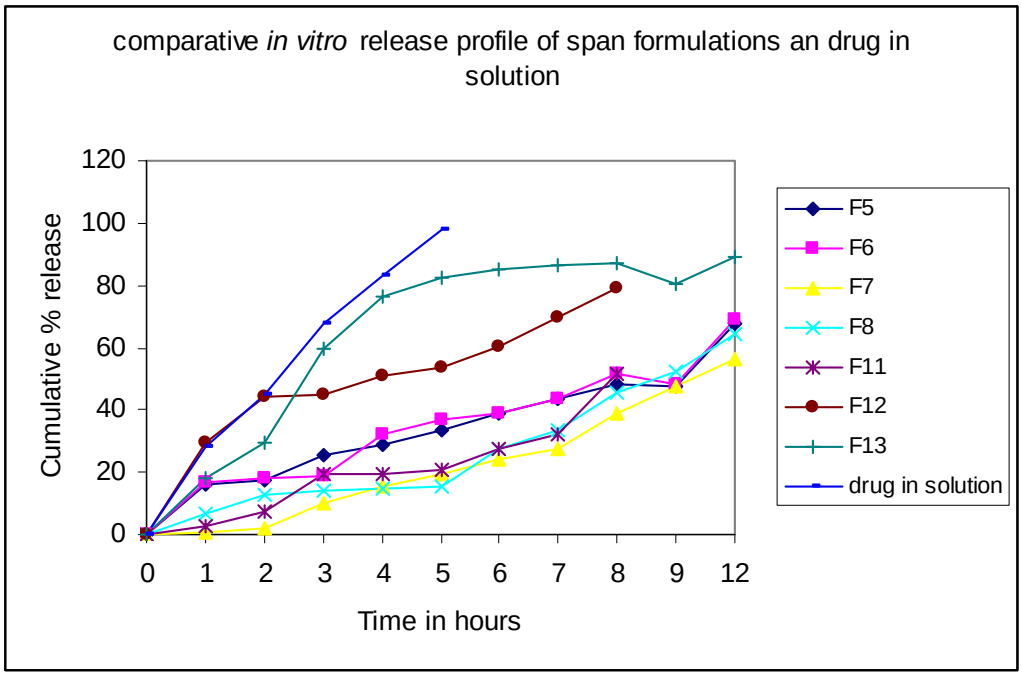
Table 22: Release data for formulation F13 (Span 60 + DCP)

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.03246	3.56826568	0.003568	0.356827	18.29±1.64
2	0.04443	5.77675277	0.005777	0.577675	29.62±1.86
3	0.07648	11.6900369	0.01169	1.169004	59.94±2.42
4	0.09412	14.9446494	0.014945	1.494465	76.63±1.38
5	0.10059	16.1383764	0.016138	1.613838	82.76±0.66
6	0.10291	16.5664207	0.016566	1.656642	84.95±0.52
7	0.10437	16.8357934	0.016836	1.683579	86.33±0.62
8	0.10535	17.0166052	0.017017	1.701661	87.26±0.48
9	0.09842	15.7380074	0.015738	1.573801	80.70±2.14
12	0.10732	17.3800738	0.01738	1.738007	89.12±1.36

Table 23: Release data for drug in solution

Time in hours	Absorbance	Concentration in µg/ml	Concentration in mg/ml	Total concentration (mg)	Cumulative percentage release
1	0.04304	5.520295	0.00552	0.55203	28.30921
2	0.06049	8.739852	0.00874	0.873985	44.81976
3	0.08492	13.24723	0.013247	1.324723	67.93453
4	0.10096	16.20664	0.016207	1.620664	83.11098
5	0.11682	19.13284	0.019133	1.913284	98.11714

Fig 27: Comparative *In vitro* release profile of Span formulations and drug in solution



Determination of drug release kinetics

Drug release kinetic models such as Higuchi, Peppas, Zero order and First order were predicted for two best formulations from *in vitro* data's. The regression coefficient and slope values were determined (fig 28– fig 35).

Fig 28

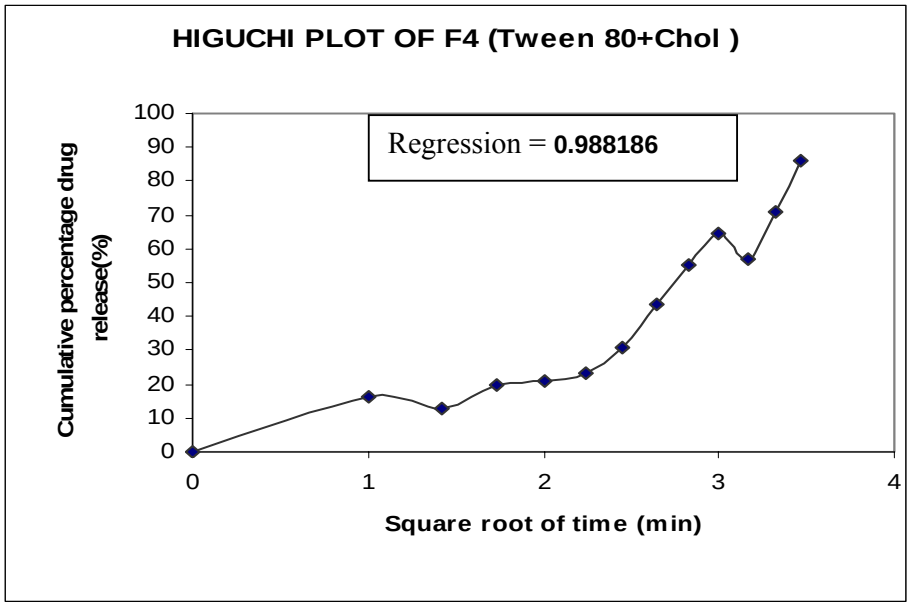


Fig 29

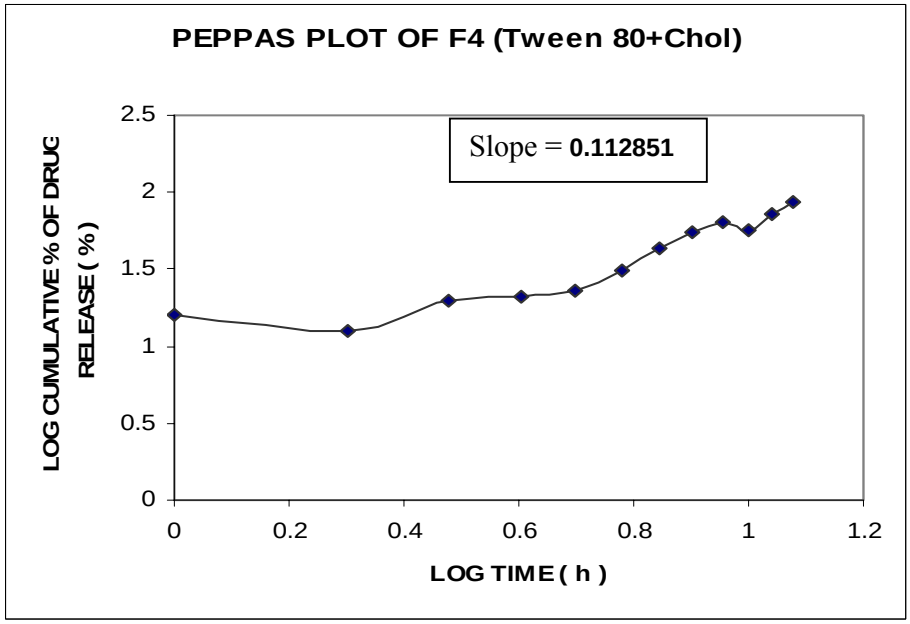


Fig 30

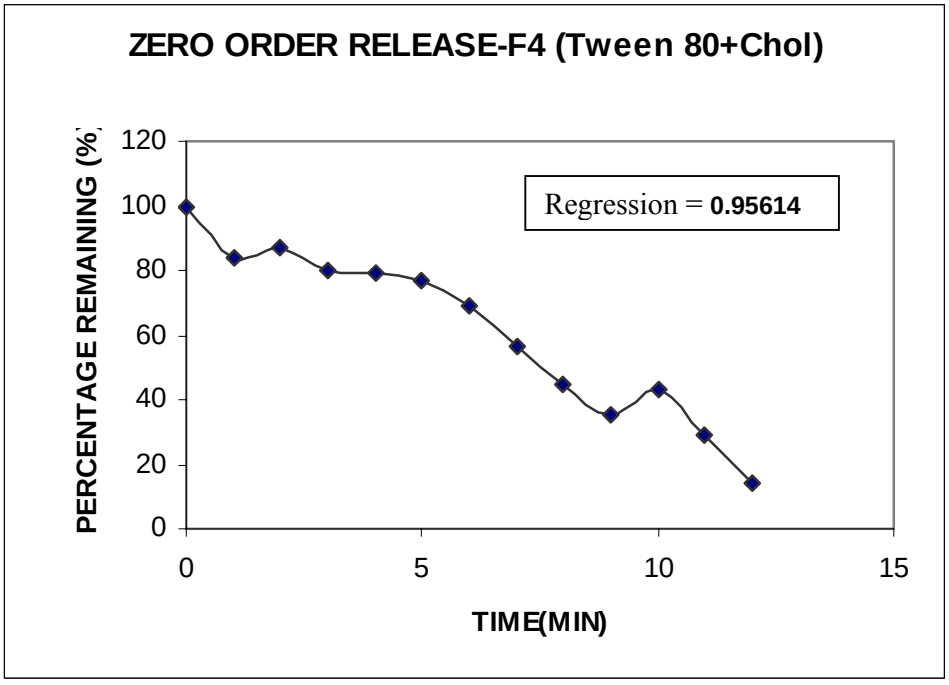

ZERO ORDER RELEASE-F4 (Tween 80+Chol)
PERCENTAGE REMAINING (%)
120
100
80
60
40
20
0
0
5
10
15
TIME(MIN)
Regression = 0.95614

Fig 31

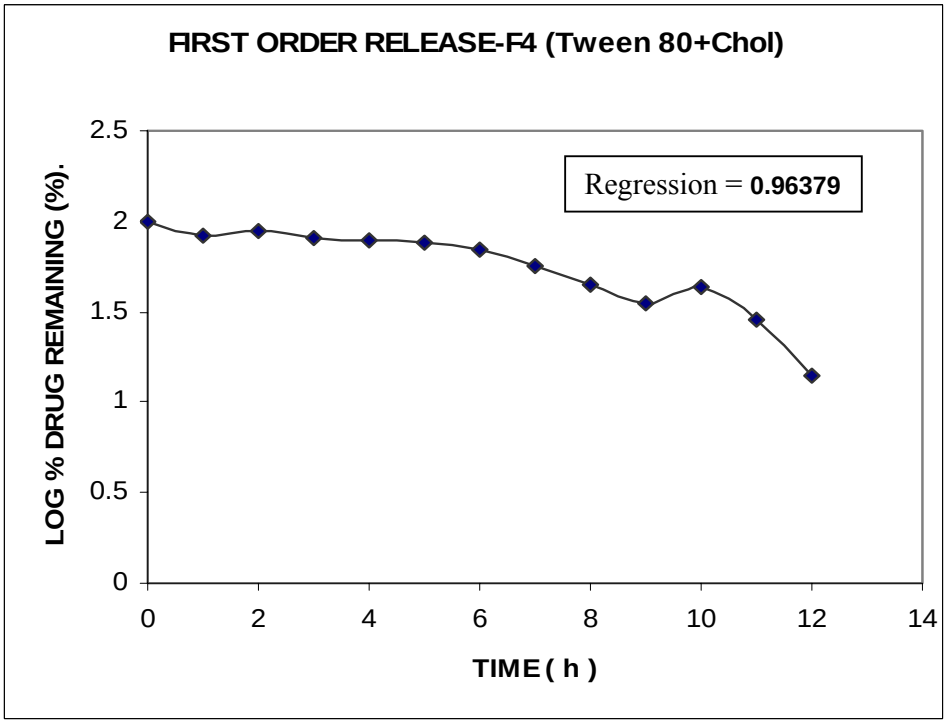

FIRST ORDER RELEASE-F4 (Tween 80+Chol)
LOG % DRUG REMAINING (%).
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
14
TIME (h)
Regression = 0.96379

Fig 32

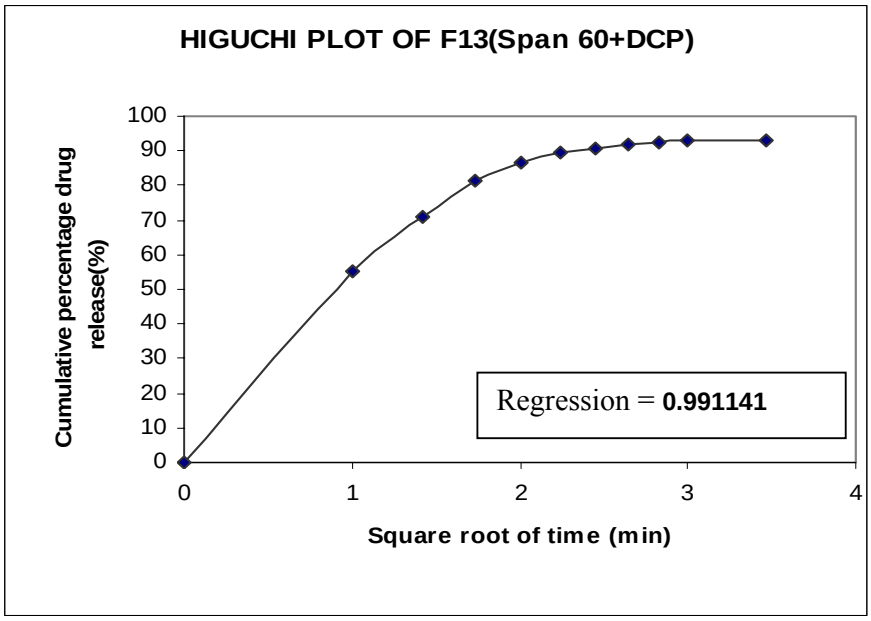

HIGUCHI PLOT OF F13(Span 60+DCP)
Cumulative percentage drug release(%)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
Square root of time (min)
Regression = 0.991141

Fig 33

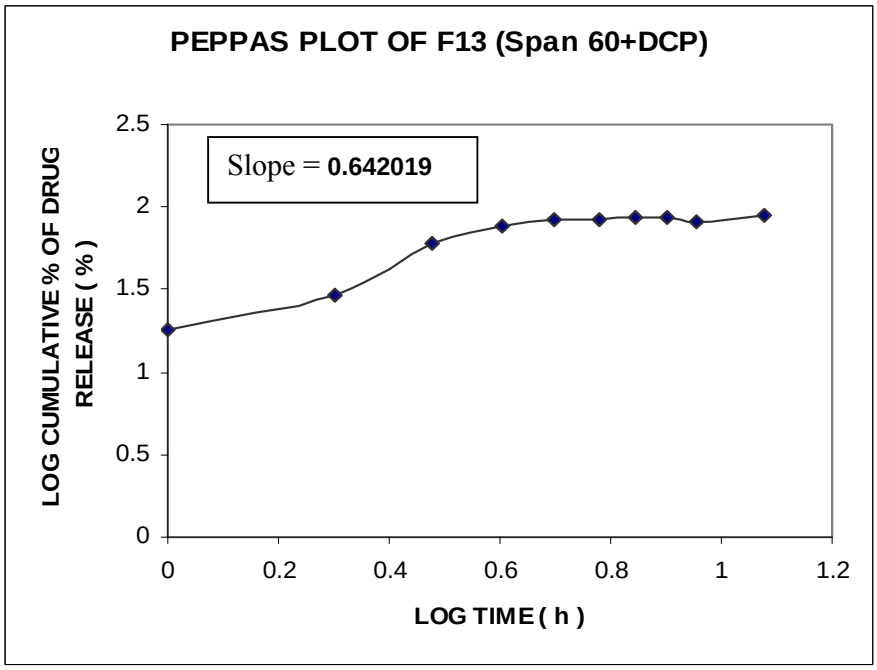

PEPPAS PLOT OF F13 (Span 60+DCP)
LOG CUMULATIVE % OF DRUG RELEASE (%)
2.5
2
1.5
1
0.5
0
0
0.2
0.4
0.6
0.8
1
1.2
LOG TIME (h)
Slope = 0.642019

Fig 34

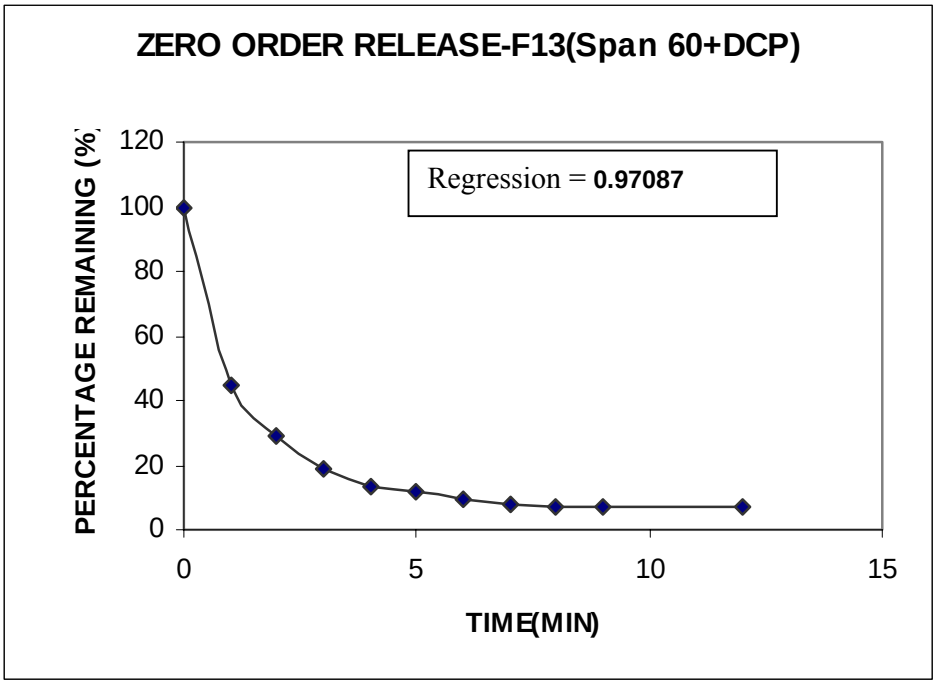
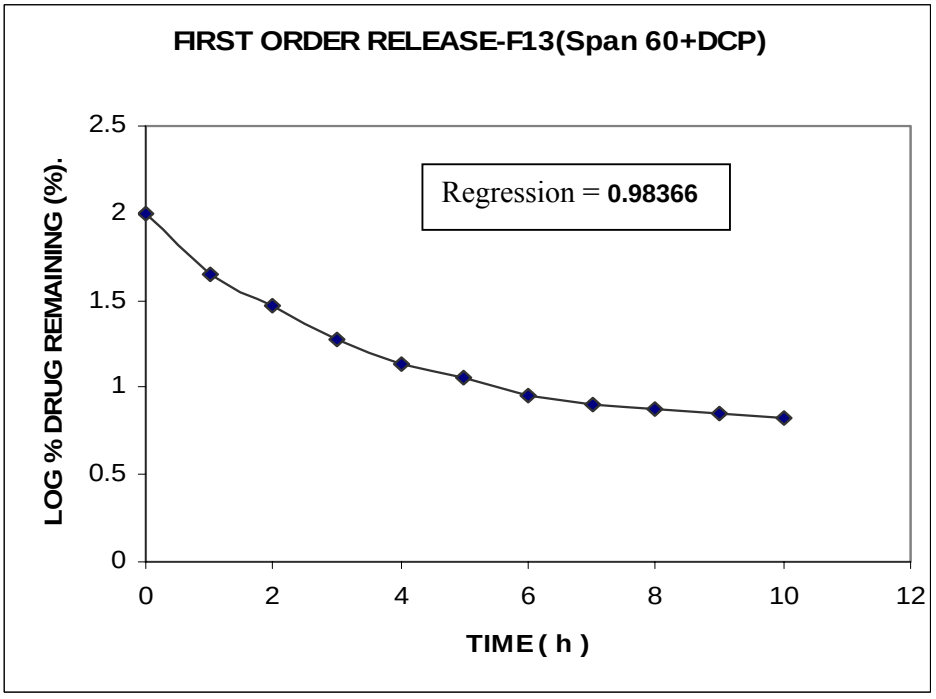

ZERO ORDER RELEASE-F13(Span 60+DCP)
PERCENTAGE REMAINING (%)
120
100
80
60
40
20
0
0
5
10
15
TIME(MIN)
Regression = 0.97087

Fig 35


FIRST ORDER RELEASE-F13(Span 60+DCP)
LOG % DRUG REMAINING (%).
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
TIME (h)
Regression = 0.98366

6. Stability studies[36]

Stability study was carried out at accelerated condition as per ICH guidelines with slight modification. Two ml of each formulation was taken in a 10 ml ampoule, sealed and kept at room temperature. Relative humidity of atmosphere was noted each day and it was found to be 65 ± 5% during study period. Formulations with cholesterol was studied for 90 days and formulations without cholesterol was studied for 60 days.

Parameters to be analyzed during storage

Turbidity, colour, pH, leakage rate and vesicle size

In accelerated condition, the products are usually stored up to 3 months. The sampling is done at short intervals of 15, 30, 60 and 90 days after storage.

The collected samples were subjected to analysis for above said parameters.

a. Colour

The change in colour of the formulations was visually analyzed by keeping the sample at the dark background (fig 36 and fig 37).

b. Turbidity

Percentage transmittance was analyzed to determine the turbidity nature of the formulation with different non-ionic surfactants. 100 µ litres of niosomes formulations were taken and diluted with PBS pH 7.4 in order to reduce the lipid concentration. After rapid mixing for 5 minutes, the percentage transmittance was measured as the absorbance at 276 nm with an ultraviolet – visible spectrophotometer. Higher the percentage transmittance shows less turbid the nature of formulation (table 24).

c. pH

pH changes on storage was measured using LABINDIA μp controlled pH analyser at each period interval (table 25).

d. Drug leakage from vesicles

Drug leakage from vesicles on accelerated storage condition was determined by analyzing the supernatant solution after ultra centrifugation at 13, 000 rpm for 90 minutes at 4^{o} C. Percentage of drug remaining vesicles were shown in table 26.

e. Vesicle size

Changes in the vesicle size on storage were determined at 40 X magnification using optical microscopy with pre-calibrated eye piece micrometer (table 27).

Fig 36: Colour observed in Tween formulations

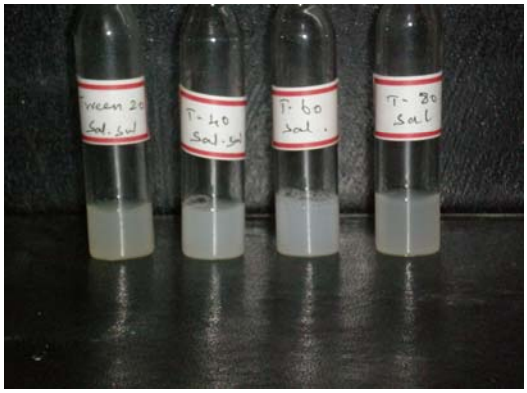


Fig 37: Colour observed in Span formulations

Table 24: % Transmittance on accelerated storage condition

S.No	Code	First day	After 15 days	After 30 days	After 60 days	After 90 days
1	F1	89.49	89.46	89.16	89.24	89.02
2	F2	92.22	91.78	91.64	91.62	91.36
3	F3	92.93	90.94	90.94	91.28	91.14
4	F4	92.16	88.37	88.46	90.32	90.28
5	F5	88.78	85.64	84.72	85.34	84.97
6	F6	90.43	86.96	87.02	89.42	89.24
7	F7	84.96	81.48	81.46	83.27	82.92
8	F8	89.83	86.14	85.89	87.74	87.53
9	F9	81.24	80.94	-	-	-
10	F10	82.16	81.56	-	-	-
11	F11	94.28	94.12	94.08	94.10	-
12	F12	92.64	92.38	92.26	92.24	-
13	F13	36.36	35.41	34.96	34.82	-

Table 25: pH on accelerated storage condition

S.No	Code	First day	After 15 days	After 30 days	After 60 days	After 90 days
1	F1	7.41	7.32	7.26	7.24	7.26
2	F2	7.08	6.98	6.96	7.02	7.00
3	F3	6.94	6.92	6.94	6.90	6.84
4	F4	7.15	7.01	7.12	7.06	6.98
5	F5	7.44	7.22	7.18	7.24	7.14
6	F6	7.33	7.16	7.10	7.12	7.16
7	F7	7.41	7.24	7.16	7.20	7.18
8	F8	7.19	7.08	7.01	7.0	6.92
9	F9	7.12	7.06	-	-	-
10	F10	6.96	6.84	-	-	-
11	F11	7.29	7.21	7.24	7.20	-
12	F12	7.48	7.32	7.30	7.24	-
13	F13	7.24	7.16	7.14	7.10	-

Table 26: Percentage drug remaining (leakage) in vesicles on accelerated storage condition

S.No	Code	First day	After 15 days	After 30 days	After 60 days	After 90 days
1	F1	81.4	75.2	72.4	63.3	59.4
2	F2	86.9	79.6	76.9	64.8	60.9
3	F3	89.6	80.4	84.2	71.3	67.4
4	F4	79.2	71.9	66.1	57.8	54.3
5	F5	87.7	81.2	76.9	64.3	59.9
6	F6	90.5	82.6	78.9	71.2	67.3
7	F7	90.0	83.4	79.6	72.6	68.4
8	F8	77.7	70.7	64.2	57.8	54.7
9	F9	64.8	61.2	-	-	-
10	F10	69.2	64.6	-	-	-
11	F11	76.1	72.3	68.2	61.6	-
12	F12	78.4	73.8	69.1	61.8	-
13	F13	89.6	87.2	84.8	82.7	-

Table 27: Vesicles size (µm) variations on accelerated storage condition

S.No	Code	First day	After 15 days	After 30 days	After 60 days	After 90 days
1	F1	3.04	3.21	3.26	3.20	3.25
2	F2	3.62	3.73	3.82	3.62	3.60
3	F3	2.75	2.92	3.12	3.00	3.15
4	F4	2.37	2.68	4.16	4.10	4.15
5	F5	4.12	4.31	4.42	4.32	4.3
6	F6	3.58	3.40	3.48	3.34	3.35
7	F7	3.12	3.36	3.41	3.26	3.25
8	F8	2.50	2.76	2.97	2.90	2.90
9	F9	3.91	4.12	-	-	-
10	F10	2.91	3.24	-	-	-
11	F11	5.25	5.48	5.64	5.5	-
12	F12	4.75	4.88	5.02	5.15	-
13	F13	2.95	3.28	3.42	3.4	-

7. Tissue distribution studies[32, 49]

Two best formulations were selected from the *in vitro* release data and the tissue distribution nature was compared with the drug in solution after administered to mice through intravenous route. Animal ethical committee clearance obtained from PSGIMSR (Registration number: 158/1999/CPCSEA; dated on 5th January 2009).

Male mice with weight of 20 – 25 g were divided in to 4 groups comprising of 3 animals each to carryout tissue distribution profile. Animal dose was calculated from human dose according to the animal body surface area with respect to weight of the animal[53]. Salbutamol sulphate equivalent to 50 – 55 µg were dosed intravenously via the tail vein with formulations. Group 1 receives formulation F4 (Tween 80 + CHOL), group 2 receives formulation F13 (Span 60 + CHOL + DCP), group 3 receives drug in solution and group 4 was treated as control (fig 38).

One hour after the injection of the drug, mice were sacrificed after placing in tank containing anesthetic ether dipped cotton swab. Blood was collected by cardiac puncture into tubes containing 30 µl of 10 % trisodium citrate. Plasma was separated from other blood components by centrifugation at 3000 rpm for 20 minutes and it was stored below zero degree temperature. Different organs like heart, lungs, liver, spleen and kidney were removed from each and washed with PBS pH 7.4 (fig 39).

The organs were homogenized with 4 ml of PBS pH 7.4 using tissue homogenizer and it was ultra centrifuged at 13, 000 rpm for 30 minutes. Then the clear supernatant was separated and kept at refrigerator until analysis.

Drug concentration in different organs and plasma were analyzed by HPLC technique. Drug was extracted from plasma and other organs by liquid-liquid extraction technique using ethyl acetate as solvent.

Calibration of standard curve

Accurately, about 10 mg of Salbutamol Sulphate was weighed and transferred to a 10 ml standard flask. The drug was dissolved in 5 ml of PBS pH 7.4 with shaking and then the volume was made up to the mark with PBS pH 7.4. Drug in stock solution was extracted with equal volume of ethyl acetate with vigorous shaking. Then the mixture was allowed to separate and the organic layer was completely evaporated at 100^{o} C. Then the residue was reconstituted with 10 ml of buffer which is considered as the standard stock solution containing 1000 µg/ml.

From the stock solution further dilutions was made to get different concentrations. Linearity was obtained in the concentration between 50 - 150 µg/ml. The standard samples were analyzed at 276 nm using HPLC with acetonitrle:water:glacial acetic acid:triethyl amine (55:45:01:0.1) as mobile phase. Retention time was found to be 4.2 minutes. Then the standard graph was plotted against concentration vs area (fig 40).

Analysis of samples

100 µl of plasma was taken and to this 1 ml of ethyl acetate was added, mixed well for 5 min and kept aside for 15 min for the separation of organic layer. The organic layer was separated using micropipette and allowed to evaporate at 100^{o} C to get the residue. The residue was then reconsituted with 1 ml of PBS pH 7.4. Buffer solutions were then injected into 250 x 4.6 mm, phenomenex Luna C18(2) 100-A column and eluted with an acetonitrle:water:glacial acetic acid:triethyl amine (55:45:01:0.1) mobile phase. The peak area was determined at 276 nm.

Drug present in the supernatant layer obtained from different organs (fig 39) was extracted with equal volume of ethyl acetate and analysis was performed as described above (Table 28 - Table 30).

The area obtained from the analysis of different organs was calibrated using the standard curve. Chromatogram was shown in fig 41 – fig 43.

Drug distributed in different organs were calculated using the area obtained from the HPLC analysis. Comparative drug distribution profile was shown in Fig 44.

Comparison between the drug distribution in lungs for Formulation F4 (Tween 80+Chol) , Formulation F13 (Span 60+DCP+Chol) and drug in solution was performed by analysis of variance (one way ANOVA with Turkey's multiple comparison post test) with graph pad prism (version 3.0)software.

Fig 38: Formulation administering through tail vein

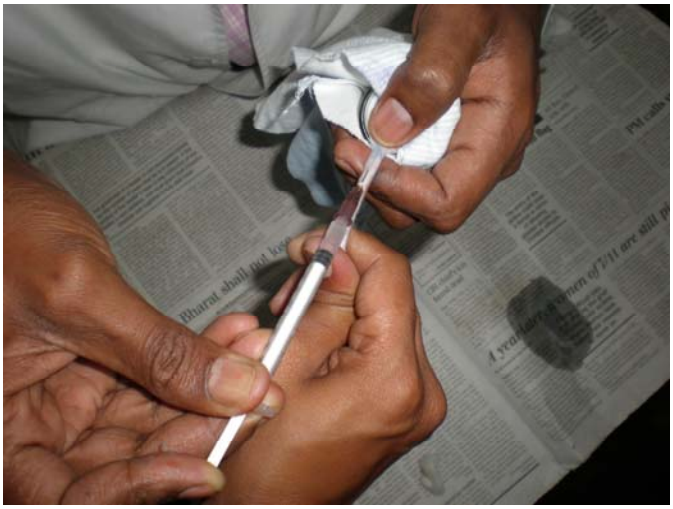


Fig 39: Different organs isolated from injected mice

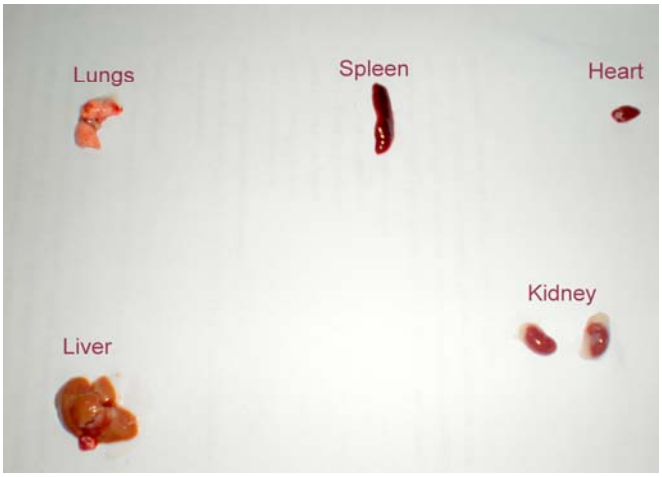


Fig 40: HPLC Calibration curve

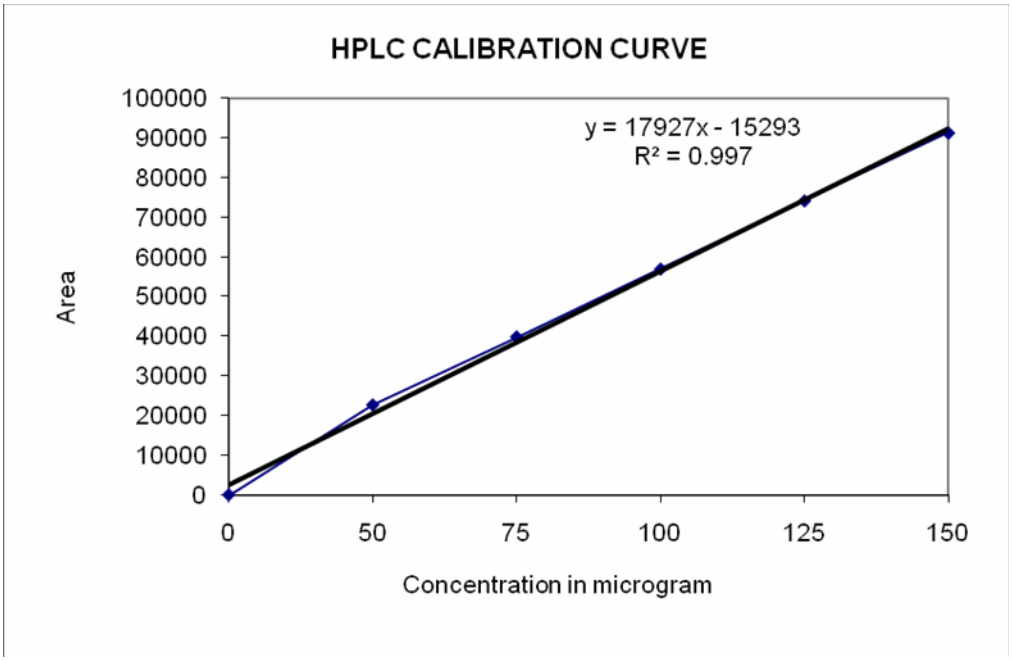


Table 28: Distribution of drug in different organs for F4 (Tween 80 + CHOL) [n=3]

Organs	Area	Concentration in µg/ml	Total concentration in 5 ml	% of drug in organs
LUNGS	21238	2.03776427	10.18882	14.17±0.83
LIVER	8162	1.30836169	6.541808	9.09±0.9
SPLEEN	10144	1.41892118	7.094606	9.86±1.2
KIDNEY	23292	2.15234005	10.7617	14.96±1.4
HEART	15771	1.73280527	8.664026	12.05±0.3
PLASMA	19240	1.92631227	9.631561	13.39±1.36

Table 29: Distribution of drug in different organs for F4 (Span 60 + CHOL + DCP) [n=3]

Organs	Area	Concentration in µg/ml	Total concentration in 2 ml	% of drug in organs
LUNGS	9501	1.38305349	2.766107	4.19±0.34
LIVER	10359	1.43091426	2.861829	4.33±0.6
SPLEEN	18055	1.86021086	3.720422	5.63±0.9
KIDNEY	8505	1.32749484	2.65499	4.02±0.76
HEART	14505	1.66218553	3.324371	5.03±0.7
PLASMA	113325	7.17454119	14.34908	21.74±1.8

Table 30: Distribution of drug in different organs for drug in solution [n=3]

Organs	Area	Concentration in µg/ml	Total concentration in 2 ml	% of drug in organs
LUNGS	15587	1.72254142	3.445083	4.64±0.46
LIVER	44725	3.34791097	6.695822	9.02±0.7
SPLEEN	0	0	0	0
KIDNEY	0	0	0	0
HEART	33766	2.73659843	5.473197	7.37±1.4
PLASMA	12133	1.52987114	3.059742	4.12±0.92

Fig 41: Chromatogram for F4 (Tween 80 + CHOL) formulation

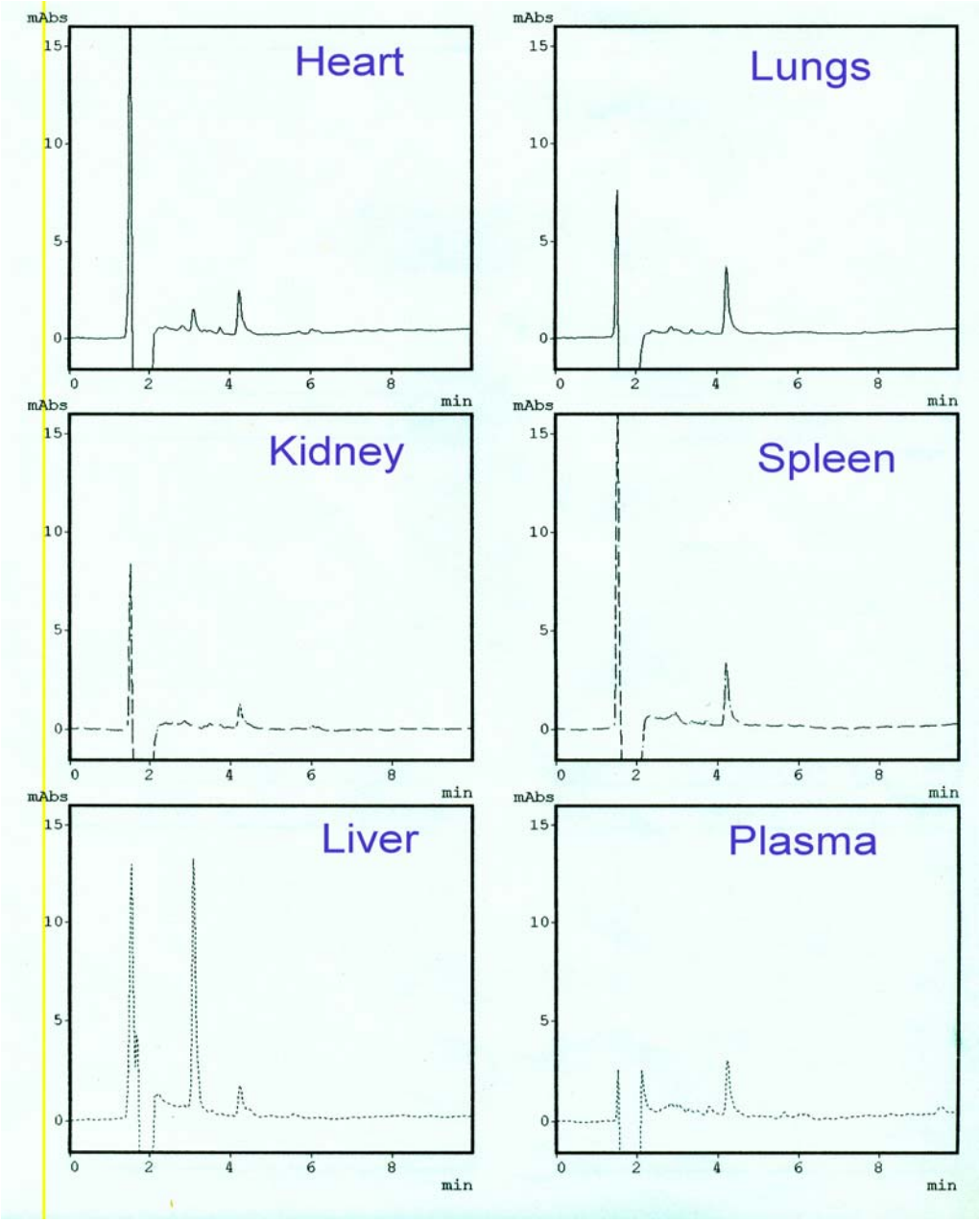


Fig 42: Chromatogram for F13 (Span 60 + CHOL + DCP) formulation

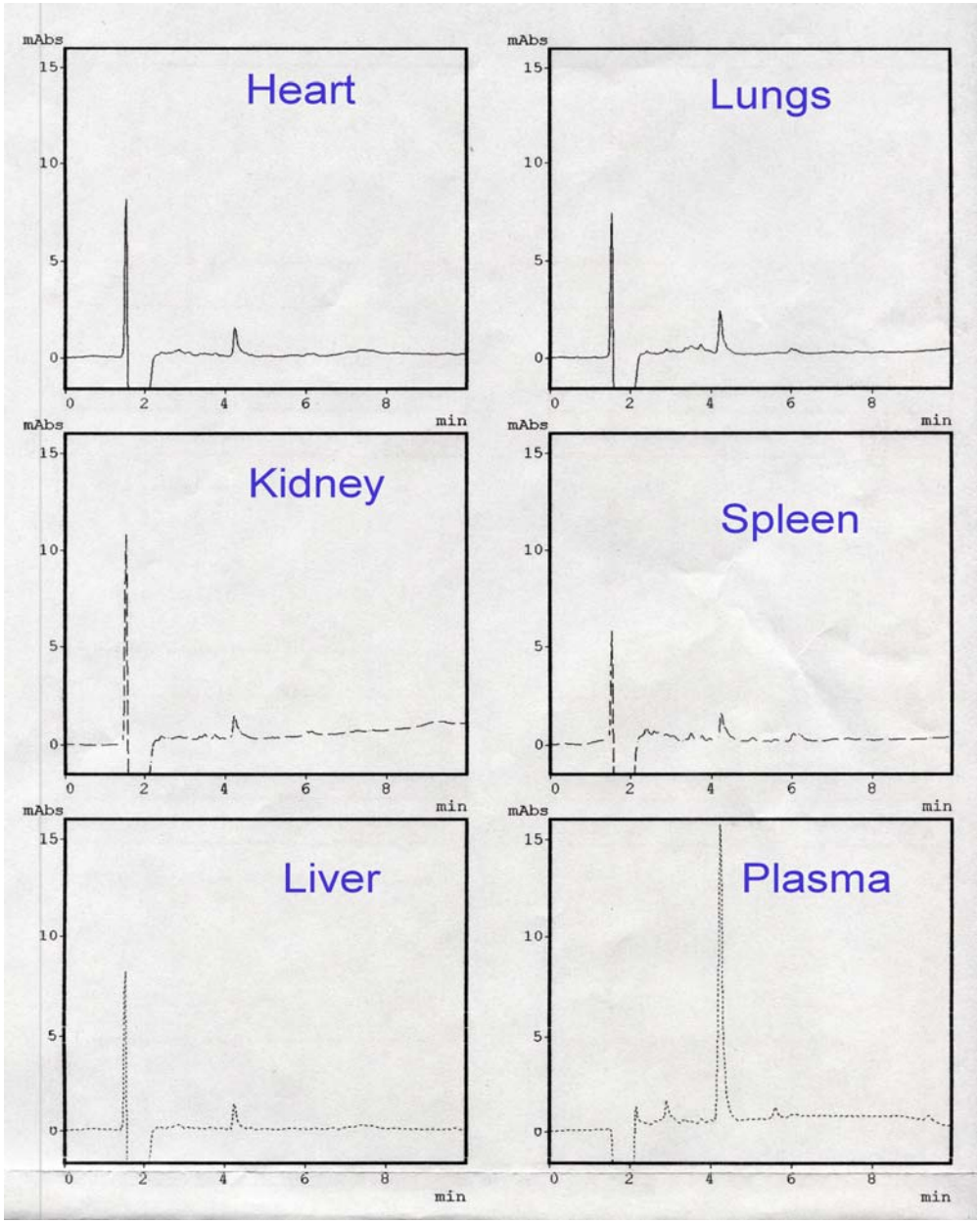


Fig 43: Chromatogram for drug in solution

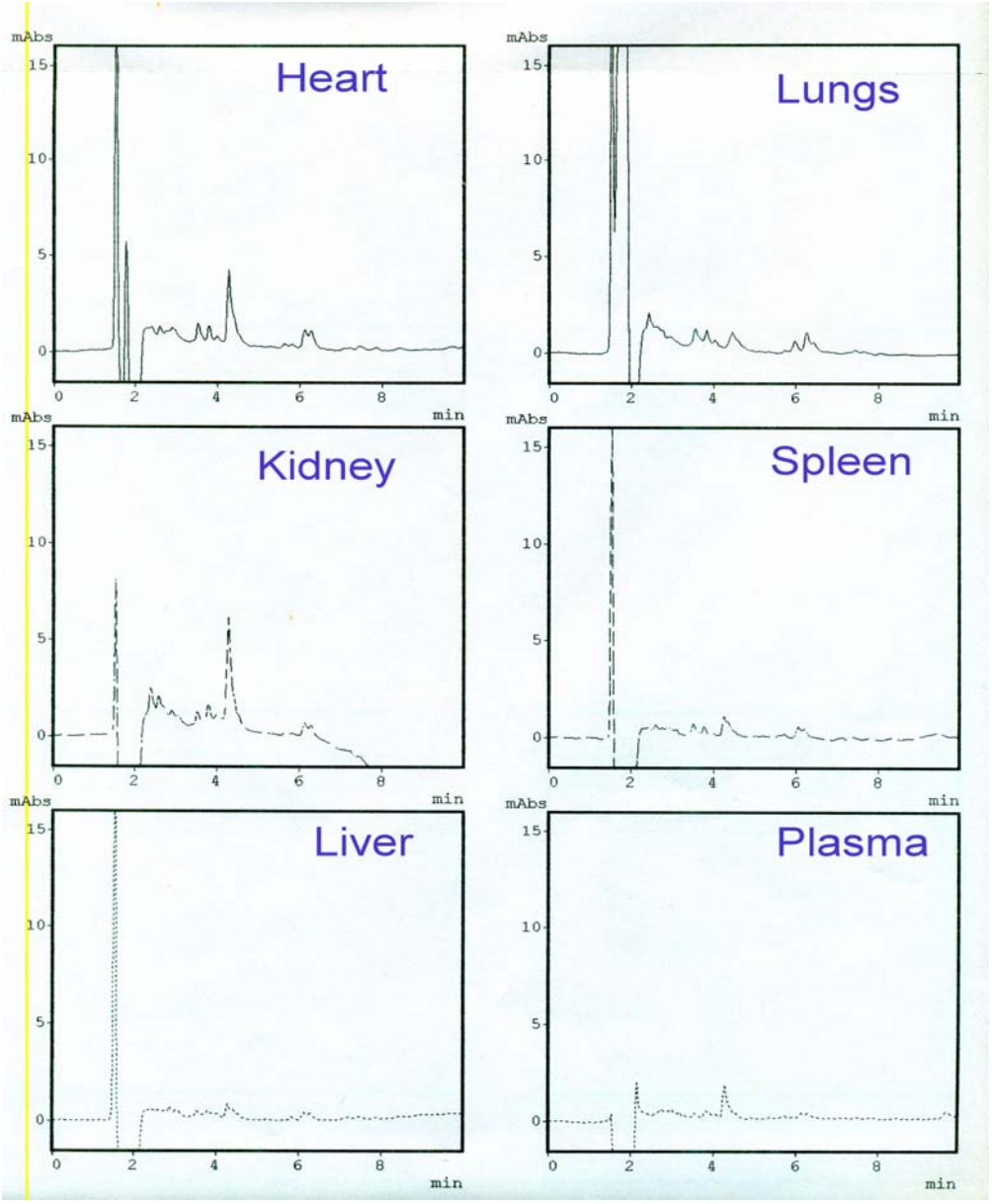
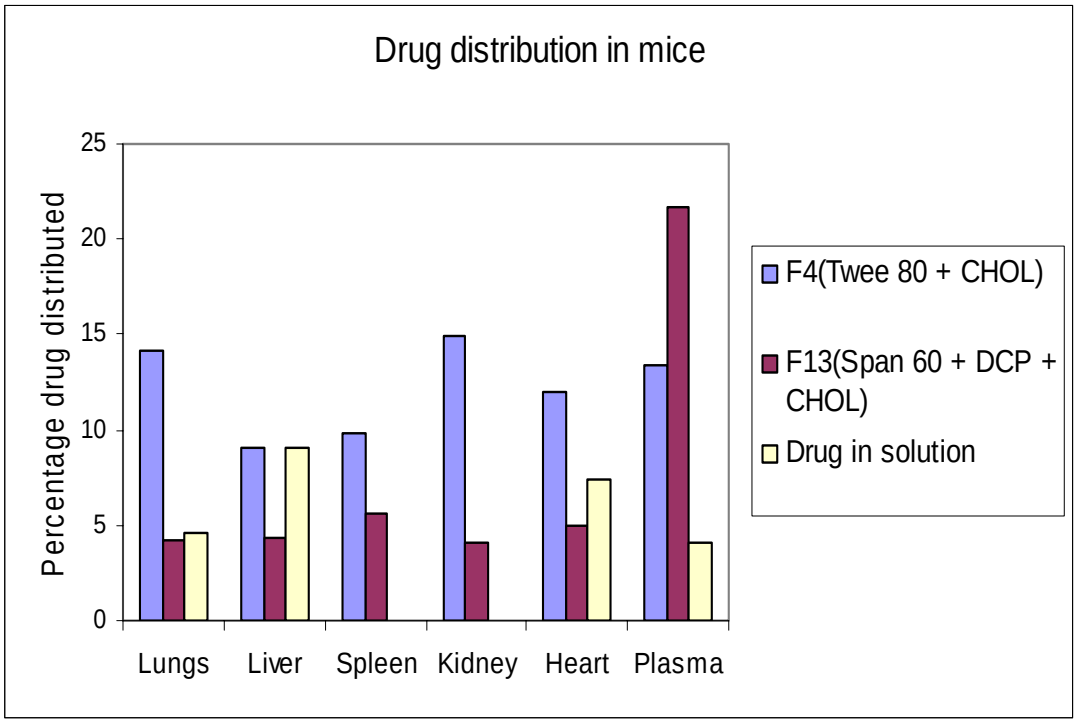


Fig 44: Comparative drug distribution in different organs and plasma



Pharmacokinetic evaluation

Pharmacokinetic evaluation was done using rabbits in order to determine the kinetics of Tween, Span with DCP niosomal formulations and drug in solution. Rabbits weighing around 1.5 to 2.0 kg were divided in to 3 groups each containing 3 animals. Drug equivalent to 1.12 – 1.5 mg was administered. Group 1 receives formulation F4 (Tween 80 + CHOL), group 2 receives formulation F13 (Span 60 + CHOL + DCP) and group 3 receives drug in solution. Drug was administered intravenously through ear vein. 0.5 ml of the blood was collected into the tubes containing 30 µl of 10 % trisodium citrate through ear vein puncture. Blood samples was collected every 30 minutes time interval up to 2 hours and every 1 hour interval up to 5 hours from the time of injection (fig 45).

Fig 45: Blood sample collection from ear vein

The collected blood samples were centrifuged at 3000 rpm for 20 min to separate the plasma. 100 µ litre of the plasma samples was taken and treated with ethyl acetate for extracting the drug from plasma. Organic layer separated and allowed to dry at 100^{o} C. The residue was then reconstituted with 1 ml of PBS pH 7.4 and analysed using HPLC as said in tissue distribution studies.

Concentration of the drug was calculated and the parameters such as maximum concentration (Cmax), time maximum to reach maximum concentration (Tmax), rate of elimination (Kel), half-life ($t_{1/2}$), area under the curve (AUC), area under the first moment curve (AUMC) and mean residence time (MRT) were determined using the formulas [54]. Data's were given in table 31.

Table 31: Pharmacokinetic data's

S.No	Parameter	Results	
		F4 (Tween 80+CHOL)	F13 (Span 60+CHOL+DCP)
1	C_{max}	3.36 µg/ml	2.36 µg/ml
2	T_{max}	1 hr	1 hr
3	K_{el}	1.99 hrs^{-1}	0.621 hrs^{-1}
4	$t_{1/2}$	0.35 hrs	1.1 hrs
5	AUC_{0-t^*}	3.82 µg. hr/ml	4.98 µg. hr/ml
6	$AUC_{0^*-\alpha}$	4.44 µg. hr/ml	7.76 µg. hr/ml
7	$AUMC_{0-t^*}$	10.76 µg. hr^2/ml	4.291 µg. hr^2/ml
8	$AUMC_{0^*-\alpha}$	12.63 µg. hr^2/ml	17.139 µg. hr^2/ml
9	MRT	2.84 hrs	2.2 hrs

CHAPTER - VII

RESULT AND DISCUSSION

Selection of analytical wavelength & calibration of standard curve

The diluted stock solution was scanned for λ_{max} and it was found to be 276 nm (fig 1). In standard graph linearity was obtained between 20-200 µg/ml concentration of Salbutamol Sulphate and the regression value was found to be r^2=0.99957

Preformulation and optimization of formulation

According to some reports (S.Baskaran et al., 2001; Himanshu M. Varshney et al., 2007) non-ionic surfactant can form bilayer vesicles in the ratio of 1:1 (CHOL:Surfactant).

We found that the thin film of cholesterol and surfactant in the ratio 1:1 did not form vesicles with Salbutamol Sulphate. Table 2 shows the vesicle formation nature of different ratios of Non-ionic surfactant Tween 80. 20:90 µ molar ratio of cholesterol:Tween 80 shows the spherical vesicle formation without any crystals and found to be stable more than 150 days on refrigerated condition.

Preparation of niosomes

Niosomes were prepared by thin film hydration technique. Formulations were made with and without the addition of cholesterol. It was also confirmed that non-ionic surfactants (Tween and Span) are capable of forming vesicles any concentration without the addition of cholesterol.

Formulation was also tried with the addition of drug to the cholesterol and non-ionic surfactant which is dissolved in the chloroform. Since Salbutamol Sulphate is insoluble in chloroform which leads to the distribution of drug particles throughout the thin film layer of cholesterol and surfactant mixture. On hydration it shows no proper vesicle formation. This result states that, drugs which are freely soluble in water can be dissolved in hydration medium for the better vesicle formation.

Vesicle size, photomicrographs and SEM

M. Marconi et al., (2002), studied the influence of the loaded drug on vesicle size. Both empty and drug loaded vesicles shows difference in the vesicle size.

In our study, drug loaded vesicles was found to be bigger in size (table 5) when compared to empty vesicles. This confirms that the drug has been incorporated into vesicular system.

The vesicle size in the formulations without cholesterol was bigger size when compared to formulations with cholesterol (table 5). It may be due to the accommodation of cholesterol in the molecular cavities of the surfactant. Inclusion of DCP has not increased the vesicle size significantly.

Gopi N. Devaraj (2002) reported that cholesterol alters the fluidity of chain in bilayer of non-ionic surfactants and abolishes the bilayer and surface pressure of surfactants/cholesterol mixture thereby causes decrease in the vesicle size.

Vesicle size was found to be greater in span formulations when compared to Tween.

Vesicles seen through optical microscopy were photographed using Olympus 8 mega pixel camera. It proves that thin film hydration technique results in the multi-lamellar vesicles (fig 15).

Bath sonication was carried out for 2 minutes. Multi-lamellar vesicles get converted to uni-lamellar vesicles which make the vesicle size less than a micron.

SEM shows the spherical nature of the vesicles. The size was found to be more than 10 μm (Fig 19). It shows much variation with the vesicle size with respect to size determined through optical microscopy (table 5). The difference may be due to the aggregation of vesicles while drying at 20^{o} C before coating with platinum. The loss of water molecules in the vesicles leads to the aggregation with the adjacent vesicles

and forms bigger vesicles.

Osmotic shock

The addition of KI in the formulation containing DCP causes shrinkage due to the K^+ counter ion interaction with the negative charge of DCP (D. Gopinath et al., 2004). But in our study it was observed that the formulation with DCP as well without DCP shows shrinkage in vesicles when incubated with KI at 37^o C. It proves that, shrinkage of vesicles was not due to the interaction of K^+ ion with negative charge in DCP.

In the formulations without cholesterol vesicles was found to be low in number when compared to formulation with cholesterol on incubation with hypertonic medium (KI). After 3 hours of incubation, the vesicles found to disappear in formulations without cholesterol. This confirms the membrane stabilizing nature of cholesterol.

Formulations incubated with 0.9% NaCl (Saline) shows no much vesicle difference (Table 7). It indicated that, the niosomal formulations can be very well diluted with normal saline for the parentral use.

Formulations incubated with 0.5% NaCl (Hypotonic medium) shows higher increase in vesicle size when compared to formulation incubated at normal saline (table7). Vesicle size difference of formulation without cholesterol, incubated in hypotonic medium was found to be higher when compared to formulations with cholesterol. This result further confirms the stabilizing nature of cholesterol.

Viscosity

Presence of cholesterol in the formulation shows difference in the viscosity.

Formulations with cholesterol found to be less viscous when compared to formulations without cholesterol (Table 8).

Tween 60 viscosity was found to be high and turbidity is low. Span 60 has less viscosity showing more turbidity. This shows that high viscosity reduces the fusion or aggregation of vesicles during storage thereby producing minimal turbidity. The other Tween and Span shows no correlation between the viscosity and turbidity.

Entrapment efficacy

Entrapment efficacy was found to high in span 60 formulation. In both Tween and Span formulations, surfactant containing stearate fatty acid entraps more amount of drug when compared to surfactant containg Oleate fatty acid.

In Span formulations, entrapment was low in least HLB value (Span 80; HLB - 4.3) surfactant and at the same time the highest HLB value (Span 80, HLB – 8.6; Span 40, HLB – 6.7) surfactant entraps less amounts of drug. This result explains that the highest and lowest lipophillic nature of Span does not show good entrapment but the intermediate lipophillicity (Span 60, HLB – 4.7) confirms the best entrapment of drug (table 8).

Our results was also similar to M.Manconi et al., (2002) in which Sorbitan monostearate (C18) always shows the high entrapment efficiency with respect to other formulations.

In Tween formulations, surfactant with low HLB value (Tween 60, HLB – 14.9) shows best entrapment when compared with high HLB value surfactants (Tween 20, HLB – 16.7; Tween 40, HLB – 15.6; Tween 80, HLB – 15.0)

SPAN Index

Size distribution profile is shown in table 10. Small SPAN indicates a narrow

size distribution. Formulation F9 (Tween 40) and F12 (Span 60) shows minimum SPAN value which confirms the uniform size distribution of vesicles.

***In vitro* release**

In vitro release from pure drug shows release for 5 hours but the formulations with cholesterol extends the drug release upto 12 hours (Fig 26 and Fig27).

Tween and Span formulations with cholesterol shows release upto 12 hours but the formulations without cholesterol has a release of 7-9 hours (fig 26 and fig 27). This may be due to the adsorption of drug in the lipophillic region of niosomes. Hence during *in vitro* release with dialysis membrane, the adsorbed drug comes out fastly moreover the amount of drug released is also very low (50 – 79 %) in the formulations without cholesterol and high (upto 86 %) in formulations with cholesterol (fig 26 and fig 27). This result confirms that, presence of cholesterol in the bilayer alters the rate of release of Salbutamol Sulphate.

Span 60 formulation with DCP shows 89% of drug release which is higher than the span 60 formulation without DCP (fig 27). Thus presence of DCP in the formulation also alters the release of drug.

Eventhough the Span 60 formulation has greater entrapment efficiency, Tween 80 formulation shows maximum amount of drug release. It may be due to the fatty acid chain length difference in the polyoxy ethylene sorbitan esters. The maximum release in Tween 80 may be due to the presence of unsaturated alkyl chain. In Tween formulations it was conformed that higher the alkyl chain length of surfactant shows lesser the release. The release from Tween formulations can be ranked has Tween 80 > Tween 20 > Tween 40 > Tween 60 which is having the alkyl chain length of $C_{9=9}$, C_{12}, $C_{16,}$ C_{18} respectively (fig 26). The unsaturated bond present in the Tween 80 increases the fluidity & permeability of the drug.

The release from Span formulations can be ranked as Span 40 > Span 20 > Span 80 > Span 60. It shows no correlation between the alkyl chain length and release of drug.

Drug release kinetics

Drug release kinetics was derived for two best formulations from the *in vitro* profile. Result from Higuchi plot shows that both Tween 80 and Span 60 formulation follows diffusion mechanism where the regression value was above 0.95 (fig 28 and fig 33)

Slope value for formulation F4 (Tween 80 + CHOL) from Peppas plot was found to be less than 0.5 (Slope - 0.112; fig 29), which confirms that formulation F4 follows Fickain's diffusion mechanism. But in formulation F13 (Span 60 + DCP), the value was above 0.5 (Slope - 0.642; fig 33) which confirms Non-Fickian diffusion mechanism.

Regression value of zero order and First order kinetics for both formulations were above 0.9 (fig 30, fig 31, fig 34 & fig 35). Thus it was confirmed that both formulations follow mixed order release kinetics.

Stability Studies

Stability of niosomes was evaluated for all the formulations. No change in colour was observed on 90 days storage at room temperature (approximately 28 ± 3^{o} C; RH – 65 ± 5 %)(fig 36 & fig 37).

J.Y. Fung et al., (2001), reported that the presence of DCP shows higher turbidity value due to the high degree of hydrophilicity & also increase in the vesicle size.

In our study also, inclusion of DCP shows higher turbid value & increase in the vesicle size (Table 24).

Stability result states that cholesterol also increases the turbidity of the formulations. There is no much difference in the turbidity value of the formulations during storage (table 24).

There is no much difference in the pH was observed during storage (table 25).

Drug leakage from vesicles was observed in all the formulations. Leakage was associated with the increase in vesicle size (table 26 & table 27). Leakage was found to be more in formulations without cholesterol (table 26).

These results confirm that formulations are not stable on accelerated storage and it should be stored only in refrigerated condition.

Bath sonicated vesicles were kept for stability assessment. The result obtained on 15^{th} day shows that vesicles are in micron range (table 27) which can be measured using optical microscopy. It confirms that, formulation on storage leads to the aggregation of vesicles. This shows that bath sonication for 2 min converts multi-lamellar vesicles to uni-lamellar vesicles for a shorter period only.

After 30 days storage, vesicles was disappeared in Tween formulations without cholesterol but the Span formulation without cholesterol was found to be stable up to 60 days (table 27).

Gradual increase in the vesicle size associated with leakage of drug from vesicles was observed. The formulation without cholesterol shows more increase in size when compared to formulations with cholesterol and drug leakage was higher in formulations without cholesterol (Table 27).

Vesicle size was found to be increasing up to 60 days and 90^{th} day result shows that no much difference in size when compared previous data. This shows that aggregation of vesicles occurs up to certain period of time.

Tissue distribution studies

Tissue distribution of drug in organs was compared for formulations and drug in solution. Formulation F4 (Tween 80 + Chol) shows three folds increase in drug distribution to lungs (table 28 – table 30) when compared to Formulation F13 (Span 60 + DCP) and drug in solution. In formulation F4 (Tween 80 + Chol), drug reaching to liver was less than the amount of drug distributed in lungs. Thus the metabolite product of salbutamol sulphate i.e. inactive 4'-O-sulphate, will be minimum in niosomal formulation.

In formulation F4 (Tween 80 + Chol), uptake of salbutamol sulpahte by β_1 receptor was less than that of the lungs. But the formulation containing span 60 + DCP and drug in solution shows more distribution of drug in heart with that of lungs (fig 44). Thus it can be concluded that the undesirable effects produced by the stimulation of β_1 receptor can be reduced in niosomal formulations. Drug in plasma was found to be higher in Span 60 when compared to Tween 80 formulation and drug in solution.

Formulation F4 (Tween 80 + CHOL) show better distribution to specific organs when compared to drug in solution. This confirms the targeting nature of the niosomal formulation.

Pharmacokinetic studies

Kinetics between the F4 (Tween 80 + Chol) and F13 (Span 60 + DCP) formulations were determined. Drug in solution injected to rabbit via IV route does not show any concentration of salbutamol sulphate after 30 minutes. It may due to the rapid excretion of drug when given through IV. Even though the excretion was found to be high, niosomal formulation shows drug concentration in plasma upto 90 minutes (table 31). Maximum concentration was found in F4 (Tween 80 + Chol) formulation. Even the concentration is high, the half life was found to be very low in F4 when

compared to F13. Rate of elimination was found to be less in Span 60+DCP formulation.

From the data's (table 31) obtained from pharmacokinetic studies it was proved that the concentration and mean residence time is more for Tween 80+Cholesterol formulation and also the distribution of drug was more in F4 (Tween 80+Chol) formulation.

Level of significance of Tween 80 Vs Span 60 formulation, Tween 80 Vs Drug in solution and Span 60 Vs Solution was found to be $p<0.001$, $p<0.001$ and $p>0.05$ respectively. The value shows that formulation F4 (Tween 80 + CHOL) was found to be the best.

CONCLUSION

Stable noisome formulation were prepared by thin film hydration technique using Tween 20, 40, 60, 80 and Span 20, 40, 60, 80 in the ratio of 20:90 (cholesterol:surfactant). Drug entrapment was found to be high in Span 60 and Tween 60 formulations. Good *in vitro* release was found to be in Tween 80+CHOL and Span 60+DCP+CHOL formulations. Tween 80 formulation shows three fold increased drug concentration in lungs and found to have higher plasma concentration as well as mean residence time.

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