FORMULATION, EVALUATION AND LYOPHILIZATION OF CISPLATIN LOADED LIPOSOMES

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CERTIFICATES

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LIST OF SYMBOLS/ABBREVIATIONS

AUC	Area under curve
AVE	Artificial viral envelopes
CDR	Cumulative Drug Release
CDRR	Cumulative drug release remaining
CONC	Concentration
DNA	De oxy ribose nucleic acid
DSPC	Distearoyl phosphatidyl choline
EE	Encapsulation efficiency
FTIR	Fourier Transform Infrared
GUV	Gaint unilamellar vesicles
gm	Gram
HPLC	High performance liquid chromatography
IPA	Iso propyl alchol
IR	Infrared spectroscopy
KS	Kaposis sarcoma
LUV	Large unilamellar vesicles
MLV	Multi laminar vesicles
MPS	Mononuclear phagocytic system
MVV	Multi vesicular vesicles

NDDS	Novel Drug Delivery System
OLV	Oligolamellar vesicles
PBS	Phosphate buffer saline
PC	Phosphatidyl choline
PDI	Poly dispersive index
PEG	Polyethylene glycol
RES	Reticulo Endothelial system
RNA	Ribonucleic acid
RPM	Rotations per minute
SEM	Scanning Electron Microscopy
SUV	Small unilamellar vesicles
TEM	Transmission electron microscopy
USP	United States Pharmacopoeia

INTRODUCTION

INTRODUCTION

1. INTRODUCTION TO TARGETED DRUG DELIVERY SYSTEMS

The concept of designing specified delivery system to achieve selective drug targeting has been originated from the perception of Paul Ehrlich, who proposed drug delivery to be as a "magic bullet".

Controlled & Novel drug delivery envisages optimized drug delivery in the sense that the therapeutic efficacy of a drug is optimized, which also implies nil or minimum side effects. It is expected that the 21st century would witness sea changes in the area of drug delivery. The products may be more potent as well as safer. Target specific drug delivery is likely to overcome much of the criticism of conventional dosage forms. The cumulative outcome could be summarized as optimized drug delivery that encompasses greater potency & greater effectiveness, lesser side effects and toxicity, better stability, low cost hence greater accessibility, ease of administration and best patient compliance.

1.1 RATIONALE OF DRUG TARGETING[1]

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 controlled rate & mode of drug delivery to pharmacological receptor and specific binding with target cells; as well as bioenvironmental protection of the drug in route to the site of action are specific features of targeting. Invariably, every event stated contributes to higher drug concentration at the site of action and resultant lowers 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(s) with effective accessibility to the target site(s) could maximize the benefits of targeted drug delivery.

1.1.1 APPROACHES[1,2]

The various approaches of vectoring the drug to the target site can be broadly Classified as:

- Passive targeting
- Inverse targeting
- Active targeting (Ligand mediated targeting and Physical targeting)
- Dual targeting
- Double targeting
- Combination targeting

➢ **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 physiochemical 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(s) 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. This category of targetable devices includes drug bearing bilayer vesicular systems as well as cellular carriers of micron or sub micron size range.

- **INVERSE TARGETING:**

It is essentially based on successful attempts to circumvent and avoid passive uptake of colloidal carriers by reticuloendothelial system (RES). This effectively leads to the reversion of bio distribution 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 pre-injection of a large amount of blank colloidal carriers or macromolecules like dextran sulphate. This approach leads to RES blockade and as a consequence impairment of host defense system. Alternate strategies include modification of the size, surface charge, composition, surface rigidity and hydrophilicity of carriers for desirable biofate.

- **ACTIVE TARGETING:**

Conceptually, active targeting exploits modification or manipulation of drug carriers to redefine its biofate. The natural distribution pattern of the drug carrier composites is enhanced using chemical, biological and physical means, so that it approaches and is identified by particular biosites. The facilitation of the binding of the drug-carrier to

target cells through the use of ligands or engineered homing devices to increase receptor mediated (or in some cases receptor independent but epitopes based) localization of the drug and target specific delivery of drug(s) is referred to as active targeting. This targeting approach can further be classified into three different levels of targeting:

(i)First Order Targeting: It refers to restricted distribution of the drug-carrier system to the capillary bed of a predetermined target site, organ or tissue. Compartmental targeting in lymphatic, peritoneal cavity, plural cavity, cerebral ventricles, lungs, joints, eyes, etc., represents first order targeting (it could also be categorized as a level of passive targeting).

(ii)Second Order Targeting: The selective delivery of drugs to a specific cell type such as tumor cells and not to the normal cells is referred as second order drug targeting. The selective drug delivery to the Kupffer cells in the liver exemplifies this approach.

(iii)Third Order Targeting: The third order targeting is defined as drug delivery specifically to the intracellular site of target cells. An example of third order targeting is the receptor based ligand-mediated entry of a drug complex into a cell by endocytosis, lysosomal degradation of carrier followed by release of drug intracellularly or gene delivery to nucleolus.

➢ **LIGAND MEDIATED TARGETING:**

Targeting components, which have been studied and exploited are pilot molecules themselves (bioconjugates) or anchored as ligands on some delivery vehicle (drug-carrier system). All the carrier systems, explored so far, in general, are colloidal in nature. They can be specifically functionalized using various biologically relevant molecular ligands including antibodies, polypeptides, oligosaccharides (carbohydrates), viral proteins and fusogenic residues. The ligands afford specific avidity to drug carrier. The engineered carrier constructs selectively deliver the drug to the cell or group of cells generally referred to as target.

The cascade of events involved in ligand negotiated specific drug delivery is termed as ligand driven receptor mediated targeting.

➢ **PHYSICAL TARGETING (Triggered Release):**

The selective drug delivery programmed and monitored at the external level (*ex vivo*) with the help of physical means is referred to as physical targeting. In this mode of targeting, some characteristics of the bioenvironmental are used either to direct the carrier to a particular location or to cause selective release of its content.

➢ **DUAL TARGETING:**

This classical approach of drug targeting employs carrier molecules, which have their own intrinsic antiviral effect thus synergizing the antiviral effect of the loaded active drug. Based on this approach, drug conjugates 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.

- **DOUBLE TARGETING:**

For a new future trend, drug targeting may be combined with another methodology, other than passive and active targeting for drug delivery systems. 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 therapeutic index by the following two effects. First, if drug release or activation is occurred locally at therapeutic sites, selectivity is increased by multiplication of the spatial selectivity with the local release/activation. Second, the improvement in the therapeutic index by a combination of spatially selective delivery and a preferable release pattern for a drug, such as zero order release for a longer time period of the drugs. When these two methodologies are combined, it may be called

"Double targeting"

- **COMBINATION TARGETING:**

Petit and Gombtz, 1998 have suggested the term combination targeting for the site-specific delivery of proteins and peptides. 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 favor targeting the specific compartments, organs or their tissues within the vasculature.

1.1.2 LIMITATIONS OF TARGETED DELIVERY SYSTEMS [3]

Several problems have been identified which require alterations in targeting Strategies particularly, *in- vivo*. These include:

- Rapid clearance of targeted systems especially antibody targeted carriers.
- Immune reactions against intravenous administered carrier systems.
- Target tissue heterogeneity
- Problems of insufficient localizations of targeted systems into tumor cells.
- Down regulation and sloughing of surface epitopes.
- Diffusion and redistribution of released drug leading to no-specific accumulation.

1.1.3 CARRIERS USED IN TARGETED DRUG DELIVERY SYSTEMS. [4,5,6]

Carrier is one of the most important entities essentially required for successful transportation of the loaded drug(s). They are drug vectors, which sequester, transport and retain drug *en route*, while elute or deliver it within or in vicinity of target.

(I)Colloidal carriers:

(a) Vesicular systems: Liposome□s; Niosomes; Pharmacosomes; Virosomes; Immunoliposomes.

(b) Micro particulate systems: Microparticles; Nanoparticles; Magnetic microspheres;

Nanocapsules.

(ii)Cellular carriers

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

(iii)Cellular delivery systems

Micelles; reverse micelles; mixed micelles; polymeric micelles; liquid crystal; lipoproteins (chylomicron; VLDL; LDL) synthetic LDL mimicking particles (Supramolecular biovector system)

(iv)Polymer based systems

Signal sensitive; muco-adhesive; biodegradable; bioerodible; solute synthetic polymeric carriers.

(v)Macromolecular carriers

Proteins, glycoproteins; neo glycoproteins and artificial viral envelopes (AVE) Glycosylated water-soluble polymers (poly-L-lysine)

Mabs; Immunological Fab fragments; antibody enzyme complex & bispecific Abs Toxins, immunotoxin & rCD4 toxin conjugates

Lectins (Con A) & polysaccharides.

1.2 LIPOSOMES[7]

Liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer. Liposomes were discovered by A. Bangham and his team about 45 years ago and ever since then they developed into versatile tools in biology, medicine and biochemistry. Liposomes are the smallest artificial vesicles of spherical shape that can be produced from natural untoxic

phospholipids and cholesterol. Liposomes can be defined as a particular kind of vesicles or any structure formed by lipid bilayers which are capable to enclose a volume of the substance.

Liposomes are microscopic vesicles with diameter between 20nm to 20µm, which consist of one or more concentric bilayers phospholipids surrounding an aqueous membrane. When phospholipids are dispersed in an aqueous medium, liposomes form spontaneously as a result of interaction of water and phospholipid molecules which are amphiphilic i.e. they possess hydrophilic and hydrophobic regions. This allows a wide range of materials to be incorporated since hydrophilic drugs are entrapped in the aqueous region and hydrophobic materials are located in the hydrophobic regions.

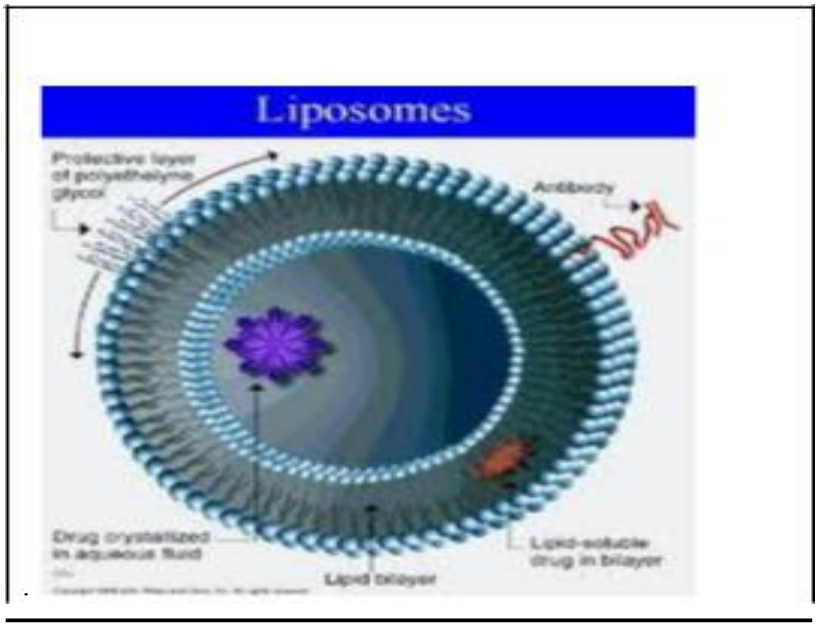


Figure No: 1 Structure of liposome

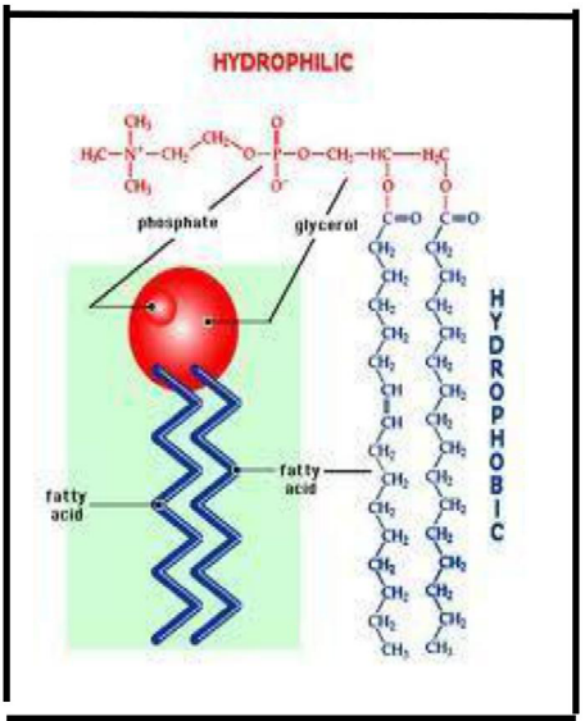


Figure No. 2 Shape of phospholipids molecule

1.2.1 LIPOSOMES DELIVERED INTERCELLULARLY[8]

Liposomes deliver their contents to the cytoplasm of cells in culture either by fusion with the outer cell membrane or by endocytosis where upon they are concentrated in acidic lysozymal sacs.

1) Inter membrane transfer

Inter membrane transfer of lipid component can take place upon close approach of the two phospholipid bilayers without the need for disruption of the

liposome. Only specific phospholipids exchange (PC &PE) via intermediate of a specific cell surface exchange protein. Similarly Liposomes and lipoproteins (HDL) interactions is important liposomal stability in circulation. Addition of cholesterol retards immediate destabilization of Liposomes.

2) Contact release

The liposome content with cell causes an increase in permeability on the entrapped content through bilayer membrane, curiously, cell induced leakage of solutes have been observed to be greater in membranes with cholesterol concentration above 30%. This process strengthened by means of receptor/ligand between the two membranes.

3) Adsorption

The adsorption of Liposomes takes place either as a result of physical attractive forces, or as a result of binding by specific receptors to ligands on the vesicle membranes. The attraction depends on the specific cell surface protein. This interaction was more in case of gel phase of liposome.

4) Fusion

Close approaching of Liposomes and cell membranes can lead to fusion of the two resulting in complete mixing of liposomal lipids with those of the plasma membrane

of the cell and the liposomal content released in to cytoplasm. This process takes place after phagocytosis and endocytosis of Liposomes. Inside the Endosome, Liposomes were affected by acidic pH. There by this will completely fuse and deliver the drug content in to cytoplasm.

1.2.2 STABILITY OF LIPOSOMES.[9,10]

The stability of Liposomes is of major concern in their development for pharmaceutical applications. A drug containing Liposomes can be unstable because of physical or chemical stability. The stability studies could be broadly covers under two main sections.

Stability *in-vitro* mainly covers the stability aspects prior to the administration of the formulation and with regard to the stability of the constituted lipids.

Stability *invivo,* which covers the stability aspects once the formulation, is administered via various routes of biological fluids.

1.3 COMPOSITION

1.3.1. Phospholipids. [10,11,12]

Phospholipids are the main component of naturally occurring bilayers. These phospholipids include phosphatidylcholines (PC), phosphatidylethanolamines (PE) and phosphatidylserines (PS]. The key common feature that bilayer-forming compounds share is their amphiphilicity i.e., they have defined polar and non-polar regions. This is the reason the non-polar regions orientate themselves

towards the interior away from the aqueous phase, the polar regions being in contact with it .

The phospholipid composition and the capability of binding to other chemical species is one of the main features of their use in liposome formation (Rachev *et al.,* 2001; Gomez-hens and Fernandez-Romero, 2005). Phospholipids are part of all cell membranes and play an important role in the signal transduction mechanism. The phospholipid structure consists of phosphoric residue, hydrophilic head and hydrophobic tail. The hydrophilic head consists of the phosphate group, glycerol back bone, choline, ethanolamine and inositol, with different chemical properties. When exposed to an aqueous environment, phospholipids tend to form unique assemblies called "bilayers". The hydrophilic heads of the phospholipids turn towards the water molecules while the hydrophobic tails hide from water molecules.

There are two basic sources of phospholipids: synthetic and natural. Natural lipids are generally either egg-derived or bovine-derived. Regardless of the regulatory issues, animal-derived products do not offer any advantages to synthetic lipids. They are inherently less stable due to the polyunsaturated fatty acids, and in most cases the synthetic counterpart costs the same or less than the tissue-derived product. Synthetic lipids from different sources are not necessarily equal either. Synthetic lipids can be prepared from glycerol or glycero-3-phosphocholine (GPC) derived from a plant or animal source. The latter is sometimes referred to as semi-synthetic lipid because a portion of the molecule is derived from a natural source. Lipids derived from glycerol require the chiral centre be synthetically prepared which may lead to stereo chemical impurities present in the final product.

The typical plant source for GPC is soybean lecithin. This type of compound is synthesized by converting a natural PC into an acyl group through a chemical reaction. A highly pure, high-quality hydrogenated soy phospholipids that can assist you in developing new products is generally obtained from carefully selected, non-genetically modified soybeans as a source of raw materials.

There are five main groups of phospholipids that are available that can be used for liposome preparation.

1. Phospholipids from natural sources.

2. Phospholipids modified from natural sources.

3. Semi-synthetic phospholipids.

4. Fully-synthetic phospholipids,

5. Phospholipids with non-natural head groups

Table No 1: The most common glycerophospholipids

The esterified group	Name of the Phospholipid	Abbreviation	Net charge on pH 7
-H	Phosphotidicacid	PA	Negative
$-CH_2CH_2N^+(CH_3)_3$	Phosphotidylcholin	PC	Zwitterionic
$-CH_2CH_2NH_3^+$	Phosphotidylethanolamine	PE	Zwitterionic
$-CH_2CHNH_3^+COO^-$	Phosphotidylserine	PS	Negative
$-CH_2CHOHCH_2OH$	Phosphotidylglycerol	PG	Negative
$-HC_6H_5(OH)_5$	Phosphotidylinositol	PI	Negative

The charge of the lipid used in liposome formation dictate the surface charge of the liposomes. The surface charge of liposomes can be tailored by replacing phosphatidyl choline (PC) partly with negatively or positively charged (phospho)lipids (Table 1.1), which induces electrostatic repulsion and stabilization against liposome fusion. The surface characteristics of liposomes may also be altered by modifying lipids with hydrophilic moieties e.g. polyethylene glycol (PEG) to membrane bilayers.

1.3.2. STEROIDS[14]

Cholesterol, is an alternative habitual component of the liposome structure and a natural constituent of the biological membranes, whereas stearic acid is a saturated form

of fatty acid, cholesterol and stearic acid play an important role in controlling the fluidity and the permeability of the artificial vesicle i.e. it makes the membranes more rigid thus avoiding leakage of the encapsulated compound. In order to improve the rigidity of the liposome, cholesterol and stearic acid were combined with other phospholipids to form liposome formulations. Cholesterol incorporation increases the separation between the choline head group and eliminates the normal electrostatic and hydrogen bonding interactions. Its inclusion in liposomal membrane has three recognized effects[31].

1. Increasing the fluidity or micro viscosity of the bilayer.

2. Reducing the permeability of the membrane to water-soluble molecules.

3. Stabilizing the membrane in the presence of biological fluids such as plasma.

Liposomes without cholesterol are known to interact rapidly with plasma proteins such as albumin, transferrin and micro globulins. These proteins tend to extract bulk phospholipids from liposomes, thereby depleting the outer most layer of the vesicles leading to physical instability.

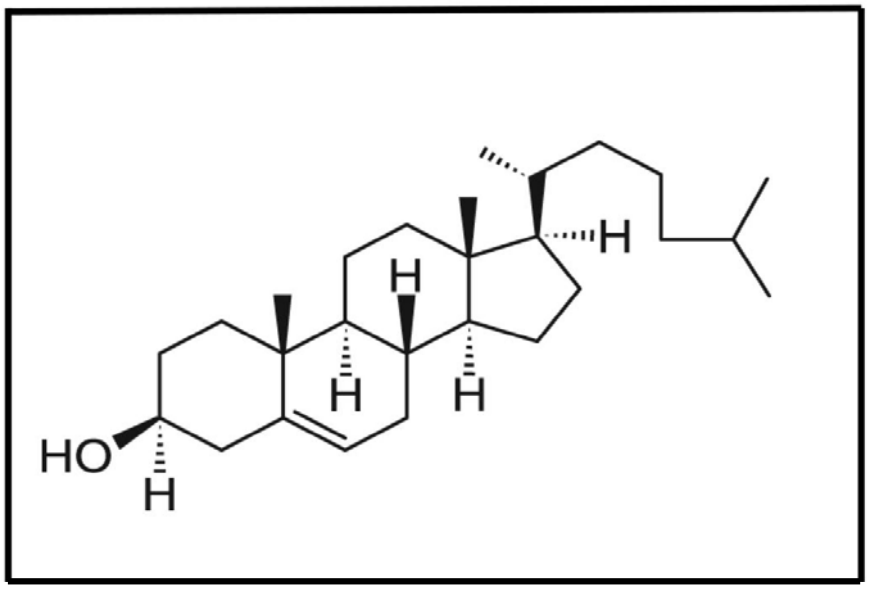


Figure No:3 General Structure Of Cholesterol (Chol)

1.4. CLASSIFICATION OF LIPOSOMES[15]

(A) Based on structural parameters:

Liposomes can be classified by the number of bilayers present. Single bilayers (lamella) liposomes are called unilamellar vesicles (ULVs), while liposomes containing more than one bilayer are multilamellar vesicles (MLVs). Other liposomes consisting of small vesicles incorporating large vesicle are known a multivesicular vesicles (MVVs) (Gomez-hens and Fernandez-Romero, 2005). The entrapment of active compounds also depends on the different sizes of liposomes ranging from nanometer (nm) to micrometer (μm), such as small unilamellar vesicles (SUVs, 25-50 nm), large unilamellar vesicles (LUVs, 100 nm-1 μm), giant unilamellar vesicles (GUVs, 1.0-200 μm), multilamellar

vesicles (MLVs, 0.1-15 μm), and multi-vesicular vesicles (MVVs, 1.6-10.5 μm) (Silva *et al.,* 2010). Liposomes can usually be stored in a buffer at pH 7.4 and at temperature approximately 4 °C for 5-7 days.

Vesicle types	**Abbrev**	**Diameter Size**	**Number of lipid bilayers**
Small unilamellar vesicles	SUV	20-100nm	One lipid bilayer
Large unilamellar vesicles	LUV	>100nm	One lipid bilayer
Multi lamellar vesicles	MLV	>0.5nm	5-20 lipid bilayers
Oligolalamellar vesicles	OLV	0.1-1nm	Approximately 5 lipid bilayers
Multivescular vesicles	MMV	>1μm	Multi compartmental structure

Table No:2 Classification of liposomes

(B) Based on method of preparation

(1) Single or oligolamellar vesicles made by reverse phase evaporation.

(2) Multilamellar vesicles made by the reverse phase method.

(3) Stable plurilamellar vesicles.

(4) Frozen and thawed multilamellar vesicles.

(5) Vesicles prepared by extrusion method.

(6) Vesicles prepared by French press.

(7) Vesicles prepared by fusion.

(8) Dehydration-rehydration vesicles.

(C) Based on materials used for preparation:

(1) Conventional liposomes

- Stabilized natural lecithin or phosphatidyl choline (PC) mixtures.
- Synthetic identical-chain phospholipids.
- Glycolipid containing liposomes.

(2) Specialty liposomes

- Bipolar fatty acids
- Antibody directed
- Methyl/Methylene linked
- Lipoprotein coated
- Carbohydrate coated
- Multiple encapsulated
- Emulsion compatible

(D) Based upon composition and applications:

(1) Conventional liposomes: Neutral or negatively charged phospholipids and Cholesterol.

(2) Fusogenic liposomes: Reconstituted sendai virus envelopes.

(3) Cationic liposomes: Cationic lipids with dioleoyl phosphotidyl ethanolamine (DOPE).

(4) Long circulatory: Neutral high T^0C, cholesterol and (stealth) liposomes 5-10% of Poly ethylene glycol-distearoyl ethanolamine (PEG-DSPE).

(5) Immuno liposomes: conventional liposomes (CL) or long circulating liposomes (LCL) with attached monoclonal antibody or recognition sequence

1.5 THE ROLE OF LIPOSOME SIZE[16, 17]

The rate of the opsonisation and clearance by the reticuloendothelial system (RES) of the injected liposomes from the blood circulation is dependent on the composition and size. RES is part of the immune system and their main function is to eliminate foreign materials from the body. RES consists of cells such as blood monocytes and macrophages found mainly in the Kupffer cells in liver, the lung and the spleen. Shortly after i.v injection, the liposomes become coated by serum proteins called opsonins. Once they are opsonised, they will rapidly be phagocyted by the RES cells, and the major part of the injected liposomes will be accumulated in the liver and spleen (Maurer et al., 2001). Large liposomes (>200 nm in diameter) are rapidly opsonised and taken up by the (RES) disappear from the blood circulation within short time and

primarily end up in the spleen. Opsonisation decreases with a decreasing in liposome size.

Small liposomes have a relatively larger surface area, and will have a lower density of opsonins on the membrane surface which results in lower uptake by the macrophages. Liposomes with a size of 70 to 200 nm will have a greater chance to escape from RES and remain in the circulation longer and then reach the target. Due to extravasations through the fenestrated capillary walls in the liver, the small liposomes (< 70 nm in diameter) show shorter circulation time. The structure and architecture of the blood capillary walls varies in different organs and tissues. There are structure differences between healthy and tumour capillaries and blood supply to the organs and tissues is somewhat different .

1.6 THE ROLE OF SURFACE CHARGE AND MEMBRANE CHARACTERESTICS[18, 19]

Lipid organization in the liposome membranes has a major role on the physical membrane properties such as permeability, membrane elasticity, surface charge and binding properties of proteins, and is of equal importance for clearance as compared to liposome size. Neutral charged liposomes with tightly packed membranes tend to remain longer in the circulation and exhibit increased drug retention, compared to charged systems. Protein opsonisations onto the liposome surface are reduced due to the tightly packed and rigid membrane. The presence of Chol in liposome formulations may change the packing of the phospholipids to a more ordered and rigid membrane and may stabilize to avoid drug leakage. Moreover, this may reduce binding of opsonins on the liposomes

and may improve stability and retention of liposomes *in vivo*. Certain plasma proteins have an affinity for liposomes, and the affinity is enhanced if the liposome is charged. In particular cationic systems are expected quickly interaction with various components in systemic circulation and thus having shorter half life *in vivo*. It is also known that anionic liposomes containing negatively charged lipids such as phospatidylserine (PS), phosphatidicacid (PA) and phosphatidylglycerol (PG) are quickly taken up by macrophages and thus disappear from the circulation in short time.

1.7 APPLICATIONS OF LIPOSOMES[20, 21]

Liposomes are mainly used as delivery systems for many substances which can easily be entrapped or act as anchor to various substituents. Liposomal delivery systems are well-known procedures used for encapsulation of anticancer and antimicrobial drugs, enzymes and antigens. The Application of Liposomes in the delivery systems aid in processes such as transport, distribution, controlled release, protection and localization of encapsulated drugs.

In order to improve the performance of the enclosed drug, it is useful to consider the basic reasons for applying liposome as drug carriers.

1. Liposomes act as carriers for drug both in vitro and in vivo.
2. In targeted delivery system, liposomes are used as carriers for anticancer drugs such as methotrexate, actinomycin-D etc.
3. Liposomes encapsulation has been used as a step in enzyme purification.
4. The use of liposomes as encapsulated insulin and growth hormone for oral

administration has been reported.

5. The most important use of liposomes in cell biology is to manipulate the status of membrane lipid.
6. Liposomes can be used to insert important regulatory molecules such as C_{GMP},C_{AMP} and enzymatic cofactors into cells.
7. Liposome collagen gels show slow release of a variety of bioactive drugs into the circulation thus having an obvious advantage over multiple dosage regimens.
8. This technique may also have topical application in the treatment of wounds and burn.
9. Inclusion of antimicrobials or cells growth factors within the liposomes can facilitate enhanced cell growth and prevention of infection.
10. As research tool for modeling those cellular functions, which are determined at the membrane level.
11. In the field of micro analysis (membrane immuno assay).
12. Liposomes are used as carriers of radiopharmaceuticals for diagnostic imaging.
13. Used industrially in the rapidly developing field of recombinant DNA technology for the synthesis of exotic chemicals and drugs.
14. Applications in cosmetics.

Liposomes are used as carriers of substances such as natural moisturizing factor (NMF), alpha hydroxyl acid (AHA), proteins, vitamins (A and E), oils, phytosterols and antioxidants.

15. Liposomes in gene delivery:
    - Gene and antisense therapy.
    - Genetic(DNA)vaccination.
16. Liposomes in immunology
    - Immunoadjuvant
    - Immunomodulator
    - Immunodiagnostic
17. Liposomes as artificial blood surrogates

1.8 METHODS OF PREPARATION[20,21]

There are several methods to prepare liposomes each depending on the type of liposome desired (SUV, MLV or LUV). Some commonly used procedures are:

1. Thin film hydration
2. Sonication
3. Extrusion
4. French pressure
5. Injection of water immiscible solvent
    - Ether injection
    - Fluorocarbon injection
6. Injection of water miscible solvents
    - Ethanol injection

7. By alternative water miscible solvents
8. By pH adjustment
9. Detergent Dialysis method
    - Dialysis
    - Column chromatography
    - Centrifugation
    - Bio-beads
    - Reverse phase evaporation

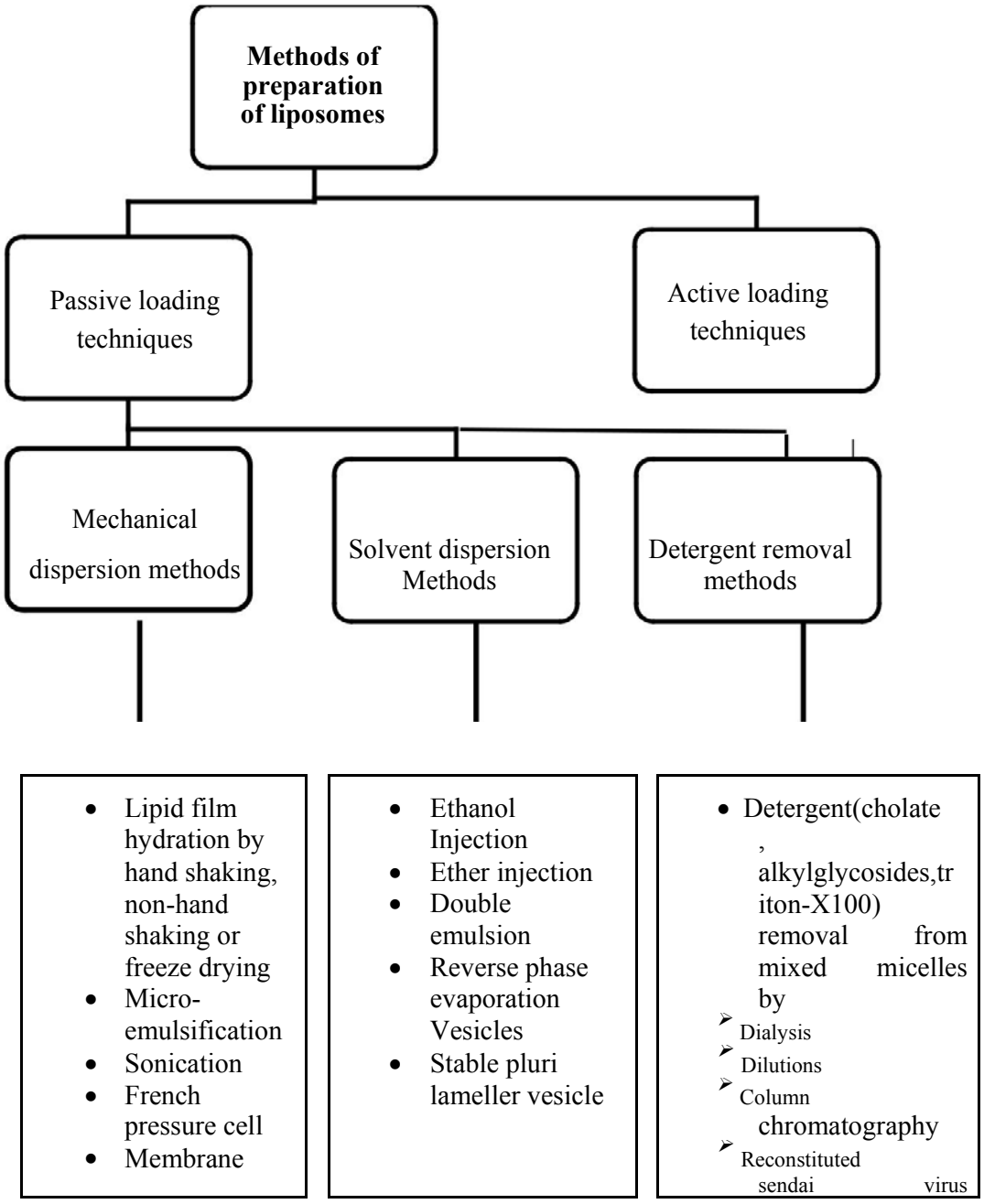


Fig.no 4 Liposomes preparation methods.

1.9 MECHANISM OF LIPOSOME FORMATION[23]

In order to understand why liposomes are formed when phospholipids are hydrated, it requires a basic understanding of psycho-chemical features phospholipids.

Phospholipids are amphipathic (having affinity for both aqueous and polar moieties) molecules as they have a hydrophobic tail and a hydrophilic or polar head. In aqueous medium the molecules in self-assembled structures are oriented in such a way that the polar portion of the molecule remains in contact with the polar environment and at the same time shields the non-polar part. Molecules of phosphatidyl choline (PC) are not soluble (rather dispersible) in aqueous medium in the physical chemistry sense, as in aqueous media they align themselves closely in planer bilayer sheets to minimize the unfavorable interactions between the bulk aqueous phase and long hydrocarbon fatty acyl chain. Such interactions are completely eliminated when the sheets fold over themselves to form closed, sealed and concentric vesicles. Phosphatidylcholine preferably orient to form bilayer sheets rather than micellar structures. Lipid vesicles are formed when thin lipid films or lipid cakes are hydrated and stacks of liquid crystalline bilayers become fluid and swell. The hydrated lipid sheets detach during agitation and self close to form large, multilameller vesicles (MLVs). Once these vesicles are formed, a change in the vesicle shape and morphology requires energy input in the form of sonic energy (sonication to get small unilamellar vesicles, SUVs) and mechanical energy (extrusion to get large unilamellar vesicles, LUVs).

1.9.1 Rigidization of fluid phase vesicles with cholesterol:

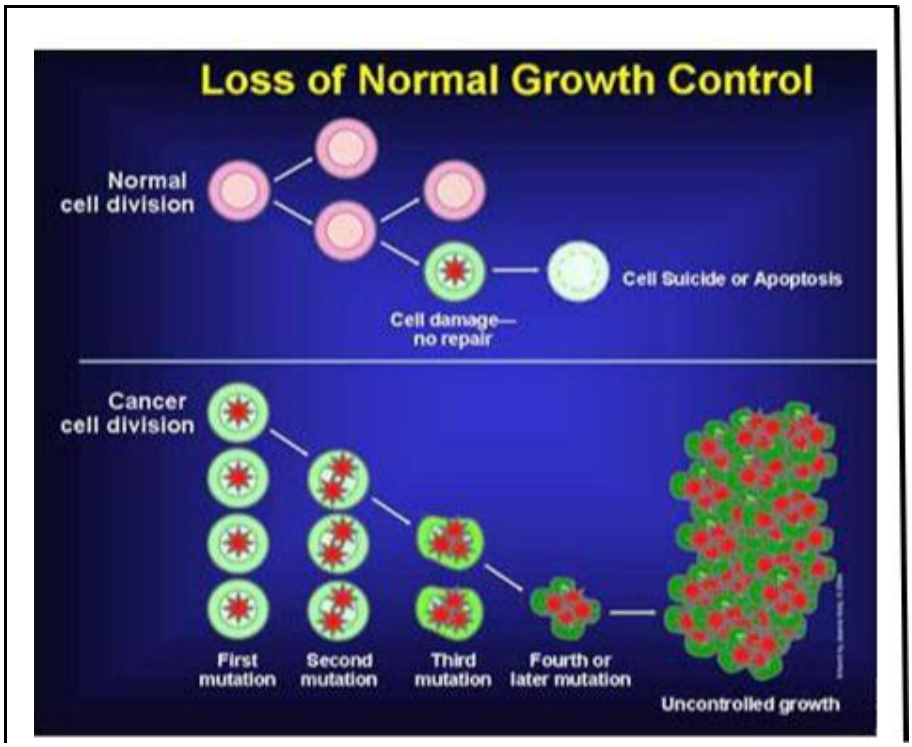
Cholesterol acts as "Fluidity buffer" since below the phase trans ition it tends to make the membrane less ordered while above the transition it tends to make the membrane more ordered, thus suppressing the tilts and shift in membrane structure specifically at the phase transition. Though cholesterol itself does not form bilayers, but it can be incorporated into phospholipid membrane in very high concentrations up to 1:1 or even 2:1 molar ratio of cholesterol to PC. Being an amphipathic molecule, cholesterol inserts into the membrane with its hydroxyl group oriented towards the aqueous surface and the aliphatic chain aligned parallel to acyl chains in the center of the bilayer. This could be the possible mechanism for phospholipid membranes with high levels of cholesterol that retard the chain tilt.

1.10 CANCER CHEMOTHERAPY[22]

Cancer (Sydney. Basic clinical pharmacology (3rd edition)) is a group of neoplastic diseases that occur in human of all age groups and races as well as in all animals species. The incidence, geographic distribution and behavior of specific types of cancer are related to multiple factors include sex, age, race, genetic predisposition and exposure to environmental carcinogen.

Cancer is a disease of uncontrolled cell division, invasion and metastatic. It is generally considered to be due to the clonal expansion of a singe neoplastic cell. However there may be additional somatic leading to heterogeneous cell population.

Fig No:5 Understanding series of Cancer and normal Cell division



Cancer chemotherapy has been under intensive development for the past 30 years, resulting in cures of certain types of disseminated cancers that were previously fatal. Even patients with advanced disease have improved dramatically with chemotherapy.

Mode of action of chemotherapeutics

Most antineoplastic agents are regarded as "cell-cycle specific". They act specifically on processes such as DNA synthesis, transcription, or the function of

mitotic spindle.

All slow growing and fast growing tumor cells display a similar pattern during the division. This may be characterized as follows:

1. There is pre synthetic phase
2. The synthesis of DNA occurs
3. An interval follows the termination of DNA synthesis, the post-synthetic phase.
4. Mitosis ensures, the G2 cell, containing a double complement of DNA, divides in to 2 daughter GI cells, may immediately re-enter to the cell cycle.

Antitumor drugs are better at killing cells during DNA synthesis and active division. When a tumor is young, most of its cells are making DNA. This is defined as large growth function in this state, tumors are destroyed by drugs because the majority of their cells are making DNA and dividing.

The major problems in cancer chemotherapy are the toxic drugs effects on normal cells and the rapid clearance of the drug from the tumor cells. Useful drugs without side effects do not at exist. Rapidly dividing normal cell such as hair follicles, cells lining the gastrointestinal tract and bone marrow cells involved in the immune defense system are also destroyed by the present day chemotherapy. Nausea, hair loss, increased susceptibility to infection and many others comprising a discouraging list.

Liposomes: An ideal "Drug Carrier" for anticancer drugs

Anticancer drugs are known to produce serious side effects to other healthy tissues. The more serious effects are myocardiopathy and pulmonary toxicity.

Therefore targeting such type drugs to the cancerous cell is essential because these drugs are new for the treatment of different type of carcinomas effectively. Usually a therapeutically profitable target agent relationship is far from ideal and undesirable side effects are usually observed. The alternative is to use simple functional molecules which transport the drug to the specific site and release it to perform task. Liposomes are non-toxic, biodegradable microcapsule made up of one or multiple lipid bilayers membranes

Chemicals of interest can be entrapped inside the aqueous compartment of Liposomes or can be incorporated in to the lipid bilayer. Covalent attachment of functional group to lipid molecules adds flexibility to liposomes. Liposomes have been proved a suitable vehicle for selective drug delivery and controlled drug release.

Important characteristics of drug carrier include protection of the encapsulated compound. Selective delivery of the entrapped material to specific tissues with minimal losses of drug during transit, regulation of the drug delivery rate, reduction of toxicity and removal of unused drug. All these function can co-exist in a single liposome preparation which makes it an ideal carrier of drug. Liposomes have proved to be suitable vehicles for antitumor dr

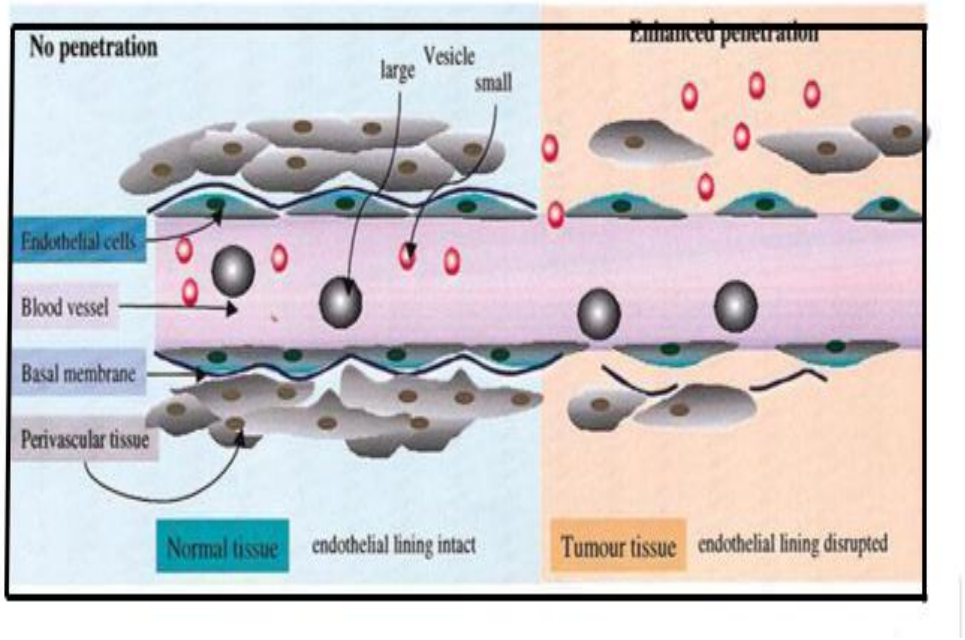


Figure No: 6 Accumulations of Liposome's within Solid Tumors

If liposomes carrying a drug are exposed to oxygen radicals, the hydro peroxides formed will affect the permeability, behavior of the vesicles, thus causing premature release of the drugs targeting to a specific site. The targeting of compounds attached to proteins of polymers, which may be modified by radical attack, could provoke an adverse response thus amplifying the initial damage. Eg: In microbial infections with excessive phagocyte action or in disease involving redistribution and de-compartmentalization of iron. However optimization of a particular function is possible by modifying liposome composition, charge and size.

1.11 LYOPHILIZATION[24]

Lyophilization is a process which extracts the water from foods and other products so that the foods or products remain stable and are easier to store at room temperature (ambient air temperature). Lyophilization is carried out using a simple principle of physics called sublimation. Sublimation is the transition of a substance from the solid to the vapour state, without first passing through an intermediate liquid phase. To extract water from foods, the process of lyophilization consists of:

1. Freezing the food so that the water in the food becomes ice
2. Under a vacuum, sublimating the ice directly into water vapour;
3. Drawing off the water vapour;
4. Once the ice is sublimated, the foods are freeze-dried and can be removed from the machine.

Processing:

The fundamental process steps are:

1. Freezing: The product is frozen. This provides a necessary condition for low temperature drying.

2. Vacuum: After freezing, the product is placed under vacuum. This enables the frozen solvent in the product to vaporize without passing through the liquid phase, a process known as sublimation.

3. Heat: Heat is applied to the frozen product to accelerate sublimation.

4. Condensation: Low-temperature condenser plates remove the vaporized solvent from the vacuum chamber by converting it back to a solid. This completes the separation

process.

The first step in the lyophilization process is to freeze a product to solidify all of its water molecules. Once frozen, the product is placed in a vacuum and gradually heated without melting the product. This process, called sublimation, transforms the ice directly into water vapor, without first passing through the liquid state. The water vapor given off by the product in the sublimation phase condenses as ice on a collection trap, known as a condenser, within the lyophilizer's vacuum chamber.

To be considered stable, a lyophilized product should contain 3% or less of its original moisture content and be properly sealed.

Lyophilization Equipment :

A lyophilizer consists of a vacuum chamber that contains product shelves capable of cooling and heating containers and their contents. A vacuum pump, a refrigeration unit, and associated controls are connected to the vacuum chamber.

Chemicals are generally placed in containers such as glass vials that are placed on the shelves within the vacuum chamber.

Cooling elements within the shelves freeze the product. Once the product is frozen, the vacuum pump evacuates the chamber and the product is heated. Heat is transferred by thermal conduction from the shelf, through the vial, and ultimately into the product.

Lyophilization Container Requirements:

The container in which a substance is lyophilized must permit thermal conductivity, be capable of being tightly sealed at the end of the lyophilization cycle, and minimize the amount of moisture to permeate its walls and seal. The enclosed reagents can only remain properly lyophilized if the container in which they are processed meets these requirements.

Advantages:

Lyophilization has many advantages compared to other drying and preserving techniques.

1. Lyophilization maintains food/ biochemical and chemical reagent quality because they remains at a temperature that is below the freezing-point during the process of sublimation; The use of lyophilization is particularly important when processing lactic bacteria, because these products are easily affected by heat.

2. Food/biochemicals and chemical reagents which are lyophilized can usually be stored without refrigeration, which results in a significant reduction of storage and transportation costs.

3. Lyophilization greatly reduces weight, and this makes the products easier to transport. For example, many foods contain as much as 90% water. These foods are 10 times lighter after lyophilization.

4. Because they are porous, most freeze-dried products can be easily rehydrated. Lyophilization does not significantly reduce volume; therefore water quickly regains its place in the molecular structure of the food/ bio chemicals and chemical reagents.

LITERATURE REVIEW

2. LITERATURE REVIEW

Amarnath Sharma *et al.,*[25] described that liposomes are micro particulate lipoidal vesicles which are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. In this they have discussed the potential applications of liposomes in drug delivery with examples of formulation approved for clinical use, and the problems associated with further exploitation of these drug delivery systems.

Antoneta *et al.,*[26] studied effect of cholesterol and other sterols are important components of biological membranes and are known to strongly influence the physical characteristics of lipid bilayers. Although this has been studied extensively in fully hydrated membranes, little is known about the effects of cholesterol on the stability of membranes in the dry state.

Chien *et al.,*[27] have described the concept of designing specific delivery systems to achieve selective drug targeting. In this they compared between the conventional and targeted drug delivery systems. Also described principle,advantages and disadvantages of target drug delivery systems, and rationale of targeted drug delivery systems.

Eugenia *et al.,*[28] have reported the advantages and application of liposome with respect to anti-cancer, anti-inflammatory and anti-microbial agents. In this they described advantages of encapsulation of macromolecules like enzymes into liposomes. In this study they incorporated the enzymes into liposomes by dried film hydration method and studied the therapeutic activity of enzymes.

Fresta *et al.,*[29] have loaded of 5FU in liposomes by different methods and characterized encapsulation efficacy, storage stability and fusogenic properties. They concluded that the most suitable liposome preparation was the SPL that

showed both better drug loading and stability parameters than others.

Ganesh *et al.,*[30] have studied Inclusion of docetaxal in liposomal formulation. Studied the effect of drug antitumor activity. They have formulated docetaxal liposomes using Phosphatidyl choline (soybea lecithin), cholesterol and various stabilizers by dried thin film hydration method. They have evaluated Particle size analysis, drug content and entrapment efficiency in charged Liposomes were strongly affected by the different stabilizers, and stability of the lyophilized docetaxel Liposomes were evaluated after stored at 40C and room temperature for 3 months. The Liposomes stored at 40C were found to be stable for duration of 3months. Hence it can be concluded that stabilizers like Stearylamine and Dicetylphosphate along with cholesterol were suitable carrier for the preparation of liposomal docetaxel.

Gautam Vinod *et al.,*[31] have developed a long circulating non-pegylated liposomes consisting of doxorubicin for parenteral administration. The circulation time in Swiss albino mice was measured for both formulations (conventional and non-pegylated). The non-pegylated liposomes are stable, exhibit low toxicity and have been found to be efficacious in different tumor models.

Gregoiadis *et al.,*[32] have developed liposomal formulation using (egg phosphatidly choline). In this they described usage of Triton-X 100 for finding out the percentage of drug loading in liposome. They found that Triton-X 100 can be used to disrupt the lipid bilayer in liposome.

Jain *et al.,*[33] described the target drug delivery systems were likely to overcome much of the criticism of conventional dosage forms. They summarized that the optimized drug delivery that encompasses greater potency & effectiveness, lesser side effects and toxicity, better stability, low cost hence greater accessibility, ease of administration and best patient compliance. They

also described about carriers used in targeted drug delivery systems.

Jian-chiou su *et al.,*[34] have established a solid phase synthesis to prepare peptide-poly(ethylene glycol)-lipid(peptide-PEG-lipid) conjugates. In octreotide-PEG2000-DSPE(OPD2000) used as model drug. The OPD2000 obtained had confirmed structure, activity, and purity providing a targeting molecule for preparation of well-defined drug delivery systems, such as targeted liposomes, for further studies.

Lasic *et al.,*[35] have described about the recent discoveries in the field of liposomes and latest application of liposomes. and explained the usage of different types of liposomes and their advantages. They also studied the efficacy of DNA encapsulation in liposomes. In the treatment of colon carcinoma and AIDS related Kaposi sarcoma. And they concluded that the liposomes can be used as carrier for biological like RNA, DNA, etc.

Luigi Cattel *et al.,*[36] have synthesized a series of increasingly lipophilic pro drugs of gemcitabine by linking the 4-amino group with valeroyl, heptanoyl, lauroyl and stearoyl linear acyl derivatives and studied their stability at storage, in plasma and with the lysosomal intracellular enzyme cathepsins. They also studied incorporation of these lipophilic prodrugs in liposomes, and concluded that encapsulation efficiency (EE) closely depends on the length of the saturated 4-(N)-alee chain, the phospholipids chosen and the presence of cholesterol.

Marc Ostro *et al.,*[37] have developed liposomal preparation of different drugs and evaluated in different disease states and they concluded that liposomes are better dosage form than conventional dosage forms. They have also stated the drug can be targeted by active and passive targeting mechanism using liposomes.

Sayed Daoud *et al.,*[38] have described the challenge of chemotherapy in this they discussed about liposomes in cancer therapy and liposomes an ideal "drug carrier" for anti cancer drugs. They described the preclinical studies, clinical trials-phase 1 and 2 and drug resistance of anthracyclines and also described alkylating agents and platinum compounds.

Sydney *et al.,*[39] have described the cancer chemotherapy, in this they discussed incidence, geographic distribution and behavior of specific types of cancer. In that they described the mode of action, adverse effects, clinical use and dose administration of different chemotherapeutic agents.

Tyrrell *et al.,*[40] have described the general consideration and method of preparation of liposomes and also described about liposome-protein interaction and protein entrapment in liposomes. They summarized about uptake of liposomes in vivo and interaction of liposomes with cell culture and immunological aspects of liposomes.

Wollina *et al.,*[41] have studied the usage of two drugs namely, doxorubicin and daunorubicin in liposomal formulation for treatment of skin cancer like cutaneous T- cell lymphoma, malignant melanoma, AIDS related Kaposi□s sarcoma. They formulated liposomes with synthetic phospholipids and stabilizing agents like stearylamine and cholesterol. They also found that the efficacy of drug is improved in liposomal formulation than that of free drug.

Xiaojuan Yang *et al.,*[42] have described liposomes, comprising hydrogenated soy phosphatidylcholine (HSPC),cholesterol and poly (ethylene glycol) 2000-distearoyl phosphatidylethanolamine (PEG-DSPE), were preparedby

polycarbonate membrane extrusion and then loaded with flavopiridol by a pH-gradient driven remote loading procedure. The liposomes had a mean diameter of 120.7nm and a flavopiridol entrapment efficiency of 70.4%.

. **A. Laouini *et al.*,**[43] Liposomes, spherical-shaped nanovesicles, were discovered in the 60ies by Bangham. Since that,they were extensively studied as potential drug carrier. Due to their composition variability andstructural properties, liposomes are extremely versatile leading to a large number of applications including pharmaceutical, cosmetics and food industrial fields. This bibliographic paper offers a general review on the background and development of liposomes with a focus on preparation methods including classic (thin film hydration, reverse-phase evaporation, ethanol injection…) and novel scalable techniques. Furthermore, liposome characterization techniques including mean size, zetapotential, lamellarity, encapsulation efficiency, in vitro drug release, vesicles stability and lipid analysis synthesized from different published works are reported.

Maharshi, et al.[44] Repoted that Doxorubicin is an effective anticancer drug used in the treatment of several cancers such as osteosarcoma, kaposis sarcoma. The usage of the drug is limited because of its adverse effects on the heart. To reduce the adverse effects and to increase the release rate doxorubicin is formulated into liposomal dosage form. The liposomes are prepared by the thin film hydration method. Using soya lecithin as the phospholipid. This study mainly explains about the effect of concentration of soyalecithin, cholesterol and DSPE-MPEG2000 on the particle size of formulated liposomes which ranges in between 0.766 ± 0.03µm to 13.56 ± 0.10 µm drug entrapment efficiency of different formulations in which maximum entrapment efficiency was determined as 96.45 ± 0.95 % and minimum was 24.89 ± 1.18 % , zeta potential which is determined as -0.271 mV, *in vitro* drug release in which the the maximum sustain release was found as 41.45 ± 1.06 % and stability studies at different temperatures and maximum drug retention was found in refrigerated temperature 2-8 oC.

Arvind, et al. [46] Amphotericin B is a polyene antifungal drug used intravenously for systemic fungal infections. Liposomal formulation of amphotericin B for injection, prepared by thin film hydration technique was selected in the present study. Different formulations variables (solvents ratio and pH of complex formation) and process variable (numbers of homogenization cycles) were carried out to control the impurities levels and particle size of liposomes. Formulation prepared at pH 3.0 with 1:2 solvent ratio (Methanol: Chloroform) was given least impurities. Formulation prepared at 1400 bar pressure with 15 homogenization cycles was shown desire particle size. The optimized formulation was exhibited more than 90% release of drug for a period of 7 days. The stability study (40±2°C/ 75±5% RH) of the Amphotericin B liposomes was evaluated for 3 months and it was found to be stable.

A.D. Carvalho Júnior[47], Encapsulated cisplatin into stealth pH-sensitive liposomes and studied their stability, cytotoxicity and accumulation in a human small-cell lung carcinoma cell line (GLC4) and its resistant subline(GLC4/CDDP). Since reduced cellular drug accumulation has been shown to be the main mechanism responsible for resistance in theGLC4/CDDP sub line, we evaluated the ability of this new delivery system to improve cellular uptake. The liposomes were composed of dioleoyl phosphatidyl ethanolamine (DOPE), cholesteryl hemisuccinate(CHEMS), and distearoylphosphatidylethanolamine-polyethyleneglycol2000 (DSPE-PEG2000) and were characterized by determining the encapsulation percentage as a function of lipid concentration.

DRUG AND EXCIPIENTS PROFILE

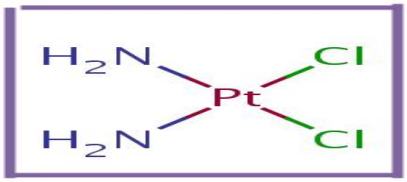
3. DRUG AND EXCIPIENTS PROFILE

3.1 DRUG PROFILE

Cisplatin:

Cisplatin, is an anticancer drug, belongs to alkylating agents class. Cisplatin is commonly used for the treatment of a wide range of cancers, majorly used to testicular and ovarian cancers and sarcomas many types of carcinoms.

Chemistry[49]:



(STRUCTURE OF CISPLATIN)

Iupac Name (*SP*-4-2)-diammine dichloridoplatinum

Molecular formula: $H_6Cl_2N_2Pt$

Molecular weight: 301.1gm/mol.

Melting point: 270-300 ^{O}C.

Physical description: Yellow powder or Orange yellow crystals**.**

Solubility: Sparingly soluble in dimethylformamide, slightly soluble in water and

practically Insoluble in ethanol (95%).

Bioavailability: 95%.

Half-life:20-30 min.

Mechanism of action[50]:

Alkylating agents work by three different mechanisms: 1) attachment of alkyl groups to DNA bases, resulting in the DNA being fragmented by repair enzymes in their attempts to replace the alkylated bases, preventing DNA synthesis and RNA transcription from the affected DNA, 2) DNA damage via the formation of cross-links (bonds between atoms in the DNA) which prevents DNA from being separated for synthesis or transcription, and 3) the induction of mispairing of the nucleotides leading to mutations.

Pharmacokinetics[50]:

After intravenous administration Cisplatin disappears from the plasma in a biphasic manner and half-lives of 25 to 49 minutes and 58 to 73 hrs have been reported for total platinum.

The majority of the platinum from a dose is rapidly bound to plasma protein. Cisplatin is concentrated in liver, kidney, and large and small intestines. Penetration into the CNS appears to be poor. Excretion is mainly in the urine but is incomplete and prolonged, up to about 50% of a dose has been reported to be excreted in urine over 5 days and platinum may be detected in tissues for several months afterwards. Cisplatin is well absorbed following intra peritoneal administration

Pharmacodynamics:

Cisplatin is an antineoplastic in the class of alkylating agents and is used to treat various forms of cancer. Alkylating agents are so named because of their ability to add alkyl groups to many electronegative groups under conditions present in cells. They stop tumor growth by cross-linking guanine bases in DNA double-helix strands - directly attacking DNA. This makes the strands unable to uncoil and separate.

As this is necessary in DNA replication, the cells can no longer divide. In addition, these drugs add methyl or other alkyl groups onto molecules where they do not belong which in turn inhibits their correct utilization by base pairing and causes a miscoding of DNA. Alkylating agents are cell cycle-nonspecific. Alkylating agents work by three different mechanisms all of which achieve the same end result - disruption of DNA function and cell death

The following adverse reactions are discussed in more detail in other sections of the labeling.

- Nephrotoxcity
- Neuro toxicity
- Ototoxicity

The most common adverse reactions observed with Cisplatin

- Fatigue
- Fever
- Nausea
- Stomatitis

- Vomiting
- Diarrhea
- Constipation
- Anorexia
- Rash and Neutropenia
- Thrombocytopenia and anemia

3.2 LIPID PROFILE:

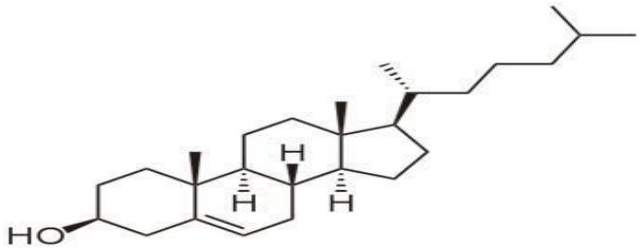
3.2.1 Cholesterol:

Cholesterol is a waxy steroid of fat that is manufactured in the liver or intestines. It is used to produce hormones and cell membranes and is transported in the blood plasma of all mammals. It is an essential structural component of mammalian cell membranes. It is required to establish proper membrane permeability and fluidity. In addition cholesterol is an important component for the manufacture of bile acids, steroid hormones and vitamin D.

Description:

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

CHEMISTRY[49]:



(STRUCTURE OF CHOLESTEROL)

IUPAC name : (3β)-Cholest-5-en-3-ol

Synonyms: Cholestrin

Molecular Formula: C27H46O

Molar mass: 386.65 g/mol

Appearance: white

crystalline powder

Density: 1.052 g/c

Melting point: 148-150 ^{O}C

Boiling point: 360 ^{O}C (decomposes)

Solubility in water: 0.095 mg/L (30 ^{O}C)

Solubility: Soluble in acetone, benzene, chloroform, ethanol, ether,

hexane, isopropyl myristate and methanol.

Cholesterol is oxidized by the liver in to a variety of bile acids. These in turn are conjugated with glycine, taurine, glucuronic acid, or sulphate. A mixture of conjugated and non-conjugated bile acids along with cholesterol itself is excreted from the liver in to the bile.

Approximately 95% of the bile acids are reabsorbed from the intestine and the remainder lost in the feces. The excretion and re-absorption of bile acids forms the basis of the entero-hepatic circulation which is essential for the digestion and the absorption of dietary fats. Under certain circumstances, when more concentrated, as in the gallbladder, cholesterol crystallizes and is the major constituent of most gallstones, although lecithin and bilirubin gallstones also occur less frequently.

Cholesterol is required to build and maintain cell membranes it regulates membrane fluidity over a wide range of temperatures. The liver produces about 1 gram of cholesterol per day, in bile. The hydroxyl group on cholesterol interacts with the polar. Head groups of the membrane phospholipids and Sphingolipids, while the bulky steroid and the hydrocarbon chain is embedded in the membrane, alongside the non-polar fatty acid chains of the other lipids. Bile which is stored in the gallbladder and helps digest fats is important for the absorption of the fat soluble vitamins, vitamins A, D, E and K. It also reduces the permeability of the plasma membrane. In myelin, it envelops and insulates nerves, helping greatly to conduct nerve impulses. It also reduces the permeability of the plasma membrane to protons (Positive hydrogen ions) and sodium ions.

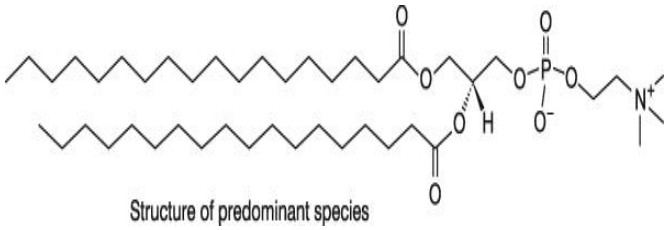
Stability and storage Condition

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

3.2.2 Hydrogenated Soya phosphatidyl Choline

Phospholipid molecule generally consists of hydrophobic tails and a hydrophilic head. Biological membranes in eukaryotes also contain another class of lipid, sterol, interspersed among the phospholipids and together they provide membrane fluidity and mechanical strength. Purified phospholipids are produced commercially and have found applications in nanotechnology and materials science.

Chemistry[49]



Structure of predominant species

Synonyms[50]**:** Hydro Soy PC.

Molecular Formula: C27H46O.

Molar mass: 783.774gm/mol.

Appearance: white powder.

Boiling point: 50-60 ^{O}C

Solubility in water: insoluble in water.

Solubility: Soluble in acetone, benzene, insoluble in triglycerides,alcohol and water.

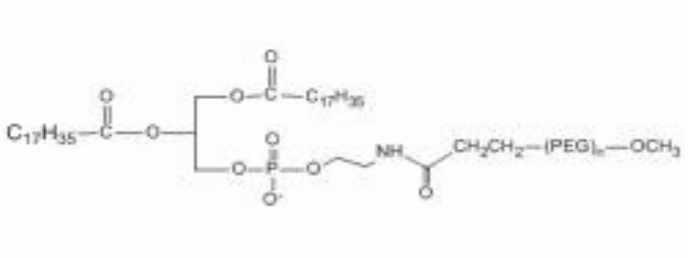
Storage: Keep container tightly closed in a dry and well-ventilated place.

3.2.3 Mpeg-dspe (Methoxyl polyethylene glycol DSPE)

Description

1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine(DSPE) conjugated Polyethylene Glycol, DSPE PEG is a phospholipid PEG conjugate which has both hydrophilicity and hydrophobility. Pegylated phospholipids are excellent liposome formation materials that can be used for drug delivery, gene transfection and vaccine delivery as well. Pegylation of phospholipids significantly improves the blood circulation time and stability for encapsulated drugs. These materials can also be used for targeted drug delivery by modifying their surfaces with targeting ligands such as antibodies, peptides.

Chemistry[49]



(STRUCTURE OF Mpeg-dspe)

Physical Properties[50]**:**

- Off-white/white solid or viscous liquid depends on molecule weight
- Soluble in regular aqeous solution as well as most organic solvents

Storage :

Store at -20 ^{0}C, desiccated Protect from light, Avoid frequent thaw and freeze

AIM & PLAN OF WORK

4. AIM OF THE WORK

The aim of the present study was to

- ✓ Compatibility study of API with lipids & cholesterol.
- ✓ Development of liposomal formulation.
- ✓ In-vitro characterization of liposomal formulation.
- ✓ Selection of best formulation based on the In-vitro characterization data.
- ✓ Lyophilization of the best formulation.

Scope of the study

Cisplatin, an anticancer drug, belongs to alkylating agent□s class. Though it has great importance in treating the cancers, its importance is decreasing due to its high protein binding character (which decreases bioavailability).

Recent literature is substantiating that Liposomal technology is one of the prominent technology for targeting drug delivery. Also stating that, by means of this technology one can increase the safety, efficacy of the drug molecules, achieves better compliance, bioavailability, etc.

Hence an attempt has-been made to avoid the protein binding of cisplatin by decreasing the exposure of the drug to proteins by enclosing the drug in liposomes.

PLAN OF WORK

STAGE 1:

1. Preformulation studies
    a. Standard calibration curve of Cisplatin in UV
    b. Compatibility studies

STAGE 2:

1. Preparation of Plain Liposomes with Ammonium sulphate and stabilizers.
2. Preparation of drug loaded liposomes with Stabilizers by Dried Thin Film Hydration Technique.

STAGE 3:

1. Physical characterization of liposomes
    a. Particle size analysis
    b. Zeta potential
    c. Transmission electron microscopy
    d. Scanning electron microscopy
    e. Polydispersity index

STAGE 4:

1. *In- vitro* charactarization
    a. Encapsulation efficiency
    b. Assay

c. Study on *in-vitro* drug release

d. Release kinetics

STAGE 5:

1. Short term stability studies

MATERIALS & METHODS

5. MATERIALS & METHODS

5.1 LIST OF CHEMICALS

Table No: 3 List of Chemicals used for the work

S.No	Ingredients	Manufactures
1.	Cisplatin	Nicholas and Piramal.Indore.Cipla Bangalore.
2.	Hspc	Lipoid Pvt. Ltd., Mumbai.
3.	Cholesterol	Lipoid Pvt. Ltd., Mumbai.
4.	Mpeg –dspe	Sigma Aldrich, Mumbai.
5.	Ammonium sulphate	Triveni chemicals, Mumbai.
6.	Sucrose	Triveni chemicals, Mumbai.
7.	Histidine	Merck chemicals, Mumbai.
8.	Chloroform	Fisher scientific, Mumbai.
9.	Sodium hydroxide	Merck chemicals, Mumbai.
10.	Triton X-100	Merck chemicals, Mumbai.
11.	Acetonitrile	Merck chemicals, Mumbai.
12.	Methanol	Merck chemicals, Mumbai.
13.	Sodium lauryl sulphate	Merck chemicals, Mumbai.
14.	Isopropyl alcohol	Merck chemicals, Mumbai.

5.2 LIST OF EQUIPMENTS

Table No: 4 List of Equipment used in the work

S.No	Equipment	Manufacturer
1.	UV- Visible spectrophotometer	PerkinElmer□s
2.	Infra Red spectroscopy	Bruker
3.	Rotary vacuum evaporator	Buchi
4.	Homogenizer	Panda
5.	Peristaltic pump	Electro lab
6.	Electronic balance	Sartorius
7.	Centrifuge	Remi Instruments
8.	Bath Sonicator	Jeken Ultrasonic
9.	Electronic Microscope	Motic
10.	HPLC	LC20AD, Shimadzu
11.	Magnetic stirrer	Remi Instruments
12.	Zeta Sizer version 6.00	Malvern
13.	Scanning Electron Microscopy	Field Instruments

5.3 STANDARD CALIBRATION CURVE

Standard calibration curve of Cisplatin was developed in phosphate buffer pH 7.4 and estimated by UV-Visible spectrophotometer at 301nm.

5.3.1 General Procedure for the preparation of calibration curve by UV

A stock solution of (1mg/ml) of standard drug was prepared, later required dilutions were made with a phosphate buffer pH 7.4. From the stock solution a serial dilutions made to obtain the re write solutions having concentration 10 –80 µg/ml, using phosphate buffer pH 7.4 as makeup solution, the absorbance were recorded at 301nm using UV- Visible spectrophotometer. Absorbance values were plotted against respective concentration to obtain standard calibration curve.

5.3.2 Preparation of Phosphate Buffer pH 7.4

Take accurately 1.564 gm of sodium hydroxide and 6.804gm potassium dihydrogen phosphate and dissolved in small volume of distilled water and made volume up to 1000 ml. The resulting solution is phosphate buffer having pH 7.4.

5.4 COMPATIBILITY STUDIES

5.4.1 FT-IR

IR spectroscopy was used to investigate and predict any physicochemical interactions between API and excipients in a formulation. Based on the results suitable chemically compatible excipients were chosen.

The following compounds IR spectroscopy were recorded

- Cisplatin
- Hydroxy soya phosphatidyl choline
- Cholesterol
- Mpeg –Dspe

- Mixture of Hspc and drug
- Mixture of cholesterol, and drug
- Mixture of mpeg-Dspg and drug

One part of the sample and three parts of potassium bromide were taken in a mortar and triturated. A small amount of triturated sample was taken in to a pellet maker and was compressed at 10kg/cm^2 using hydraulic press. The pellet was kept on to the sample holder and scanned from 4000cm^{-1} to 400cm^{-1} in Bruker IR spectrophotometer. Then it was compared with original spectra.

IR spectra was compared and checked for any shifting in functional peaks and non-involvement of functional group. From the spectra it is clear that there is no interaction between the selected carriers, drug and mixtures. Hence the selected carrier was found to be compatible in entrapping the selected cisplatin with carriers without any mutual interactions.

5.5 PREPARATION OF CISPLATIN LIPOSOMES

5.5.1 Procedure for the preparation of Cisplatin liposome

Liposomes were prepared using soya phosphatidyl choline (HSPC) by dried thin film hydration technique using rotary evaporator.

Accurately weighed quantities of Hspc, cholesterol, Mpeg-dspe and are dissolved in chloroform and methanol rotated in a rota-evaparator by applying vaccum of about 25mmHg at 25^0c, until it forms a thin film. Required quantities of ammonium sulphate and sucrose (0.3%) are dissolved in W.F.I and it is added to the above thin film in R.B flask and rotated until it forms a milky white suspension. The above solution is homogenized for 15 cycles to reduce particle size of liposomes.

The above solution is undergone for 25 cycles of dialysis, by using sucrose solution (10%) to remove free ammonia and sulphate from the lipid solution.

Drug solution is prepared by adding the required quantities of Drug and Tocoferol in a W.F.I and drug solution is added to the solution in a R.B flask (lipid solution) and rotated for 1hr.

Table No: 4 The composition of Drug, Soy Hspc, Cholesterol, Mpeg-dspe, Tocoferol and Ammonium sulphate.

Formulation code	**Drug (cisplatin)**	**Hspc** (gm/100ml)	**Cholesterol** (gm/100ml)	**Mpeg-dspe** (gm/100ml)	**tocoferol** (ml/100ml)	**Ammonium, Sulphate** (gm/100ml)
F1	40mg/ 15ml	3.5	1.75	1.5	0.1	3.96
F2		3.85	1.75	1.5	0.1	3.96
F3		4.0	2.0	1.85	0.1	3.96
F4		4.25	2.0	1.85	0.1	3.96
F5		4.25	2.25	2.0	0.1	3.96
F6		4.5	2.5	2.0	0.1	3.96

5.6 CHARACTERIZATION OF LIPOSOMES

All the liposomal formulation was evaluated for physicochemical properties like

- Particle size analysis
- Polydispersity index
- Zeta potential analysis
- SEM analysis
- TEM
- Determination of free drug
- Assay
- Invitro drug release
- Stability studies

5.6.1 Determination of particle size distribution

Determination of average vesicle size of liposomes was very important. Hence it was carried out by using MALVERN INSTRUMENT in JAWAHARLAL NEHRU TECHNOLOGICAL UNIVERSITY.

5.6.2 Polydispersity Index:

Polydispersity was determined according to the equation,

Polydispersity = D (0.9) - D (0.1) / D (0.5)

Where,

D (0.9) corresponds to particle size immediately above 90% of the sample.

D (0.5) corresponds to particle size immediately above 50% of the sample.

D (0.1) corresponds to particle size immediately above 10% of the sample.

5.6.3 ZETA POTENTIAL ANALYSIS

Zeta potential is a physical property which is exhibited by any particle in suspension. It can be used to optimize the formulations of suspensions and emulsions. It is an aid in predicting long-term stability. The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repeal each other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating.

A value of 25mV (positive or negative) can be taken as the arbitrary value that separates low-charged surfaces from high-charged surfaces.

The zeta potential was analyzed by MALVERN ZETASIZER in JAWAHARLAL NEHRU TECHNOLOGICAL UNIVERSITY.

5.6.4 TRANSMISSION ELECTRON MICROSCOPY

TEM (Hitachi, H-7500, Germany) was a method of probing the microstructure of rather delicate systems such as micells, liquid crystalline phases, vesicles and emulsions. The shape and size of optimized liposomal formulation is observed by TEM investigation.

5.6.5 SCANNING ELECTRON MICROSCOPY

Determination of surface morphology (roundness, smoothness and formation of aggregates) of cisplatin Liposomes was carried out by scanning electron microscopy (**SEM**).

The Scanning Electron Microscopy (SEM) was analyzed by FIELD INSTRUMENT in OSMANIA UNIVERSITY

5.7 *IN-VITRO* CHARACTERIZATION

5.7.1 Determination of free drug by Triton-X100 method

Take 1ml of liposomal solution in 10ml volumetric flask and make it up to the mark with 5%sucrose and determine the absorbance. Since the absorbance value is above 1 for 10 dilituion sample , 1ml from the dilution sample is take and made upto the mark with 5% sucrose and determine with uv-visible spectrophotometer. From the absorbance, determine the concentration of the free drug in liposomal solution.

Again take 1 ml of liposomal sample and add 1ml of triton- x100 and make it with 5% sucrose solution in a 10ml volumetric flask. From the absorbance amount of free drug in liposomal sample is determined.

5.8 Cisplatin Assay

A standard and sample solution were prepared, Inject separately 20 microlitre of the standard and sample solution in chromatographic condition and record the chromatogram. Calculate the content of drug per ml in liposomal injection as follows.

Assay= A/B×W/200×5/50×C/100×100-D/100×50/5×100/5

Where,

A = Area corresponding to Cisplatin in sample.

B = Area corresponding to Cisplatin in working standard.

C = % purity of Cisplatin in working standard.

D = % water content of working standard

. W = Weight of working standard in mg.

Chromatographic conditions:

Column	:	C_{18} BDS (250×4.6mm)
Mobile phase	:	Buffer + Acetonitrile + Methanol (47ml+48ml+ 5ml)
Buffer	:	2.8% w/w sodium lauryl sulphate + 2.3% w/v Phosphoric Acid.
Wave length	:	301nm.
Flow rate	:	1.7 sml/mn.
Solvent	:	Acidified IPA (90mlIPA+ 0.68ml Hcl+ Make up to 100mlwith(water)

5.9 *In vitro* diffusion studies of Cisplatin liposome

The *in vitro* release studies of all liposomal suspension were performed using exhaustive dialysis method. Two side open ended glass tube was taken and one side has been closed with semi permeable membrane. The fabricated tube was used as donor compartment in which 2 ml of liposomal suspension was taken and placed in receptor compartment containing 200 ml phosphate buffer (PBS, Ph 7.4) with acetonitrile. The dialysis was carried out at 50 rpm at 37^{o} C and stirred with the help of a magnetic stirrer. Aliquots (4ml) of release medium was withdrawn at different time intervals and the same volume was replaced with fresh phosphate buffer pH 7.4, to maintain sink condition volume and then the samples were analyzed by UV-Visible spectrophotometry at a λ max of 301nm.

5.10. Release kinetics

The release data fitted in various release kinetic models to know the mechanism of drug release kinetics. The zero order rate describes the systems where the drug release rate is independent of its concentration. The first order rate describes the release from system where release rate is concentration dependent. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on fickian diffusion

Various release models

1. Zero - order kinetic model – Cumulative % drug released versus time.
2. First – order kinetic model – Log cumulative percent drug remaining versus time.
3. Higuchi□s model – Cumulative percent drug released versus square root of time.
4. Korsmeyer equation / Peppa□s model – Log cumulative percent drug released versus log time.

a. Zero order kinetics:

Zero order release would be predicted by the following

equation: At = A0 – K0t

Where

At = Drug release at time,,t□

A0 = Initial drug concentration.

K0 = Zero- order rate constant (hr^{-1})

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys Zero – order kinetics and its slope is equal to Zero order release constant K0**.**

b. First order kinetics:

First - order release could be predicted by the following equation:

Log C = log C0 – Kt /

2.303

Where,

C = Amount of drug remained at time,,t□

C0 = Initial amount of drug.

K = First - order rate constant (hr^{-1}).

When the data plotted as log cumulative percent drug remaining versus time, yields a straight line, indicating that the release follow first order kinetics. The constant „K1□ can be obtained by multiplying 2.303 with the slope value.

c. Higuchi's model:

Drug release from the matrix devices by diffusion has been described by following

Higuchi□s classical diffusion equation: $Q = [D\epsilon / \tau(2A - \epsilon Cs)\ Cst]^{1/2}$

Where,

Q = Amount of drug release at time,,t□

D = Diffusion coefficient of the drug in the matrix. A = Total amount of drug in unit volume of matrix. Cs = Solubility of drug in the matrix.

Є = Porosity of the matrix. τ = Tortuosity.

t = Time (hrs at which q amount of drug is released.

Above equation can be simplified as if we assumes that „D□, „Cs□ and „A□ are constant. Then equation becomes:

$$Q = Kt^{1/2}$$

5.10. Release kinetics

The release data fitted in various release kinetic models to know the mechanism of drug release kinetics. The zero order rate describes the systems where the drug release rate is independent of its concentration. The first order rate describes the release from system where release rate is concentration dependent. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on fickian diffusion.

Various release models

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4. Korsmeyer equation / Peppa□s model – Log cumulative percent drug released versus log time.

a. Zero order kinetics:

Zero order release would be predicted by the following equation: $At = A0 – K0t$

Where

At = Drug release at time,„t□

A0 = Initial drug concentration.

K0 = Zero- order rate constant (hr^{-1})

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys Zero – order kinetics and its slope is equal to Zero order release constant K0**.**

b. First order kinetics:

First - order release could be predicted by the following equation:

$$\text{Log } C = \log C0 - Kt /$$

$$2.303$$

Where,

C = Amount of drug remained at time,,t

C0 = Initial amount of drug.

K = First - order rate constant (hr^{-1}).

When the data plotted as log cumulative percent drug remaining versus time, yields a straight line, indicating that the release follow first order kinetics. The constant ,,K1 can be obtained by multiplying 2.303 with the slope value.

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Drug release from the matrix devices by diffusion has been described by following

Higuchi s classical diffusion equation: $Q = [D\epsilon / \tau(2A - \epsilon Cs) Cst]^{1/2}$

Where,

Q = Amount of drug release at time,,t□

D = Diffusion coefficient of the drug in the matrix. A = Total amount of drug in unit volume of matrix. Cs = Solubility of drug in the matrix.

Є = Porosity of the matrix. τ = Tortuosity.

t = Time (hrs at which q amount of drug is released.

Above equation can be simplified as if we assumes that „D□, „Cs□ and „A□ are constant. Then equation becomes:

$$Q = Kt^{1/2}$$

When the data is splitted according to equation i.e. cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to „K□ (Higuchi□s 1963).

d. Korsmeyer equation / Peppa's model:

To study the mechanism of drug release from the liposomal solution, the release data was also fitted to the well-known exponential equation (Korsmeyer equation/ Peppa□s law equation), which is often used to describe the drug release behavior from polymeric systems.

$$Mt / M\alpha = Kt^{n}$$

Where,

Mt / Mα = The fraction of drug released at time,,t□.

K= Constant incorporating the structural and geometrical characteristics of the drug / polymer system.

n = Diffusion exponent related to the mechanism of the release.

Above equation can be simplified as follows by applying log on both sides, Log Mt / Mα = Log K+ n Log t

5.11 Lyophilization

Processing:

The fundamental process steps are:

1. Freezing: The product is frozen. This provides a necessary condition for low temperature drying

2. Vacuum: After freezing, the product is placed under vacuum. This enables the frozen solvent in the product to vaporize without passing through the liquid phase, a process known as sublimation.

3. Heat: Heat is applied to the frozen product to accelerate sublimation.

4. Condensation: Low-temperature condenser plates remove the vaporized solvent from the vacuum chamber by converting it back to a solid. This completes the separation process.

Wet samples can be frozen by placing them in a vacuum. The more energetic molecules escape, and the temperature of the sample falls by evaporative cooling. Eventually it freezes. About 15% of the water in the wet material is lost.

The simplest form of lyophilizer would consist of a vacuum chamber into which wet sample material could be placed, together with a means of removing water vapor so as to freeze the sample by evaporative cooling and freezing and then maintain the water-vapor pressure below the triple-point pressure. The temperature of the sample would then continue to fall below the freezing point and sublimation would slow down until the rate of heat gain in the sample by conduction, convection, and radiation was equal to the rate of heat loss as the more energetic molecules sublimed away were removed.

RESULTS

6 .RESULTS

6.1: CALIBRATION CURVE OF CISPLATIN

Table No 7: Standard Readings of Cisplatin in phosphate buffer pH 7.4

S. No.	Concentration	Absorbance at 301nm
1.	0	0
2.	10	0.101
3.	20	0.224
4.	30	0.311
5.	40	0.425
6.	50	0.540
7.	60	0.645
	80	0.830

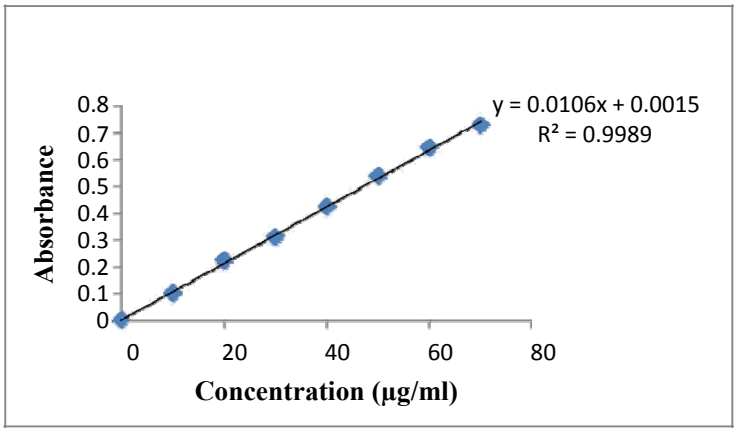

Figure no:7 Standard graph of Cisplatin in phosphate buffer pH 7.4

DISCUSSION

The UV absorbance of Cisplatin standard solution in the range of 10-80 µg/ml of drug in phosphate buffer pH 7.4 showed linearity at λ max 301nm. The linearity was plotted for absorbance against concentration with R^2 value 0.9989 and with the slope equation y = 0.0106x + 0.0015.

6.2: PREFORMULATION STUDIES OF CISPLATIN

6.2.1: FT-IR STUDIES

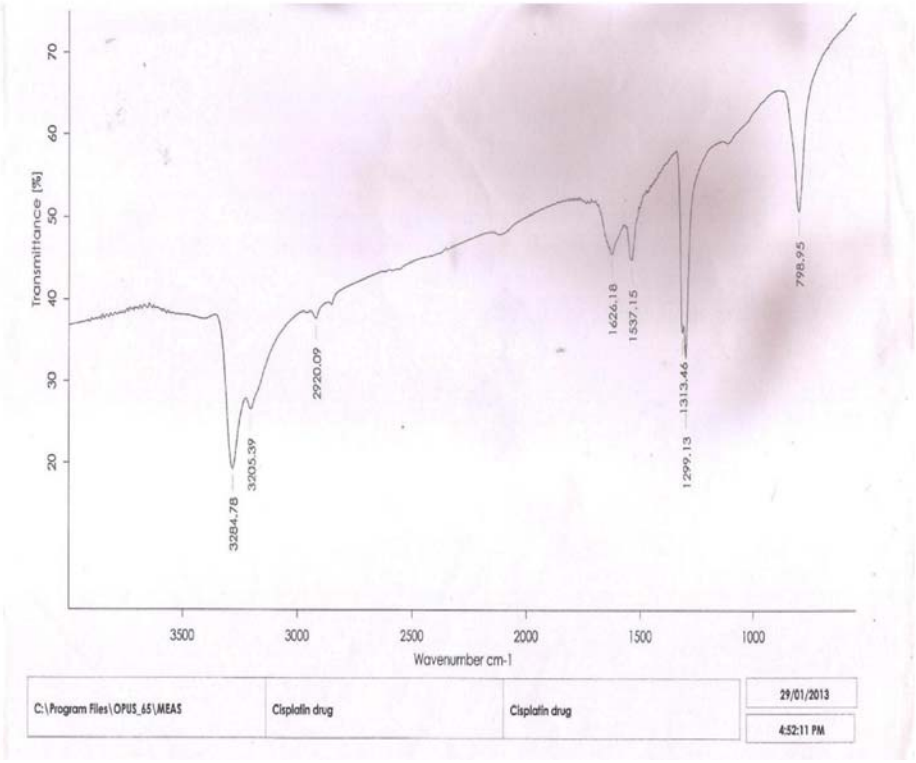

Figure No: 8 FTIR of cisplatin drug

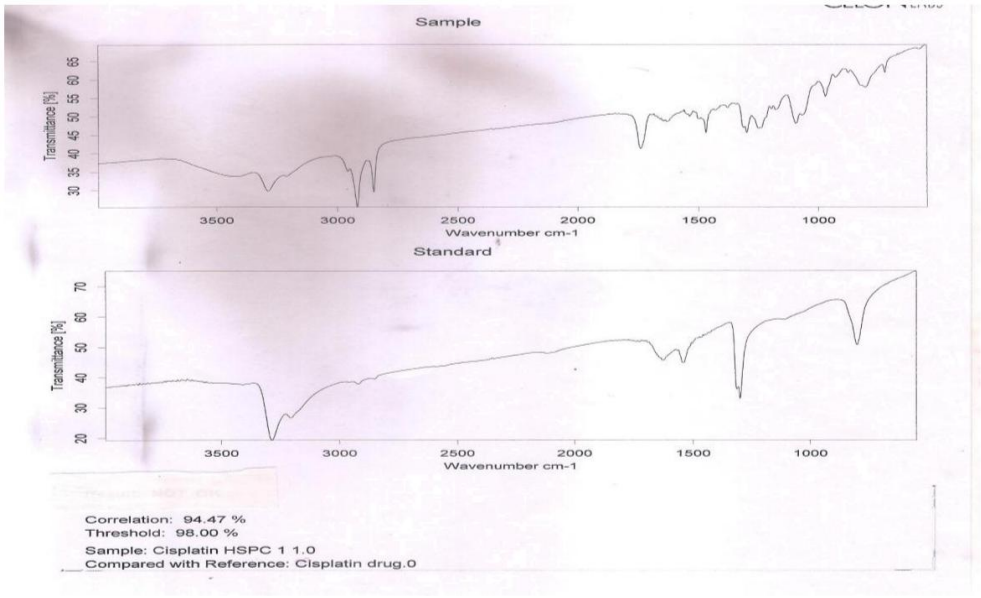

Figure No: 9 FTIR of drug and Hspc.(1:1)

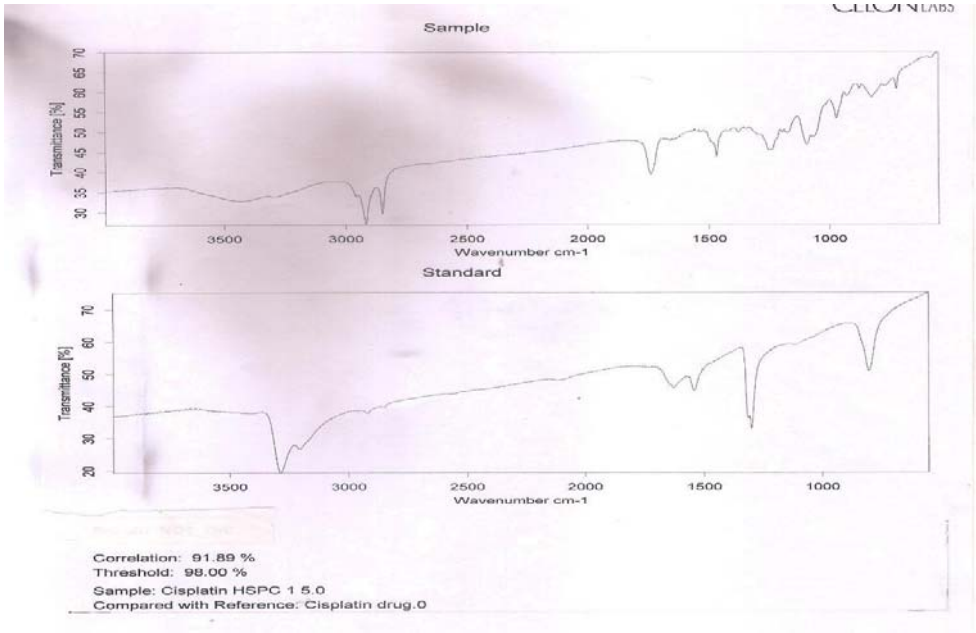

Figure No: 10 FTIR of drug and Hspc.(1:5).

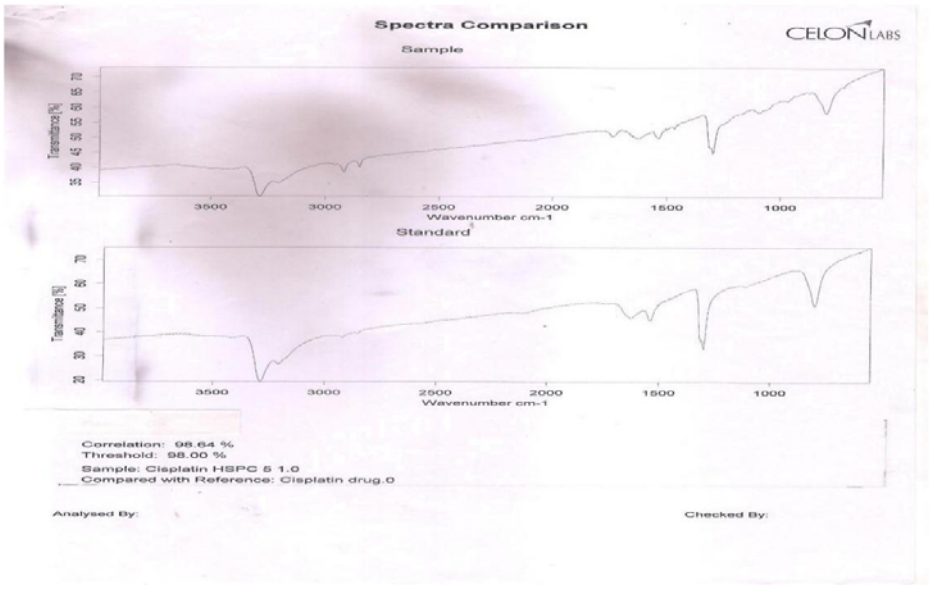

Figure No: 11 FTIR of Cisplatin and Hspc (5:1)

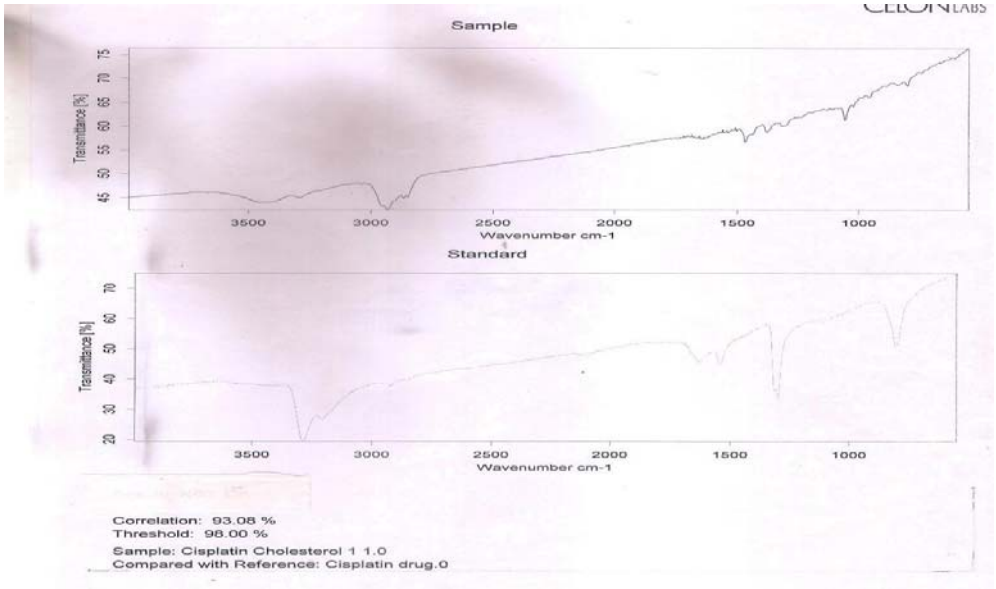

Figure No : 12 FTIR of Cisplatin and Hspc (1:1)

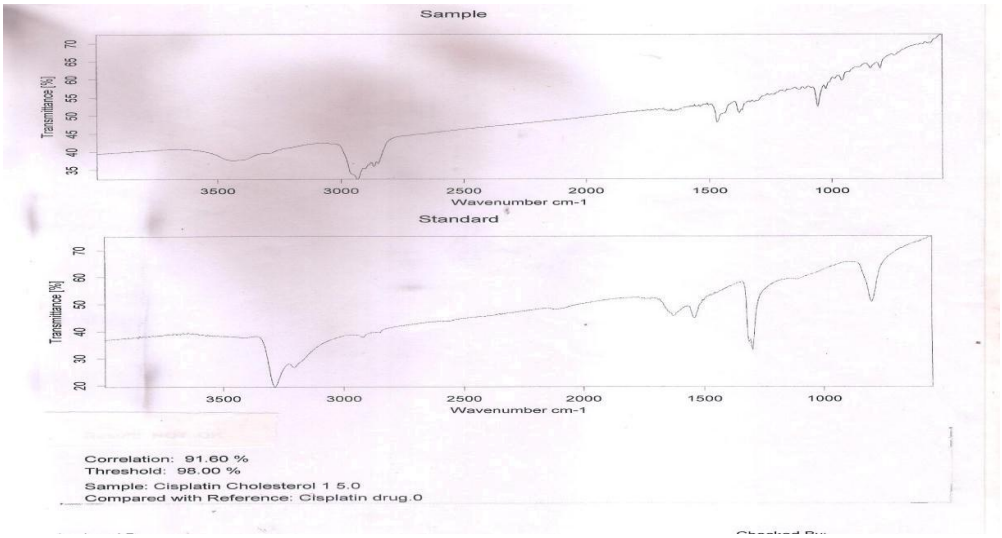

Figure No: 13 FTIR of Cisplatin and cholesterol (1:5)

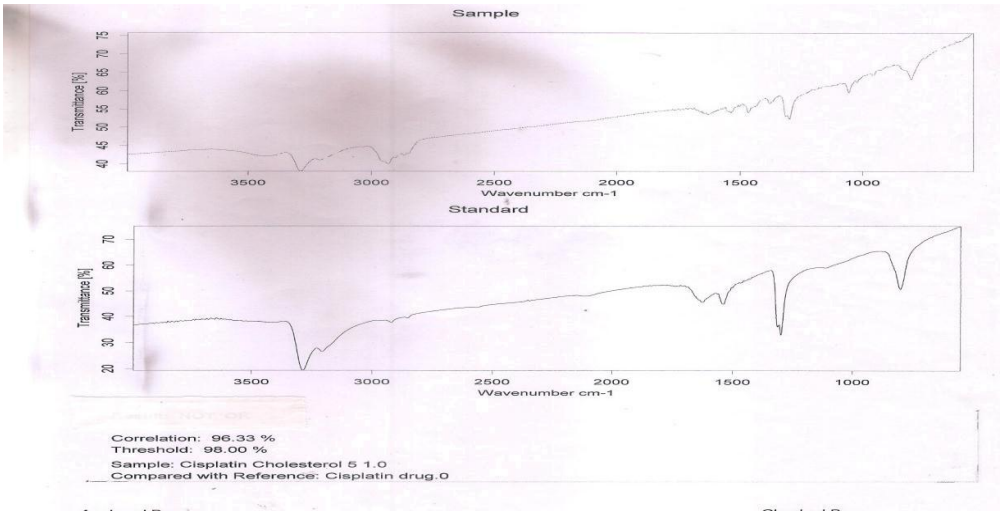

Figure No: 14 FTIR of Cisplatin and mpeg-dspe (5:1)

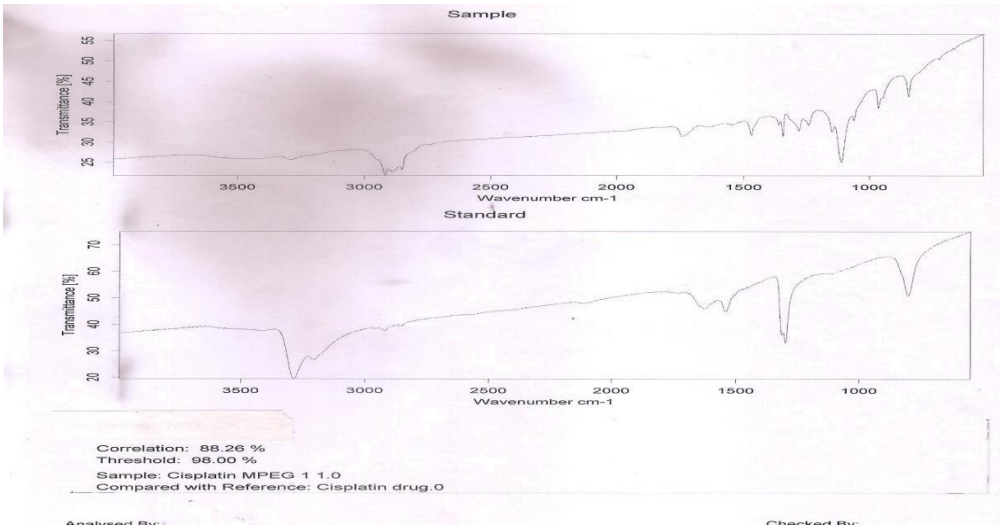

Figure No: 15 FTIR of Cisplatin and mpeg-dspe (1:1)

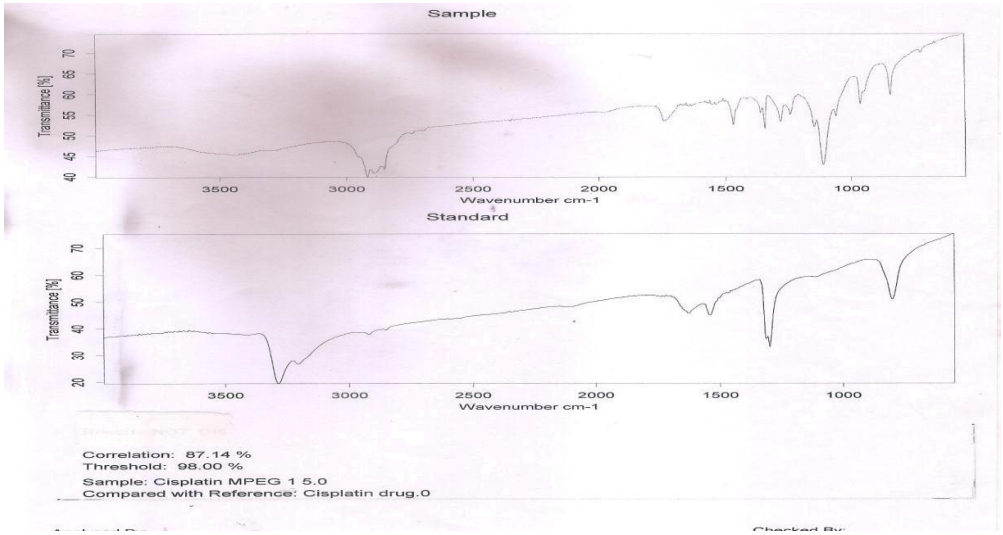

Figure No: 16 FTIR of Cisplatin and mpeg-dspe (1:5)

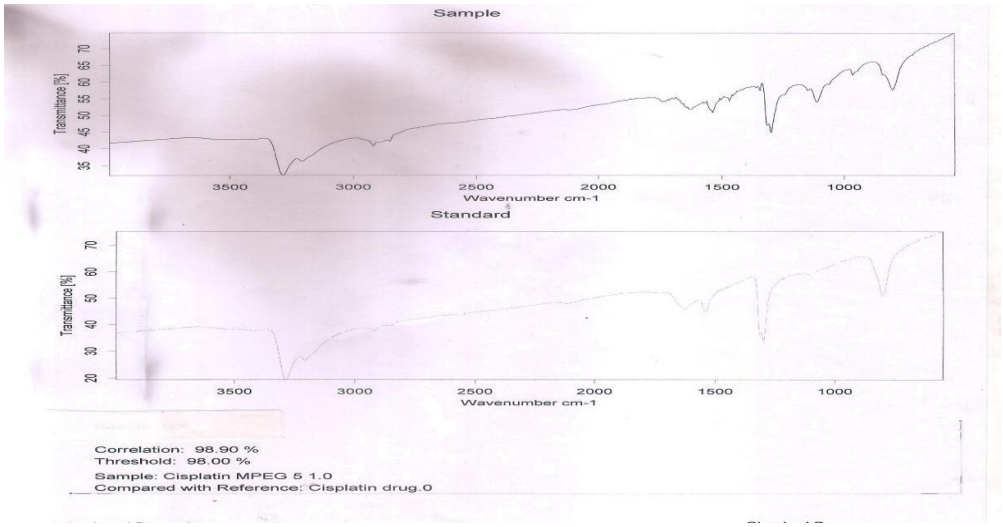

Figure No: 17 FTIR of Cisplatin and mpeg-dspe (1:5)

Table no 8: Percentage correlations of drug and lipid mixtures

S.no	Formulation code	Percentage compatibility ratio of drug and excipients
1	Drug and Hspc.(1:1)	94.47
2	Drug and Hspc.(1:5)	91.89
3	Drug and Hspc.(5:1)	98.64
4	Drug and cholesterol (1:1)	93.08
5	Drug and Cholesterol (1:5)	91.60
6	Drug and cholesterol (5:1)	96.33
7	Drug and mpeg-dspe (1:1)	88.26
8	Drug and mpeg-dspe (1:5)	87.14
9	Drug and mpeg-dspe (5:1)	98.50

DISCUSSION

The compatibility between the drug and the selected lipid and other excipients was evaluated using FTIR peak matching method. The percentage correlations were evaluated and the results showing that there was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid and other chemicals.

6.3 DIFFERENTIAL SCANNING CALORIMETRY (DSC) RESULTS

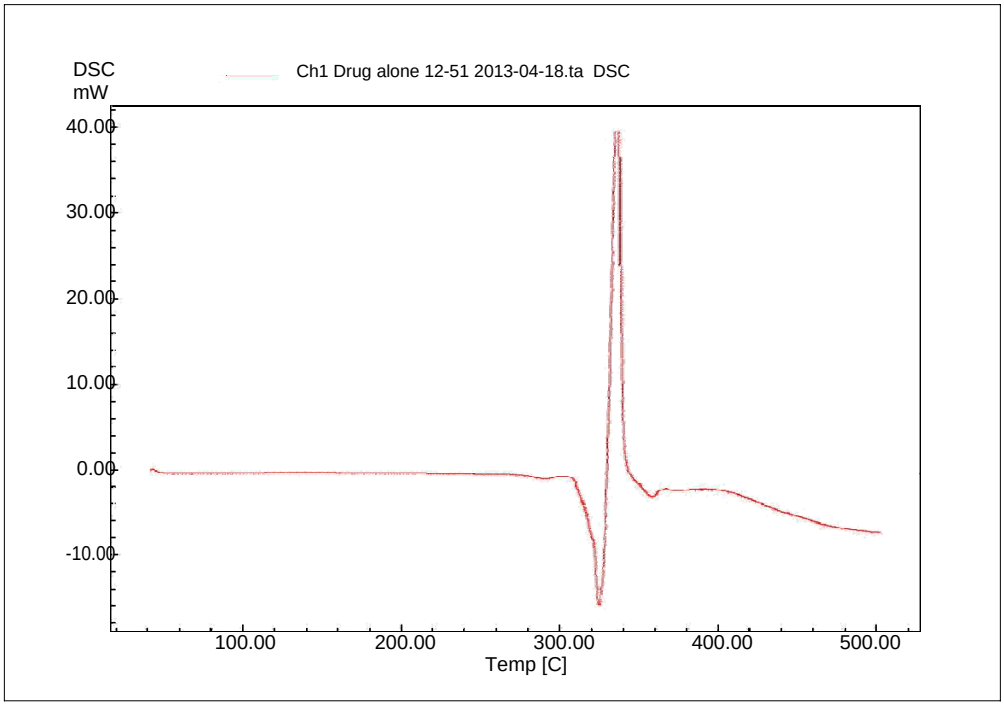

Figure No: 18 D.S.C of Cisplatin drug

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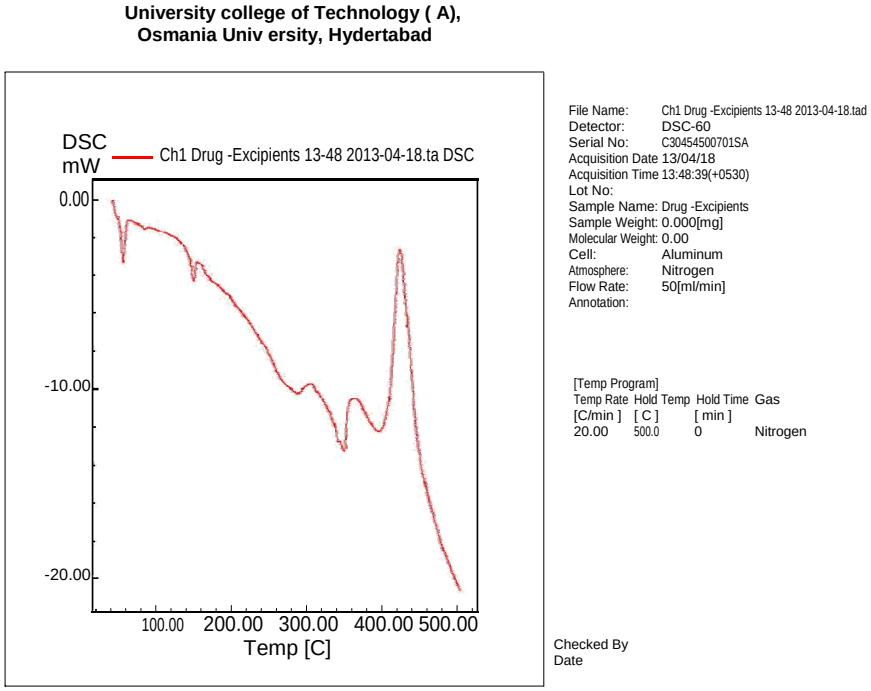


Figure No: 19 D.S.C: Cisplatin, Hspc, Mpeg-dspe and Cholesterol

DISCUSSION

The DSC curve of pure drug Cisplatin exhibits a sharp endothermic peak at 320.1°C.The thermogram of drug-lipid mixture displayed endothermic peaks at 32.1°C, 120.0°C,400.5°C. DSC measurements showed that drug-lipid mixture was having less ordered arrangement of crystals, and this was favorable for increasing the drug loading capacity.

6.4 DETERMINATION OF PARTICLESIZE DISTRIBUTION AND POLYDISPERSITY INDEX:

Table no 9: particle size determination

S.No	Formulation code	Average vesicular size(nm)	Poly dispersive index (pdi)
1	F2	289.2	0.247
2	F6	108	0.461

DISCUSSION

The Liposomes were prepared by dried thin film hydration technique using rotary evaporator with drug and carrier (hydrogenated soya phosphatidyl choline).The formulation containing Cisplatin were prepared with stabilizer like tocoferol and all other parameters like temperature, vacuum and RPM were kept constant. The particle size distribution was analyzed for F2, F6 formulations of Cisplatin Liposomes by wet method. The particle size was optimum in F6 Formulation. Results were shown in figure no.20, 21.

CISPLATIN.nsz

Measurement Results

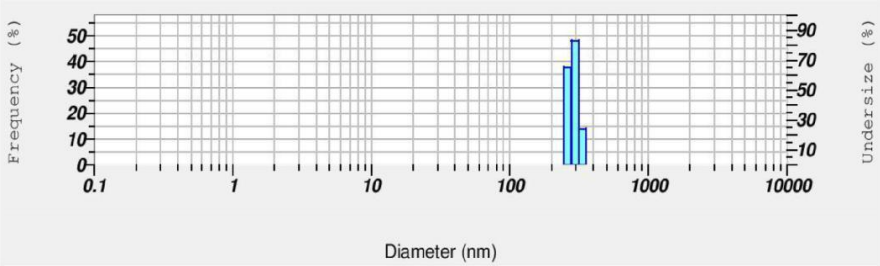
Date	:	Tuesday, March 05, 2013 9:49:31 AM
Measurement Type	:	Particle Size
Sample Name	:	CISPLATIN
Scattering Angle	:	90
Temperature of the holder	:	25.2 ℃
T% before meas.	:	28629
Viscosity of the dispersion medium	:	0.892 mPa·s
Form Of Distribution	:	Standard
Representation of result	:	Scattering Light Intensity
Count rate	:	0 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	289.1 nm	24.3 nm	287.8 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	289.1 nm	24.3 nm	287.8 nm

Histogram Operations

% Cumulative (2)	:	10.0 (%) - 255.1 (nm)
% Cumulative (6)	:	50.0 (%) - 287.8 (nm)
% Cumulative (10)	:	90.0 (%) - 326.4 (nm)



No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation
1	0.34	0.000	0.000	22	4.40	0.000	0.000	43	57.09	0.000	0.000	64	740.89	0.000	100.000
2	0.38	0.000	0.000	23	4.97	0.000	0.000	44	64.50	0.000	0.000	65	837.07	0.000	100.000
3	0.43	0.000	0.000	24	5.61	0.000	0.000	45	72.87	0.000	0.000	66	945.74	0.000	100.000
4	0.49	0.000	0.000	25	6.34	0.000	0.000	46	82.33	0.000	0.000	67	1068.52	0.000	100.000
5	0.55	0.000	0.000	26	7.17	0.000	0.000	47	93.02	0.000	0.000	68	1207.24	0.000	100.000
6	0.62	0.000	0.000	27	8.10	0.000	0.000	48	105.10	0.000	0.000	69	1363.97	0.000	100.000
7	0.70	0.000	0.000	28	9.15	0.000	0.000	49	118.74	0.000	0.000	70	1541.04	0.000	100.000
8	0.80	0.000	0.000	29	10.34	0.000	0.000	50	134.16	0.000	0.000	71	1741.10	0.000	100.000
9	0.90	0.000	0.000	30	11.68	0.000	0.000	51	151.57	0.000	0.000	72	1967.14	0.000	100.000
10	1.02	0.000	0.000	31	13.20	0.000	0.000	52	171.25	0.000	0.000	73	2222.51	0.000	100.000
11	1.15	0.000	0.000	32	14.91	0.000	0.000	53	193.48	0.000	0.000	74	2511.05	0.000	100.000
12	1.30	0.000	0.000	33	16.84	0.000	0.000	54	218.60	0.000	0.000	75	2837.04	0.000	100.000
13	1.47	0.000	0.000	34	19.03	0.000	0.000	55	246.98	0.000	0.000	76	3205.35	0.000	100.000
14	1.66	0.000	0.000	35	21.50	0.000	0.000	56	279.04	37.874	37.874	77	3621.48	0.000	100.000
15	1.87	0.000	0.000	36	24.29	0.000	0.000	57	315.27	48.137	86.011	78	4091.63	0.000	100.000
16	2.11	0.000	0.000	37	27.45	0.000	0.000	58	356.20	13.989	100.000	79	4622.81	0.000	100.000
17	2.39	0.000	0.000	38	31.01	0.000	0.000	59	402.44	0.000	100.000	80	5222.96	0.000	100.000
18	2.70	0.000	0.000	39	35.03	0.000	0.000	60	454.69	0.000	100.000	81	5901.02	0.000	100.000
19	3.05	0.000	0.000	40	39.58	0.000	0.000	61	513.71	0.000	100.000	82	6667.10	0.000	100.000
20	3.45	0.000	0.000	41	44.72	0.000	0.000	62	580.41	0.000	100.000	83	7532.65	0.000	100.000
21	3.89	0.000	0.000	42	50.53	0.000	0.000	63	655.76	0.000	100.000	84	8510.56	0.000	100.000

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Figure No: 20 Particle size distribution of F2 liposomal formulation.

CISPLATIN.nsz

Measurement Results

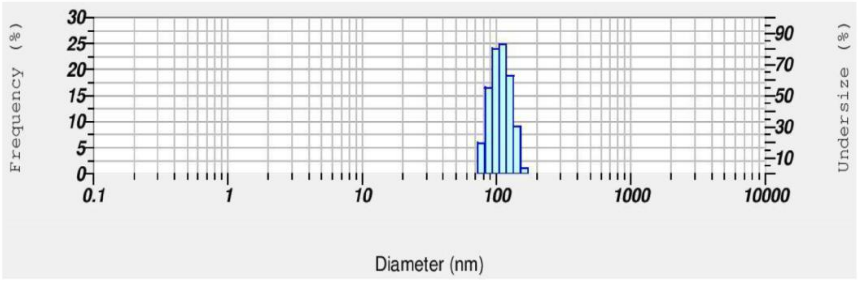
Date	: Thursday, May 09, 2013 11:08:30 AM
Measurement Type	: Particle Size
Sample Name	: CISPLATIN
Scattering Angle	: 173
Temperature of the holder	: 25.1 ℃
T% before meas.	: 2041
Viscosity of the dispersion medium	: 0.893 mPa·s
Form Of Distribution	: Standard
Representation of result	: Scattering Light Intensity
Count rate	: 4 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	108.6 nm	18.6 nm	110.3 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	108.6 nm	18.6 nm	110.3 nm

Histogram Operations

% Cumulative (2)	: 10.0 (%) - 84.9 (nm)
% Cumulative (6)	: 50.0 (%) - 106.9 (nm)
% Cumulative (10)	: 90.0 (%) - 134.2 (nm)



No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation
1	0.34	0.000	0.000	22	4.40	0.000	0.000	43	57.09	0.000	0.000	64	740.89	0.000	100.000
2	0.38	0.000	0.000	23	4.97	0.000	0.000	44	64.50	0.000	0.000	65	837.07	0.000	100.000
3	0.43	0.000	0.000	24	5.61	0.000	0.000	45	72.87	0.000	0.000	66	945.74	0.000	100.000
4	0.49	0.000	0.000	25	6.34	0.000	0.000	46	82.33	5.902	5.902	67	1068.52	0.000	100.000
5	0.55	0.000	0.000	26	7.17	0.000	0.000	47	93.02	16.518	22.420	68	1207.24	0.000	100.000
6	0.62	0.000	0.000	27	8.10	0.000	0.000	48	105.10	24.050	46.470	69	1363.97	0.000	100.000
7	0.70	0.000	0.000	28	9.15	0.000	0.000	49	118.74	24.749	71.219	70	1541.04	0.000	100.000
8	0.80	0.000	0.000	29	10.34	0.000	0.000	50	134.16	18.776	89.995	71	1741.10	0.000	100.000
9	0.90	0.000	0.000	30	11.68	0.000	0.000	51	151.57	8.999	98.994	72	1967.14	0.000	100.000
10	1.02	0.000	0.000	31	13.20	0.000	0.000	52	171.25	1.006	100.000	73	2222.51	0.000	100.000
11	1.15	0.000	0.000	32	14.91	0.000	0.000	53	193.48	0.000	100.000	74	2511.05	0.000	100.000
12	1.30	0.000	0.000	33	16.84	0.000	0.000	54	218.60	0.000	100.000	75	2837.04	0.000	100.000
13	1.47	0.000	0.000	34	19.03	0.000	0.000	55	246.98	0.000	100.000	76	3205.35	0.000	100.000
14	1.66	0.000	0.000	35	21.50	0.000	0.000	56	279.04	0.000	100.000	77	3621.48	0.000	100.000
15	1.87	0.000	0.000	36	24.29	0.000	0.000	57	315.27	0.000	100.000	78	4091.63	0.000	100.000
16	2.11	0.000	0.000	37	27.45	0.000	0.000	58	356.20	0.000	100.000	79	4622.81	0.000	100.000
17	2.39	0.000	0.000	38	31.01	0.000	0.000	59	402.44	0.000	100.000	80	5222.96	0.000	100.000
18	2.70	0.000	0.000	39	35.03	0.000	0.000	60	454.69	0.000	100.000	81	5901.02	0.000	100.000
19	3.05	0.000	0.000	40	39.58	0.000	0.000	61	513.71	0.000	100.000	82	6667.10	0.000	100.000
20	3.45	0.000	0.000	41	44.72	0.000	0.000	62	580.41	0.000	100.000	83	7532.65	0.000	100.000
21	3.89	0.000	0.000	42	50.53	0.000	0.000	63	655.76	0.000	100.000	84	8510.56	0.000	100.000

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Figure No: 21 Particle size distribution of Cisplatin F6 liposomal formulation

6.5 ZETA POTENTIAL ANALYSIS

TableNo:10 Zeta potential values of Cisplatin Liposomes for Optimized Batch.

S.No	Formulation Code	Zeta Potential(mV)
1	F2	-17.0
2	F6	-20.0

DISCUSSION

The zeta potential of the optimized formulation (F6) was found to be -20mV.Zeta potential is an important parameter that influences stability. Extremely positive or negative zeta potential values cause larger repulsive forces, whereas repulsion between particles with similar electric charge prevents aggregation of the particles and thus ensures easy redispersion. In case of a combined electrostatic and steric stabilization, a minimum zeta potential of ± 20 mV is desirable. The report was shown in fig.no.22,23.

2013.03.05 10:07:01

Measurement Results

201303051006008.nzt

Measurement Results

Date	: Tuesday, March 05, 2013 10:06:58 AM
Measurement Type	: Zeta Potential
Sample Name	: CISPLATIN
Temperature of the holder	: 25.2 °C
Viscosity of the dispersion medium	: 0.892 mPa·s
Conductivity	: 0.424 mS/cm
Electrode Voltage	: 4.8 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-17.0 mV	-0.000152 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	: -17.0 mV
Electrophoretic Mobility mean	: -0.000152 cm^2/Vs

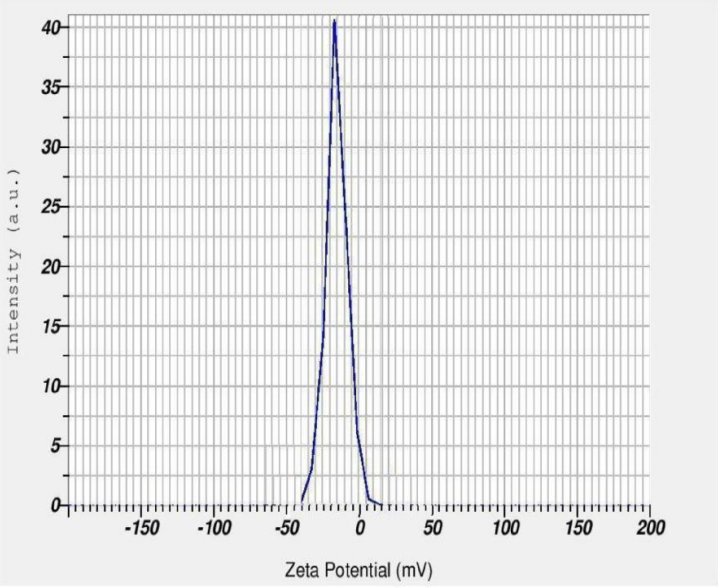

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Figure No: 22 Zeta potential for Cisplatin Liposomal F2 formulation

2013.05.09 12:07:21

Measurement Results

201303051006008.nzt

Measurement Results

Date	: Thursday, May 09, 2013 12:07:18 PM
Measurement Type	: Zeta Potential
Sample Name	: CISPLATIN
Temperature of the holder	: 25.2 ℃
Viscosity of the dispersion medium	: 0.892 mPa·s
Conductivity	: 0.424 mS/cm
Electrode Voltage	: 4.8 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-20.0 mV	-0.000104 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	: -20.0 mV
Electrophoretic Mobility mean	: -0.000104 cm^2/Vs

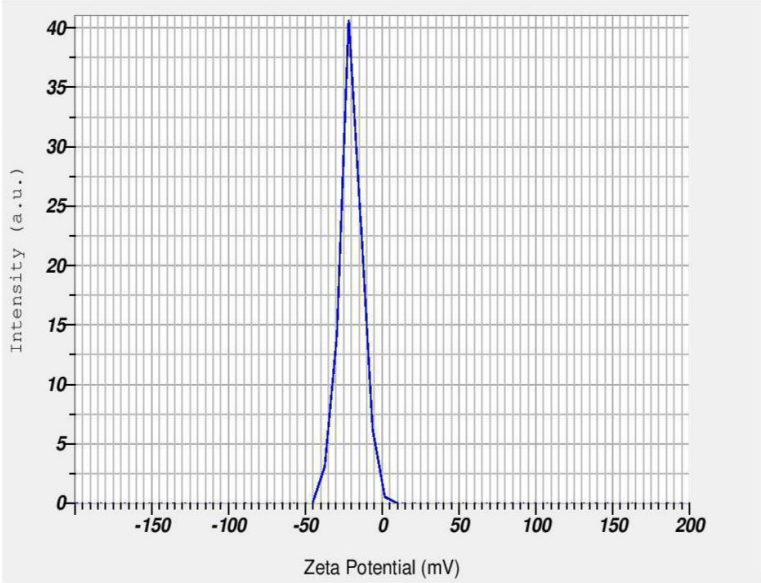

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Figure No: 23 Zeta potential for Cisplatin Liposomal F6 formulation

6.6 TRANSMISSION ELECTRON MICROSCOPY (TEM):

The morphology of optimized formulation (F6) produced was assessed by Transmission electron microscopy (TEM) and it is shown in Fig.No.24 confirming the spherical shaped particles in nanometric range.

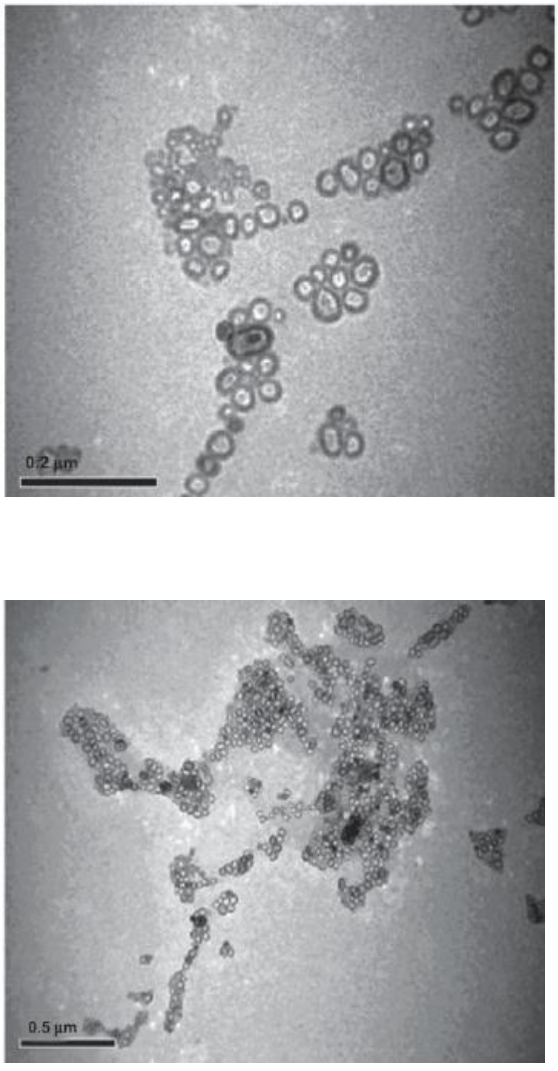

Figure no 24: TEM images of liposomes

6.7 SCANNING ELECTRON MICROSCOPY (SEM)

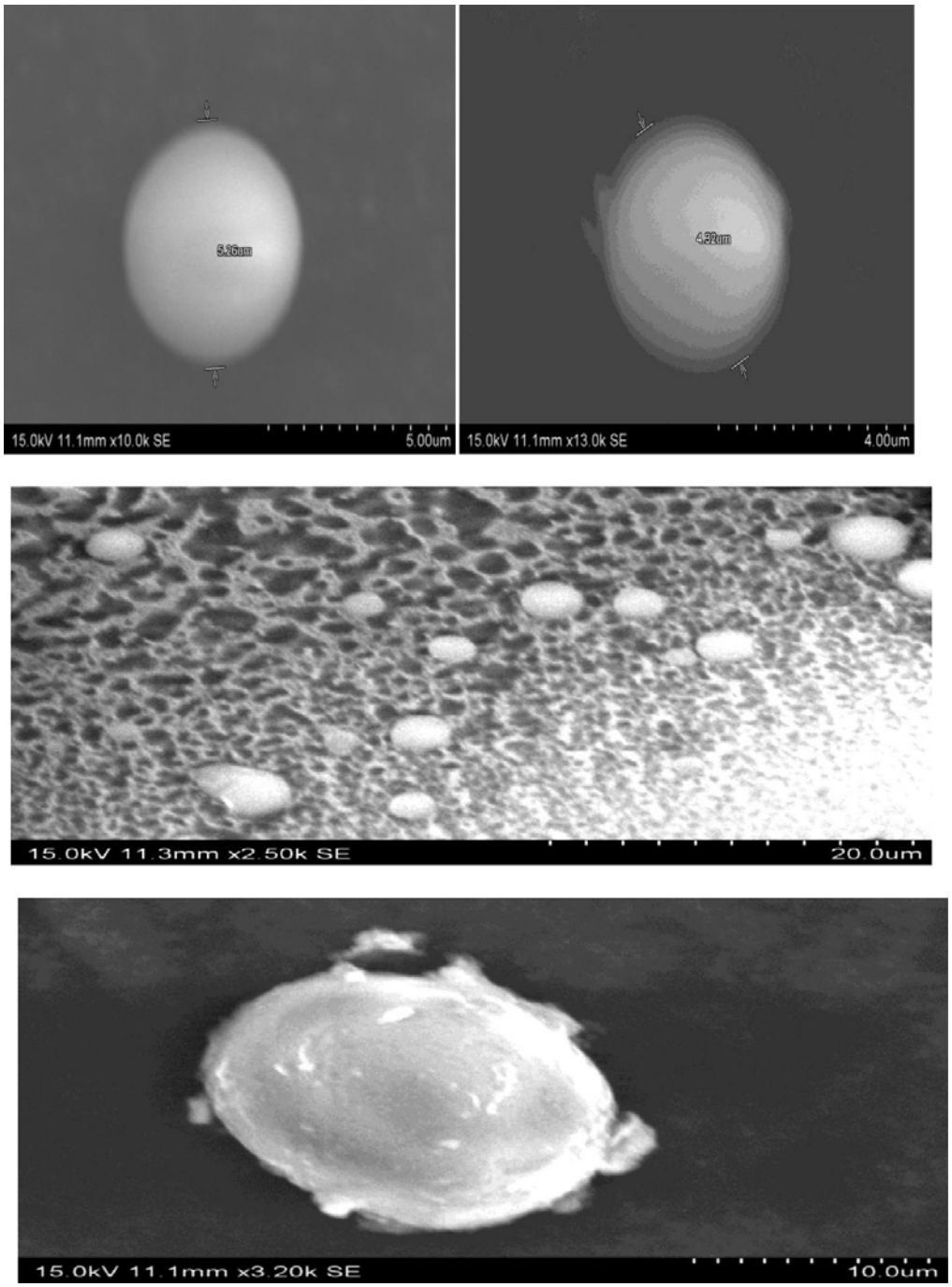

Figure no 25: SEM images of Cisplatin liposomes

DISCUSSION

The Morphology and surface appearance of Liposomes were examined by using SEM. The SEM photographs of F2 and F6 formulation showed that the particles have smooth surface. The SEM images were shown in figure no: 25.

6.8 DETERMINATION OF PERCENTAGE FREE DRUG

Table No: 11 Percent free drug of Cisplatin liposomal solution for F1, F2, F3, F4, F5 and F6 Formulations

S. No.	Formulation code	Percentage of free drug
1.	F1	45.23
2.	F2	39.3
3.	F3	30.20
4.	F4	25.31
5.	F5	17.21
6.	F6	8.72

DISCUSSION

The percent free drug is determined for all the formulations F1 to F6. The percent free drug was optimum in F6 formulation, which is within the limit (10%), the percent free drug was as shown in the table no: 11.

6.9 ASSAY RESULTS

Table No: 12 Assay of Cisplatin liposomal solution for F1, F2, F3, F4, F5, F6

Formulations.

S. No.	Formulation code	Cisplatin
1.	F1	98.8%
2.	F2	99.7%
3.	F3	95.6%
4.	F4	97.3%
5.	F5	97.2%
6.	F6	98.0 %

DISCUSSION

The percentage purity is determined for all the formulations from F1to F6. The assay value is within the limit (90%) for all the formulations, the results were shown in the table no: 12.

6.10 *IN- VITRO* DIFFUSION SYUDIES

Table No.13 Diffusion results of F1 formulation

Time (hr)	√time	log time	Absorbance (nm)	Conc. (µg/2ml)	%CDR	%CDRR	Log %CDR	Log %CDRR
2	1.414	0.301	0.0032	0.165	1.29	98.71	0.110	1.994
4	2	0.602	0.0047	0.3068	2.36	97.64	0.372	1.989
6	2.441	0.778	0.0074	0.5616	4.32	95.68	0.635	1.980
8	2.828	0.903	0.0092	0.7395	5.65	94.35	0.752	1.974
10	3.162	1	0.013	1.108	8.53	91.47	0.930	1.961
12	3.464	1.079	0.016	1.457	11.21	88.79	1.049	1.948
14	3.741	1.146	0.022	1.982	15.25	84.75	1.183	1.928
16	4	1.204	0.027	2.424	18.65	81.35	1.250	1.910
18	4.242	1.255	0.037	3.377	25.98	74.02	1.414	1.869
20	4.472	1.301	0.048	4.400	33.85	66.15	1.529	1.820
22	4.69	1.342	0.067	5.960	45.85	54.15	1.661	1.733
24	4.899	1.380	0.069	6.425	49.43	50.57	1.693	1.703

Table No.14 Diffusion results of F2 formulation

Time (hr)	√time	log time	Absorbance (nm)	Conc. (µg/2ml)	%CDR	%CDRR	Log %CDR	Log% CDRR
2	1.414	0.301	0.006	0.424	3.26	96.74	0.513	1.985
4	2	0.602	0.007	0.537	4.43	95.57	0.646	1.980
6	2.441	0.778	0.009	0.707	5.58	94.42	0.746	1.975
8	2.828	0.903	0.012	0.99	7.61	92.39	0.881	1.960
10	3.162	1	0.015	1.27	9.79	90.21	0.99	1.950
12	3.464	1.079	0.0158	1.34	11.37	88.61	1.055	1.947
14	3.741	1.146	0.028	2.5	19.23	80.77	1.283	1.907
16	4	1.204	0.035	3.16	24.30	75.70	1.385	1.879
18	4.242	1.255	0.049	4.48	34.46	65.54	1.537	1.816
20	4.472	1.301	0.063	5.8	44.62	55.38	1.649	1.743
22	4.69	1.342	0.083	7.68	52.13	47.87	1.717	1.680
24	4.899	1.380	0.085	7.90	61	38.34	1.786	1.589

Table No.15 Diffusion results of F3 formulation

Time (hr)	√time	log time	Absorbance (nm)	Conc. (µg/2ml)	%CDR	%CDRR	Log %CDR	Log %CDRR
2	1.414	0.301	0.0037	0.2145	1.65	98.35	0.217	1.992
4	2	0.602	0.0059	0.4173	3.21	96.79	0.506	1.985
6	2.441	0.778	0.0084	0.6513	5.01	94.99	0.699	1.977
8	2.828	0.903	0.0.053	0.8528	6.56	93.44	0.816	1.970
10	3.162	1	0.0105	1.280	9.85	90.15	0.993	1.954
12	3.464	1.079	0.0201	1.762	13.56	86.44	1.132	1.936
14	3.741	1.146	0.024	2.21	17.04	82.96	1.231	1.918
16	4	1.204	0.297	2.66	20.53	79.47	1.312	1.900
18	4.242	1.255	0.0423	3.85	29.62	70.38	1.471	1.847
20	4.472	1.301	0.0518	4.75	36.56	63.44	1.563	1.802
22	4.69	1.342	0.0698	6.44	49.59	50.41	1.695	1.702
24	4.899	1.380	0.0812	7.525	57.30	42.11	1.762	1.624

Table No.16 Diffusion results of F4 formulation

Time (hr)	√time	log time	Absorbance (nm)	Conc. (µg/2ml)	%CDR	%CDRR	Log %CDR	Log %CDRR
2	1.414	0.301	0.007	0.51	3.99	96.01	0.6	1.982
4	2	0.602	0.0082	0.632	5.07	94.93	0.705	1.977
6	2.441	0.778	0.015	1.27	9.79	90.29	0.987	1.955
8	2.828	0.903	0.016	1.367	10.51	89.49	1.021	1.951
10	3.162	1	0.018	1.55	11.96	88.04	1.077	1.944
12	3.464	1.079	0.02	1.74	13.42	86.58	1.127	1.937
14	3.741	1.146	0.036	3.25	25.03	74.97	1.398	1.874
16	4	1.204	0.047	4.29	33.01	66.99	1.518	1.826
18	4.242	1.255	0.059	5.42	41.72	58.28	1.62	1.765
20	4.472	1.301	0.075	6.93	53.30	46.97	1.724	1.671
22	4.69	1.342	0.083	7.68	59.30	40.7	1.771	1.609
24	4.899	1.380	0.095	8.82	67.84	32.13	1.831	1.506

Table No.17 Diffusion results of F5 formulation

Time (hr)	√time	log time	Absorbance (nm)	Conc. (µg/2ml)	%CDR	%CDRR	Log %CDR	Log %CDRR
2	1.414	0.301	0.030	0.275	2.12	97.88	0.326	1.990
4	2	0.602	0.007	0.522	4.02	95.98	0.607	1.982
6	2.441	0.778	0.009	0.795	6.12	93.88	0.786	1.972
8	2.828	0.903	0.012	1.081	8.32	91.68	0.920	1.962
10	3.162	1	0.019	1.679	12.92	87.08	1.111	1.939
12	3.464	1.079	0.025	2.278	17.53	82.47	1.243	1.916
14	3.741	1.146	0.031	2.78	21.45	78.55	1.331	1.895
16	4	1.204	0.036	3.295	25.35	74.65	1.403	1.873
18	4.242	1.255	0.051	4.67	35.95	64.05	1.555	1.806
20	4.472	1.301	0.063	5.83	44.86	55.14	1.651	1.741
22	4.69	1.342	0.077	7.182	55.25	44.75	1.743	1.650
24	4.899	1.380	0.087	8.14	62.64	37.36	1.796	1.572

Table No.18 Diffusion results of F6 formulation

Time (hr)	√time	log time	Absorbance (nm)	Conc. (µg/2ml)	%CDR	%CDRR	Log %CDR	Log %CDRR
2	1.414	0.301	0.005	0.330	2.5	97.5	0.397	1.989
4	2	0.602	0.008	0.613	4.71	95.29	0.673	1.979
6	2.441	0.778	0.011	0.896	6.89	93.11	0.836	1.968
8	2.828	0.903	0.017	1.462	11.24	88.76	1.05	1.948
10	3.162	1	0.019	1.65	12.64	87.31	1.103	1.941
12	3.464	1.079	0.025	2.21	17.05	82.95	1.231	1.918
14	3.741	1.146	0.04	3.63	27.9	72.1	1.445	1.857
16	4	1.204	0.59	5.42	41.72	58.28	1.62	1.765
18	4.242	1.255	0.73	6.74	51.84	48.16	1.714	1.682
20	4.472	1.301	0.85	7.87	61.31	38.69	1.787	1.587
22	4.69	1.342	0.98	9.10	70.02	29.98	1.845	1.476
24	4.899	1.380	0.120	11.17	85.92	14.08	1.934	1.148

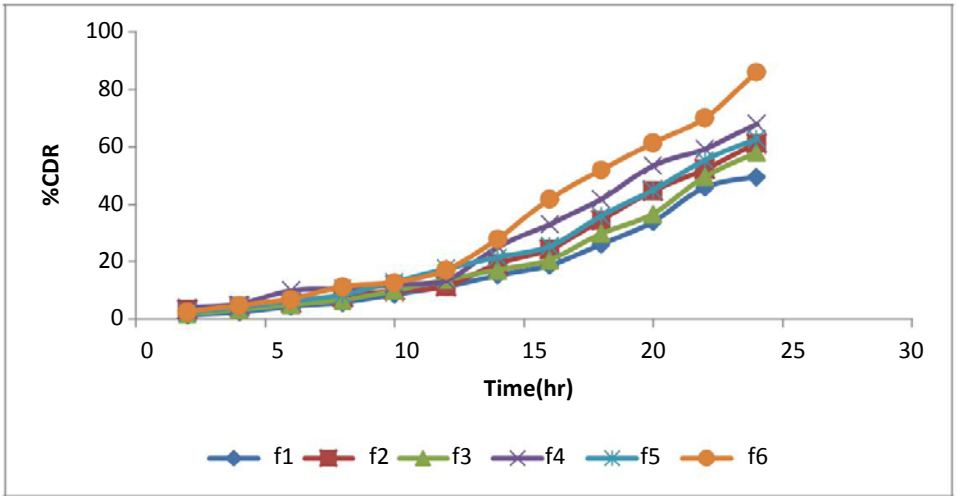

Figure No: 26 In-vitro drug release of F1 to F6 Formulations

DISCUSSION

The *in-vitro* diffusion profile of prepared formulations was carried out by membrane diffusion method. The diffusion was carried out for a period of 24 hrs in Phosphate buffer pH 7.4.

The cumulative percent release of F1 to F6 formulations at various time intervals was calculated and tabulated in table no: 11 to 16. The cumulative percent drug release in all formulations was plotted against time in figure no: 26. The Maximum percent of drug release was found in F6 formulation which contains maximum drug entrapment.

6.11 RELEASE KINETICS

Table No: 19 Curve fitting data of release rate profile of Formulations F1, to F6.

Type of	Zero-order	First-order	Higuchi	Korsmeyer –
F1	0.9058	0.8554	0.8089	0.9668
F2	0.9029	0.8473	0.801	0.8938
F3	0.901	0.8331	0.8029	0.9635
F4	0.9234	0.8256	0.8315	0.9094
F5	0.9338	0.8662	0.8453	0.9718
F6	0.9312	0.8013	0.8402	0.9552

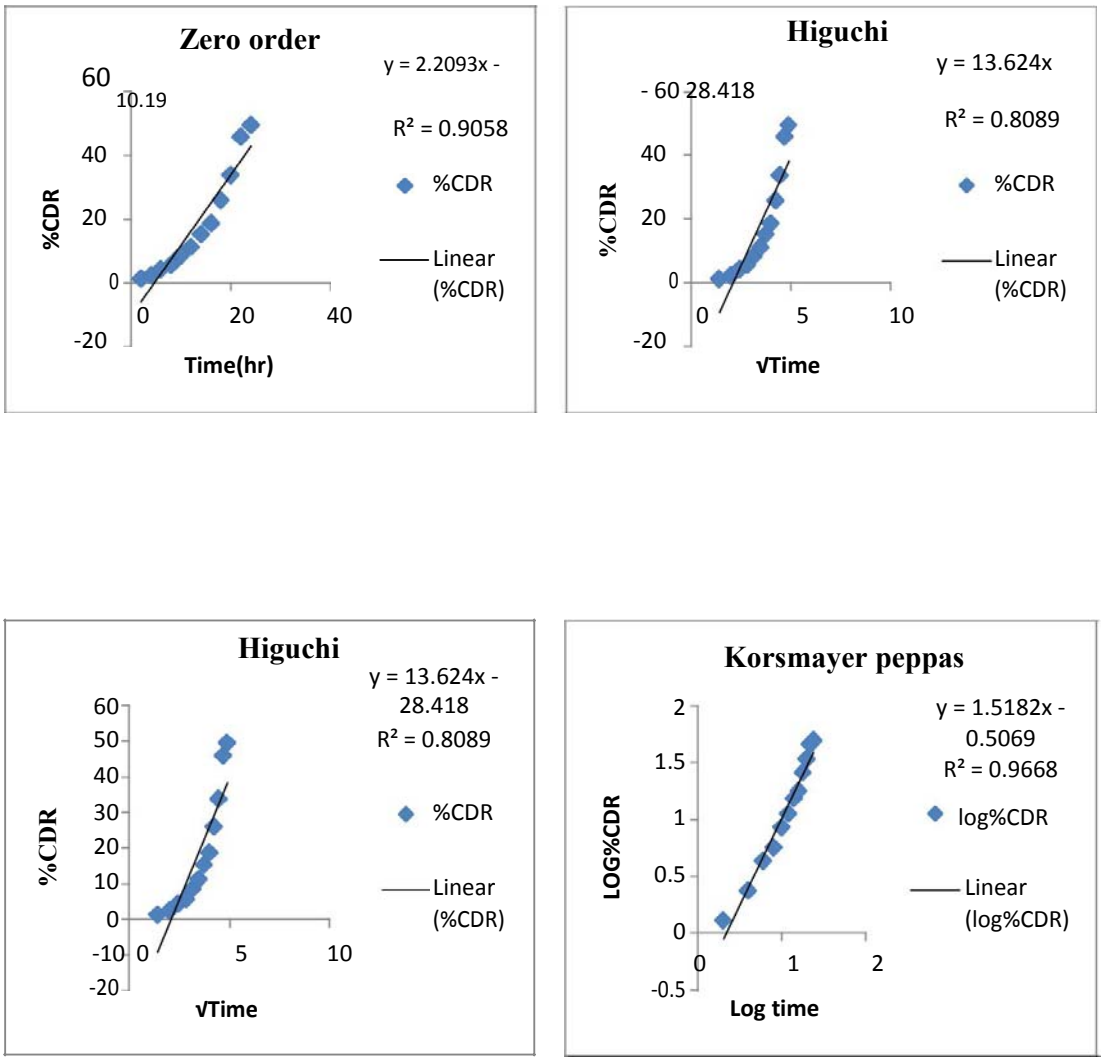

Figure No: 27 Release kinetics from F1 Formulation

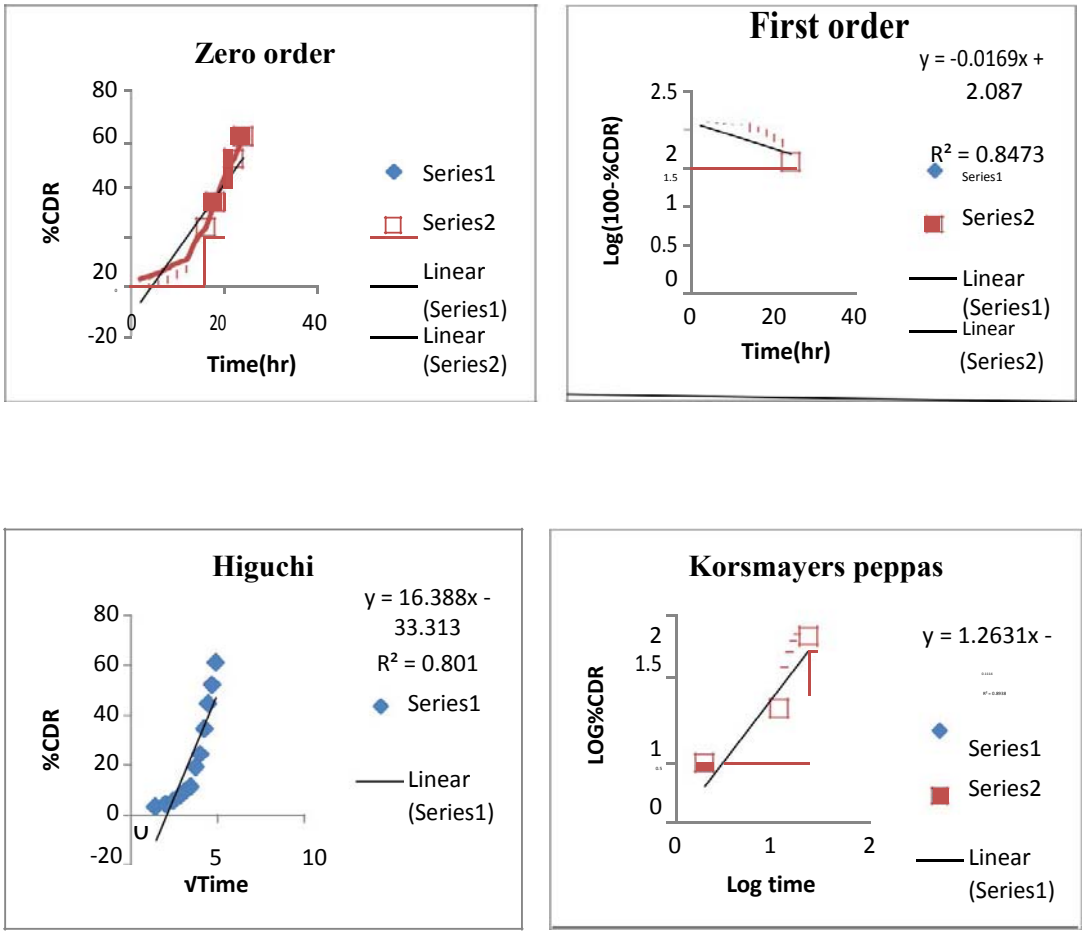

Figure No: 28 Release kinetics from F2 Formulation

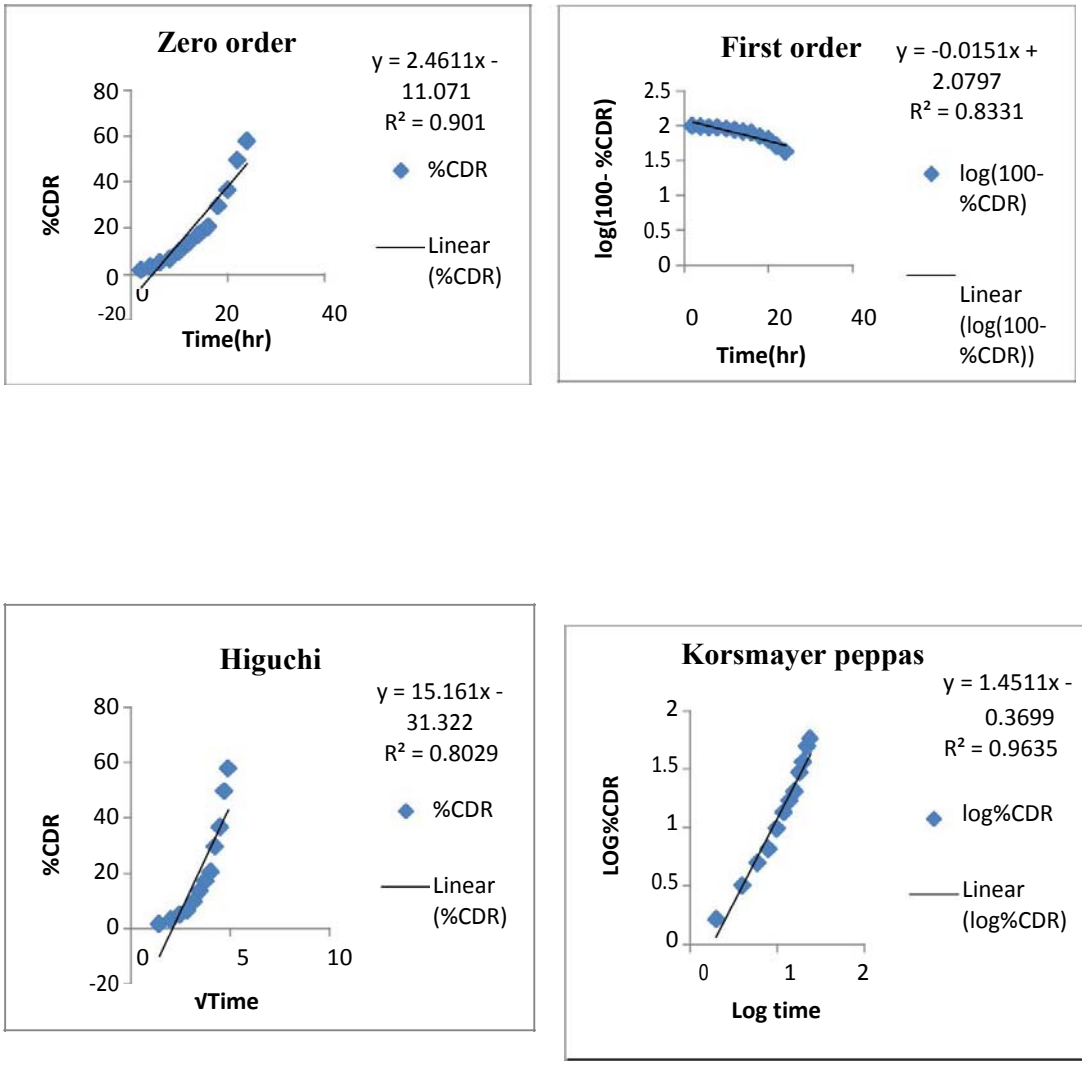

Figure No: 29 Release kinetics from F3 Formulation

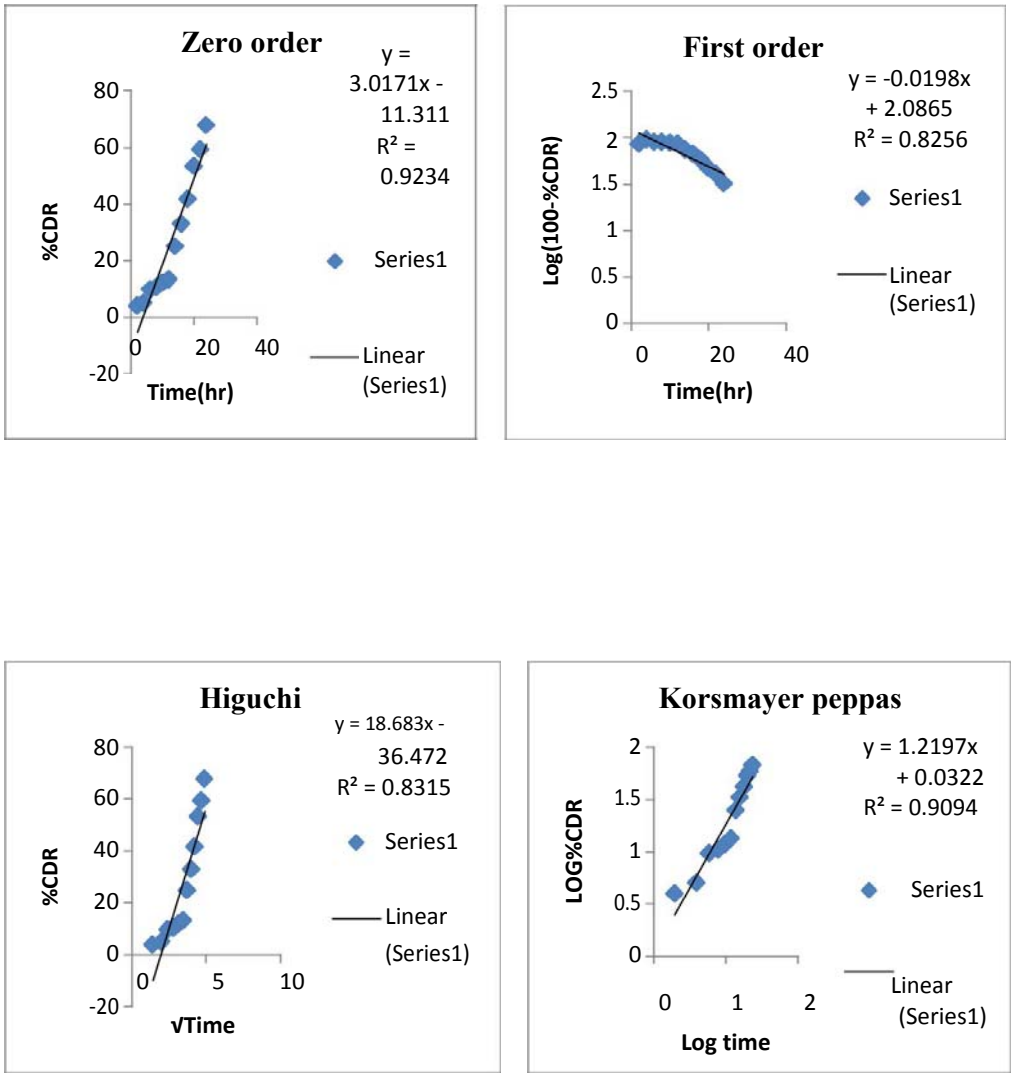

Figure No: 30 Release kinetics from F4 Formulation

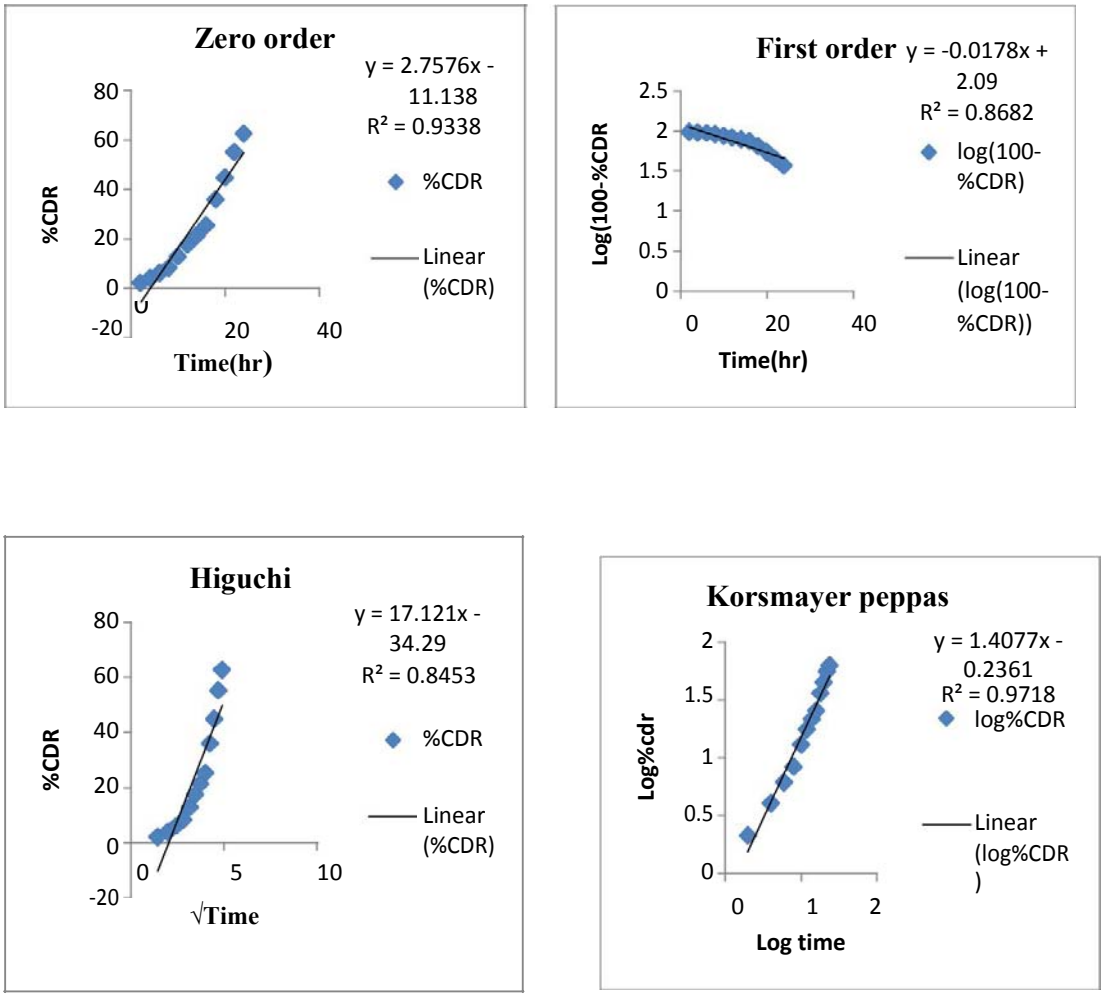

Figure No: 31 Release kinetics from F5 Formulation

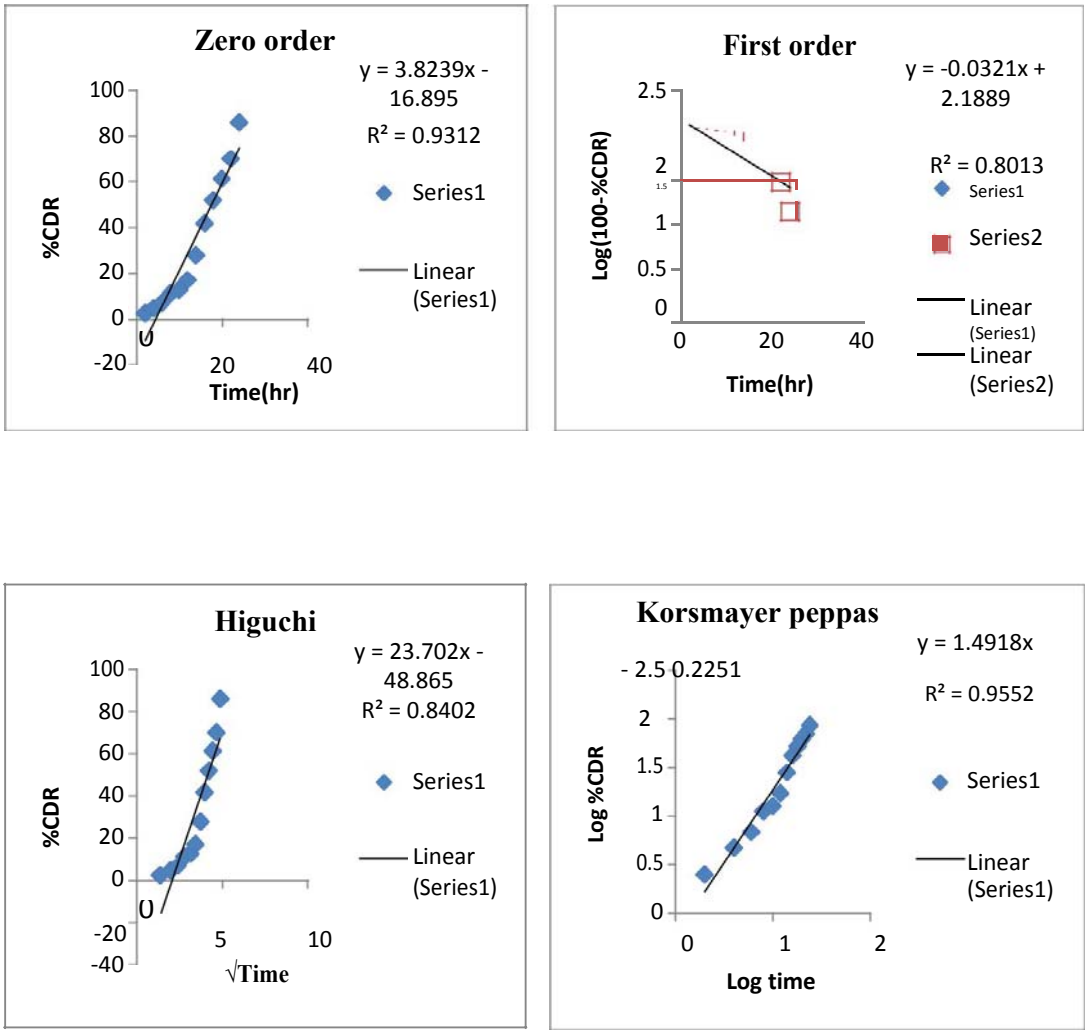

Figure No: 32 Release kinetics from F6 Formulation

DISCUSSION

The release kinetics of F1 to F6 formulations were studied. All formulations follow Zero order release kinetics and follow case II transport when it applied to the Korsmeyer-Peppa□s Model for mechanism of drug release. F6 formulation has better kinetic results when compared to other formulations. The results are shown in table no: 10 to 15 and graph no: 25 to 30.

6.12 LYOPHILIZATION

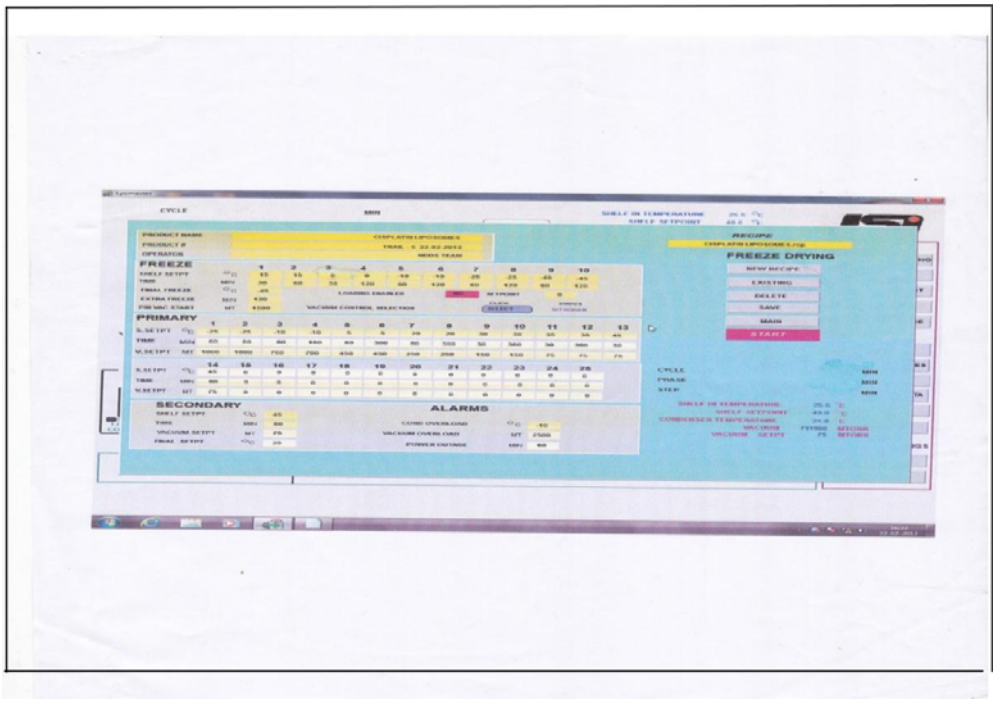

Figure no : 33 Lyophilization process

Figure No : 34 Lyophilized formulation of Cisplatin liposomes

DISCUSSION

The optimized formula was lyophilized for 5-6 trails keeping the liposomal suspension at various primary, secondary temperature and 5th trial was optimized and optimized sample cake stored at 4^{0} c. Results of lyophilized formulation were shown in figure no.33,34.

6.12 STABILITY STUDIES

Table No: 20 Effect of temperature on Assay of Cisplatin at $4^{o}c$ and room temperature

S. No	Days	**Assay at $4^{o}c$**	**Assay at room temperature**
1	0	98	98%
2	15	97.5%	97.8%
3	30	96.7%	95.9%
4	40	96.2%	92.5%
5	60	95.7%	90.2%

DISCUSSION

The stability of the Cisplatin Liposomes was evaluated after storage at $4^{0}c$ and room temperature for 60 days. The assays of the samples were determined as a function of the storage time. The Liposomes stored at $4^{0}c$ were found to be stable for duration of 60 days. The results were shown in table no 20.

DISCUSSION

DISCUSSION

CALIBRATION CURVE OF CISPLATIN

The UV absorbance of Cisplatin standard solution in the range of 10-80 µg/ml of drug in phosphate buffer pH 7.4 showed linearity at λ max 301nm. The linearity was plotted for absorbance against concentration with R^2 value 0.9989 and with the slope equation y = 0.0106x + 0.0015.

PREFORMULATION STUDIES OF CISPLATIN

The compatibility between the drug and the selected lipid and other excipients was evaluated using FTIR peak matching method. The percentage correlations were evaluated and the results showing that there was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid and other chemicals.

DIFFERENTIAL SCANNING CALORIMETRY (DSC) RESULTS

The DSC curve of pure drug Cisplatin exhibits a sharp endothermic peak at 320.1°C.The thermogram of drug-lipid mixture displayed endothermic peaks at 32.1°C, 120.0°C,400.5°C. DSC measurements showed that drug-lipid mixture was having less ordered arrangement of crystals, and this was favorable for increasing the drug loading capacity.

DETERMINATION OF PARTICLESIZE DISTRIBUTION AND POLYDISPERSITY INDEX:

The Liposomes were prepared by dried thin film hydration technique using rotary evaporator with drug and carrier (hydrogenated soya phosphatidyl choline).The formulation containing Cisplatin were prepared with stabilizer like tocoferol and all other

parameters like temperature, vacuum and RPM were kept constant. The particle size distribution was analyzed for F2, F6 formulations of Cisplatin Liposomes by wet method.

ZETA POTENTIAL ANALYSIS

The zeta potential of the optimized formulation (F6) was found to be -20mV.Zeta potential is an important parameter that influences stability. Extremely positive or negative zeta potential values cause larger repulsive forces, whereas repulsion between particles with similar electric charge prevents aggregation of the particles and thus ensures easy redispersion. In case of a combined electrostatic and steric stabilization, a minimum zeta potential of ± 20 mV is desirable.

TRANSMISSION ELECTRON MICROSCOPY (TEM):

The Morphology and surface appearance of Liposomes were examined by using SEM. The SEM photographs of F2 and F6 formulation showed that the particles have smooth surface.

DETERMINATION OF PERCENTAGE FREE DRUG

The percent free drug is determined for all the formulations F1 to F6. The percent free drug was optimum in F6 formulation, which is within the limit (10%),

ASSAY RESULTS

The percentage purity is determined for all the formulations from F1to F6. The assay value is within the limit (90%) for all the formulations

IN- VITRO DIFFUSION SYUDIES

The *in-vitro* diffusion profile of prepared formulations was carried out by membrane diffusion method. The diffusion was carried out for a period of 24 hrs in Phosphate buffer pH 7.4.

The cumulative percent release of F1 to F6 formulations at various time intervals was calculated and tabulated in table no: 11 to 16. The cumulative percent drug release in all formulations was plotted against time in figure no: 26. The Maximum percent of drug release was found in F6 formulation which contains maximum drug entrapment.

RELEASE KINETICS

The release kinetics of F1 to F6 formulations were studied. All formulations follow Zero order release kinetics and follow case II transport when it applied to the Korsmeyer-Peppa□s Model for mechanism of drug release. F6 formulation has better kinetic results when compared to other formulations.

LYOPHILIZATION

The optimized formula was lyophilized for 5-6 trails keeping the liposomal suspension at various primary, secondary temperature and 5^{th} trial was optimized and optimized sample cake stored at 4^{0} c.

STABILITY STUDIES

The stability of the Cisplatin Liposomes was evaluated after storage at 4^{0} c and room temperature for 60 days. The assays of the samples were determined as a function

of the storage time. The Liposomes stored at 4^{0} c were found to be stable for duration of 60 days.

SUMMARY & CONCLUSSION

SUMMARY & CONCLUSION

Cisplatin, is an anticancer drug, belongs to alkylating agent□s class. Cisplatin is commonly used for the treatment of a wide range of cancers, majorly used in testicular and ovarian cancers and also in many types of sarcomas and carcinomas.

The main objective of this work was designed to prepare and evaluate the Cisplatin Liposomes. This formulation can target the site of action with effect of stabilizer on drug entrapment efficiency, and to reduce the side effects by formulating pegylated Liposomes. This liposomal formulation was formulated using the hydrogenated soya phosphatidyl choline and cholesterol which has lesser toxicity.

The Liposomes were prepared by dried thin film hydration technique using rotary evaporator with drug, carrier, ammonium sulphate and stabilizer. The parameters like temperature, vacuum and RPM were maintained accordingly. After preparation, the Liposomes were stored in freezed condition, and given for further evaluation.

The prepared Liposomes of F1, F2, F3, F4, F5 and F6 formulations were evaluated for physical and chemical characteristics like average vesicle size, shape and zeta potential. The evaluated batches showed good physicochemical characteristics in F6 formulation.

The prepared Liposomes of F1 to F6 were evaluated for % free drug and

Assay, The % Free drug was optimum in F6 formulation when compared to other formulations.

This developed liposomal drug delivery system was also evaluated for diffusion study by phosphate buffer pH7.4 using membrane diffusion method. The release of drug from F6 formulation was found to be sustained to certain extent when compared to other formulations.

The release kinetics of F6 Formulation were optimized. F6formulation follow Case II transport when it is applied to the Korsmeyer model for mechanism of drug release. F6 formulation has better kinetic results when compared to other formulations.

The stability of the Cisplatin Liposomes was evaluated at 4°c and room temperature for 60 days. The assay of the samples was determined as a function of the storage at different time intervals. The Liposomes stored at 4^{o}c were found to be stable for duration of two months.

From the results of physical characterization, *in-vitro* evaluation, release kinetics and stability studies, it was found that negative charged Liposomes containing Cisplatin might be used for the treatment of a testicular and ovarian cancer when compared to the normal drug and neutral Liposomes.

CONCLUSION:

To conclude the liposomal delivery of cisplatin would act as a suitable technique to enhance bioavailability of cisplatin than the conventional dosage form. It is also confirmed that liposomes will sustain the drug release, from the executed experimental results, it could be concluded that the stabilizer and along with hydrogenated soya phosphatidyl choline and cholesterol, were suitable carriers for the preparation of Cisplatin Liposomes

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