TRANSFERSOMES AS A CARRIER FOR TRANSDERMAL DELIVERY OF ACARBOSE

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In partial fulfillment for the degree of

MASTER OF PHARMACY

IN

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Submitted by

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Under the guidance of

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CERTIFICATE

This is to Certify that this dissertation entitled **"TRANSFERSOMES AS A CARRIER FOR TRANSDERMAL DELIVERY OF ACARBOSE" by V.Dinesh Kumar** for the award of "**Master of Pharmacy"** degree, comprises of the bonafide work done by him in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli, under my supervision and guidance and to my full satisfaction.

Place: Tiruchirappalli

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I recommend this research work for acceptance as project for the partial fulfillment of the degree of "**Master of Pharmacy"** of the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli, for the year March 2008.

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CONTENTS

1. INTRODUCTION

NOVEL DRUG DELIVERY SYSTEM

[1]For many decades treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms including tablets, capsules, pills, suppositories, creams, ointments, liquids, aerosols and injectables, as drug delivery systems are the primary pharmaceutical products commonly seen in the market, even though these drug delivery system ensure a prompt release of drug, it is necessary to take this type of drug several times a day to achieve as well as to maintain the drug concentration with in the therapeutically effective range needed for the treatment. This results in significant fluctuations in drug level.

In the past two and a half decades several advancements have been made. They have resulted in the development of new techniques for drug delivery. These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and targeting the delivery of drug to a cell or tissue. Recently pulsatile drug delivery system is gaining importance.

These advancements have led to the development of several novel drug delivery systems that could revolutionalise the method of medication and provides a number of therapeutic benefits.

[2]Novel drug delivery system can be broadly divided into two classes:

1. Sustained release drug delivery system.
2. Controlled release drug delivery system.

SUSTAINED RELEASE DRUG DELIVERY SYSTEM:

Sustained release drug delivery system is described as a pharmaceutical dosage form formulated to retard the release of a therapeutic agent such that its appearance in the systemic circulation is delayed and/or prolonged and its plasma profile is sustained in duration. The onset of its pharmacological action is often delayed and the duration of its therapeutic effect is sustained (e.g. coated granules).

CONTROLLED RELEASE DRUG DELIVERY SYSTEM:

Controlled release drug delivery system has a meaning that goes beyond the scope of sustained drug release. It implies a predictability and reproducibility in the drug release kinetics. The release of drug ingredients from a controlled release drug delivery system proceeds at a rate profile that is not only predictable kinetically but also reproducible from one unit to another.

Controlled release drug delivery system can be classified into four categories:

1. Rate-Preprogrammed drug delivery system.
2. Activation-Modulated drug delivery system.
3. Feedback-Regulated drug delivery system.
4. Site-Targeting drug delivery system.

[1] RATE-PREPROGRAMMED DRUG DELIVERY SYSTEM

In this system, the release of drug molecules from the drug delivery system has been preprogrammed at specific rate profiles. This was achieved by system designing which controls the molecular diffusion of drug molecules in and/or across the barrier medium with in or surrounding the delivery system. (e.g.) implants, transdermal system[3].

[2] ACTIVATION – MODULATED DRUG DELIVERY SYSTEM

The release of the drug molecule from this delivery system is activated by some physical, chemical or biochemical process and/or facilitated by the energy supplied externally. The rate of drug release is then controlled by regulating the process applied or energy input.

Based on the nature of the process applied or the type of energy used, these activation modulated drug delivery system can be classified in to three categories:

1. Physical–e.g.: Osmotic pressure activated drug delivery system-
   osmotic pump[4], iontophoresis activated drug delivery system
2. Chemical- e.g.: pH activated drug delivery system[6]
3. Biochemical-e.g.: Enzyme activated drug delivery system[7]

[3] FEED BACK –REGULATED DRUG DELIVERY SYSTEM

The release of the drug molecule from the delivery system is activated by a triggering agent, such as biochemical substance in the body and regulated by its concentration viz. some feed back mechanisms. The rate of drug release is then controlled by the concentration of triggering agent detected by a sensor in the feed back regulated mechanism. E.g. bio-responsive drug delivery system, glucose triggered insulin delivery system[8].

[4] SITE-TARGETING DRUG DELIVERY SYSTEM

In this system the drug molecules are circumventing the other tissues and moving towards the specific diseased site and get released. This will enhance the therapeutic effectiveness and reduces the toxicity to other healthy tissues and improve the treatment spectrum e.g. Niosomes, Microspheres.

MERITS OF NOVEL DRUG DELIVERY SYSTEM

1. Improved treatment of many chronic illness where symptom break through occurs when the plasma level of drug drops below the minimum effective level.(e.g.) asthma, arthritis

2. Increased bioavailability

3. Reduction in the incidence and severity of untoward systemic side effects related to high peak plasma drug concentration.

4. Maintenance of the therapeutic action of a drug during overnight; no dose periods.

5. Reduction in the total amount of drug administered over the period of drug treatment. This contributes to the reduced incidence of systemic and local side effects.

6. Production from first pass metabolism and gastro- intestinal tract degradation.

7. Improved patient compliance resulting from the reduction in the number and frequency of doses required to maintain the desired therapeutic response.

8. Targeting the drug molecule towards the tissue or organ reduces the toxicity to the normal tissues

9. Pulsatile and pH dependent systems release the drug whenever the body demands.

10. Biocompatibility & Economic saving are claimed to be made from better disease management achieved with this system.

LIMITATIONS OF NOVEL DRUG DELIVERY SYSTEM

Though there are so many advantages in this system there are few factors that limit its usage.

1. Variable physiological factors such as gastro intestinal pH enzyme activities, gastric and intestinal transit rates, food and severity of patients disease which often influence drug bioavailability of conventional dosage forms may also interfere which the precision of control release and absorption of drug from this system.

2. The products which tend to remain intact may become lodged at some sites. If this occurs slow release of drug from the dosage form may produce a high-localized concentration of drug, which causes local irritation.

3. Drugs having biological half-life of 1 hr or less are difficult to be formulated as sustained release formulations. The high rate of elimination of such drugs from the body needs an extremely large maintenance dose which provides 8-12 hrs of continuous therapy.

4. These products normally contain a large amount of drug. There is a possibility of unsafe over dosage, if the product is improperly made and the total drug contained there in is released at one time or over too short time of interval.

5. If it is once administered it may be difficult to stop the therapy for reasons of toxicity or any other.

6. It may be unwise to include potent drugs in such systems.

TARGETED DRUG DELIVERY SYSTEM (TDDS)

THE CONCEPTS OF TARGETING

- Targeted drug delivery as an event where, a drug-carrier complex/conjugate, delivers drugs exclusively to the pre-selected target cells in a specific manner.
- Targeted drug delivery implies for selective and effective localization of pharmacologically active moiety at pre-identified target in therapeutic concentration, while restricting its access to non-target normal cellular linings, thus minimizing toxic effects and maximizing therapeutic index.

RATIONALE OF DRUG TARGETING

The site specific targeted drug delivery negotiates an exclusive delivery to specific pre-identified compartments with maximum intrinsic activity of drugs and concomitantly reduced access of drug to irrelevant non-target cells. The targeted delivery to previously in-accessible domains, e.g. intracellular sites, virus, bacteria and parasites offers distinctive therapeutic benefits.

The controlled rate and mode of drug delivery to pharmacological receptor and specific binding with target cells; as well as bioenvironmental protection of the drug en route to the with of action are specific features of targeting. Invariably, every event stated contributes to higher drug concentration at the site of action and resultant lower concentration at non-target tissue where toxicity might crop-up. The high drug concentration at the target site is a result of the relative cellular uptake of the drug vehicle, liberation of drug and efflux of free drug from the target site.

Targeting is signified if the target compartment is distinguished from the other compartments, where toxicity may occur, and also if the active drug could be placed predominantly in the proximity of target site.

The restricted distribution of the parent drug to the non-target site with effective accessibility to the target site could maximize the benefits of targeted drug delivery.

- [9]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 sub cellular 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 therapeutic index of the drugs.
- Earlier work done between late 1960s and the mind 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 of metabolism in liver or clearance by the kidneys. These approaches generally were not focused to achieve site specific or targeted delivery such as getting a cyto-toxic 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 year.
- 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.
- [10]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, niosomes, microshpheres, nanoparticles, 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 systems 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 content. The ideal drug delivery systems delivers drug at a rate.

1. Nanoparticles[11, 12]

Nanoparticles are one or several types of systems known collectively as colloidal drug delivery systems. Also included in this group are microcapsules, nanocapsules, macro molecular complexes, polymeric beads, micropsheres and liposomes.

A nanoparticle is a particle containing dispersed drug with a diameter of 200 to 500 nm. Materials used in the preparation of nanoparticles are sterilisable, non-toxic and biodegradable. They usually are prepared by a process similar to the coacervation method of micro encapsulation. Nanoparticles are also called as nanospheres or nanocapsules depending upon whether the drug is in a polymer matrix or encapsulated in a cell. The polymers used are the usual bio degradable ones. The main advantage of this system is that it can be stored for up to one year and can be used for selective target via reticuloendothelial system to liver and to cells that are active phagocytically.

2. Niosomes

Nonionic surfactant vesicles known as niosomes are used as carriers to delivery drugs to target organs and modify drug disposition.

Niosomes are found to improve therapeutic efficacy of drugs in cancer therapy, parastic, viral and microbial diseases. Many non- ionic surfactants like cetrimide, sodium dodecyl sulphate are used with cholesterol to entrap drugs in vesicles.

Livers can act as a depot for many drugs where niosomes containing drug may be taken up by the liver where they are broken down by lysosomal lipase slowly to release the free drug to the circulation. Niosomes slowly degraded providing a more sustained effect.

Niosomes are capable of releasing entrapped drug slowly. Niosomes are found to have selective drug delivery potential for cutaneous application of 5- α – dihydro testerione triamcinolone acetamide and intravenous administration of methotraxate for cancer treatment and sodium stilbogluconate in the treatment of lishmaniasis etc.

3. **Resealed Erythrocytes**

When erythrocytes are suspended in a hypotonic in a hypotonic medium, they sell to about one and half times their normal size and the membrane ruptures resulting in the formation of pores with diameters of 200- 5000A^0. The pores allow equilibration of the medium then is adjusted to iso-tonicity and the cells are incubated at 37^0C, the pores will close and cause the erythrocytes to reseal. Using this technique with a drug present in the extra cellular solution, it is possible to entrap up to 40% of the drug inside the resealed erythrocyte and to use this system for targeted delivery via intravenous injection.The advantage of using resealed erythrocytes as drug carrier is that they are biodegradable, fully biocompatible and non -immunogenic, exhibit flexibility in circulation time depending on their physiochemical properties, the entrapped drug is shielded from immunologic detection and chemical modification of drug is not required. Resealed erythrocytes can be targeted selectively to either the liver or spleen, depending on their membrane characteristics.

The ability of resealed erythrocytes to deliver drugs to the liver or spleen can be viewed as a disadvantage in that other organs and tissues are inaccessible.

4. Microspheres

Microspheres are free flowing powders consisting of spherical particles of size ideally less than 125 microns that can be suspended in a suitable aqueous vehicle and injected. Each particle is basically a matrix of drug dispersed in polymer from which release occurs by a first order process. The polymers used are biocompatible and biodegradable ex. Polylactic acid, poly lactidecoglycolide etc. Drug release is controlled by dissolution / degradation of matrix. The system is ideally suited for controlled release of peptide/ protein drugs.

In order to overcome uptake of intraveneousaly administered microspheres by the reticuloendothelial system and promote drug targeting to tumours with good perfusion, magnetic microspheres were developed. They are prepared from albumim and magnetite and have size of 1μ g to permit intravascular injection.

5. Monoclonal antibodies

Monoclonal antibodies are exceptionally high quality antibodies which consist of one molecular species and which may be obtained in a virtually homogeneous state.

Kohler and Milstein in 1975 showed that somatic cell hybridization could be used to produce a continuous hybrid cell line producing a single type of anti body. The basic principle was to b-lymphocyte from an antigen primed mouse, having the ability to secrete a specific antibody and to fuse this with a suitable mouse derived plasmacytoma (often called myleom) line. The outcome was hybrid cell line (hybridoma) which had the phenotypic properties of both parental cells, that is malignancy and specific antibody secretion indefinitely one b- lymphocytes or plasma cell is committed to one antibody specificity.

The discovery of hybridoma technology has been more dramatic than arrival of new scientific theory and has revolutionized immunology in a matter of few years.

6. Liposomes

It is defined as spherule vesicle of lipid bilayers enclosing an aqueous compartment. The lipid most commonly used is phospholipids, sphingolipids, glycolopids and sterols have been used to prepare liposomes.

In recent year, liposomes have been extensively studied for their potential to serve as carriers for delivery of drugs, antigens, hormones, enzymes and other biologicals. Because liposomes are composed of naturally occurring substance they have the distinct advantage of being nontoxic and biodegradable. Biologically active materials encapsulated withing liposomes are protected to various extends from immediate dilutions or degradations *in vivo*. This protective property promotes the delivery of entrapped drugs to the target organ by preventing a premature drug release after administration.

Liposomes have two standard forms.Multilamellar vesicles (MLV's) made up of several lipid bilayers separated by fluid. Unilamellar vesicles (ULV's) consisting of single bilayer surrounding an entirely fluid core. The ULV's are typically characterized as being small (SUV's) or large (LUV'S).

[13]Limitations of TDDS

TDDS such as liposome's, 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 shows high specificity to superficially located organs and tissues but cannot be targeted to deep seated organs.
- Monoclonal antibodies may sometimes cause unwanted antigen antibody reaction which leads to serious consequences.
- Microspheres of particle size more than 50 μg can lead to problem of thromboembolisms in general circulation.
- One 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, nonirritating, biocompatible and biodegradable and biodegradable in to nontoxic compounds within an appropreiate time, preferably close to duration of action.
- The products which tend to remain intact may become lodged at some sites. If these occur slow release of drug from dosage form leads to a high localized concentration of drug which caused 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 8-12 hrs of continuous therapy.
- As these products normally contain large amount of drug there is possibility of unsafe over dosage if the products is improperly made.
- If it is once administered it may be difficult to stop the therapy due to toxicity or any other reasons.

Merits of TDDS

Targeting of the drug molecule towards the issue or organ reduces the toxicity to the normal tissues.

- Increase bioavailability.
- Improved treatment of many chronic illnesses where symptom breaks 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 dose administered.
- Bio compatibility can be well achieved.
- Maintenance of therapeutic action of the drug over night.
- 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.

Applications of TDDS:

- Red blood cells, leukocytes, lymphocytes and fibroblasts have also 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 possible carriers for Methotrexate 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 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 in the assemble polymers. These particulate system are best utilized as sustained release vehicles.

- Bovine album in 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 micro-spheres sustained the drug release over a period of time.

[14]CARRIER SYSTEMS USED FOR TARGETED DRUG DELIVERY

1. Colloidal carriers

a) Vesiclular system

Liposomes; Niosomes; Pharmacosomes; Virosomes; Immunoliposomes.

b) Microparticulate systems

Micro particles; Nano particles; Magnetic-micro spheres;

Albumin micro-spheres; Nano-capsules.

2. Cellular carriers

Resealed erythrocytes; serum albumin; antibodies; platelets; leukocytes.

3. Supramolecular delivery systems

Micelles; reverse micelles; mixed micelles; polymeric micelles;

Liquid crystals; Lipoproteins (chylomicron; VLDL; LDL)

Synthetic LDL mimicking Particles (supramolecule biovector system).

4. Polymer based systems

Signal sensitive; Muco-adhesive; Biodegradable; Bio-erodible;

Soluble synthetic polymeric carriers.

5. Macromolecular carriers

a) Proteins, glycoproteins; neo glycoproteins and artificial viral envelopes (AVE).

b) Glycosylated water soluble polymers (poly-L-lysine).

c) Mabs; Immunological Fab fragments; antibody-enzyme complex & bispecific Abs.

d) Toxins, immunotoxin & rCD4 toxin conjugates.

e) Lectins (Con A) & polysaccharides

LEVELS OF DRUG TARGETING

Targeted drug delivery may be achieved by using carrier systems, where reliance is placed on exploiting both, intrinsic pathway(s) that these carriers follow, and the bio-protection that they can offer to drugs during transit through the body. The various approaches of vectoring the drug to the target site can be broadly classified as:

1. Passive targeting
2. Inverse targeting
3. Active targeting (Ligand mediated targeting and physical targeting)
4. Dual targeting
5. Double targeting
6. Combination targeting

1) Passive Targeting

Systems that target the systemic circulation are generally characterized as "passive" delivery systems (i.e. targeting occurs because of the body's natural response to the physicochemical characteristics of the drug or drug-carrier system. It is a sort of passive process that utilizes the natural course of (attributed to inherent characteristics) bio-distribution of the carrier system, through which, it eventually accumulate in the organ compartment of body.

The ability of some colloids to be taken up by the RES especially in liver and spleen has made them as ideal vectors for passive hepatic targeting of drugs to these compartments. Passive capture of colloidal carriers by macrophages offers therapeutic

opportunities for the delivery of anti-infective for disease conditions that involve macrophage cells of the reticuloendothelial system (RES) e.g. leishmaniasis, brucellosis and candidiasis. Delivery into lysosomal compartment can also be affected for the treatment of certain lysosomal storage diseases, macrophage neoplasms and macrophage activation.

This category of target able devices includes drug bearing bi-layer vesicular systems as well cellular carriers of micron or submicron size range. The passive target ability of micro particulate drug carriers is due to the recognition of these exogenous particulates either in the intact or in the opsonized form, by the phagocyte cells of the RES and this sensing behavior is exploited to target MPS associated diseased cell lines.

A major disadvantage of micro particulate carriers is that they cannot pass the endothelial cell lines; as a result extravasations are generally poor. Although some investigation claim that slow Tran cellular (vesicular) transport of liposome's and micro spheres is possible through endothelia. The practical applications of micro particulate carriers are largely restricted to intravascular targets. Attempts have been made for targeting them to intravesicular non-RES cell lines and to increase circulation half life by exploiting strategies that involve modification of size, surface charge, composition, surface rigidity and surface hydrophilicity.

These long circulatory modules can then be relied as carrier base. However, to endow them with target specificity some site directing ligands could be appended on their surface site specific ligands can be immobilized with optimal targeting efficiency.

2) Inverse Targeting

It is essentially based on successful attempts to circumvent and avoid passive uptake of colloidal carriers by reticuloendothelieal system (RES). This effectively leads to the reversion of biodistribution trend of the carrier and hence the process is referred to as inverse targeting.

One strategy applied to achieve inverse targeting is to suppress the function of RES by a pre-injection of a large amount of blank colloidal carriers or macromolecules like dextran sulphate.This approach lead to RES blockade and as a consequence impairment of host defence system.

3) **Active Targeting**

Active targeting exploits modification or manipulation of drug carriers to redefinr its biofate. The natural distribution pattern of the drug carrier composites is enhanced using chemical, biological and physical means, so that it approaches and identified by particular biosites.The facilitation of the binding of the drug-carrier to target cell through the use of ligands or engineered homing devices toincrease receptor mediated (or in some cases receptor independent but epitope based) localization of the drug and target specific delivery of drug is referred to as active targeting.

4) **Dual Targeting**

This classical approach of the drug targeting employs carrier molecules, which have their own intrinsic antiviral effect thus synergies the antiviral effect the loaded active drug. Based on this approach, drug conjucates can be prepared with fortified activity profile against the viral replication. A major advantage is that the virus replication process can be attacked at multiple points, excluding the possibilities of resistant viral strain development.

5) **Double Targeting**

Drug targeting may be combined with passive and active targeting for drug delivery system. The combination is made between spatial control and temporal control of drug delivery.

The temporal control of drug delivery has been developed in terms of control drug release prior to the development of drug targeting. If spatial targeting is combined with temporal control release results in an improved therapeutics index by the following two effects. First, if drug release or activation is occurred locally at therapeutic sites, selectivity with the local release/activation. Second, the improvement in the therapeutic index by a combination of a spatially selective delivery and a preferable release pattern for a drug, such as zero order release for a longer time period of drugs.

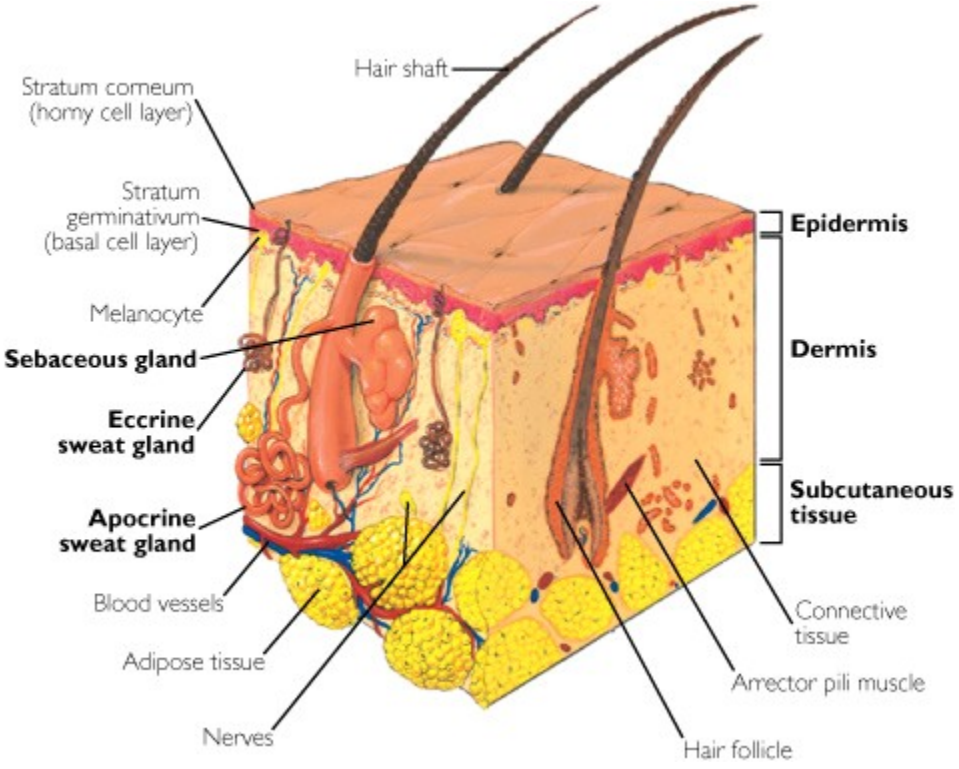
6) Combination Targeting

These targeting systems are equipped with carriers, polymers and homing devices of molecular specificity that could provide a direct approach to target site. Modification of proteins and peptides with natural polymers, such as polysaccharides, or synthetic polymers, such as poly (ethylene glycol), may alter their physical characteristics, and favour targeting the specific compartments, organs or their tissues within the vasculature. Further vectorization of these modified proteins and peptides into vesicular or micro-particulate carriers may take advantage of the intrinsic of inherited (through homing devices) properties of carrier to achieve a site specific active targeting of encapsulated contents.

THE PENETRATION BARRIER OF THE INTACT SKIN

In the field of the dermal or transdermal drug delivery the skin represents the application site and sometimes also the target, but it is the main obstacle for efficient drug and/or carrier penetration. The main barrier is the so-called horny layer, or stratum corneum.

Figure No. 1 Structure of skin



The skin (cutis) consists of two histologically and functionally different parts:

The inner part, the dermis, is 10 to 20 times thicker than the outer part, the epidermis that is usually approximately 4 mm thick. The dermis encompasses a variety of specialized cells, tissues, blood vessels, lymph ducts, glands, hair follicles, and sensory and immunocompetent cells. Each of these fulfills a range of important tasks.

One of the important functions of the dermis is to nourish the cells of the epidermis. The main function of the epidermis is to render the skin mechanically stable and chemically and environmentally resistant; the low permeability is part of this.

The predominant cell type in the epidermis is the keratinocyte. Other cells, such as melanocytes (for UV protection), Langerhans cells (for immune response), and Merkel cells (part of sensory system) etc., play an important role in the function of the skin as well.

The keratinocytes at the basal membrane of the epidermis continuously produce new cells. These then gradually move toward the skin surface and thus replace the shedding cells of the stratum corneum.

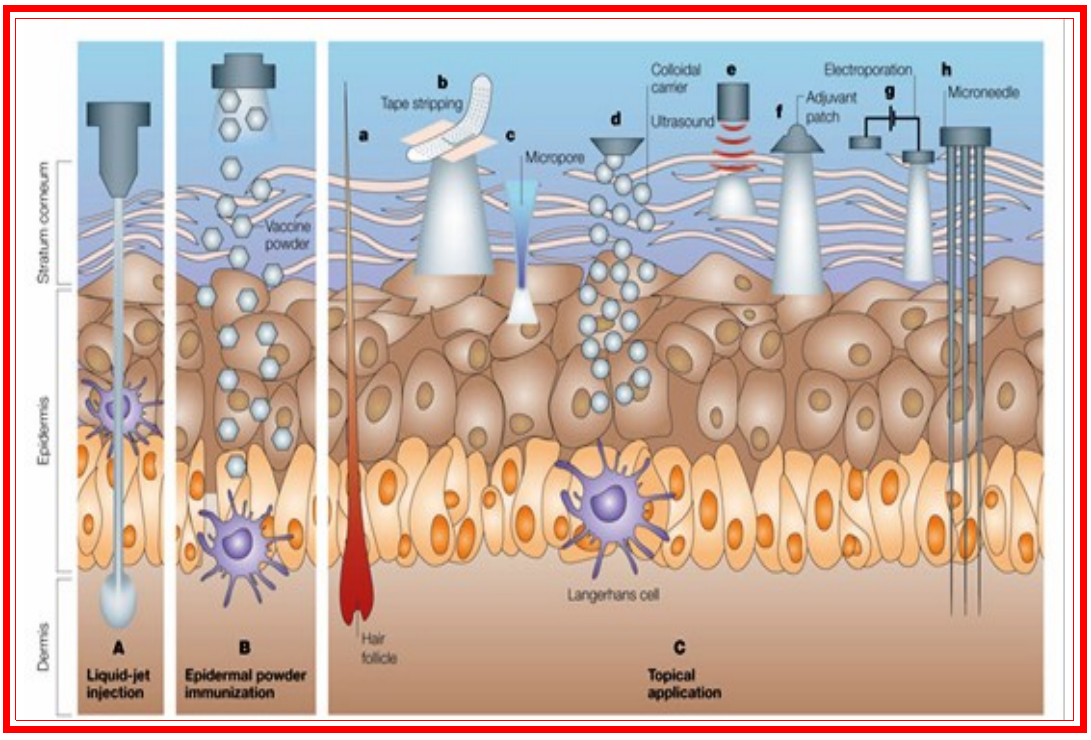
The stratum corneum consists of several layers of dead, flattened cells (corenocytes) embedded into a quasi-lamellar lipid matrix[15]. Cells originating from the same keratinocyte stem cell remain organized in a columnar stack during the terminal differentiation. Corneocytes of neighboring stacks interdigitate at their edges. This structure is often referred to as the “brick and mortar” model of stratum corneum;

NOVEL METHODS IN TRANSDERMAL DRUG DELIVERY SYSTEMS

New dosage forms and drug delivery systems providing excellent improvement in drug therapy are termed as novel drug delivery systems .These are 'novel' due to recent development with satisfactory results in field of drug delivery[16] Some of these novel advanced transdermal technologies include[17]:

1. Penetration enhancers
2. Iontoporesis
3. Electroporation and Sonophoresis
4. Micro fabricated Micro needles and Microchips
5. Vesicular approaches

Figure No – 2 Novel Methods in Transdermal Drug Delivery Systems



1. PENETRATION ENHANCERS

Transdermal drug delivery has been a subject of research interest since first introduction of transdermal patch of scopolamine for motion sickness[18] .The most recent developments center on methodologies to increase molecular transport across the skin. Much effort has been directed towards the penetration enhancer.

An ideal enhancer should be pharmacologicaly inactive, non-irritant and should not damage skin irreversibly. The effects on the permeation of a drug usually depend upon the physio-chemical characteristics of the permeant as well as the enhancer molecule. The penetration of the enhancer in to stratum corneum is a basic requirement for their efficacy. It is possible to facilitate the penetration of the drug by appropriate treatment of the skin with penetration enhancer[19], the main reason for enhancements include:

- Interaction with the intercellular lipids and intracellular keratin
- Increased penetration of high amounts of enhancers or co-solvents in to

the stratum corneum due to the improved dissolving capacity of the barrier to the drugs. Many of the chemical enhancers such as dimethyl sulfoxide, surfactants, alcohols urea and its derivatives have been screened for their penetration enhancement. terpenes isolated from natural essential oils are currently under investigation as safe and non irritating penetration enhancer

Terpene enhancers such as L-methanol, D-limonene, methone, carvone, and 1-8cineole have been used to enhance the transdermal delivery of drugs including 5-flououracil[20], indomethacin, zidovudine and diclofinac sodium. These chemicals have exhibited low cutaneous irritation potential and reversible alterations of skin barrier function.

2. IONTOPHORESIS

The drawbacks associated with chemical enhancers include the unsuitability for delivery of new biotechnological products like peptides, small proteins, oligonucleotides. Hence a renaissance of interest was shown towards iontophoresis developed over the last decade

Iontophoresis is a process or technique involving the transport of ionic or charged molecules in to a tissue by the passage of direct or periodic electric current through an electrolyte solution containing the ionic molecules to be delivered using an appropriate electrode polarity[21]. The process involves the transfer of ions into the body by an electromotive force.

Ions with positive charge are driven in to the skin at anode and those with negative charge at the cathode. In the conventional topical treatment by iontophoresis, the drug is administered through an electrode having the same charge as the drug and return electrode opposite in charge to the drug is placed at a neutral site on the body surface. The operator then selects a current intensity below the pain threshold level of the patient and allows the current to flow for an appropriate period of time[22].

The current intensity should be increased slowly maintained for the length of the treatment and decreased slowly at the end of the treatment. the current must be within comfortable toleration of the patient. Interposition of a moist pad between the electrode plate and the skin is necessary for making a perfect contact, preventing any skin burns, overcoming skin resistance and protecting skin from absorbing any caustic metallic compound formed on the metal plate surface. It is critically important that the drug be applied through the electrode with correct polarity, since any reversal of the polarity may result in no penetration of the drug. The electrode must not come in any direct contact with skin as it may cause burns[23].

In-spite of its extensive application, the drawbacks associated with the technology include the possibility of electric shock, skin irritation, burns and cost of treatment. Recent efforts in this technology have resulted in the design of iontophoretic electrodes, which avoids burns. The technique has gained acceptance for local therapy. Its application for systemic medication will require further research to elucidate simple means of drug delivery. The development of iontophoresis can broaden the scope of transdermal delivery to the absorption of poorly absorbed ionic drugs. User acceptance will probably depend on success in miniaturization of the assembly.

3. ELECTROPORATION

The drawbacks associated with chemical enhancers and iontophoresis can be overcome to a certain extent by electroporation technology developed in recent years. In a more futuristic way, the technology has been developed to overcome the most daunting challenges of transdermal drug delivery. The process involves the application of transient high voltage electrical pulse to cause rapid dissociation of the stratum corneum through which large and small peptides, oligonucleotides and other drugs can pass in significant amounts. The degree of enhancement achieved in vitro is related to the applied voltage, number and duration of pulses offering the possibility of a controllable phenomenon.

Electroporation is a technique in which the drug encapsulated in vesicles or particles is delivered into the skin by applying a pulse causing a breakdown of the stratum corneum[24]. Pressure mediated electroincorporation has been used to deliver leuprolide acetate microspheres into hairless mouse skin and human skin xenograflted on immunodeficient nude mice. It has been shown that application of continuous low voltage resulted in a calcein flux with three orders of magnitude.

Beside the model compound calcein, other drugs investigated for transdermal delivery by electroporation include metaprolol, flurbiprofen, cyclosporine, heparin, fentanyl and oligo-nucleotides[25].

The studies with model compounds have given excellent mechanistic insights into the magnitude of flux enhancement. These reports may not be applicable for all drugs. Hence each drug needs to be studied as a separate entity. More human clinical data is however required before the technology can be commercialized.

4. SONOPHORESIS

Another technique besides electroporation attempting to overcome the challenges of transdermal drug delivery involves the usage of high frequency ultrasound waves. The application of low frequency ultrasound was shown to increase the permeability of human skin to many drugs including high molecular weight proteins by several orders of magnitude, thus making transdermal administration of these molecules potentially feasible Low-frequency ultrasound is thus a potential, non-invasive technology for transdermal drug delivery.

Despite the excitement these findings have provoked, it is necessary to maintain an appropriate perspective until several basic questions are answered with respect to mechanism of action, toxicity, economical and technological feasibility. Furthermore, optimal parameters such as frequency, pulse length and intensity should be observed carefully to ensure a safe and efficacious application[26].

5. MICRO FABRICATED MICRONEEDLES AND MICROCHIPS

Recently a novel method has been developed for enhancing transport of molecules across the skin. The microfabricated microneedles technology employs micron-sized needles made from silicon. These micro-needle arrays after insertion into the skin create conduits for transport of drug across the stratum corneum. The drug after crossing the stratum corneum diffuses rapidly through deeper tissue and

taken up by capillaries for systemic administration. Micro-needles penetrate the skin about 10-15 mm deep inside the skin but do not reach the nerves found in deeper tissue, so are painless[27].

The micro-needles were made using the micro fabrication technology is simple for cheap and mass production of micron sized structures. For the drug delivery, a three-dimensional array of sharp-tipped microneedles with approximately 150 mm in lengths was fabricated.

A deep reactive ion etching process was used to micro-fabricate the needles for drug delivery. The reactive ion etching technique is based on the black silicon method. Each micro-needle is about 1 mm in diameter or one hundredth of the diameter of a human hair and can be seen only under a microscope. A microprocessor is attached to a tiny pump for delivering tiny amounts of the drug. The microprocessor and pump automatically inject the right dosage of the drug. The micro-needles have extremely sharp tips with radius of curvature less than 1 mm facilitating easy piercing into the skin.

The micro-fabrication technique can be easily modified to make longer or shorter needles according to the requirement. Micro-needles after insertion into skin are found to be mechanically strong, can be removed without difficult as well as reinserted into skin multiple times. The experiments were also conducted on human volunteers by inserting micro-needles into the skin of forearm or hand.

As reported, the volunteer never felt pain, but mild wearing or a weak pressure with the feeling of a piece of tape affixed to the skin. Inspection of the site after insertion showed no erythema, edema or other reaction to micro-needles over the hours and days. Micro-needles are still a long way from being marketed. It may take some years to perfect and test the technology for safety and clinical studies before gaining approval from Food and Drug Administration (FDA) [28].

6. VESICULAR APPROACHES

The encapsulation of drug in lipid vesicles prepared from phospholipids and nonionic surfactant is used for transport of drug into and across the skin. The rationale for use of lipid vesicles as a topical drug carrier is as follows[29].

- Vesicles may serve as rate-limiting membrane barrier for systemic absorption of drug.Because of the amphiphilic nature of the vesicles, these vesicles may serve as non-toxic penetration enhancer for drugs.
- They may serve as "organic solvent" for the solubilization of poorly soluble drugs.
- Vesicles can incorporate both hydrophilic and lipophilic drugs.

The vesicular approach e.g. liposomes in transdermal drug delivery systems have been studied for many purposes, but their unstable nature limits their use at clinical and industrial levels. In order to increase the stability of liposomes concept of proliposomes has been proposed. This approach has been extended to niosomes which exhibit superior stability compared to liposomes and attempts have been made to further stabilize them and overcome their limitation by proniosomal approach.

But all approaches because of their poor skin permeability, breaking of vesicles, leakage of drug, aggregation and fusion of vesicles are not much successful for effective transdermal delivery[30].

TRANSFERSOMES

Poor patient compliance is a frequent problem in daily clinical practice. The unfavorable pharmacokinetic of the drug, the inconveniences of the standard form of such drug application and the side effects due to the administration route often are the reasons for this. Consequently much effort has been put into the development of strategies that could improve the patient compliance with new modes of drug application. Delivery via the transdermal route is an interesting option in this respect because transdermal route is convenient and safe.

This offers several potential advantages over conventional routes like avoidance of first pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter-and intra-patient variations, and most importantly, it provides patient convenience.

But one of the major problems in transdermal drug delivery is the low penetration rate through the outermost layer of the skin, the stratum corneum[32]. To date many chemical and physical approaches have been applied to increase the efficacy of the material transfer across the intact skin, by use of the penetration enhancers, iontophoresis, sonophoresis and the use of colloidal carriers such as lipid vesicles (liposomes and pro-liposomes) and nonionic surfactant vesicles (niosomes and pro-niosomes).

Initially various chemical additives (such as alcohols, azones, surfactants etc.) were used to increase the lipid fluidity in the outer skin layers and thus improve the skin permeability to various agents. Use of penetration enhancers has some limitations like applicable only for low molecular weight drugs(smaller than 500 to 1000 Da), skin irritation, immunogenicity etc. physical methods like electrophoresis, iontophoresis, moreover can transfer some intermediate size charged molecules, but the resulting overall material transfer efficiency is rather low(less than 10%),and applicable only for charged drug[33].

Vesicular carrier systems like liposomes and niosomes have both received lot of attention over the last decade as a means for the transdermal drug delivery. Initially the use of liposomes on the skin was reported, since then a wide range of agents loaded in liposomes have been tested on the skin, with different rationalities in mind.

In most cases transdermal drug penetration has not been achieved. To overcome all the problems mentioned above a new type of carrier system called a

"transfersomes" was introduced recently for the effective transdermal delivery of number of low and high molecular weight drugs.

Transfersomes, in the widest sense of the word, is any supramacromolecular entity that can pass spontaneously through a permeability barrier and thereby transport material from the application to the destination site. In order to meet the goal, however, a tranfersomes must adjust its properties, most notably its deformability to the shape and the size of the pores in the barrier.

Tranfersomes, in functional terms, may be described as lipid droplets of such deformability that permit its easy penetration through the pores much smaller than the droplets' size. In thermodynamic terms this typically corresponds to an aggregate in the quasi-metastable state, which facilitates the formation of highly curved bilayers. From the composition point of view, a transfersomes is a self-adaptable and optimized mixed lipid aggregate[34].

Transfersomes were developed in order to take the advantage of phospholipids vesicles as transdermal drug carrier. These self-optimized aggregates, with the ultraflexible membrane, are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with high efficiency.

These vesicular transfersomes are several orders of magnitudes more elastic than the standard liposomes and thus well suited for the skin penetration. Transfersomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipids of the stratum corneum. There is provision for this, because of the high vesicle deformability, which permits the entry due to the mechanical stress of surrounding, in a self-adapting manner.

Flexibility of transfersomes membrane is achieved by mixing suitable surface-active components in the proper ratios[35]. The resulting flexibility of transfersome membrane minimizing the risk of complete vesicle rupture in the skin and allows transfersomes to follow the natural water gradient across the epidermis, when applied under non-occlusive condition.

Transfersomes can penetrate the intact stratum corneum spontaneously along two routes in the intracellular lipid that differ in their bi-layer properties. The following figure shows possible micro-routes for drug penetration across human skin intracellular and transcellular[36].

The high and self-optimizing deformability of typical composite transfersomes membrane, which are adaptable to ambient stress allow the ultra-deformable transfersomes to change its membrane composition locally and reversibly, when it is pressed against or attracted into a narrow pore. The transfersomes components that sustain strong membrane deformation preferentially accumulate, which the less adaptable molecules are diluted at sites of great stress. This dramatically lowers the energetic cost of membrane deformation and permits the resulting, highly flexible particles, first to enter and then to pass through the pores rapidly and efficiently. This behavior is not limited to one type of pore and has been observed in natural barriers such as in intact skin[37].

Natural transdermal water concentration gradients consequently drive high number of the specially designed lipid vesicles across the hydrophobic outer skin layers. This does not pertain to all lipid vesicles, for example, the available transdermal osmotic pressure difference is too low to push the standard lipid vesicles (liposomes) through an intact mammalian stratum corneum. Dermally applied lipid vesicles can only penetrate into rather than across this region. The reason for this is the prohibitively high cost of the standard liposome deformation.

In order to increase the efficacy of vesicle penetration through skin it is therefore necessary to minimize this cost for each given vesicles type. It had been by adjusting the lipid bi-layer composition until the maximum of the tolerable vesicles surface flexibility was achieved. Such an optimization yields transfersomes. Owing to their hyperflexibility the latter can transfer as much as 0.1 mg of lipid per hour and cm^2 across the intact skin, if applied under suitable conditions. Furthermore, transfersomes can efficient transepidermal transport of the water soluble substances, such as proteins or polypeptides[38].

SALIENT FEATURES AND LIMITATIONS OF TRANSFERSOMES

1. Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with a wide range of solubility.
2. Transfersomes can deform and pass through narrow constriction from (5 to 10 times less than their own diameter) without measurable loss. This high deformability gives better penetration of intact vesicles.
3. They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin.
4. They are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes.
5. They have high entrapment efficiency, in case of lipophilic drug near to 90%.
6. They protect the encapsulated drug from metabolic degradation.
7. They act as depot, releasing their contents slowly and gradually.
8. They can be used for both systemic as well as topical delivery of drug.

9. Easy to scale up, as procedure is simple, do not involve lengthy procedure and unnecessary use of pharmaceutically unacceptable additives.

Limitation of Transfersomes

1) Transfersomes are chemically unstable because of their predisposition to oxidative degradation.
2) Purity of natural phospholipids is another criteria militating against adoption of transfersomes as drug delivery vehicles.
3) Transfersome for formulations are expensive.

TRANSFERSOMES V_S OTHER CARRIER SYSTEMS

Transfersomes appear to be remotely related to lipid bi-layer vesicle, liposomes. However in functional terms, transfersomes differ vastly from commonly used liposomes in that they are much more flexible and adaptable.

The extremely high flexibility of their membrane permits transfersomes to squeeze themselves even through pores much smaller than their own diameter. This is due to high flexibility of the transfersomes membrane and is achieved by judiciously combining at least two lipophilic/amphiphilic components (phospholipids plus bio-surfactant) with sufficiently different packing characteristics into a single bi-layer.

The high resulting aggregate deformability permits transfersomes to penetrate the skin spontaneously. On the contrary, mixed micelles stay confined to the topmost part of the stratum corneum even they are applied non-occlusively[39]. The reason for this is that mixed micelles are much less sensitive to the transepidermal water activity gradient than transfersomes. (Transfersomes of magnitude (in size) greater than standard lipid micelles. Secondly and more importantly, each vesicular transfersomes contains a water filled core whereas a micelle is just a simple fatty droplet. Transfersomes thus carry water as well as fat-soluble agent in comparision to micelles that can only incorporate lipoidal substances.

Table No. 1
Comparison of Different Approaches for Transdermal Drug Delivery

Method	Advantage	Disadvantage
Penetration enhancer	Increase penetration through skin and give both and systemic effect	Skin irritation immunogenicity Only for low molecular weight drugs.
Physical methods e.g. iontophoresis	Increase penetration of intermediate size charged molecule	Only for charged drugs transfer efficiency is low
Liposome's	Phospholipid vesicle, Provide sustained release, biocompatible, biodegradable	Less skin penetration Less stable suitable for topical delivery
Proliposomes	More stable than liposome's	Less skin penetration
Niosomes	Non-ionic surfactants vesicles, greater stability, easy handling	Less skin penetration
Transfersomes	**More stable, high penetration due to high deformability Biocompatible and biodegradable, suitable for both low and high molecular weight and also for liphophilic as well as hydrophilic and reaches up to deeper skin layers**.	**None, but for some limitations like the higher cost stability of the formulations.**

Table No. 2

APPLICATION OF TRANSFERSOMES AS A DRUG CARRIER

Drug	Results
Insulin[40]	High encapsulation efficacy Transfer across the skin with an efficacy of >50% Provide noninvasive means of therapeutic use
Interferon-ά[41] **Interleukin-2**	Efficient delivery means(because delivery by other route is difficulty Controlled release Overcome stability problem
Soluble proteins[42-46] **Gap junction protein** **Human serum albumin** **Integral membrane protein**	Permits non-invasive immunization through normal skin Antibody titer is similar or even slightly higher than subcutaneous injection
Corticosteroids[47,48] **Hydrocortisone**	Improve site specificity and overall drug safety Biologically active at dose several times lower than the currently used formulation
Triamcinolone acetonide **Topical analgesic and anesthetic agent**[49.50]	Used both for local and systemic delivery Suitable means for the noninvasive treatment of local pain on direct topical drug application. Prolonging drug action
Diclofenac,Tetracaine,Lidocaine	Improved transdermal flux Provide controlled release
Oestradiol [51,52] **Tomoxifen**[53]	Improved transdermal flux Reduce drug toxicity
Norgesterol[54]	Improved transdermal permeation Reduce side effects
Cyclosporine[55]	Improved therapeutic efficacy Prolonging drug action
Dexamethasone[56,57]	Improved transdermal flux Prolonging drug action

Table No. 3

SOME EMERGING VESICULAR SYSTEMS

S.No	Vesicular systems	Description	Application	Reference
1	Enzymosomes	Liposomal constructs engineered to provide a mini bio-environment in which enzymes are covalently immobilized or coupled to the surface of liposomes.	Targeted delivery to tumor cells	58
2	Virosomes	Liposomes spiked with virus glycoprotein , incorporated into the liposomal bilayers based on retro viruses derived lipids.	Immunological adjuvants	59
3	Ufasomes	Vesicles enclosed by fatty acids obtained from long chain fatty acids (oleic acid, linoleic acid) by mechanical agitation of evaporated films in the presence of buffer solutions.	Ligand mediated drug targeting	60
4	Cryptosomes	Lipid vesicles with a surface coat composed of PC and of suitable polyoxyethylene derivative of phosphatidyl ethanolamine.	Ligand mediated drug targeting	61
5	Emulsomes	Nanosize lipid particles (bioadhesive nanoemulsion) consisted of microscopic lipid assembly with apolar core.	Parenteral delivery of poorly water soluble drugs .	62
6	Discomes	Niosomes solubilized with non-ionic surfaccant solution(polyosyethylene cetyl ether class)	Ligand mediated drug targeting	63
7	Aquasomes	Three layered self-assembly compositions which ceramic carbon-nanocrystalline particulate core coated with glassy cellobiose.	Specific targeting, molecular shielding.	64
8	Ethosomes	Ethosomes are lipid “soft, malleable esicles” embodying a permeation enhancer and composed of phospholipids, ethanol and water.	Targeted delivery to deep skin layers	65
9	Genosomes	Artifical macromolecular	Cell specific	66

		complexes for functional gene transfer. Cationic lipids are most suitable because they possess high biodegradability and stability in the blood stream.	gene transfer	
10	Photosomes	Photolyase encapsulated in liposomes, which release the contents, by photo- triggered charges in membrane permeability characteristics.	Photodynamic therapy	67
11	Erythrosomes	Liposomal systems in which chemically crosslinked humanery throcytes cytoskeletons are used as a support to which lipid bilayer is coated.	Effective targeting of macromolecular drugs	68
12	Hemosomes	Haemoglobin containing liposomes engineered by immobilizing haemoglobin witha polymerisable phospholipids.	High capacity oxygen carrying system.	69
13	Proteosomes	High molecular weight multi-subunit enzyme complexes with catalytic activity, which is specifically due to the assembly pattern of enzymes.	Better catalytic activity turn over than non-assciated enzymes.	70
14	Vesosome	Nested bilayer compartments in vitro via the “interdigitated” bilayer phase formed by adding ethanol to a variety of saturated phospholipids.	Multiple compartments of the vesosome give protection to interior contents in serum.	71
15	Archaeosomes	Vesicles composed of glycerolipids of archaea with potent adjuvant activity.	Potent adjuvant activity.	72

DIABETES MELLITUS[73-74]

Diabetes mellitus is a condition in which the body cells are no longer able to utilize blood sugar. Blood sugar is the fuel that cells use to make energy. Symptoms of diabetes mellitus include excessive thirst and hunger, frequent urination, and tiredness.

Diabetes mellitus is a chronic health disorder. Chronic means that the condition lasts for many years. Diabetes can cause serious health problems. These problems include kidney failure, heart disease, stroke, and blindness. About fourteen million Americans have diabetes. As many as half of these people do not know they have the condition.

Diabetes Mellitus: words to know

Glucose:

A type of sugar that is present in the blood and in cells, used by cells to make energy.

Insulin:

Hormone (type of protein) produced by the pancreas than makes it possible for cells to glucose in the production of energy.

Ketoacidosis:

A condition that results from the build-up of toxic chemicals known as ketones in the blood.

Pancreas:

A gland located behind the stomach that produces insulin.

The energy your body needs:

Our bodies require a constant production of energy. We use that energy to walk, talk, think, and carry on many other activities. The energy comes from the food we eat. Certain foods contain chemicals known as carbohydrates. When

carbohydrates enter the body, they break down to form a simple sugar known as glucose. The glucose travels to cells throughout the body by way of the bloodstream.

To enter a cell, glucose may need the help of another chemical known as insulin. Insulin is produced in the pancreas. Insulin also travels through the bloodstream to all cells in the body. It acts like a key that opens cells so that glucose can enter. In a healthy body, enough insulin is produced to make sure that all cells get the glucose they need. The cells can then produce enough energy to satisfy the body's needs.

In some cases, however, this system breaks down. One problem may be that the pancreas stops producing enough insulin. There is not enough insulin for all the cells that need it. Glucose cannot get into many of the body's cells. The cells cannot produce enough energy for the body's needs. Another problem is that some cells may no longer recognize insulin. The pancreas may still produce insulin for all the body's cells, but some cells don't respond to it. Again, glucose can't get into the cells and energy is not produced to satisfy the body's needs.

TYPES OF DIABETES MELLITUS

Two types of diabetes mellitus are recognized. These two types differ in two major ways-the age at which they occur and their causes. Type I diabetes is also called juvenile diabetes. It usually begins during childhood or adolescence. In this form of diabetes, the pancreas produces little or no insulin. The condition can be treated by having a person take daily injections of insulin. For this reason, Type I diabetes is also called insulin-dependent diabetes. Type I diabetes affects about three people in one thousand in the United States.

In type 1 the pancreas undergoes an autoimmune attack by the body itself, and is rendered incapable of making insulin. Abnormal antibodies have been found in patients with type1 diabetes. It is believed that the tendency to develop these abnormal antibodies is in part genetically inherited. The gene foe developing type 1 diabetes has been identified on chromosome no11. type 1 diabetes tends to occur in young, lean individuals, usually before 30 years of age, however, older patients do present with this form on occasion. Totally 90% of the diabetic patients were affected with type2 and remaining 10% with Type1 diabetes.

Type II diabetes is sometimes called adult-onset diabetes. The name "adult-onset" comes from the fact that Type II diabetes usually does not appear until a person grows older. More than 90 percent of the disorder is not caused by low levels of insulin. Instead, the body's cells do not recognize insulin in the bloodstream. They are not able to get the glucose they need to make energy. People with Type II diabetes do not need to take insulin. Their body produces all the insulin it needs. The body just can't use it properly. As a result, Type II diabetes is sometimes called non insulin-dependent diabetes .Type II diabetes is treated with diet, exercise, and drugs.

CAUSES

The causes of diabetes mellitus are unclear. Both heredity and environment may be involved. Studies have shown that certain genetic factors may be responsible for diabetes. Genes are chemical units found in all cells, that tell cells what functions they should perform. Genes are passed down from parents to children. If parents carry a gene for diabetes, they may pass that gene on to their children.

Some researchers believe that Type I diabetes may also be caused by a virus or some other disease-causing organism. They think the organism may attack the pancreas at an early age. The pancreas may be damaged and lose its ability to produce insulin.

A number of factors have been tied to Type II diabetes. These factors include:

- Obesity (being excessively overweight, see obesity entry)
- Having relatives with diabetes mellitus
- Belonging to certain high-risk populations, such as African Americans, Native Americans, Hispanics, or Native Hawaiians having high blood pressure
- Having an excess or deficiency of certain substances in the blood, such as cholesterol or triglycerides (a form of fat)

SYMPTOMS

The classic symptoms of diabetes include being overly tired and sick, having to urinate frequently, feeling very thirsty and hungry, and losing weight. The way these symptoms develop differs for Type I and Type II diabetes. In Type I diabetes, they usually show up slowly in children or adolescents over a period of a few days or weeks. In Type II diabetes, they develop even more slowly, over a period of years, in adults over the age of forty. Adults often do not realize they have diabetes mellitus. The condition may be discovered only during a routine physical examination for some other problem.

Type I diabetes is generally a more serious condition than Type II. The most dangerous effect of Type I diabetes is a condition known as ketoacidosis (pronounced KEE-toe-ASS-ih-doe-sus), which occurs when Type I diabetes is not controlled. In ketoacidosis, chemicals that are toxic (poisonous) to the body begin to collect in the blood. These chemicals can cause abdominal pain, vomiting, rapid breathing, extreme tiredness, and drowsiness. If this condition is not treated, a person may fall into a coma and die. The most characteristic symptom of ketoacidosis is sweet-smelling breath.

The symptoms of Type II diabetes usually develop more slowly and are less serious. In the worst circumstance, they include heart disease, infections of the gums and urinary tract, blurred vision, numbness in the feet and legs, and slow-healing wounds.

DIAGNOSIS

A patient with the symptoms listed above may be suspected of having diabetes. The diagnosis can be confirmed very easily and quickly with a blood and/or urine test. The amount of glucose present in the blood or urine can be measured. If the level is unusually high, it is likely the person has diabetes.

The simplest test for diabetes uses paper strips that change colour when dipped into urine. The color of the strip is compared to a chart that comes with the strips. The chart shows how much glucose is present in the urine.

Blood tests can also be used to test for glucose. These tests tend to be more accurate than urine tests. A sample of blood is taken from the patient's arm. The sample is then analyzed in a laboratory. The amount of glucose present is determined. That amount is compared with the amount present in a healthy person's blood. A high level of glucose suggests the presence of diabetes.

People with diabetes often test their own blood many times a day. They use home glucose test kits that contain a small needle and a chart. They use the needle to produce a single drop of blood (often from their fingertip). The drop is then placed on a spot on the chart that contains a chemical that reacts with glucose. The colour produced on the spot can be compared to the chart. It shows the level of glucose in the blood.

TREATMENT

There is currently no cure for diabetes. However, the condition can be managed well enough to allow most people to live normal lives. Treatment of diabetes focuses on two goals. The first is to keep blood glucose within a normal range, and the second is to prevent complications from developing over time.

Lifestyle Changes for Treatment of Type II Diabetes

Obesity is one of the major causes of type II diabetes. Therefore, controlling one's weight is an important step in controlling the disorder. Type II diabetics are advised to have a well-balanced, nutritious diet and to follow a program of moderate exercise.

The goal in diet planning is to limit one's intake of calories.

The term calories are used to describe the energy content of foods. If one takes in too many calories, they are not used to produce energy. They are converted into fat, which is stored in the body. The number of calories a person should take in each day depends on a number of factors, such as age, weight, and level of activity. Many professional organizations have developed diet plans for people with type II diabetes. These plans insure that people get all necessary nourishment. They also insure that people do not eat more calories than needed for daily activities.

Oral Medications for Type II Diabetes

A number of drugs have been developed for the treatment of Type II diabetes. Most of these drugs belong to a class of compounds known as the sulfonylureas (pronounced SULL-fuh-nil-u-ree-uhz). They include tolbutamide, tolazamide, acetohexamide, andchlorpropamide. These drugs stimulate the pancreas to make more insulin.

These drugs all have side effects. For example, they may cause a person to gain weight. But weight gain is often the original cause of the problem for Type II diabetics. So the drugs may not be very useful. They are still not as satisfactory as a well-planned diet and program of exercise. The drugs are also not effective against Type I diabetes.

Treatment of Last Resort: Surgery

In extreme cases, a pancreas transplant may be performed. In this procedure, the patient's own pancreas is removed and a healthy pancreas substituted. If the surgery is successfully, the healthy pancreas begins producing insulin in the patient's body.

Surgery is often a treatment of last resort. Any surgical procedure has many risks involved. A doctor wants to be certain that those risks are worth the benefit the patient will gain by having a new pancreas.

Prognosis

In most patients, diabetes can be controlled by diet, exercise, and insulin injections. If the condition is not treated, however, some serious complications may result. For example, uncontrolled diabetes is the leading cause of blindness, kidney disease, and amputations of arms and legs. It also doubles a person's risk for heart disease and increases the risk of stroke. Eye problems also occur more commonly among diabetics than in the general population.

Some other long-term effects of diabetes mellitus include the following:

Loss of sensitivity in certain nerves, especially in the legs and feet.

Foot ulcers

- Delayed healing of wounds
- Heart and kidney disease

There is currently no way to prevent Type I diabetes. The risk for Type II diabetes can be reduced, however, by maintaining an ideal weight and exercising regularly.

- **Subheet Jain, Rachna Sapre et al**[75] had studied the protransfersome gel formulations of levonorgestrel were prepared and characterizied for shape, size entrapment efficiency, and drug permeation across rat skin and were evaluated for their stability results indicated that the optimized protransfersomes formulation of levonorgestrel had better skin permeation potential, sustained release characteristic, and better stability than pro-liposomal formulation.

- **Jianxin Guo, Qineng ping et al**[76] had studied two kinds of vesicles with and without the presence of sodium cholate was prepared, using cyclosporine as a drug. When applied onto the excised abdominal skin of mice non-occlusively, the enhancing effects of vesicles on the penetration of cyclorporin A were assessed by an *in-vitro* permeation technique. In conclusion, flexible vesicle is better than conventional vesicle as the carrier for transdermal delivery of cyclosporine was observed.

- **Gamal M. Brian W .Barry et al**[77] have screened lipid vesicles improved *in-vitro* skin delivery of the lipophilic drug, oestradiol, compared with saturated aqueous solution; deformable vesicles were superior to traditional liposomes. Deformable vesicles and traditional liposomes were compared as delivery systems for oestradiol to elucidate possible systems for oestradiol to elucidate possible mechanisms of drug delivery through human skin observed.

- **S.Jain, R.B. Umamaheswari, et al**[78] reported as a review article of ultra-deformable liposomes: a recent tool for effective transdermal drug delivery. In this review the theoretical prospect, basic principle behind the development, mechanism of penetration and applications of transfersomes were studied.

- **S.S.Biju, Sushama Talegaonkar et al**[79] reported as a review article of vesicular systems. The focus of this review is to bring out the application, advantages, and drawbacks of vesicular systems.

- **Christian Hofer, Roland Gobel et al**[80] have screened transfersomes are highly deformable hydrophilic lipid vesicles that are able to penetrate the skin barrier spontaneously because of their characteristics. Transfersomes are able to transport non-invasively low and high molecular weight molecules into the body. It was possible to incorporate a large amount of interleukin-2 and interferon-f in transfersomes and the incorporate interleukin-2 and interferon-f were biologically active.

- **Prem N Gupta, Vivek Mishara et al**[81] had studied proteineous antigen alone or in combination with conventional bioactive carriers penetrate through the intact skin. Hence, specially designed, deformable lipid vesicles called transfersomes were used in this study for the non-invasive delivery of tetanus toxoid. Transfersomes were prepared and characterized for shape,size, entrapment efficiency and deformability index were also discussed.

- **Mahor S et al**[82] explain about the potential cationic transfersomes as DNA vaccine carriers for effective topical immunization. Cationic transfersomes were prepared optimized for their size, shape, zeta-potentials, deformability and loading efficiency. The immune stimulating activity was studied by measuring serum anti-HB_S Ag administered intramuscularly.

- **Benson G et al**[83] had studied development and evaluation of transfersomes and elastic vesicles as topical and transdermal delivery system. Transfersomes are applied in a non-occluded method to the skin and have been shown to permeate through the stratum corneum lipid lamellar regions as a result of the hydration or osmotic force in the skin. They have been used as drug carriers for range of small molecules, peptides, proteins and vaccines, both *in-vitro*

and *in-vivo*. Using the principle of incorporating an edge-activator agent into a bi-layer structure, a number of other elastic vesicle composition have been evaluated.

- **Zhengy B et al**[84] had studied the influence of drug properties on the encapsulation efficiency and drug release of transfersomes for a proper transfersome preparation. To prepare the transfersomes of colchicines (CLC), vincristine sulfate (VCR) and mitoxancrone hydrochloride (DHAD) with the same materials and methods, and then measures their encapsulation efficiency. To perform the drug release experiments of various types of transfersomes *in-vitro*, and compare their differences. VCR and DHAD are lipophilic or hydrophilic, owing positive charges and large molecular weight, as a result, their EE is high. CLC is amphipathic, neutral and small molecular weight, it encapsulation efficiency is very low. The present study suggested; to prepare transfersomes with high EE,drugs that are lipophilic or hydrophilic, high molecular weight and opposite charges to the membrane should be chosen. Interaction between drugs and membrane will influence the rate of drug release.

- **Long XY et al**[85] had studied capsaicin transfersome prepared entrapment efficiency of capsaicin transfersomes reached 96.7%, meeting the criterion of china pharmacopic(>80%), skin penetration of capsaicin was enhanced by a capsaicin transfersomes preparation and was affected by diverse characters and levels of skin. By high shear dispersing machine and evaluated on the entrapment efficiency, drug release rate and *in-vitro* skin permeation.

- **Huy J et al**[86]**,** explains about tanshinone transfersome were prepared and evaluated for its deformability. Tanshinone transfersomes prepared by film dispersion method has good entrapment efficiency and stability. The vesicles possess high deformability in relation to the molar ratio of sodium cholate to lecithin and external pressure.

- **Lu Y , et a[87]., ** demonstrated that preparation method of vincristine transfersomes and predicts its possibility of being a new formulation of vincristine transfersomes. Orthogonal design was used to optimize the preparation methods on the basis of single factor pretests; and the permeation tests *invitro* were performed in modified Franz diffusion cells. The test *in vitro* showed that vincristine transfersomes could permeate through mouse skin at zero Srates with the cumulative penetrating quality amounting to 63.8%.

- **Gupta PN et al[88], ** reported that elastic vesicle transfersomes, non-ionic surfactant vesicles (niosomes) and liposomes were used to study their relative potential in non-invasive delivery of tetancy toxoid (TT). Tansfersomes, niosomes and liposomes were prepared and characterized for shape, size and entrapment efficiency. These vesicles were extruded through polycarbonate filter (50-nm) to assess the elasticity of the vesicles. The immune stimulating activity of transfersomes, niosomes, and liposomes were studied by measuring the serum anti-tetanus toxoid IgG titre following topical immunization. *In-vivo* study revealed that topically given tetanus toxoid containing transfersomes, after secondary immunization, could elicit immune response (anti-TT-IgG) that was equivalent to one that produced following intramuscularly alum-adsorbed tetanus toxoid based immunization. In comparision to transfersomes, niosomes and liposomes elicited weaker immune response. Thus transfersomes hold promise for effective non-invasive topical delivery of antigen.

- **Cevc G. et al**[89] had studied insulin-loaded transfersomes, for example, can deliver the drug through the non-compromised skin barrier with a reproducible drug effect that resembles closely that of an ultra-lente insulin injected under the skin; the pharmacokinetic and pharmacodynamic properties of the injected and transdermal insulin are also comparable. Systemic

normoglycaemia that lasts at least 16-hours has been achieved using a single non-invasive, epicutaneous administration of insulin in transfersomes.

- **Cevc G, Blume G et al**[90]**,** reported that Diclofenac association with ultradeformable carriers permits it to have a longer effect and to reach 10-times higher concentration in the tissues under the skin in comparision with the drug from a commercial hydrogel. The relative advantage of diclofenac delivery by means of ultra-deformable carriers increases with the treated muscle thickness and with decreasing drug close, as seen in mice, rats and pigs. When the drug is used in a hydrogel at 8 times higher dose, the average intramuscular concentration is at least three times lower and subtherapeutic. This suggests that diclofenac in transfersomes has the potential to replace combined oral/topical diclofenac administration in humans.

- **Bhatia A, et al**[91]**,** reported that multilamellar liposomes of tamoxifen were prepared by thin film hydration method. Various formulation (lipid composition, drug-lipid ratio, amount and type of surfactant etc.) and process parameter (hydration temperature, hydration time etc) were studied to obtain liposomes with desired attributes. Tamoxifen molecules could be successfully entrapped in the liposomes with reasonable drug-loading and desired vesicle specific characters. Higher rate of drug transfer across the skin with liposomal formulation of tamoxifen, suggests that the drug in its lipo-solubilised state might have found facilitated entry into the tough barrier consisting of stratum corneum. The phospholipids enriched amphiphillic nature of the vesicles can be held responsible for modifying the properties of the keratinized layer.

- **Jains C et al**[92]**,** reported that evaluating the transdermal route as an alternative to the oral route for improving the systemic bioavailability and sustaining the constant therapeutic plasma level of zidovudine(AZT). Elastic liposomal formulations of AZT were prepared and characterized. The effect of different formulation variables on transdermal delivery of AZT from elastic liposomes

was studied. These elastic liposomes increased the transdermal flux, prolonged the release, improved the site specificity of AZT and represented an attractive strategy for sustained and targeted delivery of AZT.

- **Gaspar MM et al**[93]**,** shows that biological behavior of Acylated superoxide Dimutase (AC-SOD) inserted into the lipid bi-layer of liposomes, in comparison with SOD located in the aqueous compartment of liposomes conventional liposomes presenting an unmodified external surface and long circulating liposomal formulations of AC-SOD and SOD were prepared and labeled with indium-III and their *in-vivo* fate was not influenced by the insertion of AC-SOD in the lipid bi-layers. The potential therapeutic effect of AC_SOD enzymosomes was compared with SOD liposomes in a rat model of adjuvant arthritis. A faster anti-inflammatory effect was observed for AC_SOD enzymosomes by monitoring the volume of the inflamed paws. The present results allowed us to conclude that AC-SOD enzymosomes are nano-carriers combining the advantages of expressing enzymatic activity in intact form and thus being able to exert therapeutic effect even before liposomes disruption, as well as acting as a sustained release of the enzymes.

- **Touitou E et al**[94]**,** have screened ethosomal systems been much more efficient at delivering a fluorescent probe to the skin in terms of quantity and depth, than either liposomes or hydro-alcoholic solution. The ethosomal system dramatically enhanced the skin permeation of minoxidil *in-vitro* compared with either ethanolic or hydroethanolic solution or phospholipids ethanolic micellar solution of minoxidil. In addition, the transdermal delivery of testosterone from an ethosomal patch was greater both *in-vitro* and *in-vivo* than from commercially available patches skin permeation of ethosomal components, ethanol and phospholid, was demonstrated in diffusion cell experiment. Experiments using fluorescent probes and ultracentrifugation showed that the ethosomes had a high entrapment capacity for molecules of various lyophilicities.

- **Elsayed MM et al**[95]**,** have screened deformable liposomes and ethosomes improve skin delivery of ketotifen under non-occlusive conditions were investigated. *In-vitro* permeation and skin deposition behavior of deformable liposomes and ethosomes, having ketotifen only inside the vesicles (free ketotifen separated) and having ketotifen only outside the vesicles (Ketotifen solution added to empty vesicles), was studied using rabbit pinna skin. Results suggested deformable vesicles might play a role in improving skin delivery of drugs under non-occlusive conditions, and that the penetration enhancing effect was greater importance incase of ketotifen. Regarding ethosomes, results indicated that ketotifen should be incorporated in ethosomes vesicles for optimum skin delivery.

- **Giuseppe Derosa et al**[96]**,** have screened to evaluate the expected improvement in glucose and lipid metabolism obtainable with doxazosin is or is not synergistic with standard anti-hyperglycaemic treatment using the α-glucosidase inhibitor acarbose. Evaluated 107 patients with impaired glucose tolerance (IGT) as determined by oral glucose tolerance tests. All patients took a fixed close of acarbose 150mg/day for 3 month. In addition, patients were randomized to either placebo or doxazosin 4mg/day for the entire 6 month treatment period. Parameters were evaluated during the 6-month treatment period include body mass index, glycaemic control, fasting plasma etc. it was concluded that doxazosin given in combination with Acarbose seemed to improve gly-caemic and lipid control compared with placebo in patients with IGT.

- **Cyndya snibao et al**[97]**,** reported the effectiveness of acarbose for the treatment of post-prandial hypotension in 13 patients with severe autonomic failure secondary to post ganglionic neuronal denervation. Acarbose treatment reduced the post prandial fall in systolic blood pressure by 17mm Hg and the diastolic drop by 9mmHg, the authors report. Heart rate response did not differ significantly between treatment and placebo groups. Acarbose provides

a novel pharmacological approach to treat this condition. 100mg of Acarbose taken 20 minutes before meals effectively attenuates the fall in blood pressure induced by meals in patients with severe autonomic failure.

- **Dieter Neuser et al**[98]**,** have screened safety and tolerability of Acarbose in the treatment of type I and type II diabetes mellitus. In this study 35% Acarbose and 24% of placebo patient's adverse events were the main reason for withdrawal in Acarbose recipients. The most common adverse events for Acarbose recipients were gastrointestinal (abdominal pain, flatulence and diarrhea), which were more frequent than in placebo patients. These events occurred more often early in the study and attenuated over time. It concluded that Acarbose was safe and well tolerated by the majority of diabetic patients over a 1 year treatment period.

- **Hucking k et al**[99] reported that Acarbose is able to enhance glucagons – like peptide -1 release and delay gastric emptying in normal subjects. The effect of alpha – glucosidase inhibition on glucagons like peptide -1 has been less evident in type 2 diabetic patients. The aim of this study was to investigate the possible influence of Acarbose on glucagons like peptide-1 release and gastric emptying in type 2 diabetic patients after mixed test meal. It concluded that hyper-glycaemic type 2 diabetic patients, ingestion of Acarbose with a mixed test meal failed to enhance glucogon like peptide-1 release and did not influence gastric emptying.

- **Brunkhorst M et al**[100]**,** had studied Acarbose, a pseudomaltotetrasoe, is produced by strains of genus Actionplanes. Acarbose synthesis is induced in the presence of maltose and maltotriose. The aim of the study to investigated the transport activities of maltose and maltotriose in actinoplanes sp. Results suggest that an Acarbose- insensitive maltose transporter that also accepts maltodextrins operates in Acarbose – grown cells while a maltodextrin

transporter that accepts maltose and is moderately sensitive to Acarbose is found in cells grown in maltose / maltotriose containing media.

- **Laurie Barclay, M.D,et al**[101]**,** had studied liquid chromatography and capillary zone electrophoresis, respectively compiled to an evaporate right scattering detector and a vv detector have been developed for the analysis of Acarbose without any derivatization procedure. The electrophoretic separation of acarbose anomers was achieved through the manipulation of the working temperature. Both methods were validated and showed good validation data in terms of precision, accuracy and linearity. The validated methods were successfully applied to the dosage of Acarbose in commercially available glucobay tablets.

- **Henrik Wagner, et al**[102]**,** reported that the chemistry pharmacology, pharmacokinetics, and clinical efficacy of Acarbose reviewed. It concluded that the Acarbose is efficacious in improving metabolic control in non-insulin- dependent diabetes mellitus. Further evaluation of its effects on the long – term complications of diabetes is needed.

- **Marija Glavas – Dodav et al**[103]**,** had studied liposome gels bearing an antineoplastic agent, 5-fluorouracil, intended for topical application have been prepared and drug release properties *invitro* have been evaluated.

3. Research Envisaged

Objective of the study

Diabetes mellitus continues to be one of the leading causes of death in the world particularly in India. Generally Oral anti – diabetic drug (Acarbose) have more frequency of dosing and low biological half life.

Hence reduce frequency of dosing and adverse effects without compromising the cure and relapse rates still remains a major goal for control policies.

Transfersomes prolongs the circulation of entrapped drug and protect the drug from metabolic degradation. Transfersomes can deform and pass through narrow constriction of dermal layers without measurable loss. This high deformability gives better penetration of intact vesicles.

The Acarbose transfersomes are prepared with various phospholipids concentration by film hydration method.

Further more, encapsulation of Acarbose in transfersomes may eliminate the inherent drawbacks experienced with oral administration of Acarbose.

Thus the present study was undertaken to explore the encapsulation of Acarbose, to characterize the transfersome for various physiochemical properties and their utility in diabetic treatment after topical administration

Plan of the work

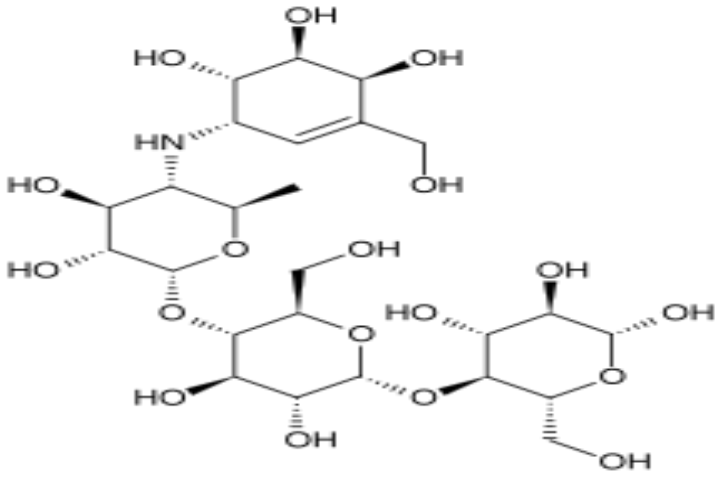
The following episodes are planned to carry out in this study.

1. To formulate the Acarbose transfersomes by film hydration method
2. To observe the particle size and size distribution of the prepared transfersomes.
3. To estimate the entrapped drug.
4. To test the stability of the formulated transfersomes.
5. To observe the compatibility of the product and excipients used in the formulation.
6. To examine the *in – vitro* diffusion study of the prepared Acarbose transfersomes gel.
7. To perform the anti- diabetic activity of the Acarbose transfersomes gel.
8. To perform the skin irritation test for the Acarbose Transfersomes gel.

4. DRUG AND EXCIPIENTS PROFILE

ACARBOSE[104]

- **Molecular formulae** : $C_{25}H_{43}NO_{18}$



IUPAC Name : *Acarbose is designated by IUPAC rules as:*

O -4, 6-dideoxy- 4-[[(1S, 4R, 5S, 6S)-4, 5, 6-trihydroxy-3- (hydroxymethyl) -2-cyclohexen-1-yl] amino]-α -D-glucopyranosyl - (1 → 4) - *O* -α-D- glucopyranosyl-(1 → 4)-D-glucose.

Acarbose is an oral alpha-glucosidase inhibitor for use in the management of type 2 diabetes mellitus. Acarbose is an oligosaccharide which is obtained from fermentation processes of a microorganism, ***Actinoplanes utahensis,***

- **Synonym:** Bay – g – 5421
- **Brand names :** Glicobase , precose
- **p.K_a:** 5.1
- **Molecular Mass:** 645.6 gm/mol
- **Category:** Anti-diabetic
- **Description :** white amorphous powder
- **Solubility :** very soluble in water, soluble in methanol, ethanol.

- **Mechanism of action:**

Acarbose inhibits enzymes (glycoside hydrolase's) needed to digest carbohydrate: specifically alpha-glucosidase enzymes in the brush border of the small intestines and pancreatic alpha-amylase. Pancreatic alpha-amylase hydrolyzes complex starches to oligosaccharide in the lumen of the small intestine, whereas the membrane-bound intestinal alpha-glycosidase hydrolyzes oligosaccharide, trisaccharide, and disaccharide to glucose and other mono-saccharide in the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of complex

carbohydrates. Less glucose is absorbed because the carbohydrates are not broken down into glucose molecules. In diabetic patients, the short-term effect of these drugs therapies is to decrease current blood glucose levels: the long term effect is a small reduction in Hb level.

Pharmacokinetic data:

- **Bio availability :** Extremely low
- **Metabolism:** G.I.T
- **Half life:** 2hrs
- **Excretion:** Renal (less than 2%)
- **Volume of distribution :** Steady state v.d is 0.32 L/Kg
- **Protein binding:**

Minimal protein binding with plasma-concentration of more than 1microgram /L and up to 98% with 0.08 microgram/L.

- **Side effects:**

Since Acarbose prevents the degradation of complex carbohydrates in to glucose, the carbohydrate will remain in the intestine; in the colon bacteria will digest the complex carbohydrates there by causing gastrointestinal side effects such as flatulence and diarrhea.
Since these effects are dose related, it is generally advised to start with a low dose and gradually increase the dose to the desired amount.

CONTRAINDICATIONS

Acarbose is contraindicated in patients with known hypersensitivity to the drug and in patients with diabetic ketoacidosis or cirrhosis. Acarbose is also contraindicated in patients with inflammatory bowel disease, colonic ulceration, partial intestinal obstruction or in patients predisposed to intestinal obstruction. In addition, Acarbose is contraindicated in patients who have chronic intestinal diseases associated with marked disorders of digestion or absorption and in patients who have conditions that may deteriorate as a result of increased gas formation in the intestine.

- **Dose:** 25, 50, 100 mg (t. i. d)

1) Polyoxyethylene 20 Sorbitan Monopalmitate[105]

Synonym: Tween 40

Molecular formula: $C_{62}H_{122}O_{26}$

Molecular weight: 1284

Description: Polysorbates have a characterstic odour and a warm, some what bitter taste, yellow oily liquid at 25^0C

Solubility: Soluble in ethanol, water

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

Application: Polysorbates are also widely used in cosmetics and fool product, emulsifying agent, solubilizing agent, wetting agent

2) Lecithin[105]

Synonym: Soybean Phospholipids

Description: Lecithin vary greatly from viscous semiliquids to powder depending upon the free fatty acid content , colour may vary from brown to light yellow depending upon the degree purity.

Solubility: Lecithins are soluble in aliphatic and aromatic hydrocarbons, mineral oil practically insoluble in water.

Storage: Lecithin should be stored in well closed container protected from light and oxidation

Application: Lecithins are mainly used in pharmaceutical prouducts as dispersing, emulsifying, and stabilizing agents and are included in intramuscular and intra venous injections.

3. Carbomer[105]

Synonym Carbopol

Description: Carbomers are white – coloured, fluffy, acidic, hygroscopic powders with a slight characteristic odour.

Melting points: 260^0C

Solubility:	Soluble in water and after neutralization, in ethanol (95%) and glycerin.
Application:	Carbomers are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity – increasing agents. Formulations include creams, gels and ointments for use in ophthalmic, rectal, and topical preparations.

5. MATERIALS AND METHODS

Table No.4 Materials Used

S. No	Materials	Suppliers
1	Acarbose	Orchid
2.	Lecithin	Himedia
3.	Carbopol – 934	Kemphasol
4.	Tween 40	S.D. Fine
5.	Potassium dihydrogen Ortho phosphate	Ranbaxy
6.	Disodium hydrogen phosphate	Ranbaxy
7.	Sodium hydroxide	Nice
8	Triton x 100	Qualigens
9	Membrane Filter	Sigma

Table No.5 Equipments used

S. No	Equipments used	Specification
1.	Electronic Balance	Sartorius
2.	Magnetic Stirrer	Remi
3.	Rotary Flash evaporator	Roteva
4.	UV- Visible Spectrophotometer	Shimadzu UV – 1700
5.	Ultra Sonic Processor	Vibronics
6.	pH meter	Elico
7.	Differential Scanning colorimetry	
8.	Laser particle counter	Spectrex
9.	Scanning electron microscopy	Hitachi
10.	FT-IR	Perkin Elmer

Preparation of reagents:

Preparation of phosphate buffer pH6.4:

The phosphate buffer pH 6.4 was prepared by dissolving 2.38g of disodium hydrogen phosphate, 0.19g of potassium phosphate and 8.0g of sodium chloride in sufficient distilled water to produce 1000ml. Adjust the pH if necessary.

Standard Curve of Acarbose:

Preparation of Standard Curve of Acarbose:

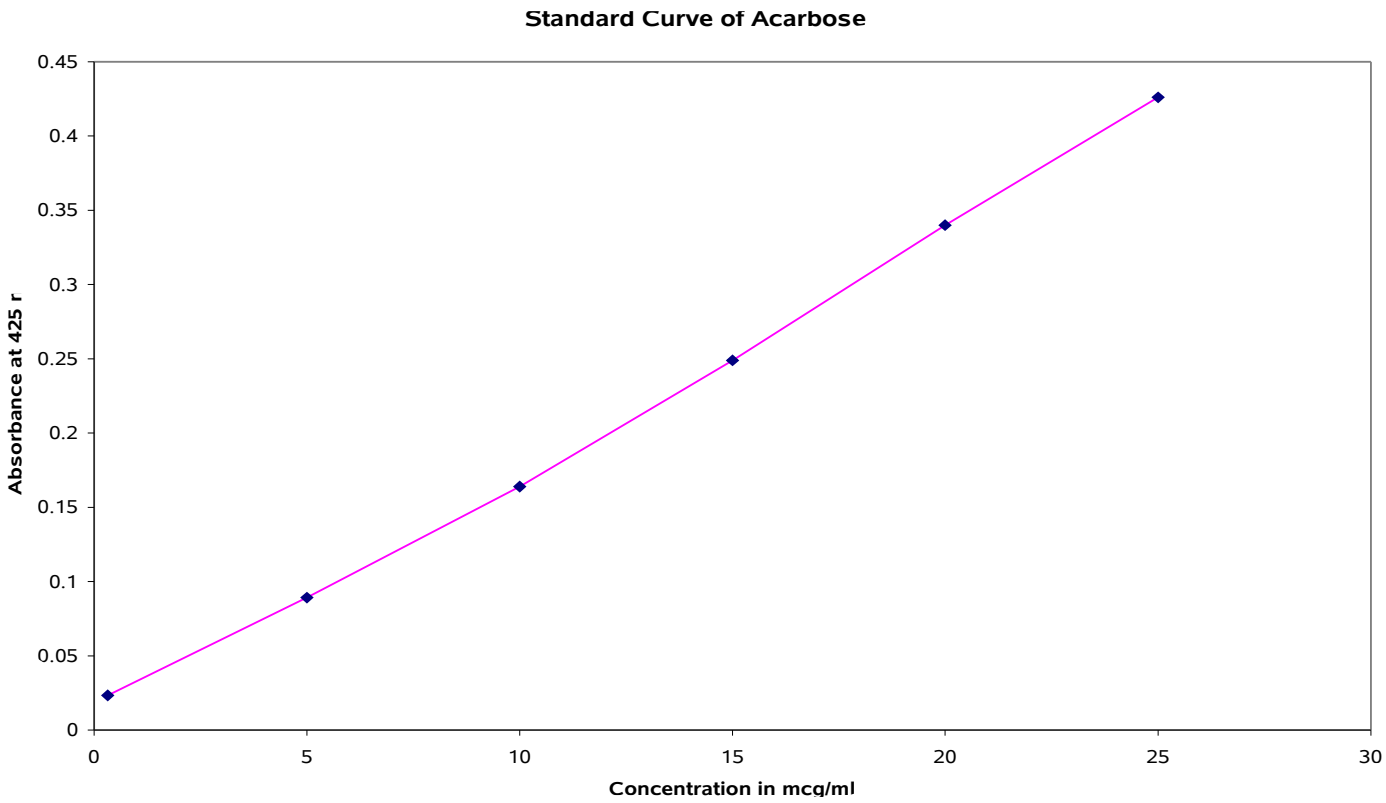
Weigh accurately about 0.1g of acarbose dissolve it in water to produce 100ml. From the stock solution serial of concentrations 5, 10, 15, 20, 25µg/ml was taken by diluting the solution to 0.5, 1, 1.5, and 2, 2.5ml to make up to 10ml with phosphate buffer pH 6.4, and measure the absorbance of the resulting solution at maximum about 425 nm. It is shown in table no. 6, figure no. 3.

Table No.6 Standard Curve of Acarbose

Concentration (mcg/ml)	Absorbance at 425nm
5	0.0892
10	0.164
15	0.236
20	0.34
25	0.426

a= 0.0088, b=0.0164, r=0.9975

Fig No. 3 Standard Curve of Acarbose



PREFORMULATION STUDIES

Drug-Excipients interaction study

The drug and excipients were analyzed by Fourier-Transform I.R spectrophotometer. The interaction of drug with the excipients was identified through interpretation of I.R spectrums.

Solubility Studies

The spontaneous interaction of two or more substance to form homogeneous molecular dispersion is called as solubility. The solubility ofAcarbose was studied in various solvents. Acarbose (10mg) was suspended separately in a 10 ml of different solvents at room temperature in tight closed test tube and shaken on wrist action. The solubility of Acarbose in various solvents are follows

- very soluble in water
- soluble in methanol & ethanol

PREPARATION OF TRANSFERSOMES[106]

All methods of preparation of transfersomes are comprised of two steps. First, a thin film is prepared, sonicated vesicles are homogenized by extrution through a membrane filter. The mixture of vesicles forming ingredients, that is phospholipids and surfactant were dissolved in volatile organic solvent (chloroform-methanol), organic solvent evaporated above the lipid transition temperature using a rotary evaporator. Final traces of solvent were removed under vaccum for overnight. The deposited lipid films were hydrated with buffer (6.4) by rotation at 60 rpm / min for 1hr at the corresponding temperature. The resulting vesicles were swollen for 2hr at room temperature .To prepare small vesicles; resulting LMVs were probe sonicated for 30 min at room temperature. The sonicated vesicles were homogenized by manual extrution through membrane filter.

Preparation of Topical Transfersome Gel Formulation:

Prepared transfersomes were incorporated in to carbopol-934 (1%) gel base in a 1:1 ratio. The carbopol-934 (1%) gel base prepared by soaking 30 min followed by continuous stirring with water.1% carbopol-934 gel base has a good consistency (gelling characteristic).

COMPATIBILITY STUDY

The stability of a formulation depends upon the compatibility of the drug with the excipients. It is of significance to detect any possible physical (or) chemical interaction, since it can affect the bio-availability and stability of the drug.

Differential scanning calorimetry is a fast and reliable method to screen drug- excipients compatibility and provide maximum interaction.

Differential Scanning Calorimetry (DSC)

Differential Scanning calorimeter (Perkin – elmer) equipped with a monitor and a Computerized Thermal Analysis System and printer were used. The instrument was calibrated with standard medium.

5-10 mg of samples were weighed and it is hermetically sealed in flat-bottomed alumininum pans. These samples were heated over a temperature range of 50-550^{0}C in an atmosphere of nitrogen (50 ml/min.) at a constant heating rate of 20^{0}C per minute, with almina being the reference standard.

DSC provide the gross idea about the possibility of interaction simply from visual comparison of the curves relevant to the individual excipient.

CHARACTERIZAITON OF TRANSFERSOMES

DETERMINATION OF PARTICLE SIZE

The particle size of Acarbose Transfersomes was viewed and photographed using Scanning electron microscopy. Acarbose Transfersomes were coated with gold by using Hitachi Vaccum evaporator. Coated samples were viewed and photographed in Hitachi s-3000H SEM.

SIZE DISTRIBUTION OF TRANSFERSOMES

Transfersomes were subjected in to laser particle counter (Spectrex make) for characterizing size distribution of transfersomes.

ENTRAPMENT EFFICIENCY [107]

The entrapment efficiency was determined by using direct method. Detergents are used to break the transfersome membranes1 ml of 0.1% Triton X-100(Triton X-100 dissolved in phosphate buffer) was added to 0.1 ml Transfersomes preparations and made up to 5 ml with phosphate buffer then it was incubated at 37^{o}C for 1.5 hrs to complete breakup of the transfersome membrane and to release the

entrapped material. The sample was filtered through a Millipore membrane filter (0.25) µm. and the filtrate was measured at 425 nm for Acarbose. The amount of Acarbose was derived from the calibration curve.

The entrapment efficiency is expressed as:

$$\text{Percentage Entrapment Efficiency} = \frac{\text{Amount entrapped}}{\text{Total amount added}} \times 100$$

TRANSFERSOMES GEL EVALUATIONS

Determination of viscosity

Viscosities of the gels were determined by using Brookfield Viscometer (model- RVTP).Spindle type, RV-7 at 20 rpm. 100gm of the gel was taken in a beaker and the spindle was dipped in it and rotated for about 5 minutes and then reading was taken.

Extrudability

It is useful empirical test to measure the force required to extrude the material from the tube. The formulations were filled in a collapsible metal tubes with a nasal tip of 5mm opening tube extrudability was then determined by measuring the amount of gel, extruded the tip when a pressure was applied on tube gel. The extrudability of the formulation was checked and the results were tabulated.

STABILITY STUDY

The formulated transfersome gels were divided in to 3 groups. These 3 groups of transfersome gel formulation was filled in to aluminium collapsible tubes and stored at

a. Room temperature
b. 37± 5 °C
c. 4-5 °C

The transfersome gel formulation was stored for a period of three months. Sample were withdrawn at every month for a period of three months and assessed for the drug content. At the end of third month they were evaluated for physical parameter and integrity of the product.

1. Physical evaluation

The physical parameters considered for the evaluation were visual appearance, nature of the product, pH, viscosity, leak, phase separation and extrudability.

2. Chemical evaluation

The drug content of the formulation was estimated by withdrawing samples from different corners of the tube. The samples were mixed together and 1gm was taken for the assay. The estimation of drug content was carried out as per the procedure.

6. IN VITRO DRUG RELEASE

Diffusion Study for Acarbose Transfersomes

The *in vitro* release of Acarbose from the transfersome formulations were studied by open ended cylinder method. This diffusion cell apparatus consist of a glass tube with inner diameter of 2.5cm, open at both ends. One end tied with artificial membrane, which serves as a donor compartment.

This study is performed for determining the permeation rate. The time needed to attain permeation flux at steady state and the information from *in vitro* studies were used to optimize the formulations. Studies of drug release from transfersomes gel formulation were performed using the *in vitro* diffusion method at37^0c, 100 rpm, within a period of 24hr. A weighed amount of prepared transfersomes gel formulation was poured in to the glass cell and diffused against phosphate buffer pH6.4 as a diffusion medium. Aliquots were taken at regular intervals and analyzed spectro-photometrically at 425nm using phosphate buffer pH 6.4 as blank.

7. ANTI DIABETIC ACTIVITY

Antidiabetic activity of formulated transfersomes was carried out by using Albino rat as a animal model. The healthy albino rats of either sex were selected. The hair on the dorsal side of rat was shaved on he previous day of the experiments. Following an over night fast rats were divided 6 groups of 6 animals each and they were treated as follows.

Group I served as a control, which received normal saline, all other groups were made diabetic by an intra peritonial injection of 2% alloxan monohydrate (150mg/kg body weight). Group 2 received normal saline act as a Diabetic control. Group – 3 received acarbose 50 mg/kg act as a standard. Group 4,5 and 6 received formulated transfersomes having entrapment efficiency 33.33% , 42.6% and 46.66% respectively which were mixed in 1gm of 1% carbopol -934 gel base. Treatment was started after 48 hrs of alloxan administration and continued up to 15 days.

After 15 days the animals were sacrificed, the blood samples were collected 1hr after the drug administration and tested for blood glucose level by using semi auto analyzer[108].

8. SKIN IRRITATION TEST

The skin irritation was carried to evaluated hypersensitivity of transfersomes. The study was carried out by using albino rats. The albino rat were divided in to 6 groups of 6 animals each. On the previous day of the experiment, the hair on the dorsal surface of rat was shaved. Group I was served as normal, without any treatment. Group II act as a control, which was applied with marketed adhesive tape. Group III act as a standard which was applied with 0.8% v/v aqueous solution of formalin (Standard irritant). Group IV, V, VI are act as a test which was applied with Acarbose trasfersomes gel formulations. Acarbose transfersomes gels and formalin solution applied in to animal's skin each day for 7 days[109].

9. RESULTS AND DISCUSSION

Encapsulation of a drug in vesicular structures can be predicted to prolong the existence of the drug in the systemic circulation and thus enhance penetration in to target tissue, and perhaps reduce toxicity if selective uptake can be achieved.

Transfersomes self-optimized aggregates, with the ultraflexible membrane,are able to deliver the drug reproducibly either into or through the skin, with high efficiency.

Acarbose was chosen as the anti-diabetic type-2 (alpha-glucosidase inhibitor) drug because it reduces blood glucose level without producing hypo-glycaemia, decreasing insulin level compared to the sulfonylurea class of anti-diabetic drugs. Acarbose, when given orally has a short elimination half life and can cause gastrointestinal irritation, abdominal disturbance, diarrhoea. Frequent oral administration of the drug is required for treatment. Encapsulation of Acarbose may eliminate the inherent drawbacks experienced with oral administration of Acarbose.

Hence Acarbose Transfersomes were formulated, evaluated, studied and concluded that long circulation time of the formulation accounts for its superior therapeutic effectiveness.

Acarbose encapsulated transfersomes were prepared by film-hydration method using rotary flash evaporator.

Vesicle size, size distribution of transfersomes, percentage drug entrapment, vesicle stability, drug with excipients interaction studies were evaluated. Also, the *invitro* release study through open tubular method with artificial membrane.

Anti-diabetic activity of transfersomes were carried out in albino-rats bearing diabetic and parameters such as blood glucose level compared with standard.

PREFORMULATION STUDY

Drug-Excipients interaction study

- The drug Acarbose and the excipients namely lecithin, tween-40, carbopol, were analyzed by Fourier-Transform I. R spectrophotometer. It is shown in the Figure no. 4, 5,6,7,8.

- The FT-IR spectrums were interpreted and it is shown there is no interaction between the drug with the excipients was conformed. It is shown in Table No. 7

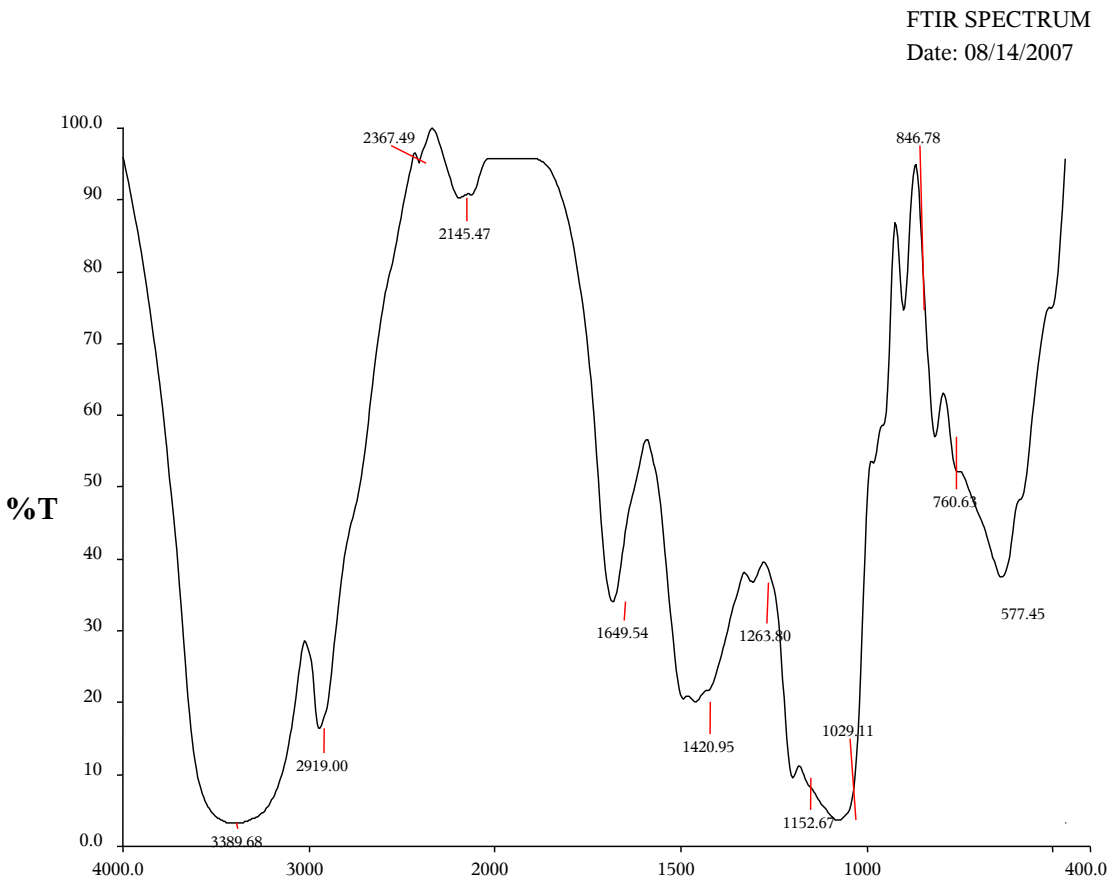
CHARACTERIZATION OF TRANSFERSOMES

PARTICLE SIZE ANALYSIS OF TRANSFERSOMES

The transfersomes were subjected to microscopic examination (S.E.M) for characterizing size and shape of the transfersomes. Microscopic examination revealed, spherical small uni-lamellar vesicles of 270-650, 360-550, 290-550 size range, for Acarbose transfersomes of 3:2:1, 3:3:1, 3:4:1 ratios respectively. The average mean particle size of formulation 1, 2 and 3 were 405nm, 455nm, 420nm respectively. Photographs were given in Figure. 9, 10, 11.

Figure No.4

Spectrum Name: Acarbose

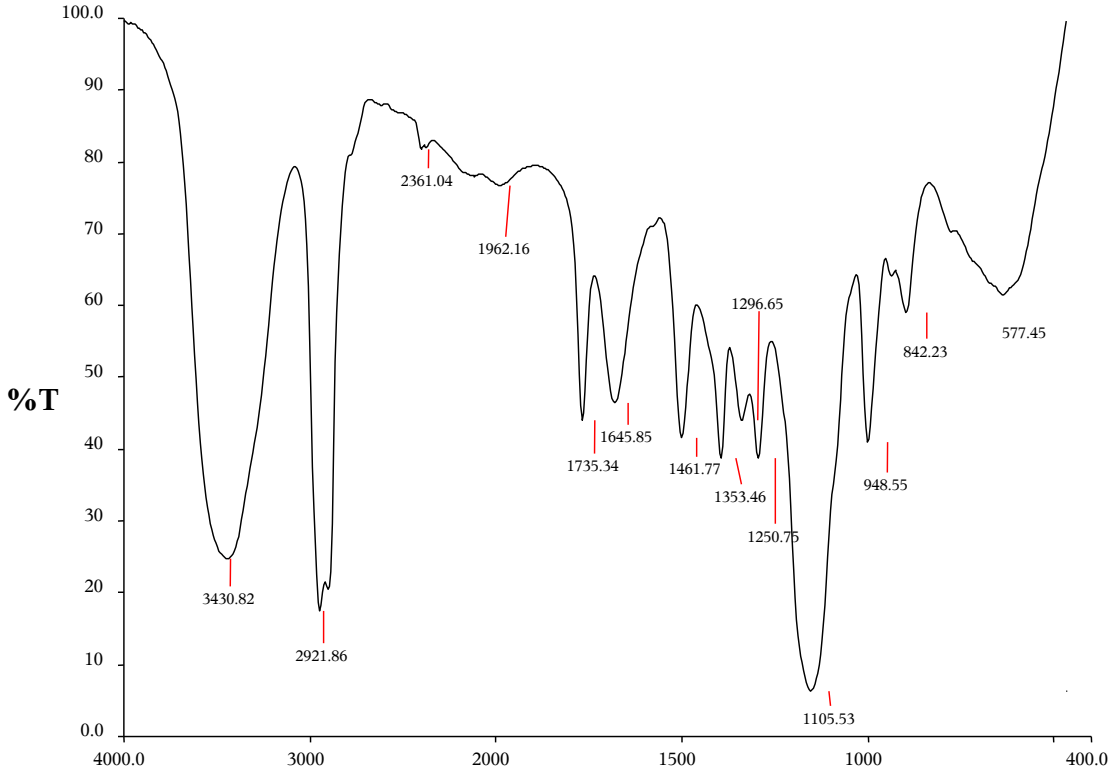


Wave number Cm^{-1}

Figure No.5

Spectrum Name: Tween-40

FTIR SPECTRUM
Date: 08/14/2007



Wave number Cm^{-1}

Figure No.6

Spectrum Name: Carbopol-934

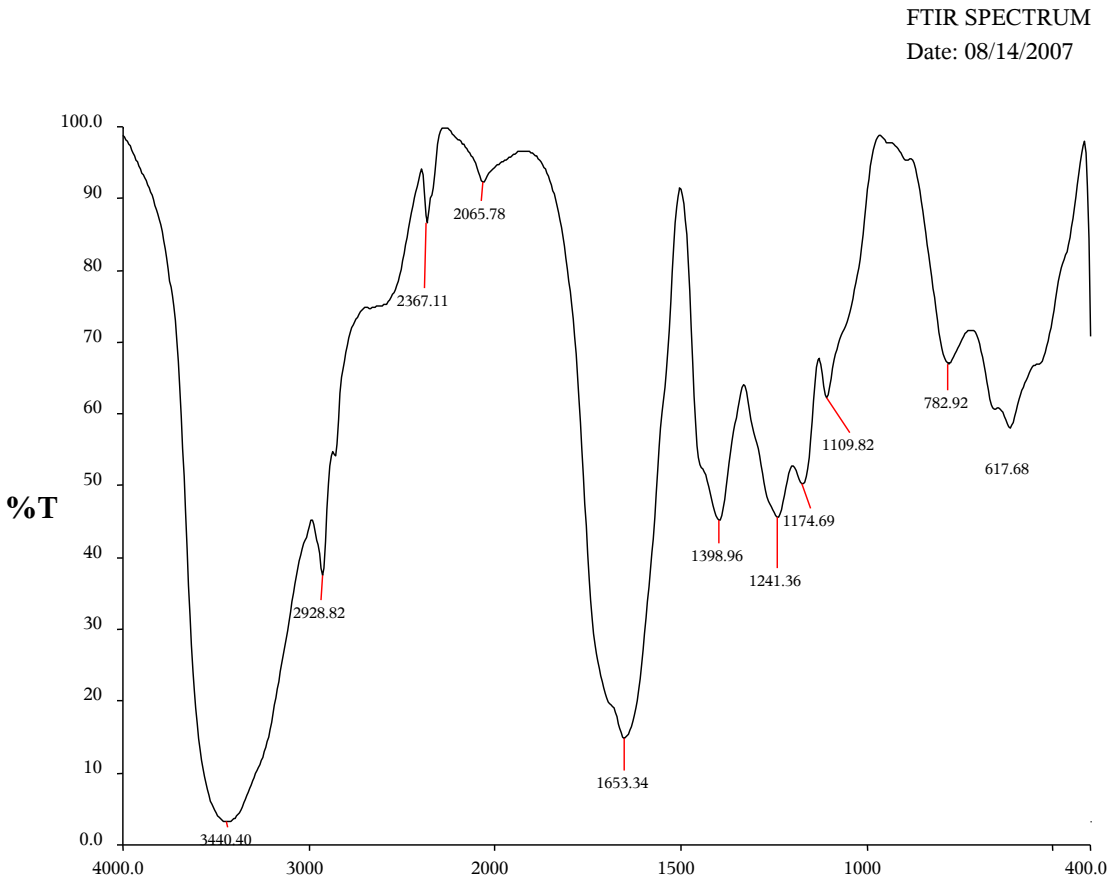


Figure No.7

Spectrum Name: Lecithin

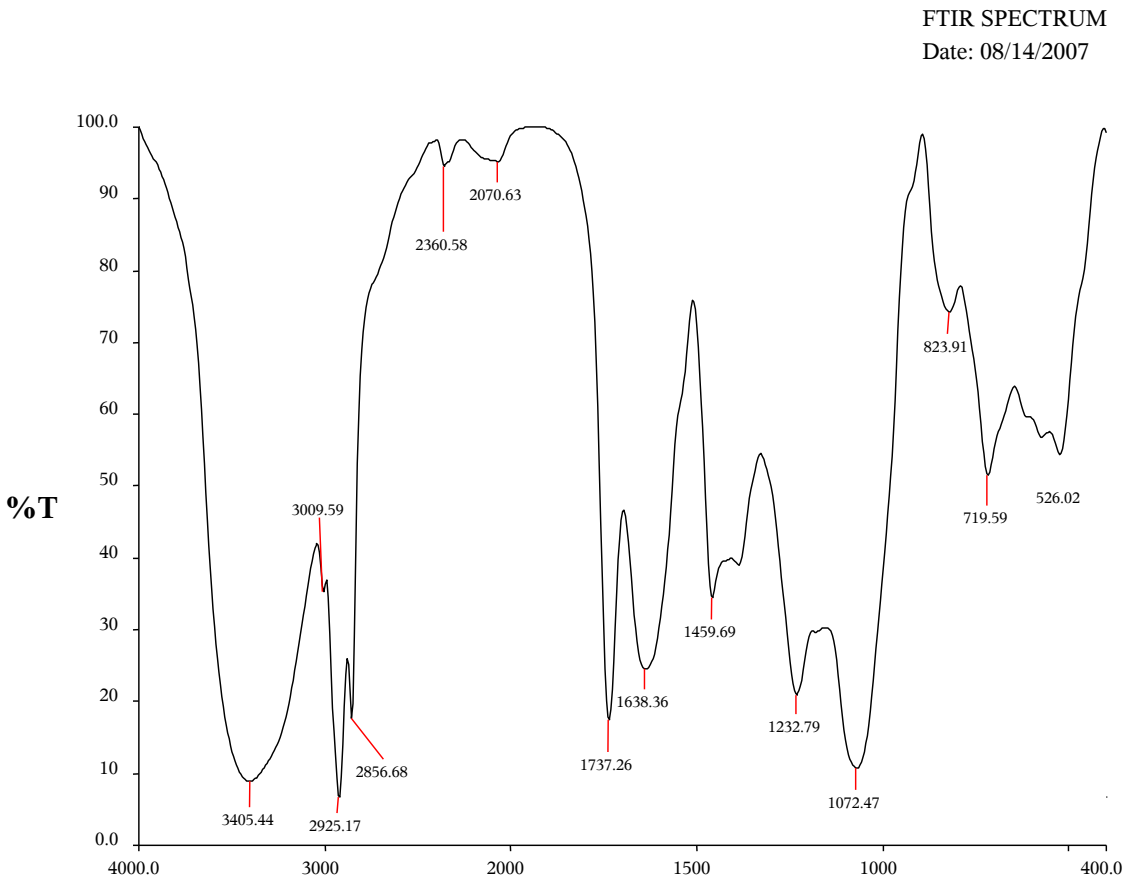
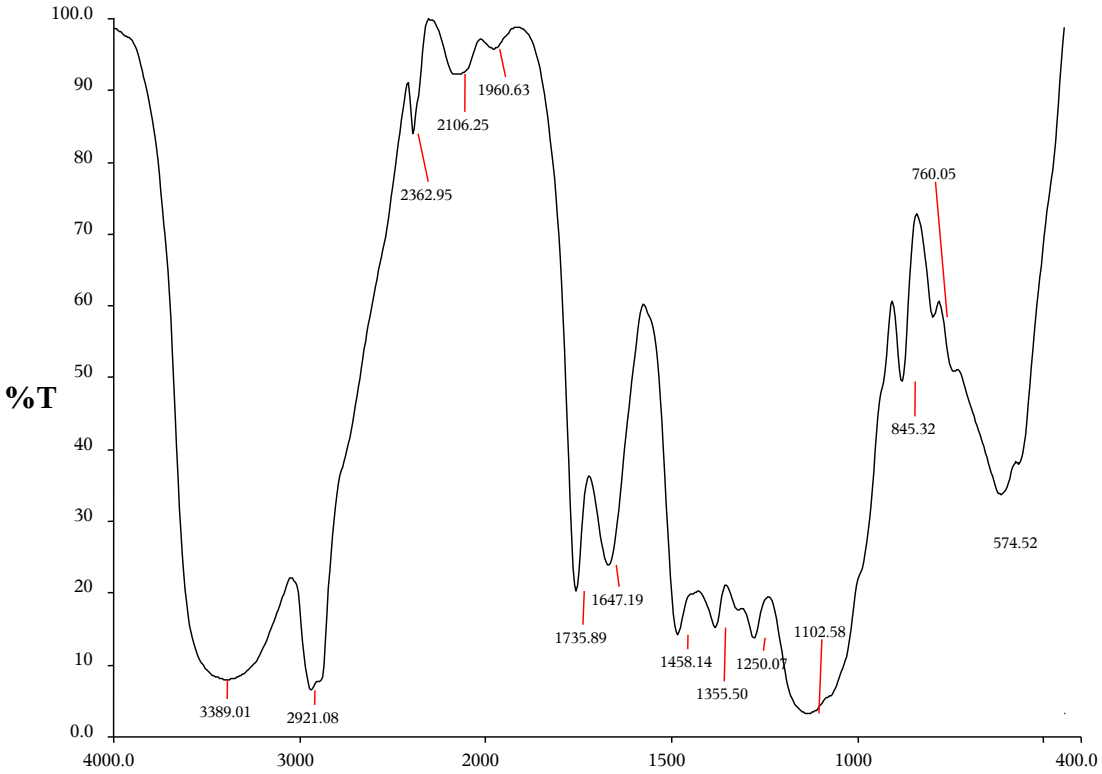


Figure No.8

Spectrum Name: Aca+Tween-40+Lec+Carbo

FTIR SPECTRUM
Date: 08/14/2007



Wave number Cm-^{1}

Table: 7

IR-INTERPRETATION

Sample	**Functional groups(1/cm)**					
	(OH) alcoholic	-CH- aromatic	C=O	C-O-C	-CH2 chain	-CH-
Acarbose	3389	2919	1649	1152	----	----
Lecithin	3405	----	----	1072	2360	2856
Tween-40	3430	2921	1645	1105	----	----
Carbopol	3392	----	1654	----	----	2920
Aca+Lec+Tw+Car	3389	2921	1647	1102	2362	2921

Compatibility Study

The DSC thermograms of acarbose, lecithin, tween-40, carbopol-934 and physical mixture of the above were obtained and compared. DSC trace combination is a simple superposition of the individual components traces, and incompatibility is highly unlikely. The appearance of extra-thermal effects in the DSC trace or the disappearance of one of the component peaks is an indication of an incompatibility.

DSC thermograms of Acarbose alone (Graph.9) shows that an onset of 290^0C and a maximum occurring at 149^0C.

DSC thermograms of Lecithin alone (Graph.10) shows that an onset of 210^0C and a maximum occurring at 160^0C

DSC thermograms of Tween -40 alone (Graph.11) shows that an onset of 150^0C and a maximum occurring at 70^0C

DSC thermograms of Carbopol alone (Graph.12) shows that an onset of 56^0C and a maximum occurring at 50^0C

The Comparative graph 13 shows that [physical mixture (1:1:1:1) of Acarbose, Lecithin, Tween-40, Carbopol] there is no incompatibility between the drug and excipients.

Figure No.9
DSC Thermogram of Acarbose

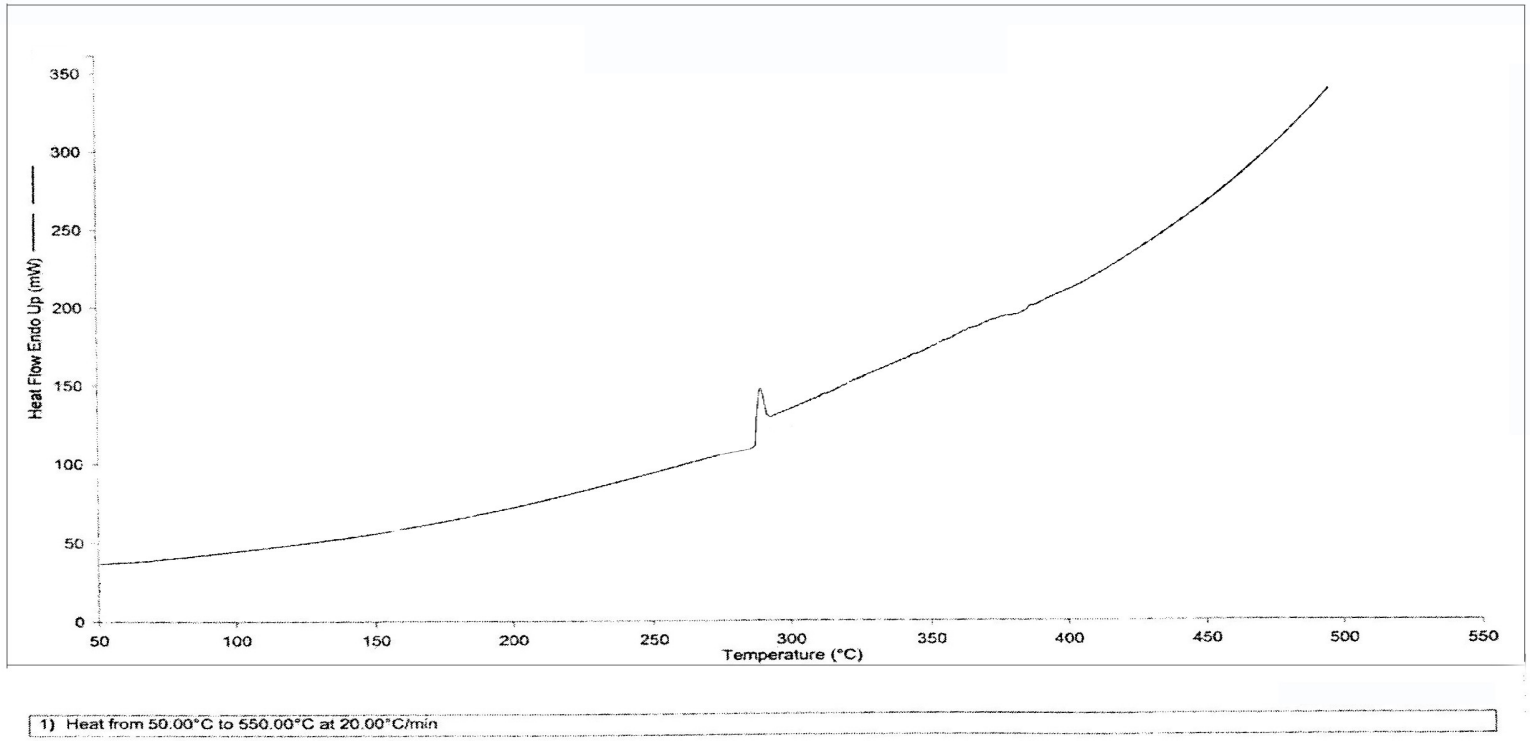


Figure No 10
DSC Thermogram of Lecithin

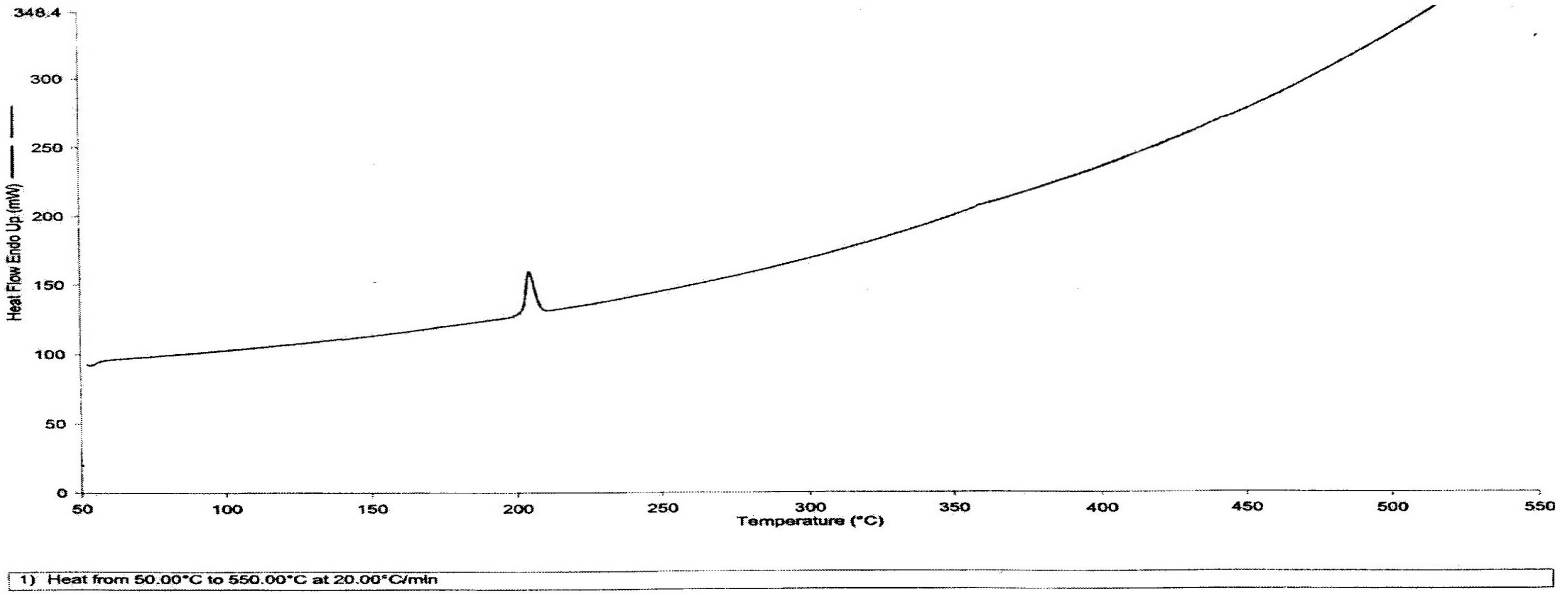


Figure No. 11

DSC Thermogram of Tween -40

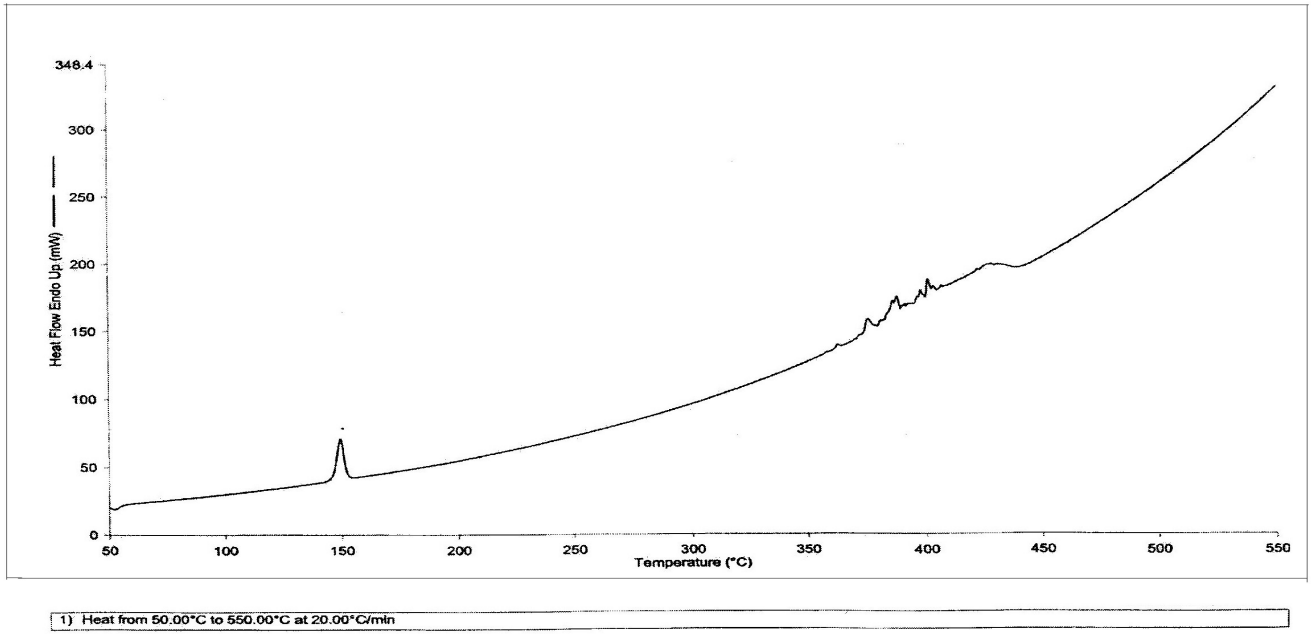


Figure No. 12

DSC Thermogram of Carbopol

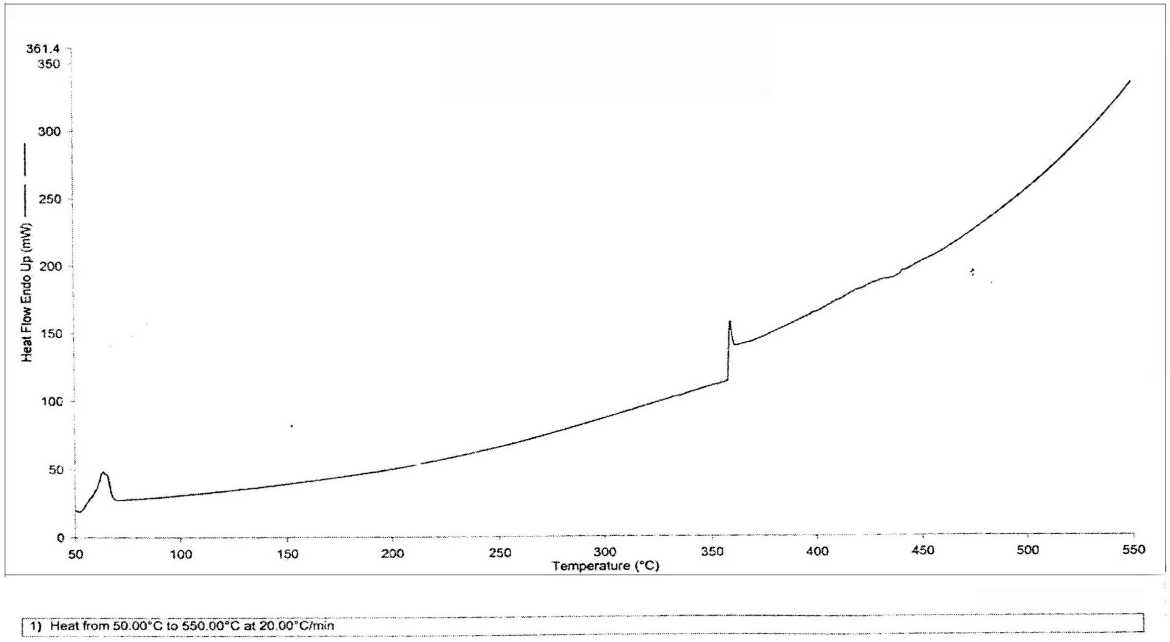


Figure No . 13
DSC Thermogram of Carbopol, Tween-40, Acarbose, Lecithin (Physical mixture 1:1:1:1)

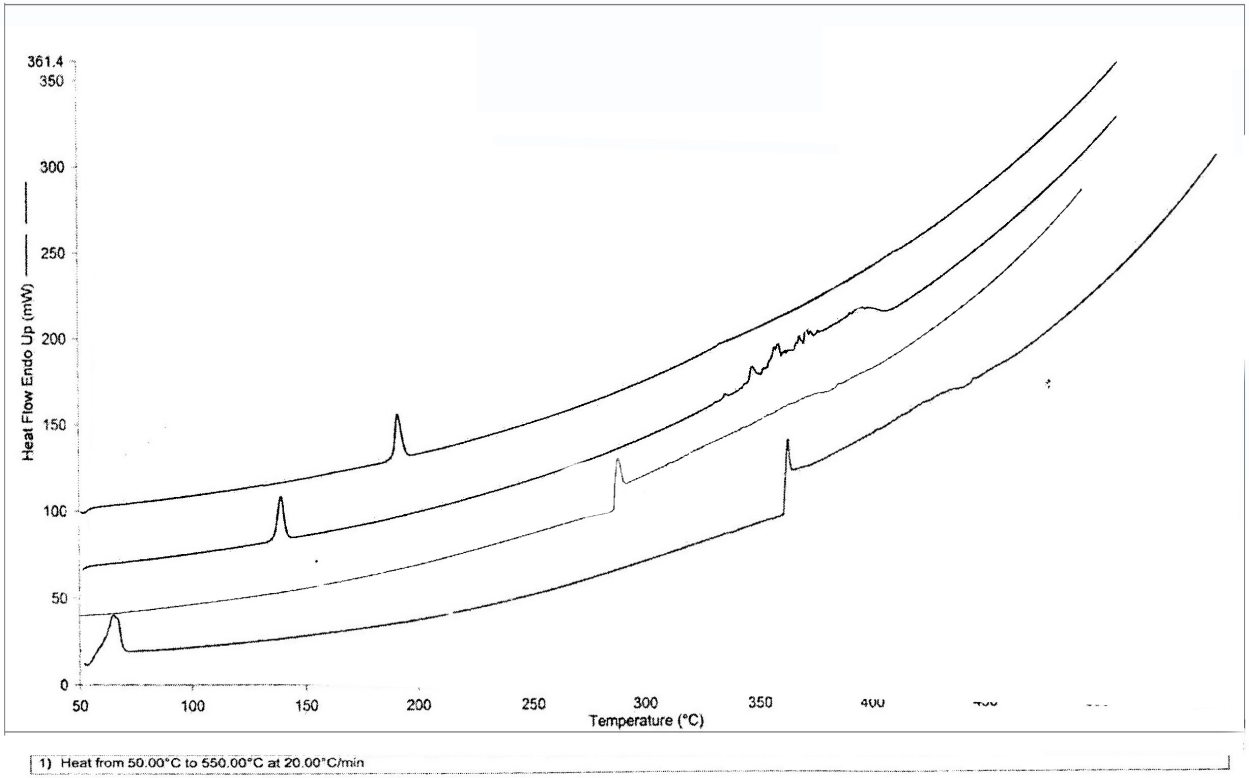

361.4
350
300
250
200
150
100
50
0
Heat Flow Endo Up (mW)
50
100
150
200
250
300
350
400
Temperature (°C)
1) Heat from 50.00°C to 550.00°C at 20.00°C/min

Figure No.14

SEM Photograph of Acarbose Transfersomes (Formulation-1)

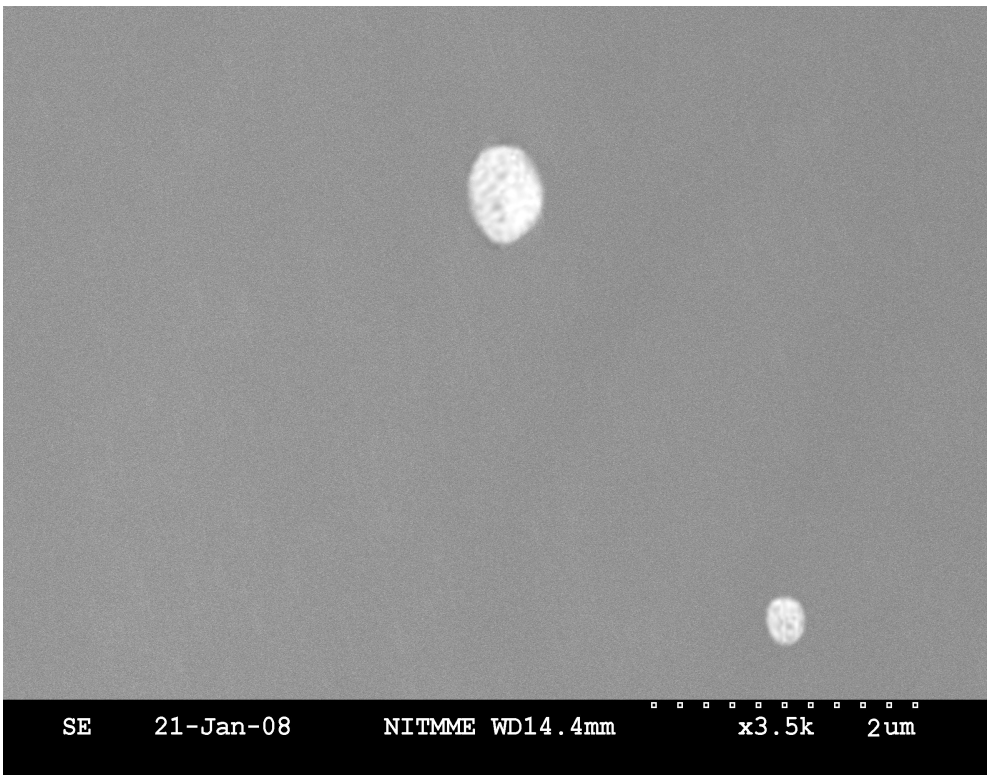


Figure No.15

SEM Photograph of Acarbose Transfersomes (Formulation-2)

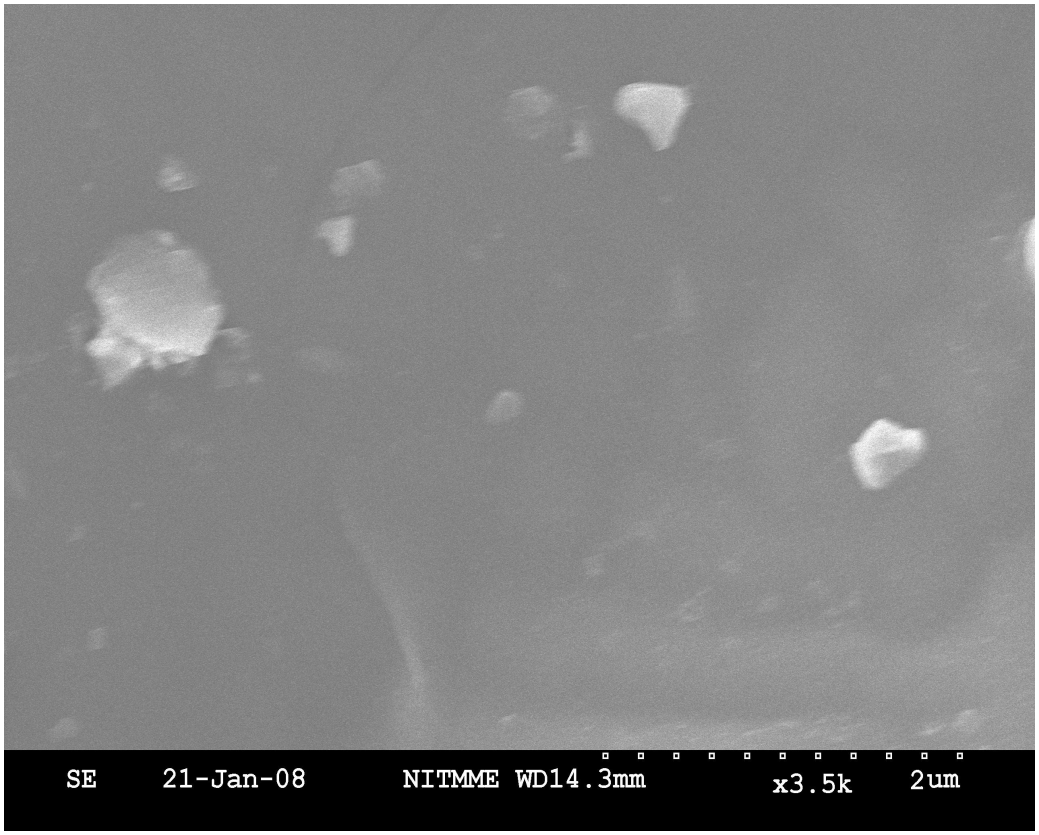
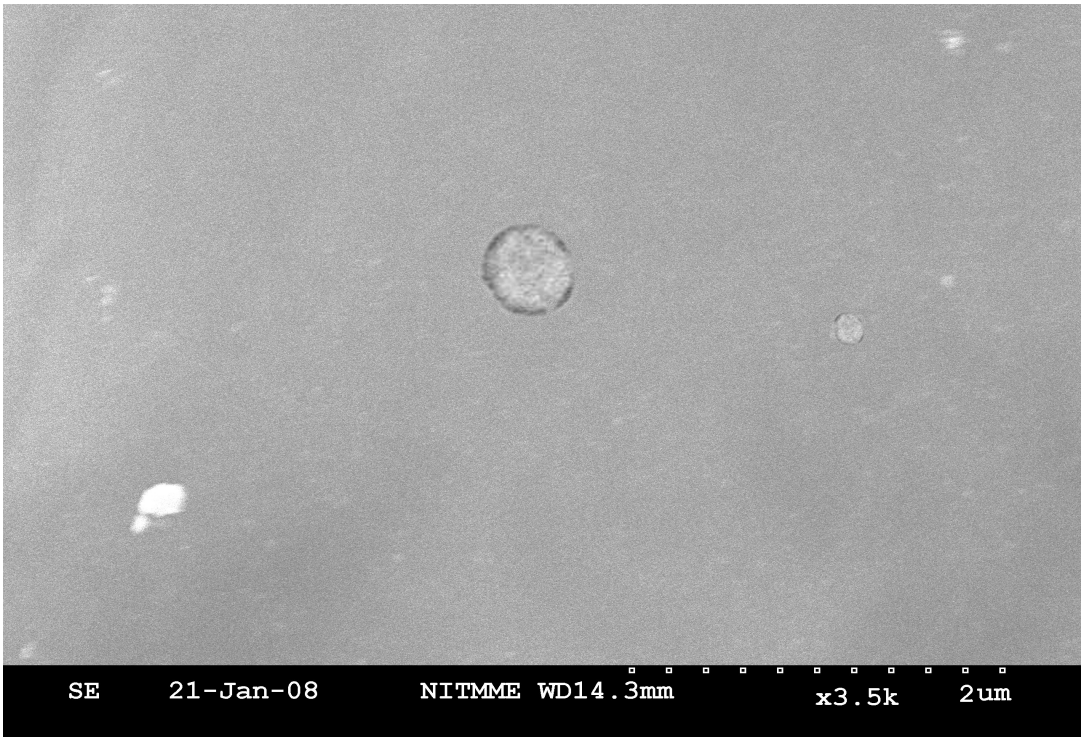


Figure No.16

SEM Photograph of Acarbose Transfersomes (Formulation-3)



SIZE DISTRIBUTION OF TRANSFERSOMES

Transfersomes were subjected in to laser particle counter (L.P.C) for characterizing size distribution of transfersomes. Its shows that the particle size range 200-700nm, 200-600nm, and 200-700 nm range for Acarbose transfersomes of 3:2:1, 3:3:1, 3:4:1 ratios respectively. The average mean particle size of formulation 1, 2 and 3 were 420nm, 450nm, 430nm respectively. It is shown in Table No 8.

Table No.8

Particle size Distribution of Acarbose Transfersomes by using Laser Particle Counter

S.NO.	Acarbose Transfersomes Formulations	Average particle Size Of Transfersomes (nm)
1	Formulation-1	420
2	Formulation-2	450
3	Formulation-3	430

ENTRAPMENT EFFICIENCY

The formulation variables were altered and optimized to obtain the transfersomes with maximum drug entrapment, desired transfersomal size and stability. Increased in the lipid concentration compared to drug entrapment with increase in quantity of lipid more number of transfersomes per ml of the transfersomal dispersion were formed, resulting in to an increased percent drug entrapment.

However, further increase in the lipid concentration had no proportionate increase in percentage drug entrapment due to approaching system saturation.

Here 3:2:1, 3:3:1, 3:4:1ratios were used to prepare transfersomes. The percentage entrapment of transfersomes was found to be 33.33%, 42.6%, 46.66% for 3:2:1, 3:3:1 and 3:4:1 respectively and 3:4:1 ratio found to have more entrapment efficiency compared to other two formulations.It is shown in Table No 9 and Figure No.12.

Increasing the sonication time resulted in to reduction in percent drug entrapment; the decrease in percent drug entrapment is due to leakage of the drug during sonication.

Sonication brings about size reduction by breaking large transfersomes to smaller ones and in doing so, leakage of small quantities of drug from the transfersomes occur. Hence sonication time was optimized to 30 min, and further reduction in the size by increasing sonication time was not attempted.

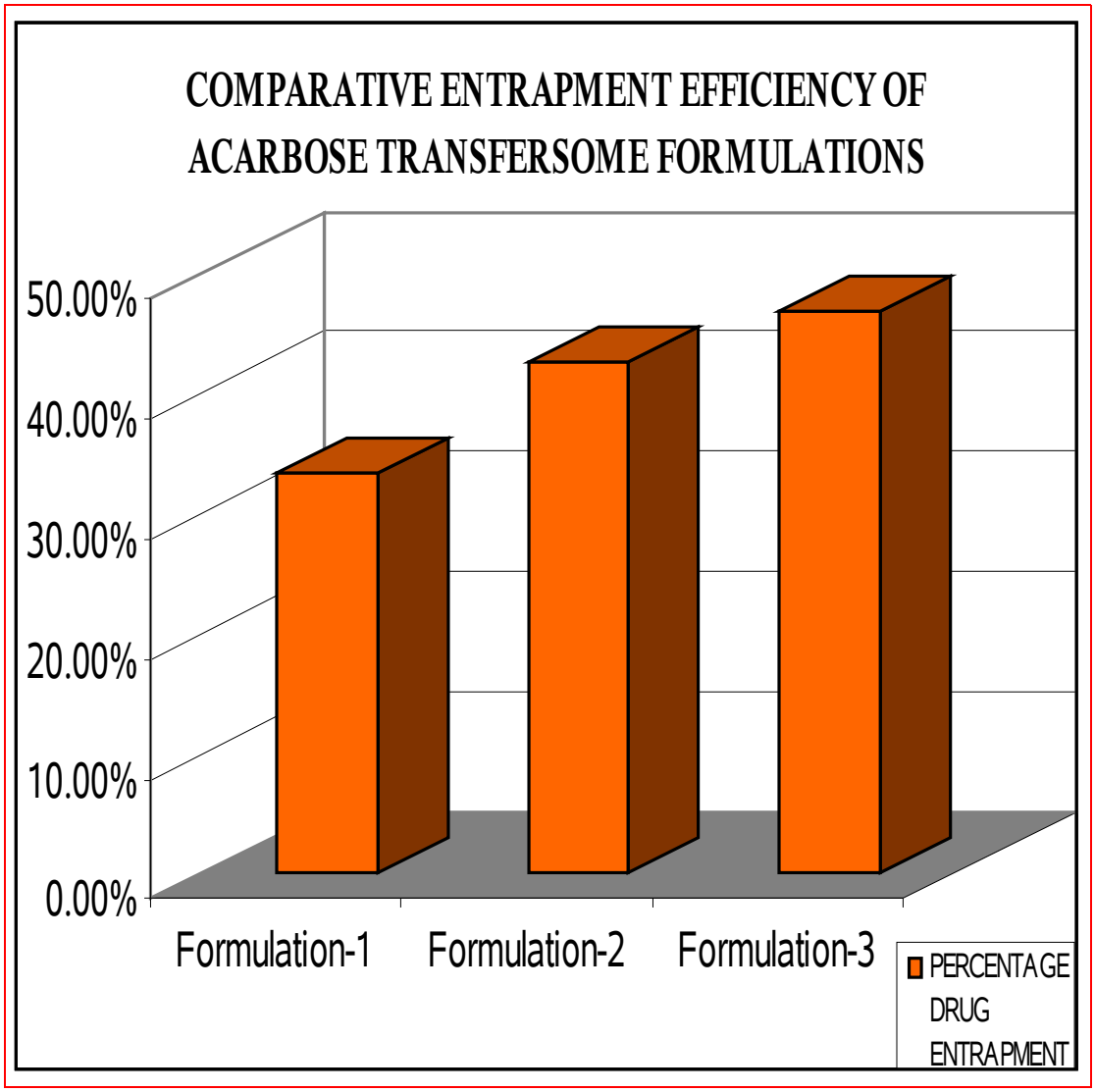
Table No.9

Percentage drug Entrapment of Acarbose Transfersomes

S.No.	Acarbose Transfersomes Formulations	Percentage of Drug Entrapment
1	Formulation-1	33.33 ±2.2%
2	Formulation-2	42.6±1.8%
3	Formulation-3	46.66±1.2%

n=3 ($P<0.05$)

Figure No.17


COMPARATIVE ENTRAPMENT EFFICIENCY OF ACARBOSE TRANSFERSOME FORMULATIONS
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Formulation-1
Formulation-2
Formulation-3
PERCENTAGE DRUG ENTRAPMENT

Transfersomes Gel Evaluation

Determination of Viscosity

The viscosity of the gels was determined by using Brookfield viscometer. The viscosity of the formulations were ranged from 46,000 to 50,000cps and the results were shown in Table no 10

Table no 10

Formulation	Viscosity in cps
Formulation-1	48,000
Formulation-2	48,500
Formulation-3	48,000

Average of three readings

Extrudability

The extrudability of the gel formulations were checked as per the procedure, extrudability of carbopol gels was excellent. It is shown in Table no. 11

Table no. 11

Formulation	Extrudability
Formulation-1	+++
Formulation-2	+++
Formulation-3	+++

+++Excellent

STABILITY STUDY

The stability studies of transfersomal formulation were carried out at refrigeration temperature (4°C), Room temperature and 37±5°C. Physical evaluation of prepared transfersomes gel shown in the Table no. 12. Leakage of the drug from the prepared transfersome were analyzed in terms of percent drug retained storage under refrigerated condition showed promising results of 98.2%, 94.5% and 95.5% for formulation 1, 2, 3 respectively after 3 month. At room temperature the percentage retained after 3 month was 94.28%, 87.98%, and 88.88% for formulation 1, 2, 3 respectively and at 37°C the percentage of drug retained was 80.82%, 78.72%, and 79.64% for formulation 1, 2, 3 respectively. These showed that the formulation found to have more stable at refrigeration temperature, where as good stability at room temperature and the drug degradation increased at 37°C. It is shown degradation increased at 37±5°C. It is shown in Table no. 13, 14 & 15.

Table No.12
STABILITY STUDYPHYSICAL EVALUATION OF FORMULATIONS (TRANSFERSOMES IN 1%OF CARBOPOL GEL BASE)

PARAMETER	ROOM TEMPERATURE	37±5^{0}C	4-5^{0}C
VISUAL APPEARANCE			
Initial	Transparent	Transparent	Transparent
1month	Transparent	Transparent	Transparent
2 month	Transparent	Transparent	Transparent
3 month	Transparent	Transparent	Transparent
pH			
Initial	6.8	6.8	6.8
1month	6.9	6.9	6.9
2 month	6.9	6.9	6.9
3 month	6.9	6.9	6.9
VISCOSITY			
Initial	48000	48000	48000
1month	48000	48000	48000
2 month	48000	48000	48000
3 month	48000	48000	48000
EXTRUDABILITY			
Initial	Satisfactory	Satisfactory	Satisfactory
1month	Satisfactory	Satisfactory	Satisfactory
2 month	satisfactory	satisfactory	satisfactory
3 month	Satisfactory	Satisfactory	Satisfactory
PHASE SEPARATION	Not found	Not found	Not found
LEAKAGE	Not found	Not found	Not found
TEXTURE			
Initial	Smooth	Smooth	Smooth
1month	Smooth	Smooth	Smooth
2 month	Smooth	Smooth	Smooth
3 month	Smooth	Smooth	Smooth

CHEMICAL EVALUATION OF FORMULATIONS (TRANSFERSOMES IN 1%OF CARBOPOL GEL BASE)

Table No.13

Drug content (Formulation-1)

Storage conditions	Intervals (3 month study)			
	Initial (%)	1st month (%)	2nd month (%)	3rd month (%)
4-5^{0}C	100	99.34	98.66	98.20
Room temperature	100	97.30	95.60	94.28
37±5^{0}C	100	90.56	84.60	80.02

Table No.14

Drug content-(Formulation-2)

Storage conditions	**Intervals (3 month study)**			
	Initial (%)	**1st month (%)**	**2nd month (%)**	**3rd month (%)**
4-5^{0}C	100	98.5	96.5	94.5
Room temperature	100	98.5	92.5	87.98
37±5^{0}C	100	90.5	86.5	78.72

Table No.15

Drug content (Formulation-3)

Storage conditions	**Intervals (3 month study)**			
	Initial (%)	**1st month (%)**	**2nd month (%)**	**3rd month (%)**
4-5^{0}C	100	97.5	96.5	95.5
Room temperature	100	92.5	90.45	88.88
37±5^{0}C	100	89.48	84.43	79.64

INVITRO RELEASE STUDIES

In vitro release study is carried through open tubular method with artificial membrane at37^{0}c, 100 rpm, within a period of 24hr.From this study we evaluated the percentage of drug diffused in the medium.The percentage of drug diffused from the Acarbose transfersomes were 70.62%, 73.35%, 84.70%, from transfersomes formulation 1, 2and 3 respectively at the end of 24hrs. It shown in Table no 15,16 and 17 Figure no. 13,14,15.

The results showed that formulation 3 having highest entrapment and maximum percentage of release. The comparative values are shown in the Table 18 and Figure no 16.

Table No.18

***Invitro* release study for Acarbose Tansfersomes Formulation-1**

S.no	Time in(hrs)	Absorbance at 425nm	Concentration in(mcg/ml)	Amount in (mg)	%drug diffused
1	0.25	0.632	0	0	0
2	0.5	0.646	0	0	0
3	0.75	0.652	0	0	0
4	1	0.658	0.023	0.119	0.850
5	1.5	0.659	0.047	0.238	1.701
6	2	0.660	0.071	0.357	2.552
7	2.5	0.666	0.214	1.071	7.658
8	3	0.672	0.357	1.785	12.76
9	4	0.678	0.5	2.5	17.86
10	5	0.684	0.64	3.21	22.97
11	6	0.692	0.83	4.16	29.78
12	7	0.693	0.85	4.28	30.63
13	8	0.698	0.97	4.88	34.88
14	9	0.702	1.07	5.35	38.29
15	10	0.712	1.30	6.54	46.80
16	11	0.718	1.45	7.26	51.90
17	12	0.720	1.5	7.5	53.60
18	14	0.726	1.64	8.21	58.79
19	16	0.728	1.69	8.45	60.41
20	18	0.732	1.78	8.92	63.82
21	20	0.736	1.88	9.40	67.22
22	22	0.738	1.92	9.64	68.92
23	24	0.740	1.97	9.88	70.62

Figure no. 18

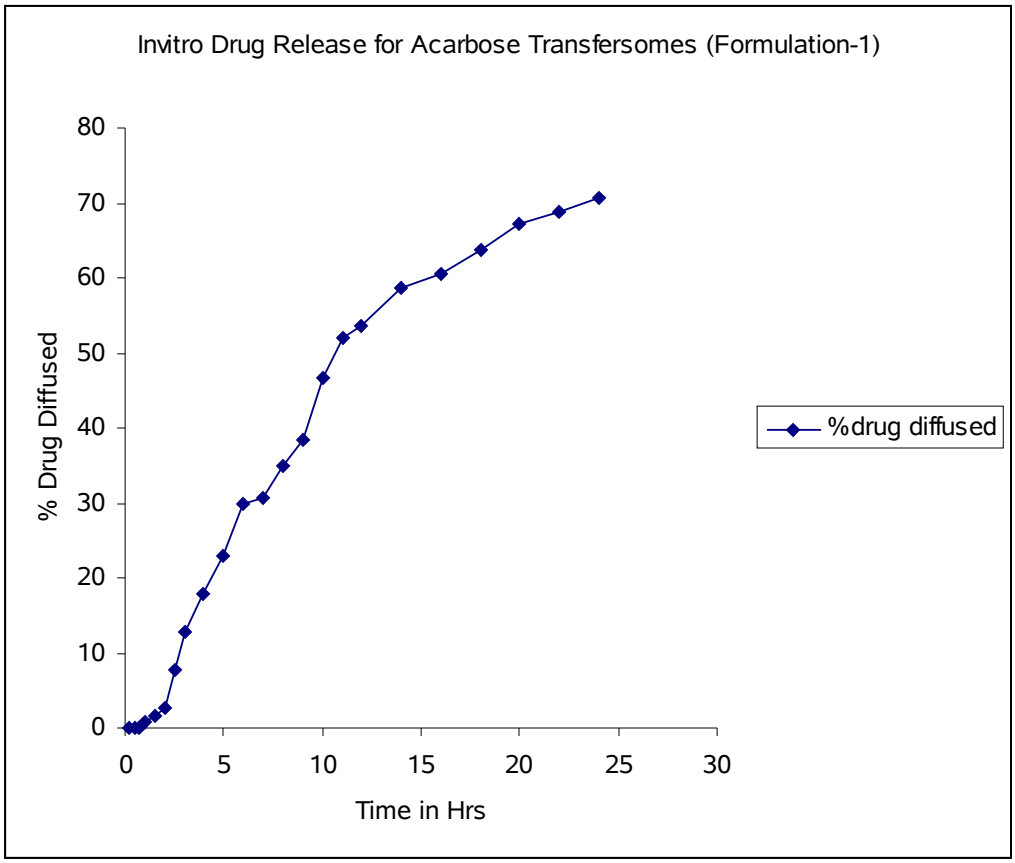

Invitro Drug Release for Acarbose Transfersomes (Formulation-1)
% Drug Diffused
Time in Hrs
%drug diffused
0
10
20
30
40
50
60
70
80
0
5
10
15
20
25
30

Table No.17

***Invitro* release study for Acarbose Transfersomes (Formulation-2)**

S.no	Time in (hrs)	Absorbance at 425nm	Concentration in (mcg/ml)	Amount in (mg)	%drug in diffused
1	0.25	0.629	0	0	0
2	0.5	0.648	0	0	0
3	0.75	0.652	0	0	0
4	1	0.656	0	0	0
5	1.5	0.660	0.0714	0.35	3.53
6	2	0.665	0.190	0.95	9.61
7	2.5	0.669	0.285	1.42	14.43
8	3	0.670	0.309	1.54	15.6
9	4	0.672	0.357	1.78	18.03
10	5	0.677	0.476	2.38	24.05
11	6	0.684	0.642	3.21	32.46
12	7	0.685	0.666	3.33	33.67
13	8	0.688	0.738	3.69	37.27
14	9	0.692	0.833	4.16	42.08
15	10	0.695	0.904	4.52	45.69
16	11	0.696	0.928	4.64	46.89
17	12	0.699	1.00	5.00	50.50
18	14	0.701	1.04	5.23	52.91
19	16	0.708	1.21	6.07	61.32
20	18	0.711	1.28	6.42	64.93
21	20	0.712	1.30	6.54	66.13
22	22	0.716	1.40	7.02	70.94
23	24	0.718	1.45	7.26	73.35

Figure No. 19

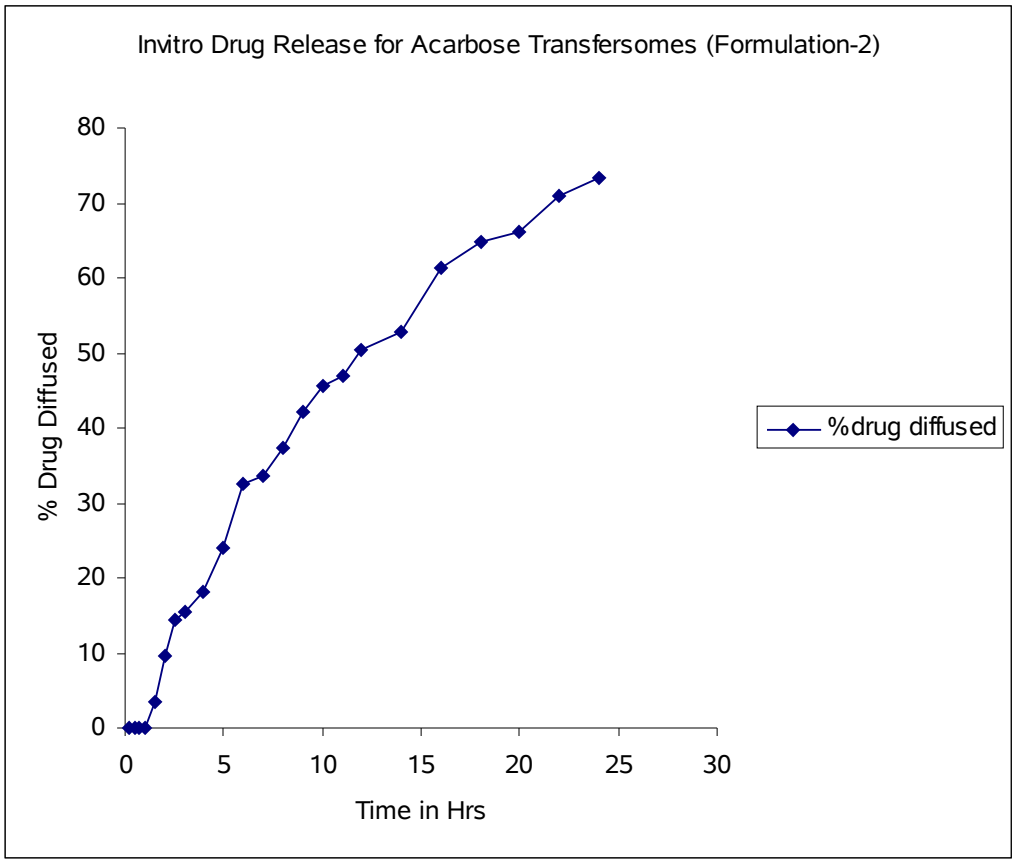

Invitro Drug Release for Acarbose Transfersomes (Formulation-2)
% Drug Diffused
Time in Hrs
%drug diffused
0
10
20
30
40
50
60
70
80
0
5
10
15
20
25
30

Table No.16
***Invitro* release study for Acarbose Tansfersomes (Formulation-3)**

S.no	Time in(hrs)	Absorbance at 425 nm	Concentration in (mcg/ml)	Amount in(mg)	%drug diffused
1	0.25	0.632	0	0	0
2	0.5	0.646	0	0	0
3	0.75	0.652	0	0	0
4	1.00	0.658	0.023	0.119	0.930
5	1.5	0.659	0.047	0.238	1.861
6	2.00	0.661	0.095	0.476	3.72
7	2.5	0.672	0.357	1.785	13.96
8	3.00	0.678	0.5	2.5	19.54
9	4	0.688	0.73	3.69	28.85
10	5	0.699	1.00	5.00	35.09
11	6	0.704	1.11	5.59	43.74
12	7	0.706	1.16	5.83	45.60
13	8	0.708	1.21	6.07	47.47
14	9	0.711	1.28	6.42	50.26
15	10	0.718	1.45	7.26	56.77
16	11	0.720	1.5	7.5	58.63
17	12	0.721	1.52	7.61	59.57
18	14	0.726	1.64	8.21	64.22
19	16	0.729	1.71	8.57	67.01
20	18	0.730	1.73	8.69	67.94
21	20	0.738	1.92	9.64	75.39
22	22	0.742	2.02	10.11	79.11
23	24	0.748	2.16	10.83	84.70

Figure No.20

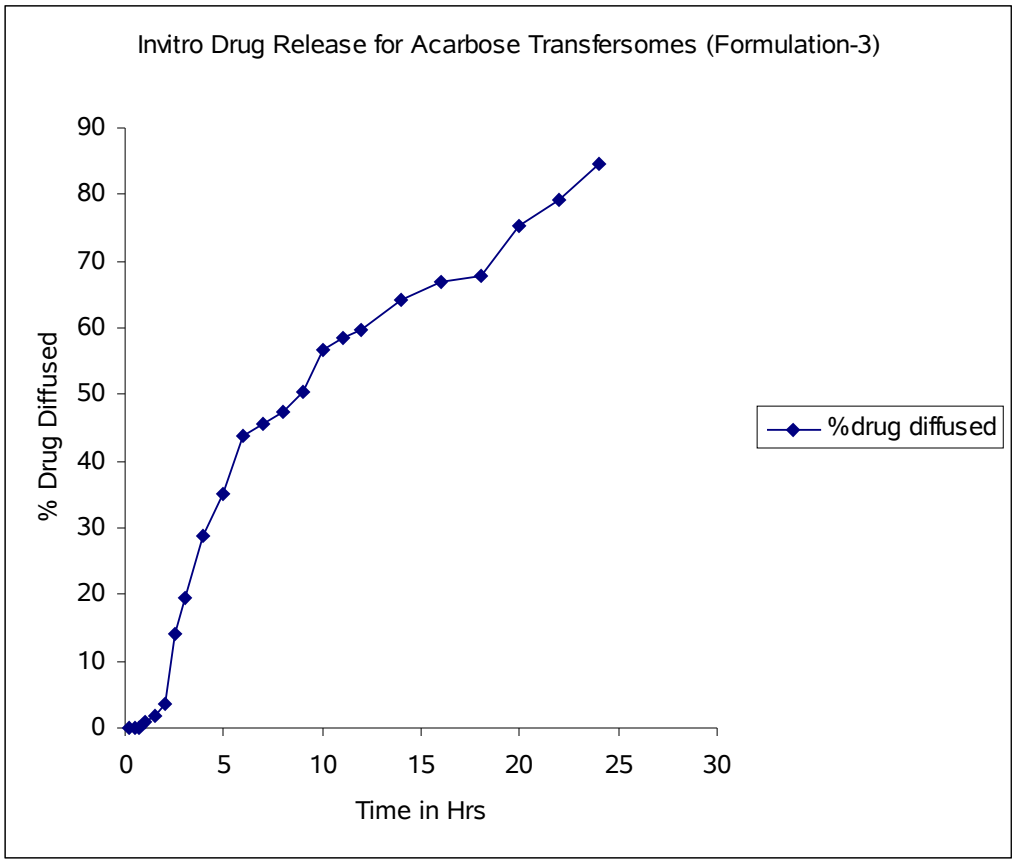
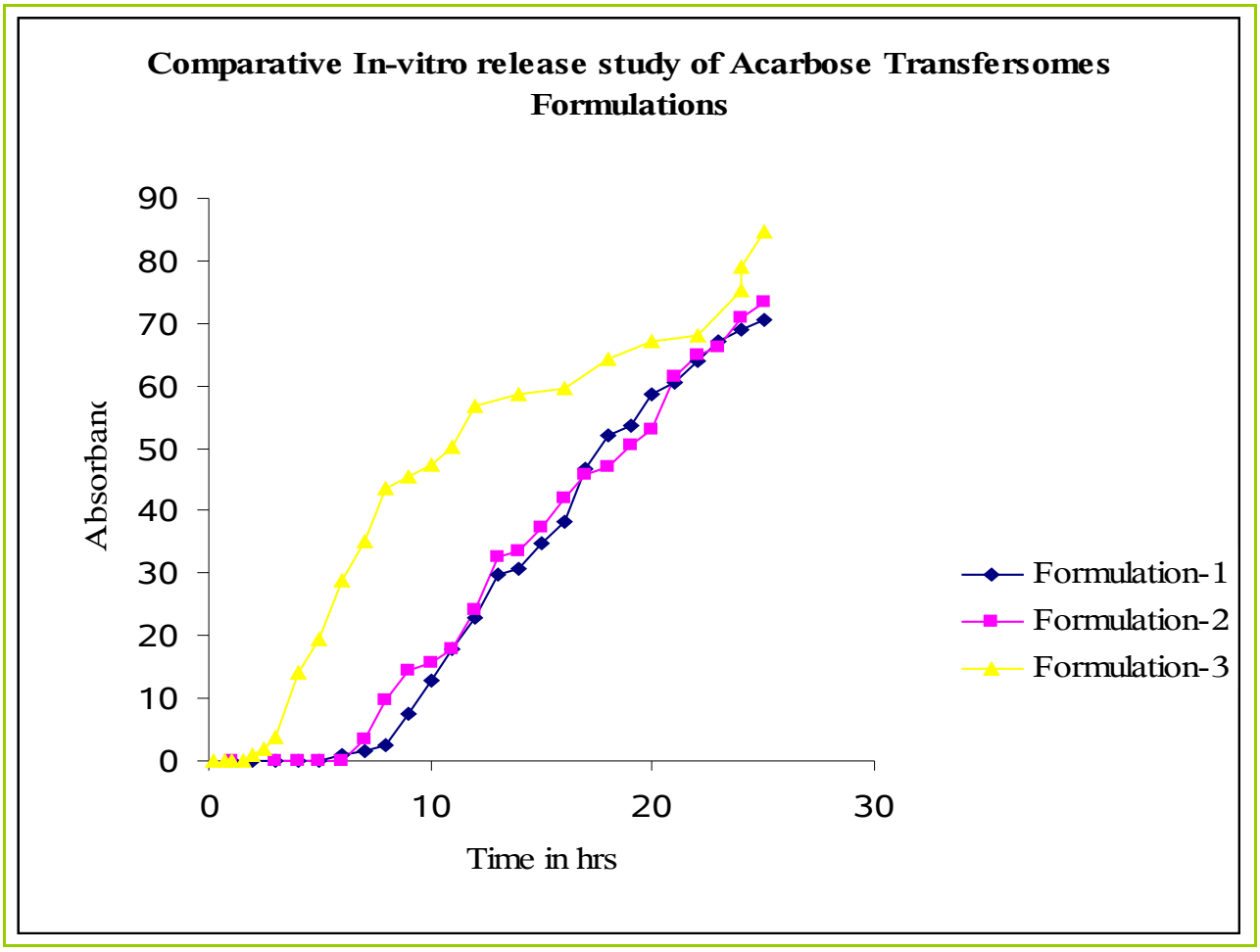

Invitro Drug Release for Acarbose Transfersomes (Formulation-3)
% Drug Diffused
Time in Hrs
%drug diffused
0
10
20
30
40
50
60
70
80
90
0
5
10
15
20
25
30

Table No.19

Comparative *In vitro* release study of Acarbose Transfersome formulations

S.No	Acarbose Transfersome Formulations	% Drug diffused
1	Formulation-1	70.62
2	Formulation-2	73.35
3	Formulation-3	84.70

Figure No.21


Comparative In-vitro release study of Acarbose Transfersomes Formulations
Absorbanc
90
80
70
60
50
40
30
20
10
0
0
10
20
30
Time in hrs
Formulation-1
Formulation-2
Formulation-3

ANTI- DIABETIC ACTIVITY

The blood glucose reducing effect was significant in standard (oral) and Acarbose transfersome treated animal groups up to 15 days, compared with control group ($P<0.05$). Acarbose (oral) produced a decrease of 98 ± 5.4 (diabetic mice, $p<0.05$ compared to diabetic control 255 ± 9.2) in blood glucose levels after 15 days. Acarbose transfersome formulations 1,2 and 3 (topical) produced a decrease of 140 ± 4.6, 125 ± 2.1 and 110 ± 7.3 in blood glucose levels after 15 days compared to diabetic control ($P<0.05$).

Table No.20

ANTI-DIABETIC ACTIVITY OF ACARBOSE TRANSFERSOMES GEL FORMULATIONS.

GROUPS	FORMULATIONS	GLUCOSE LEVEL AFTER 15 DAYS STUDY (mg/dl)
1	CONTROL	90+5.4
2	DIABETIC CONTROL	255+9.2
3	STANDARD	98+5.4
4	FORMULATION-1	140+9.6
5	FORMULATION-2	125+2.9
6	FORMULATION-3	110+5.8

Figure No.22

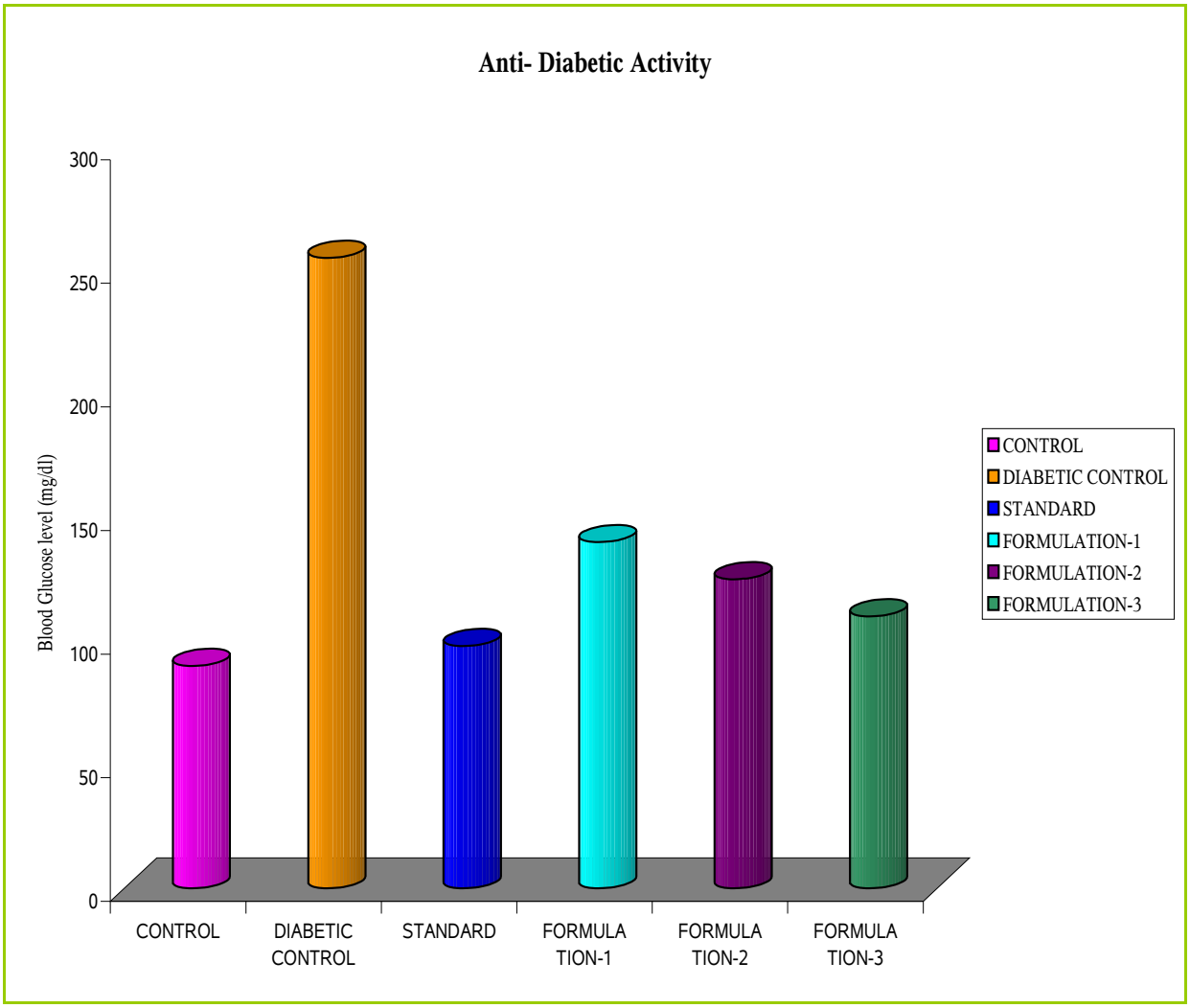
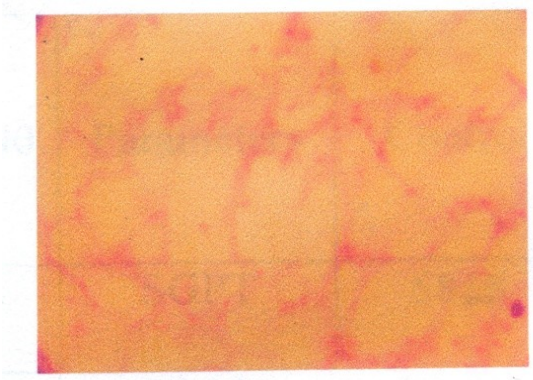
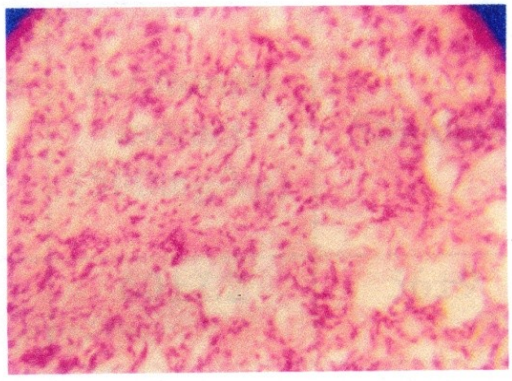

Anti- Diabetic Activity
Blood Glucose level (mg/dl)
300
250
200
150
100
50
0
CONTROL
DIABETIC CONTROL
STANDARD
FORMULATION-1
FORMULATION-2
FORMULATION-3
CONTROL
DIABETIC CONTROL
STANDARD
FORMULATION-1
FORMULATION-2
FORMULATION-3

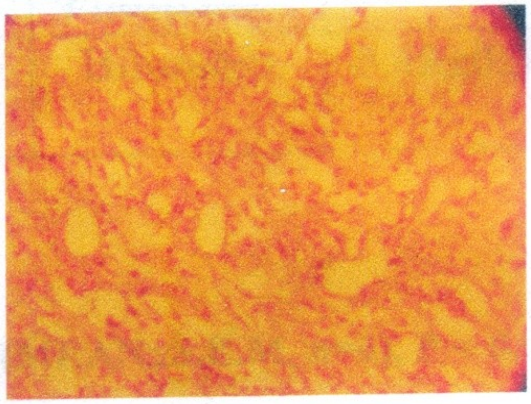
Figure No. 23 Histopathalogical Details of Anti-Diabetic Activity of Acarbose Transfersomes gel



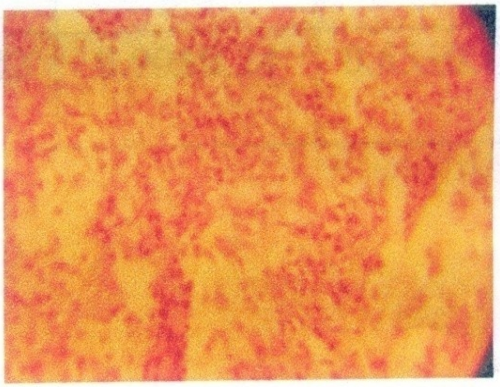
Normal



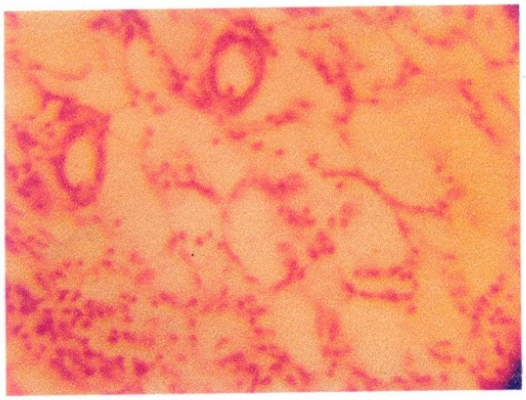
Diabetic control



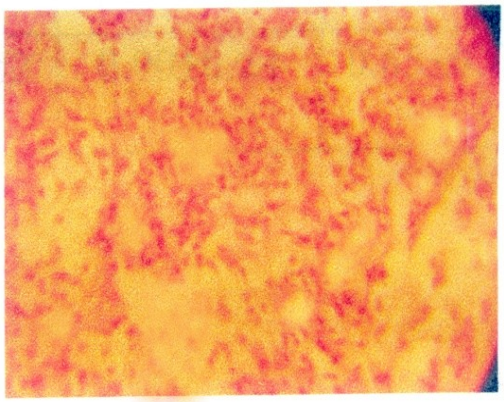
Standard (acarbose)



Formulation-1



Formulation-2



Formulation-3

SKIN IRRITATION TEST

Figure No.24 Skin Irritation Test – Before Irritation Test

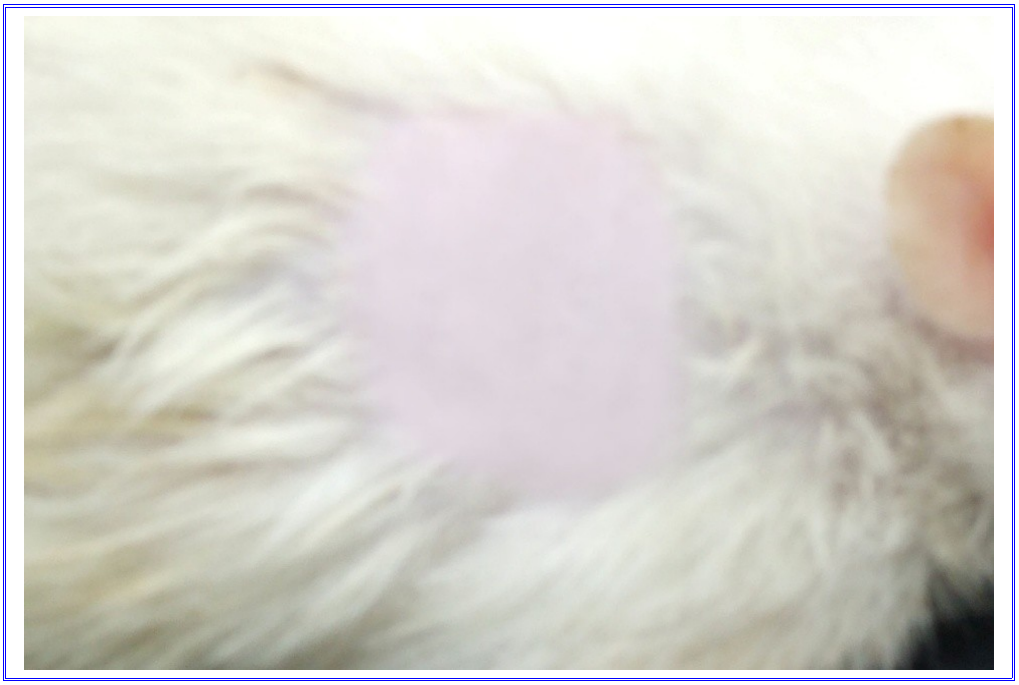


Figure No. 25 Skin Irritation Test - Standard Irritant (0.8 % v/v Formalin)

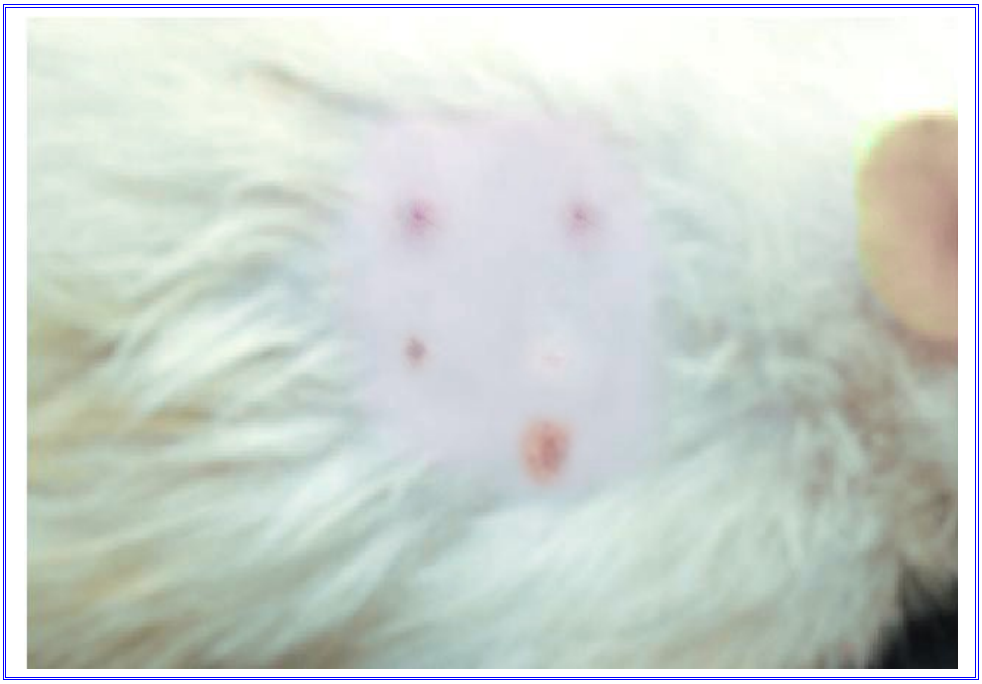


Figure No. 26 Skin Irritation Test – (USP adhesive tape)

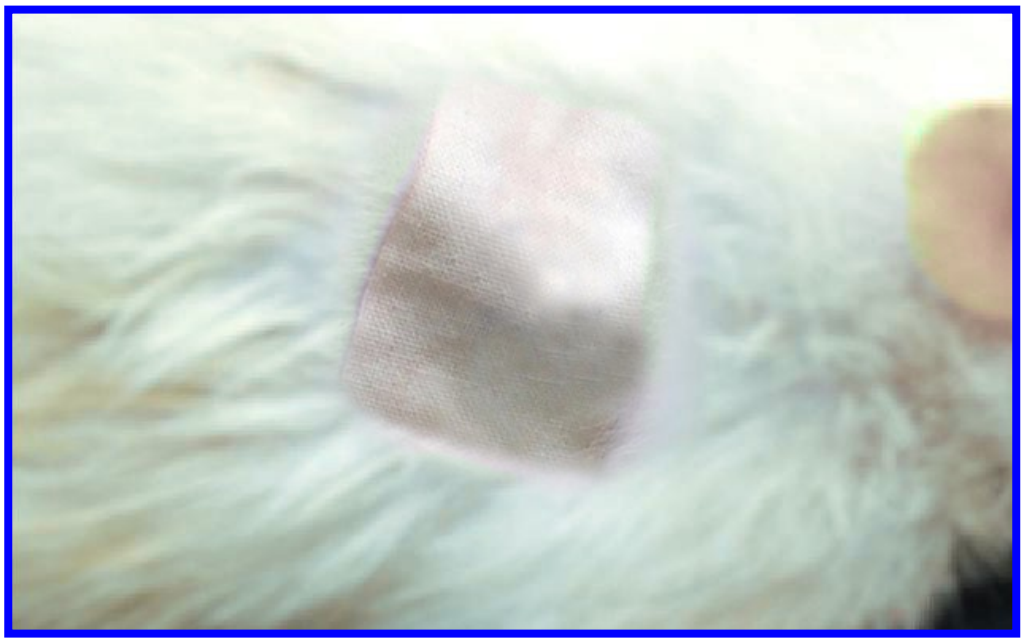
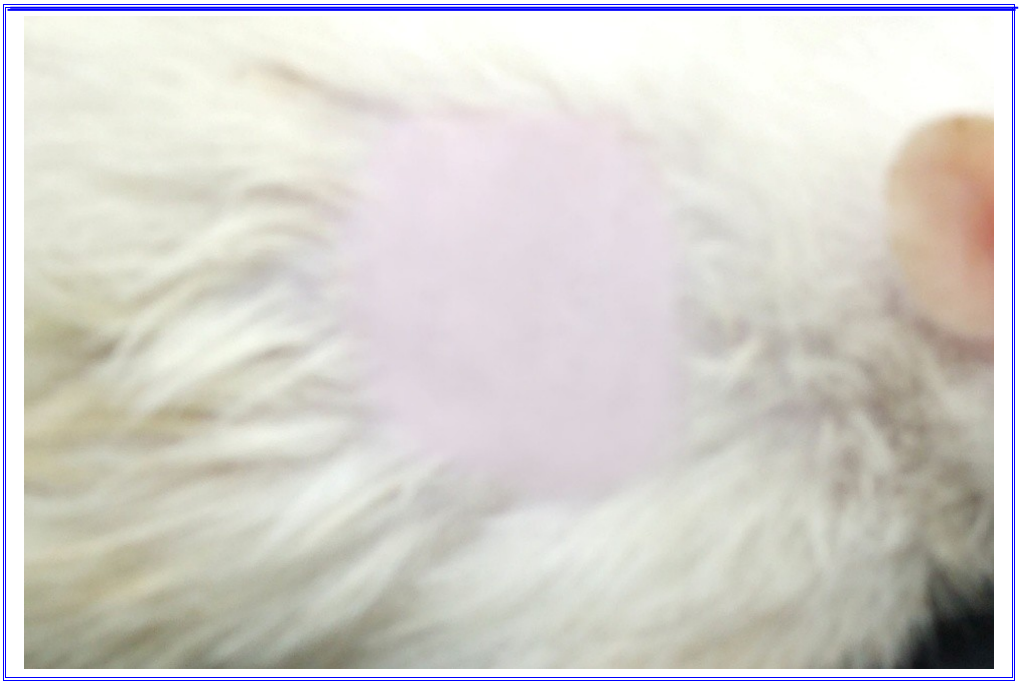


Figure No.27 Skin Irritation Test – Acarbose Transfersomes in Gel



The results revealed that the Acarbose Transfersome gel formulations and adhesive tape has not showed any signs of inflammation at the rat skin after 7 days.

10. Conclusion

On the basis of these results, the Acarbose trnasfersomes (Formulation-3) showed better characteristic behaviour.

The drug entrapment in formulation – 3 is higher than the other formulations. The diffusion study of Acarbose transfersome gave extended release of the drug which suffices to decreased dose, lesser frequent dose of treatment and more patient compliance.

In anti - diabetic studies, the formulation - 3 considerable decrease of blood glucose by topical application, when compared with other formulation. This proved its targeting efficiency. So it was concluded that Acarbose transfersomes (Formulation – 3) was a better and efficient formulation than existing formulations.

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