Utilization of nanotechnology for formulation development, optimization and evaluation of brain targeted drug delivery system

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BY

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Statement by the candidate

This is to submit that this written submission in my project report entitled "Utilization of nanotechnology for formulation development, optimization and evaluation of brain targeted drug delivery system" represents original ideas in my own words and where others' ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the School and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required.

I assure and hold full responsibility for its genuineness.

Amitoj singh

Forwarded Through

Dr. Amit Bhatia

Associate Professor

Domain: .....

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The work described in this project report entitled "Utilization of nanotechnology for formulation development, optimization and evaluation of brain targeted drug delivery system" has been carried out by amitoj singh under my supervision. I certify that this is his bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

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Certificate by School

This is certified that the work described in this project report entitled "Utilization of nanotechnology for formulation development, optimization and evaluation of brain targeted drug delivery system" has been carried out by Amitoj singh at the School of Pharmaceutical Sciences, Lovely Professional University, Punjab.

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CHAPTER 1: INTRODUCTION

1. Introduction:

1.1 **Nanotechnology**:-

In past years, growth of research and applications in the area of nanoscience and nanotechnology had been at large. Nanotechnology is defined as, engineering and manufacturing at nanometer scales, with atomic precision. Any structure less than 100 nm is a true nanostructure and unique phenomena are expected at that scale. The dimensions related to nanotechnology in understanding and control of matter is between 1 to 100 nm, where unique phenomena enable novel applications. Several attempts have been made to design the set of nanpoparticulate drug carrier systems capable of specific delivery of various pharmaceuticals.

1.1.1 Historical Background of Nanotechnology:

Back in 1959, nanotechnology embarked an unique field of science. It took abirth in December 1959, at American physical society meeting at California institute of technology. During the speech of physicist Richard Feynman. It was generated with term"there's plenty of room at the bottom" which was said by Richard Feynman. It was huge achievement to field of science that no other invention came till that period with potential of manufacturing objects with atomic precision for example: small object of size one two-hundred of inch wide can accommodate information of whole world. Since then, surface phenomenon began to dominate behavior. Later in 1974, term nanotechnology was used by Norio Taniguchi which brought technology for production to get extra high accuracy and ultra fine dimensions. In 1980, Eric Drexler gave the concept of molecular manufacturing at large scale in his book " Engines of creation".In 1985, fullerenes, or buckyballs were discovered. Since 1990, it has been shining and growing in the field of nanoscience with its wider applications.

1.1.2 Application:

1) Medicines:

Researchers are developing more and more number of nano-structured systems in order to deliver drugs directly to diseased cells in your body. Hence, overcome all the undesired effects or risks associated with chemotherapy.

Nanosponges: have been developed to prevent the toxins from the blood stream.

Carbon nanotubes: Became successful in targeting tumour cells without damaging healthy tissues.

Bismuth nanaoparticles were able to treat cancer tumors by concentrating radiation used in radiation therapy.

PEG-Nanoparticles was another unique application that came into the history to treat brain diseases. As these particles tend to absorb free redicles released after a brain injury and reduces the harm associated with disease.

2) Industrial research companies:

Exxon Mobil, uses minerals with pore size of less than 1nm for eg: zeolites , as a source of catalyst to break down large hydrocarbon molecules to form gasoline.

IBM uses nanotechnology in manufacturing Nanoscale layering of diskdrives, thus preventing resistive effect to attain highly dense data storage.

Gilead sciences also uses nanotechnology by employing liposomes as a carrier for anticancer drugs to treat AIDs-related Kaposi's sarcoma.

Carbon nanotechnologies, a company cofounded by Richard E. smalley for making carbon nanotubes.

3) Food:

Nanotechnology play vital role as good source for food safety and health benefits. Also provide strength and stability to food.

For example: Nanosensor, which detects bacteria and prevent the food from getting contaminations. Silica nanoparticles, mainly used for food packaging which prevent the gasses or moisture to enter inside. Others like packaging containing zinc oxide nanoparticles quench U.V rays to enter inside and also provide antibacterial protection.

4) Fuel:

-Nanocatalysts used in conversion of coal to liquid fuels.

-Nanoparticles cerium oxide catalyst for diesel fuel

-Nanoclusters which helps gasoline and diesel fuels burn more completely by breaking fuel into smaller droplets.

5) Water cleaner:

Nanoparticles used to convert the contaminating chemicals through chemical reactions to make it harmless.

1.2 Brain Targeting:-

The brain is one of the essential part of living system which coordinates all other parts of the living system. There have been built very efficient ways to protect it. Many commercially available formulations or pharmaceuticals tend to be ineffective in treating various diseases associated with brain due to their inability to reach to the target and function their properly due to various roadblocks .various scientists and researchers are working with full efforts in this particular field to achieve advancements in delivering drug to the main target without any degradation or side effects. Patients suffering from fatal brain diseases for example: such as brain tumors, HIV encephalopathy, epilepsy, cerebrovascular diseases and neurodegenerative disorders, far outnumbered. The failure of much potentially active formulations or pharmaceuticals is often not due to the lack of drug potency but rather to due to the shortcomings in the techniques of delivering drug to the target. Recently many researchers have achieved the advancements in developing the various ways in delivering drug to the brain with greater efficacy. Diseases associated with Brain and central nervous system disorders are the world's leading cause of disability even after vast advancements have been done in this field. This task of delivering the drug to the brain has been found very complicated due to the presence of Blood Brain Barrier (BBB). Drugs has to pass through the BBB to reach their target and to effectively produce their action againt central nervous system diseases (CNS). Various pharmaceuticals commercially available for the treatment of brain diseases are left ineffective due to their failure in effectively passing through BBB and getting sustained their within the brain. The problem is not that effective therapeutics are not potent but the methods are ineffective by which the drug is delivered. Currently about 98% of all small molecule drugs pass through the BBB in very less amount, and minuscule large molecule drugs can pass through the BBB. (Agarwal et al., 2009).

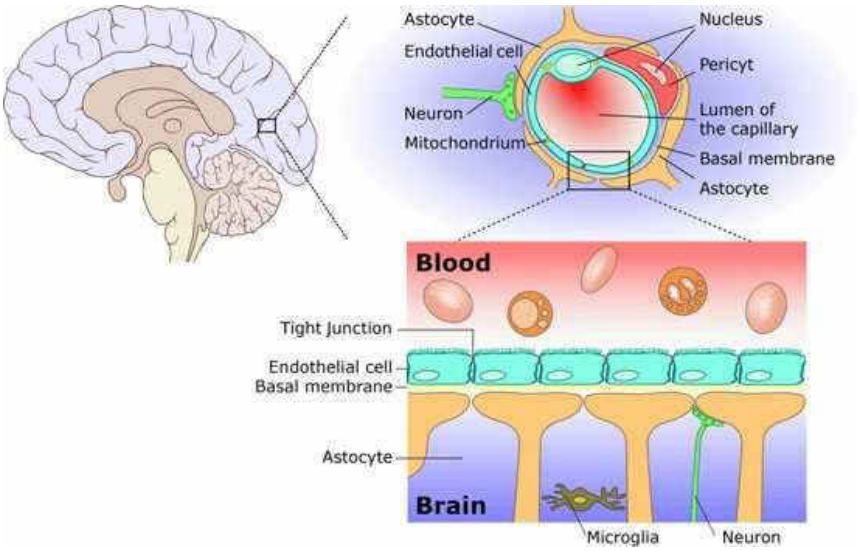

Figure 1.1: The blood brain barrier

This figure is adapted from (http://bacterial-meningitis.weebly.com/physiology.html.)

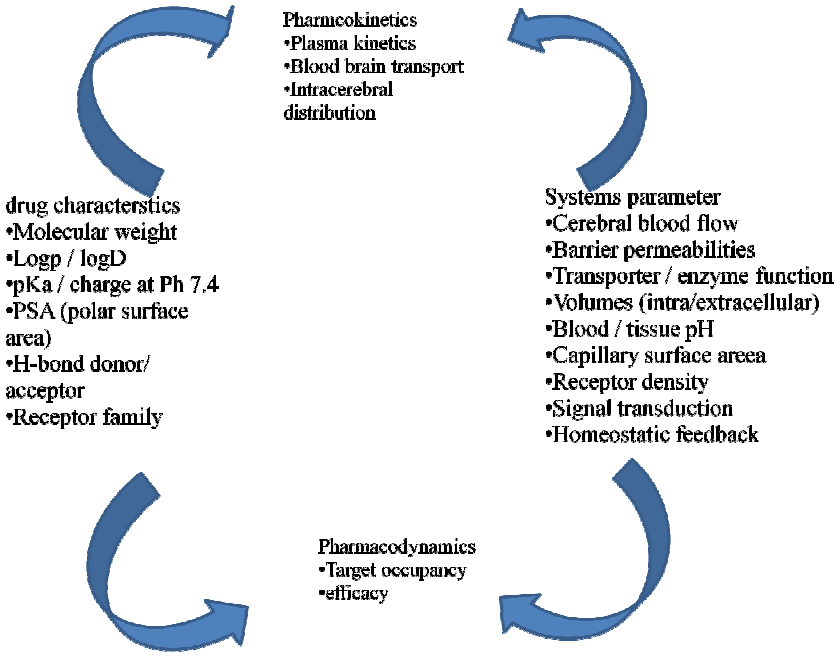

Figure 1.2: Factors affecting the pharmacokinetics and pharmacodynamics of a drug.

1.2.1 Brain drug delivery include following strategies:-

Conventional strategies such as Neurosurgical or invasive strategies (Hyperosmolar BBB disruption, Intracerebral implants, Intraventricular drug infusion)

Pharmacological strategies (Nanoparticles, Liposomes), Physiologic-based strategies

(Cationic antibodies, Pseudo-nutrients, Chimeric peptides).

Recent Advances such as Modified nanoparticles (Magnetic nanoparticles, Multifunctional nanoparticles, Dendrimers, Scaffolds, Convection-enhanced delivery, Lipoplexes and Polyplexes, Polyanhydrides). (Gupte et al., 2014).

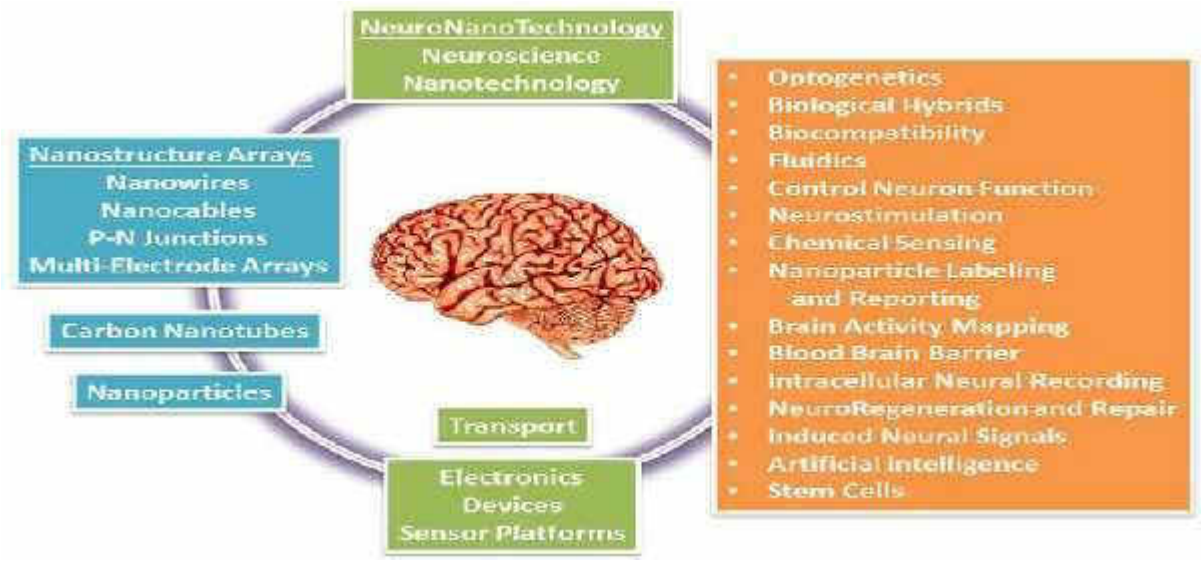

Figure 1.3:- Illustration of nanotechnology integration into the brain research.

This figure is adapted from (Vidu et al.,2014).

1.2.2 Major unmet needs in targeting drugs to the brain include:

1. Specificity in targeting drugs to particular region or cell in the brain.

2. Reduction of toxicity associated with most of the drugs and therapeutic agents employed for brain targeting.

3. Deeper knowledge of BBB transport systems.

4. *In vivo* evaluation of brain drug pharmacokinetics.

5. Potentially new brain drug targeting systems.

6. *In vivo-in vitro* correlation.

7. More patient complaint and cost effective brain drug targeting systems.

Table 1.1: Nanoparticulate drug delivery systems

Delivery system type	Typical mean particle diameter (in micrometers)
Liposomes	30-1000
Microemulsions	0.15-2
Nanoparticles	2-100
Microparticles	0.2-5
Nanocrystals	2-100
Microspheres	0.5-20

1.3 Liposomes:

Since past decades Liposomes have been employed for the purpose of delivering less amount of expensing drug with more efficacy and almost negligible side effects. Liposomes are small spherical in shape comprisisng of the aqueous compartments which are enclosed by hydrophilic and hydrophobic molecules. They comprises of single or multiple bilayers. Liposomes tend to have various applications as carrier for various bioactive agents including drugs, vaccines, cosmetics and nutraceuticals.

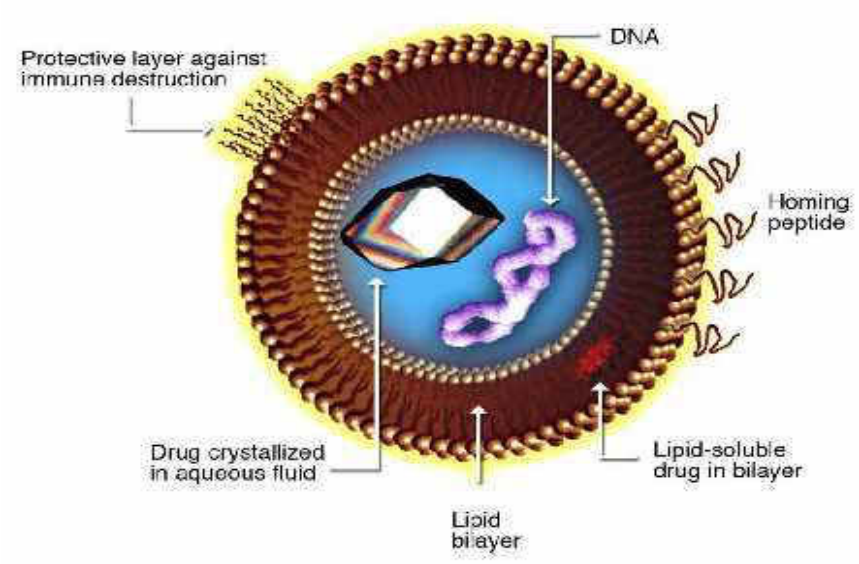

Figure 1.4:- Liposome for drug delivery

This figure is adapted from (Perche et al., 2013)

Drugs associated with liposomes have entirely different pharmacokinetic properties compared to free drugs in solution. Liposomes are also effective in lowering down the toxicity and preventing early degradation of the encapsulated drug after administration. These are pegylated, covered with polymers, polyethylene glycol (PEG) –also called stealth liposomes,by this form they prolong half life of incorporated drug. They can also be conjugated with antibodies or ligands in order enhance target-specificity. Visser et al (2005) studied pegylated horse –radish-peroxidase loaded liposomes, tagged with transferrin in passing through blood-brain barrier. The authors showed an increased efficacy in targeting the liposomes loaded with protein or peptides to the brain capillary endothelial cells and concluded that this system as an attractive approach for delivering drug to the specific target in brain. In another report, Lopez-Pinto and coworkers investigated the delivery of a lipophilic drug for example: minoxidil dermally from ethosomes versus classic liposomes by applying non-occlusively on rat skin, yet in a separate study, Ozden and Hasirci prepared small vesicles composed of phosphatidyl- choline, dicetyl phosphate and cholesterol and entrapped glucose oxidase in them. Liposomes are also examined as carriers for cells, genes or DNA fragments. Ito et al examined the effect of magnetite cationic liposomes which have positive surface charge to enrich and proliferate Mesenchymal stem cells (MSCs) in vitro. Kunisawa et al established a alternative approach for the encapsulation of nanoparticles in liposomes, which were conjugated with ultra violet-inactivated Sendai virus to compose fusogenic liposomes and noticed that fusogenic liposome proved a high ability to deliver nanoparticles carrying DNA into cytoplasm.

Foco et al stated the delivery of sodium ascorbyl phosphate (SAP), an effective oxygen species scavenger in preventing the of UV radiation degenerative effect on skin. When applied to the skin SAP was incorporated into liposomes to increase its penetration effect through the stratum corneum into the deeper layers of the skin. Sinico et al studied the transdermal delivery of tretinoin and investigated the influence of liposome composition, size, lamellarity and charge on transdermal delivery. They studied the positively or negatively charged liposomes of different types. It has been stated that negatively charged liposomes increases hydration and tretinoin retention of newborn pig skin. Arcon et al studied an encapsulated anticancer agent, cisplatin, in sterically stabilized liposomes and studied them using X-ray absorption fine structure method,

and concluded that these liposomes encapsulated anticancer drug is chemically stable and does not hydrolyze.

Table 1.2: Classification of liposomes on the basis of intracellular delivery

Classification of liposomes in terms of composition and mechanism of intracellular delivery:
1. Conventional liposomes
2. pH-sensitive liposomes
3. cationic liposomes
4. immune liposomes
5. long – circulating liposomes

Table 1.3: Classification on the basis of their size and number of bilyers

Classification on the basis of their size and number of bilayers into:
1. Small unilamellar vesicles (SUV) : 20-100nm
2. Large unilamellar vesicles (LUV) : >100nm
3. Giant unilamellar vesicles (GUV) : >1000nm
4. Oligolamellar vesicles (OLV) : 100-500nm
5. Multilamellar vesicles (MLV) : >500nm

1.3.1 Liposomes characterization:

- Lamellarity determination
- Size analysis
- Zeta potential
- Encapsulation efficiency
- Lipid analysis
- Liposomes stability
- In – vitro drug release

1.3.1.1 Lamellarity determination:

Determination of liposomes lamellarity is one of the significant parameter to be considered. Liposomes containing number of the Layers influences the encapsulation

efficiency and drug release kinetics. Liposome lamellarity is achieved by method uses visible or fluorescence signal. On adding the reagent these visible or fluorescence signal of lipid marker gets changed . it is one of the simplest method which can be easily conducted easily in standard laboratories. Several lipids can be used and results totally rely on the comparison of the total signal to the signal change which is achieved from the reaction between the lipids marker and the specified reagents.

1.3.1.2 Size analysis:

The average size and size distribution of liposomes are important parameters to be counted mainly when these liposomes are used for any therapeutic purpose via inhalation or parentral route. Several techniques are available for assessing liposome size and size distribution which include microscopy techniques, size-exclusion chromatography (SEC), field-flow fractionation and static or dynamic light scattering. A recently newly developed microscopic technique known as atomic force microscopy (AFM) has been successfully utilized for the purpose of studying liposome morphology, size and stability. AFM, provides an opportunity to visualize small liposomes without sample manipulation in natural environment.

1.3.1.3 Zeta potential:

The zeta potential is another important parameter to be considered. It is an overall charge that a particle acquires in a particular medium. It is a physical property which is exhibited by any particle in suspension. The zeta potential is a very good parameter of measuring the interaction between colloidal particles. Measurements of zeta potential are commonly used to predict the stability of colloidal systems. These large positive or negative zata potential in suspension tend to repell each other and acquire no tendency to aggregate. However, low zeta potential values of particles tend to prevent the particles flocculating as there is no force remaining. To measure the zeta potential, a laser is used which passes through the middle of the sample cell and scatter the light which is then detected. When an electric field is applied to the cell, particles moving through the measurement volume will lead to fluctuation of the detected light with a frequency proportional to the particle speed. This information is then transferred to a digital signal processor, then to a computer and hence potential zeta is calculated.

1.3.1.4 Encapsulation efficiency:

Encapsulation efficiency is one such parameter that has to be calculated while determining liposomes characterstics. The liposome preparations composed of the mixture of encapsulated and un-encapsulated drug fractions.these encapsulation efficiency is determined by the separation between the encapsulated drug (within the carrier) and the free drug. Several separation techniques have been reported in the literature.

- Minimum column
- Centrifugation method
- Ultra centrifugation method
- HPLC

1.3.1.5 Lipid analysis:

There has been enormous amount of techniques used for the determination of phospholipid content. fluorescence assay for phospholipid vesicles was reported. When phospholipid vesicles are added to an aqueous solution of 1,6-diphenyl-1,3,5-hexatriene (DPH), it was observed that a fluorescence was increased by several hundred-fold which is used for a phospholipid concentration determination.

1.3.1.6 Liposomes stability:

The liposomes stability is an important parameter to be considered while producing and administrating liposomes : from process to storage and delivery. If the dosage form is stable and well defined then its introduction will be successful. In designing a stability study, various factors are to be considered and evaluated such as: physical, chemical and microbial

1.3.1.7 In-vitro drug release profile:

In vitro drug release is carried out by dialysis tube diffusion method. In which dialysis bag membrane is used for performing this experiment. While passing the drug through it, active ingredient must pass through the membrane as it is highly permeable to the active ingradient , rest other ingradient of drug must not pass through the membrane. This dialysis bag containing liposomes suspension must tied and dropped in dissolution media. This system is kept at 37°C under continuous magnetic stirring and is closed to avoid

evaporation of the dissolution medium. Samples of the dialysate are taken at several time intervals and assayed by HPLC, spectrophotometer or any other convenient method. Every experiment is performed in triplicate and the average values are taken to determine the release profile of the drug from the liposome suspension.

1.3.2 Methods of liposome preparation

All the methods of preparing the liposomes involve four basic stages:

- Drying down lipids from organic solvent.
- Dispersing the lipid in aqueous media.
- Purifying the resultant liposome.
- Analyzing the final product.

Table 1.4: Liposomes preparation method

General method:
1. Hydration of a thin lipid film (bangham method)
2. Reverse phase evaporation technique
3. Solvent injection technique
4. Detergent dialysis
Large scale liposome technique:
5. Heating method
6. Spray drying
7. Freeze drying
8. Ethanol injection method
9. Membrane contactor

Table 1.5: Benefits of drug load in liposome

Benefits of drug loading	Examples
Improved solubility of lipophilic and amphiphilic drugs	Amphotericin B, porphyrins, minoxidil, some peptides, and anthracyclines, respectively; hydrophilic drugs, such as anticancer agent doxorubicin or acyclovir
Passive targeting to the cells of the	Antimonials, amphotericin B, porphyrins,

immune system, especially cells of the mononuclear phagocytic system	vaccines, immunomodulators
Sustained release system of systemically or locally administered liposomes	Doxorubicin, cytosine arabinoside, cortisones, biological proteins or peptides such as vasopressin
Site-avoidance mechanism	Doxorubicin andamphotericin B
Site-specific targeting	Anti-inflammatory drugs ,anti-cancer, anti-infection
Improved transfer of hydrophilic, charged molecules	Antibiotics, chelators, plasmids, and genes
Improved penetration into tissues	Corticosteroids, anesthetics, and insulin

1.3.3 Liposomes applications:

The use of liposomes has attracted attention of many research fields with their wider applications:

- Liposomes can be applied to prevent the early degradation of drug after administration. The lipid content in the liposomes are not prone to the enzymatic degradation. Thereby entrapped drug gets protected whilst the lipid vesicles circulate within the extracellular fluid.
- Liposomes can be used for drug targeting. It has been proved that distribution of drug with low dose to the specific target with increase efficacy and decrease toxicity. Liposomes have been widely applied especially in cancer treatment. It has been preffered over the effective chemotherapy which is limited by the undesired side effects of various anticancer medicines. Liposome encapsulation thus can change the distribution of the encapsulated drug molecules in the body, which thus significantly decreases undesired side effects and increases the efficacy of the treatment.
- Liposomes also used as immunotherapeutic agents: it may involve the use of antigen – presenting liposomes which is an effective approach in treating diseases like HIV infection or Herpes simplex virus genital infection. A liposomal vaccine has been launched by the Swiss Serum Institute in 1994 to

fight against hepatitis A .

- In Schmid's work, In the treatment of atopic dry skin stratum corneum liposomes have been used as vehicle in order to restore the barrier function. To penetrate through skin epidermis , composition and properties of liposomes play significant role. Liposomes functions in regenerating the skin by replenishing lipid molecules and moisture content. Lipids helps in humuidifying the skin to increase the skin elasticity and barrier function, and protect the skin from aging problems.
- The first liposomal cosmetic product to appear on the market was the anti-ageing cream "Capture" was the first liposomal cosmectic became commercially available in the market was launched by Christian Dior in 1986.
- Liposomes has their another application in the treatment of hair loss; minoxidil, a vasodilator, having active ingredient like "Regaine" that prevents the hair loss. Since 1987, several cosmetic products have been commercially available; ranging from simple liposomal pastes like creams, gels and ointments to formulations containing various extracts, moisturizers, antibiotics, etc. other Unrinsable sunscreens, long lasting perfumes, hair conditioners, aftershaves, lipsticks, make-up and similar products are also gaining their marked value in the market.
- Liposomes have recently begun to gain an importance in food products. Indeed, the ability of liposomes to solubilise compounds and to sequester compounds from potentially harmful medium, and release incorporated molecules in a sustained and effective way which can be used in food processing industry. Basides their wider application in pharmaceutical and medical uses, they have been showing their importance in food industry, as controlled drug delivery system for the delivery of various products like proteins, enzymes, vitamins, antioxidants, and flavours in the preparation of dairy products and also to stabilize these products against degradation, and to increase their efficiency.

- In the pharmaceutical field, liposomes have long been used as of great interest by offering a predictable way for both systemic and locally acting drugs employed for their therapeutic applications in humans and animals. As their vast applications in the field of drug delivery, many industries have been actively

involved in evaluation of liposome products. Most of them concern the drugs whih exhibit severe side effects when given in their free form for example: anticancer and antifungal drugs. These unwanted side effects are diminished by encapsulating these drugs into liposomal vesicles.

1.4 Depression:

Depression is an increasingly prevalent problem all over the world affecting people with some form of depressive disorders. According to World Health Organization (WHO), Depression is a usual mental disorder which is characterized by sadness, disturbed sleep or appetite, loss of interest or pleasure, guilt and tiredness feelings and loss of concentration. Depression normally affects people from leading their normal life and relationships. Women are more likely to fall depressed comparable to men. Depression can last longer or revived, which causes an individual to not function properly at work and deal with daily life. Depression may cause suicide in severe cases. People can be treated without medicines when the condition is mild but when it is severe people need professional talking treatments and medication.

Table 1.6.: Key facts regarding Depression.

There are more than 350 million people suffering from depression of all ages in the World.
Depression is the leading cause of disability worldwide. **Women are more affected than men by depression.** **Depression can lead to suicide at severe conditions.** **Depression can be treated effectively.**

Even if there are effective treatments available for depression, but still very less amount of affected people in the world receive such treatments. Various obstacles to effective care consist of lack of trained health care providers, lack of resources, social stain or mark associated with mental disorders and inaccurate assessment. There are many instances where people in depressed state are not properly diagnosed and others who are not suffering are occasionally diagnosed incorrectly and prescribed antidepressants

without any symptoms or causes of depression.

Clinical depression is characterized by a few necessary criteria and a variety of possible symptoms. The National Institute of Mental Health explained the variance of symptoms by threedimensions:(1) anxiety; (2) depressed mood-motor retardation; and (3) hostility---interpersonal sensitivity (Katz and Maas, 1994). According to this characterization, these symptoms coexist in varying proportions among severely depressed patients.

1.5 Mesembrine:

From past decades, *Sceletium tortuosum* was used by South African shepherds or herdsmen and hunter-gatherers as a source of mood-altering substance. Traditionally, dried Sceletium was often chewed as a quid, and the saliva was swallowed, but later on it has also been made into teas and tinctures. Sceletium usually with the addition of other herbs was inhaled as a snuff or smoked. It leads to decrease in anxiety, stress and tension and mood elevation. It is used as a source for treating depression and can also be utilized for rehabilitating drug addictions as it is not addictive itself. It can cause euphoria in intoxicating doses with stimulation initially and later with sedation.

The alkaloids are the active constituents of *Sceletium tortuosum*, consisting of mesembrenone, mesembrine, mesembrenol and tortuosamine. Mesembrine is considered as a major alkaloid present in *Sceletium tortuosum*. Mesembrine has been showcased to be a potent serotonin-uptake inhibitor. The neurotransmitter serotonin (also known as 5- hydroxytryptamine) is lacking in individuals who are suffering from depression. Mesembrine leads to decrease in the re-uptake process which makes it more probable that there will be more serotonin in the relevant receptors which increases the probability that there will be enough levels to set up the signal transfer in all neighbouring neurons. Mesembrine assist the brain functioning with reduced levels of serotonin which provide time for natural levels to build up, where at the mesembrine dosage can be decreased or eliminated.

Meiring stated that *Sceletium tortuosum* was used for young children, silencing them when they were suffering from “acidity.” Parents would use a few drops of fresh juice from the plants on their children, inducing a very deep rest for many hours . Hartwich and Zwicky concluded their scientific reports on *Sceletium tortuosum* by saying that the indigenous people almost used the plant medicinally (Hartwick and Zwicky, 1914).

Sceletium may have been mixed with ‘dagga,’ or *Cannabis sativa L.* as it supposedly

"induced Bushmen users to dance." (Laidler, 1928) the area where these people lived, called Little Karoo, was given the name 'Cannaland' by the white settlers for its abundance of the plant. plant had known intoxicating properties, noting that the Hottentots would ferment the root, and if they chewed it immediately afterwards, would be intoxicated. If not used immediately *a*fterwards, it would relieve thirst. The Hottentots knew it was of great value, and it was used as a form of currency to exchange for cattle and other items of value. The Bushmen made it the center of their trade, and there were numerous accounts of it being used to treat insomnia in adults, diarrhea in children, and as a mild narcotic or general intoxicant. (Gerick and viljoen , 2008) .

CHAPTER 2: REVIEW OF LITERATURE

2.1 Literature regarding the survey of depression:

In the year 2000, The Diagnostic and Statistical manual of Mental Disorders 4th Edition (DSM---IV) as per American Psychiatric Association, 2000 signified severe depressive disorder as oftenly having the depressive mood or such mood long last for two or more weeks. Such kind of mood generally arises in the morning which disturbs the rest schedule of the day. It has been listed few severe symptoms associated with this disorder are: feelings of worthlessness or guilty, lost interest in leisure activities , unable to make up the relationships, lost concentration, insomnia , fatigue, looses weight or inappropriate weight gain, pain in the body, restlessness , undesired thoughts, lack of motivation .

In the year 2011, National Institutes of Mental Health stated that women are more prone to dipressive disorders as compared to men. They listed out that men often get the symptoms of irritability, drug abuse or alcohol abuse. Many other disorders, such as post-traumatic stress disorder (PTSD), panic disorder, social phobia, and generalized anxiety disorder, tend to occur alongwith depression.

In the year 1977, 1978 Potsolt et al., conducted one test named as 'forced swim test' ,also called the'behavioral despair' test which is one of the well known test to investigate the antidepressant activity whether they have any kind of efficacy. it is generally conducted on rodents , these rodents are placed in glass cylinder containing water to its half level , in which rodents will swim and try to escape in the begening. Eventually, they will tired of doing this and at one stage they will become immobile. Immobility indicates a failure of persistence in escape which is sign of stress or helplessness. This sign arises at stage when rodents accept that escape is not possible. Learned helplessness is believed to be an analog of a diagnosis of clinical depression in humans.

2.2 Literature regarding the survey of mesembrine:

Terburg et al. 2013 reported that *Sceletium tortuosum* have soothing effect on the human brain and it also provides supporting evidence that it may have anxiolytic potential by

attenuating subcortical threat responsivity due to the dual 5-HT reuptake inhibition and PDE4 inhibition of this extract. (Terburg et al., 2013).

African Natural Health Co. have a patent (US6288104 B1) revealed that mesembrine and related compounds (e.g. mesembranol, mesembranone) can be used as scrotonin-uptake inhibitors for use in the treatment of depression, various psychiatric or psychological disorders with an anxiety component, alcohol and drug dependence, bulimia nervosa and obsessive-compulsive disorders. (http://www.google.com /patents /US6288104.)

Smith et al. 1996 reported pharmacological studies on mesembrine and other alkaloids, due to their narcotic-anxiolytic properties, very less toxicity, anti-cancer activity and strong synergism with other psychomimetics. (Smith et al., 1996).

Smith C. 2011 reported that *Sceletium tortuosum* may have positive effect on restraint- induced anxiety. (Smith C., 2011).

Schell R. 2014 reported that mesembrine alkaloids present in *Sceletium tortuosum* have antidepressant properties and can be used as an alternative for the treatment of major depressive disorder. (Schell R., 2014).

Murbach et al. 2014 reported the acceptable daily intake of 420 mg of mesembrine by a 70 kg human and suggested that it can be beneficial in terms of emotional well-being, calming effect, and stress relief. (Murbach et al., 2014).

Ujvary I. 2014 reported that Laboratory experiments with various plant preparations have revealed anti-stress, antidepressant, narcotic, anxiolytic and anti-addictive but not hallucinogenic effects of mesembrine. Screening *in vitro* a range of potential pharmacological targets revealed that mesembrine was an effective inhibitor of 5-HT reuptake, while its unsaturated derivative (*i.e.*, mesembrenone) inhibited both 5-HT reuptake and phosphodiesterase type 4 isoenzyme. (Ujvary I., 2014).

Zembrin is a patented, proprietary extract of the South African traditional medicinal plant *Sceletium tortuosum*. Patent #8,552,051 states the use of one of zembrin containing essential alkaloids as mesembrine and mesembrenone- for its property as serotonin-reuptake inhibitors. According to the point of view of Barbara Davis, Director, Medical & Scientific Affairs at PLT Health Solutions, this advancement in study of zembrin properties gives further knowledge of the use of this active agent as a dual 5-HT reuptake and PDE4 inhibitor in helping to manage stress. On studying further its mechanism it came to know that this plant species has been extensively used by San people of southern Africa" as key component of reducing stress.

According to Seth Flowerman, Director of Business Development, it is the unique 'fingerprint' of Zembrin that is an important part of clinical trials used as an extensive safety portfolio . it tends to conatin enriched alkaloidal moieties : mesembrine and mesembrenone.

As per JP71043539 to Tanabe Seiyaku Company Limited, mesembrine is known to be naturally occurring alkaloid used for Central nervous system management. Plant known as "kougoed", "channa" or "kanna" in the Cape of South Africa, are used traditionally by some communities as sedatives to elevate mood. These plants are all members of the family Mesembryanthemaceae, and contains varying amounts of (−)-mesembrine and related alkaloids.

US 6,288,104 also reported the use of mesembrine and related compounds as serotonin-uptake inhibitors, which is utilized as treatment for the depression and other psychiatric disorders.

It has also been reported that plants of the genus Sceletium, probably contain higer amount of mesembrine to produce some biological activity. However, it has also been reported that mesembrine shows unstablility while harvesting, drying, and extracting the raw material, as well as during storage and formulation of the extract (Patnala, S. and Kanfer, I. Investigations of the phytochemical content of Sceletium tortuosum following the preparation of "Kougoed" by fermentation of plant material. J. Ethnopharmacol. 2009 Jan. 12; 121 (1):86-91).

2.3 DRUG PROFILE

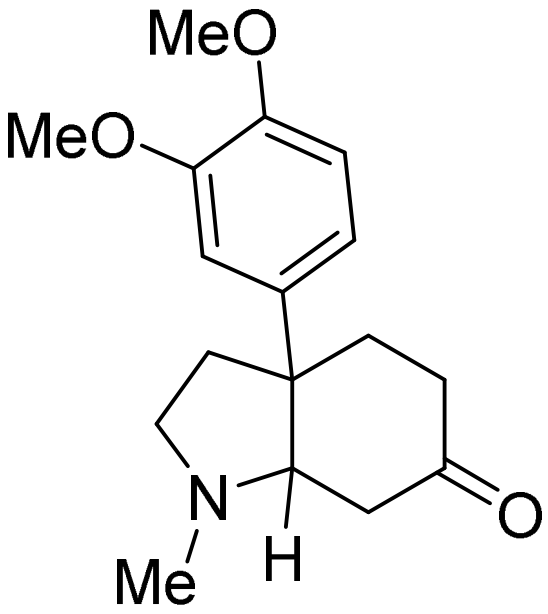

Figure 2.1: Structure of mesembrine

The active pharmaceutical ingredient chosen for the formulation and development of the medicated liposome is mesembrine.

Description:

Chemical name: 3a-(3,4-dimethoxyphenyl)-1-methyloctahydro-6H-indol-6-one.

Molecular formula: $C_{17}H_{23}NO_3$

Density: 1.1±0.1 g/cm^3

Vapour pressure: 0.0±1.0 mmHg at 25°C

Surface tension: 41.6±3.0

Molar volume: 255.2±3.0 cm^3

Molar refractivity: 81.1±0.3 cm^3

Boiling point: 419.2±45.0 °C

Pka: 8.335

LogP: 2.22

Melting point: 5302 (K)

Solubility: Free base, freely soluble in alcohol, chloroform, acetone, slightly soluble in ether, practically insoluble in benzene, petroleum ether, alkalies.

Pharmacology: Mesembrine was studied to be serotonin uptake inhibitor as well as used for the treatment of central nervous system disorder. Mesembrine is also used as phosphodiesterase-4 inhibitor (PDE4) and used in management of asthma, chronic pulmonary diseases and depression.
(http://www.chemspider.com/Chemical-Structure.167737.html.)

CHAPTER 3: RESEARCH ENVISAGED

Mesembrine is a very active drug in the treatment of depression. Liposomes have been selected as one of the promising tool to deliver mesembrine to the brain.

3.1 AIM:

The aim of study is the utilization of nanotechnology for formulation development, optimization and evaluation of brain targeted drug delivery system.

3.2 OBJECTIVE

To formulate the nanoliposomes with higher patient compliance as compared to other available formulations.

3.3 RATIONALE/SCOPE

Medicated liposomes containing mesembrine show vast applications in brain targeted drug delivery. This formulation show higher patient compliance as it has shows its significance in delivering mesembrine to the brain with less cost effectiveness, minimal side effects as compared to other formulations and making it safe to use. Patient can take its smaller dose and yet have some good relief from the depression.

CHAPTER 4: PLAN OF WORK

4.1 **Approval of research title** :

The following research title was approved through project approval committee.

4.2 **Literature survey regarding brain targeted drug delivery systems, liposome formulation and mesembrine activity**:

Literature survey has to be done to get information regarding the recent advancements and researches going on for drug loaded liposome formulations, brain targeted drug delivery system and mesembrine. Various advanced techniques for optimizing drug delivery systems will also be studied.

4.3 **Procurement of chemicals:**- The next task will be procurement of drug and various chemicals that are required for the research work such as soya lecithin, disodium hydrogen phosphate, potassium dihydrogen phosphate, mannitol etc.

4.4 **Preformulation studies:**

Preformulation studies will be done to get information about any physical and/or chemical interactions between drug and all other excipients to be added in the formulation i.e. the compatibility studies.

- Drug substance characterization
- Organolaptic properties
- Angle of repose
- Bulk density, tapped density, compressibility index and hausner ratio
- Characterization by UV-spectroscopy
- Callibartion graph using UV-visible spectroscopy
- Drugs-excipients compatibility studies

4.5 **Solubility testing of drug**:- As very less information is available regarding the solubility of drug, so the solubility testing of drug was to be done.

4.6 **Preparation of standard curve**: Preparation of standard curve of the drug based on various dilutions.

4.7 **Formulation of blank batch**:- Preparation of blank batch without drug.

4.8 **Formulation of first batch**:- Preparation of first batch using drug and characterization of the formulated nanoliposomes.

4.9 **Optimization**:- Optimization of the formulation will be done to get the best possible drug loaded liposomal formulation based on various results.

4.10 **Evaluation of formulation:**- Evaluation of formulated nanoliposomes for the antidepressant like activity in animals.

4.11 **Characterization :** Next task will be the study of drug substance characterstics for eg: organolaptic properties, particle size distribution, melting point , solubility etc.

4.12 **Compilation of observation:** After observing the all characterstics of drug substance. Compliation of result will be done.

4.13 **Preperation of thesis**: last step will be the preparation of thesis as per the guidelines.

CHAPTER 5: EXPERIMENTAL METHODOLOGY

5.1 Materials & Method:

5.1.1 Equipments

Table 5.1: Instruments used in the present investigation

Instruments	Manufacturers/Suppliers
UV Spectrophotometer	Shimadzu UV-1800 UV/Vis. double beam spectrophotometer (Kyoto, Japan).
RotaVapor	Perfit India
Microscope	Optical microscope (Labomed CX RIII, Ambala,India)
Scanning Electron Microscope	Scanning electron microscope (JSM 5610 LVSEM, JEOL, Datum Ltd, Tokyo, Japan).
pH meter	LabIndia, Mumbai
Bath Sonicator	PCI analysis
Water bath shaker	Narang scientific works, pvt ,ltd
Melting point apparatus	Perfit , india
Electronic Balance	Shimadzu
Magnetic stirrer	Remi

5.1.2 Materials

Table 5.2: Materials used in the present investigation

Material	Functional Category	Suppliers
Mesembrine	API	Xian changyue phytochemistry Co. , Ltd. China
Phospholipon 90® H	Lipid	Lipoid, Germany
Cholesterol	Lipid	Lobachem
Tween 80	Surfactant	Thomas Baker Pvt. Ltd., Mumbai
Chloroform	Solvent	Thomas Baker Pvt. Ltd., Mumbai
Ethanol	Solvent	Changshu Yanguan Chemical,China

Methanol	Solvent	Finar reagents, Ahemdabad
Dichloromethane	Solvent	Thomas Baker Pvt.Ltd.,Mumbai

5.2 Analytical method for API

5.2.1 Material and method:

The analysis of the active pharmaceutical ingredient that is mesembrine was done with the help of Ultraviolet spectrophotometer. A pair of 1cm quartz cells were used to measure the absorbance of the solutions. The de-mineralized water was used as blank.

5.2.2 Preparation of standard stock solution in water:

Accurately weighed 100mg of the drug was placed in the 100ml volumetric flask and volume was made upto 100ml using de-mineralied water. The solution of different concentration were made by taking 0.5ml , 1ml, 1.5ml, 2ml, 2.5ml, 3ml, 3.5ml, 4ml , 4.5ml and 5ml respectively making up the volume upto 10ml in the 10ml volumetric flask. The standard graph was plotted using corresponding absorbance of these concentrations measured at 276nm wavelength for mesembrine. The graph was plotted between absorbance and corresponding concentration by placing absorbances on Y-axis and concentrations on X-axis respectively.

5.2.3 Baseline correction and calibration curve:

The baseline correction was done using blank in both the quartz cells, then the calibration curve was plotted using solution of higher concentration.

5.2.4 Preparation of standard stock solution in methanol:

Accurately weighed 100mg of the drug was placed in the 100ml volumetric flask and volume was made upto 100ml using methanol. The solution of different concentration were made by taking 0.5ml , 1ml, 1.5ml, 2ml, 2.5ml, 3ml, 3.5ml, 4ml , 4.5ml and 5ml

respectively making up the volume upto 10ml in the 10ml volumetric flask. The standard graph was plotted using corresponding absorbance of these concentrations measured at 276nm wavelength for mesembrine. The graph was plotted between absorbance and corresponding concentration by placing absorbances on Y-axis and concentrations on X-axis respectively.

5.2.5 Baseline correction and calibration curve:

The baseline correction was done using blank in both the quartz cells, then the calibration curve was plotted using solution of higher concentration.

5.2.6 Preparation of standard stock solution in Ph 6.8 phosphate buffer:

Accurately weighed 100mg of the drug was placed in the 100ml volumetric flask and volume was made upto 100ml using pH 6.8 phosphate buffer. The solution of different concentration were made by taking 0.5ml , 1ml, 1.5ml, 2ml, 2.5ml, 3ml, 3.5ml, 4ml , 4.5ml and 5ml respectively making up the volume upto 10ml in the 10ml volumetric flask. The standard graph was plotted using corresponding absorbance of these concentrations measured at 276nm wavelength for mesembrine. The graph was plotted between absorbance and corresponding concentration by placing absorbances on Y-axis and concentrations on X-axis respectively.

5.2.7 Baseline correction and calibration curve:

The baseline correction was done using blank in both the quartz cells, then the calibration curve was plotted using solution of higher concentration.

5.3 Preformulation studies:

Preformulation study is the prior step in developing a formulation or any dosage form of active substance. As word preformulation itself states that pre means activities perform prior to the development of formulations. It includes the investigation of physical and chemical properties of a drug substance alone and when combined with

excipients. Thus the purpose of preformulation studies is to generate the information which is important for the formulator to design formulation with good stability and efficacy. Carrying out the preformulation studies are also important in understanding basic drug profile for example: drug's pharmacokinetic properties, efficacy of drug , bioavailability of drugs, any unwanted reactions of the drug etc.

Preformulation studies are broadly classified into two classes:

a) Fundamental properties: these are the properties which are particular properties of drug substance including its chemical nature of drug miolecule. These essential properties include- (i) Solubility – solubility is an important factor in determining drug profile which include solubility of drug substance in various solvents, (pKa) known as dissociation constant , formation of salt, partition coefficient, particle size distribution, hydrophicity and lipophilicity , drug stability, dissolution kinetics, (ii) permeability, (iii) solid state properties like solid form, polymorphism, solvated forms and amorphous form and (iv) solid state stability and solution state stability, wherein inherent stability, pH – stability profile and photo-stability are studied.

b) Derived properties : Derived preformulation are those preformulation studies which are generally done to learn about various issues related to the development of a particular dosage form like solid oral, liquid oral or parenteral. Derived preformulation properties for solid oral dosage form like tablet, include – (i) study of the particle characterstics like: morphology and particle size (ii) bulk density, (iii) flow properties and (iv) compaction behavior. As discussed, these derived preformulation properties are necessary for the development of intended dosage form. In case of a capsule dosage form, compaction behaviour has to be studied. Other important factor to be studied is the compatibility studies, wherein the physical and chemical stability of the drug molecule is studied in the presence of excipients.

5.3.1 Drug substance characterization:

5.3.1.1 Organoleptic properties:

The Organoleptic properties of Mesembrine were examined for the color, odour and appearance.

5.3.1.2 Melting point

As per the USP method, melting point of drug substance was determined by capillary tube method. Very little amount of drug was placed into a sealed capillary tube. The tube was then placed in the melting point apparatus. The temperature in the apparatus was set gradually which increases gradually and the observation of temperature was noted at point where drug starts to melt and the temperature where the entire drug gets melted.

5.3.1.3 Solubility studies:

Solubility of Mesembrine in different solvents was determined.

5.3.1.4 Partition Coefficient:

The partition coefficient determination study was performed by using shake flask method.

5.3.1.5 Chemical stability profile:

Preformulation stability studies also includes the measurement of chemical stability of a new drug. All factors affecting the drug's chemical stability are essential to be assessed. In rational dosage form, critical factors affecting chemical stability includes: temperature, pH and dosage form diluents. Various methods are available for sterilizing the poduct. These methods depends largely on the temperature stability of the drug. For example: Drugs which are less stable cannot be sterilized at higher temperature such as autoclaving but must be sterilized by other ways like filteration. pH effect on drug stability is important factor in the development of dosage form specially oral dosage form which is needed to be protected from the highly acidic environment of the stomach. Buffer selection for potential dosage forms will largely be affected by stability characteristic of the drug. Typical stress conditions are shown in table 5.3.

Table 5.3: Stress Conditions used in Preformulation Stability Assessments.

Stress Conditions used in Preformulation Stability Assessments	
Test	**Conditions**
Solid:	
Heat	(0C) 4, 20, 30 ,40, 40/70% RH, 50 and 75
Moisture uptake	30,45,60,75,and 90% RH at RTa,b
Physical Stress	Ball milling
Aqueous Solution:	
Ph	1 to 9 and 11 at RT & 370C. Reflux in 1 M HCl & 1 M NaOH
Light	UV (254&366 nm)
Oxidation	Sparging with oxygen with at RT; UV may accelerate breakdown
a) RT is ambient room temperature. Can vary between 50 and 250c b) Saturated solution of MgBr2 KNO2, NaBr , NaCl and KNO3 respectively c) At pH of maximum stability in simple aqueous solution	

5.4 Formulation development:

5.4.1 Preparation of liposome formulations:

The thin film hydration method was used to prepare the liposome formulations.Four Formulations: LI, L2, L3, L4 were prepared with Phospholipon 90 H.Four Formulations were prepared (L1, L2, L3 and L4) containing 200mg, 150mg, 100mg and 50mg respectively. Amount of Cholesterol and Chloroform was constant for all the four formulations i.e.10 mg and 10ml respectively. The formulations and the manufacturing parameters were optimized concerning drug encapsulation efficiency. Phospholipid, cholesterol and Mesembrine were dissolved in chloroform, and organic solvent was evaporated by a rotary evaporator at 60 rpm and 70 °C, thus obtaining a thin film of dry lipid on the flask wall. Evaporation was continued for 1 h after the film appeared and the flask left under the vacuum to completely remove all the traces of the solvent. Subsequently, the resulting thin film was hydrated in 10 ml water and kept on water bath

shaker at 70°C for one hour. The formulations were allowed to cool and drug entrapment and pH of each formulation was calculated. Further, three formulations (L5, L6, L7) were prepared using 0.1% Tween 80 of total formulation. Other components i.e. Phospholipon 90 H, Cholesterol and Chloroform were same as in previous formulation. Same procedure was followed for three formulations (L5, L6, L7). Drug entrapment and pH was calculated.

5.4.2 Evaluation Parameters:

5.4.2.1 pH:

The pH of each formulation was calculated using pH meter.

5.4.2.2 Infrared Spectroscopy:

The change if any, in drug properties was observed through IR spectroscopy. The IR spectra was obtained for drug as well as drug loaded liposomes.

5.4.2.3 Particle Size and Zeta Potential

Size of particles, their shape and surface area affects the flow property of drug substance. Bulk flow, formulation homogeneity, and surface-area controlled processes such as dissolution and Surface morphology of the drug particles. In general, during the preformulation studies each new drug candidate should be tested , drug substance with the smallest particle size is of greater interest as it essential to facilitate preparation of homogeneous samples and maximize the drug's surface area for interactions. Various physicochemical properties of drug substances are highly affected by the size distribution and shapes of the particles. It not only effect the physical beaviour of drug molecule also shows some effect on their biopharmaceutical behavior. It is generally believed that the drugs which are poorly water soluble will greater bioavailabilty. As these poorly water soluble drugs has dissolution- rate limiting step in the absorption process and when they are administered in a subdivided form tend to produce more bioavailabilty than the coarse material. These two important properties of prepared liposomes were determined by zeta sizer.

5.4.2.4 In-vitro drug release studies:

In vitro release studies were performed to select formulation having best entrapment value. The dialysis bag diffusion technique was used to study the in vitro drug release of Mesembrine.1 ml of liposome formulations were placed in the dialysis bag (cellulose membrane, molecular weight cut off 12–14,000 D), hermetically sealed and immersed into 100 ml of phosphate buffer (pH-6.8) in 250ml beaker. The entire system was kept on continuous magnetic stirring at 400 rpm/min.Samples (5 ml) were withdrawn from the receptor compartment at predetermined time intervals (every 1hour) and replaced by fresh medium (5 ml). The amount of drug released was determined by UV spectroscopy.UV absorbance was noted at 275 nm.

CHAPTER 6: RESULT AND DISCUSSION

6.1 Result of Preformulation study of drug (Mesembrine):

6.1.1 Organoleptic properties:

Organoleptic properties of drug Mesembrine found to be as per I.P. monograph. The Organoleptic properties of Mesembrine were found to the given table (6.1).

Table 6.1: Organoleptic Properties of Mesembrine

S.No.	Test	Specification	Observation
1.	Colour	Yellowish Brown	Yellowish Brown
2.	Odour	Odourless	Odourless
3.	Appearance	Powder	Powder

6.1.2 Melting Point:

Melting point of drug was determined by capillary fusion method.

Table 6.2: Melting Point of Mesembrine

Drug	Specification	Observation
Mesembrine	205-206 °C	205-206°C

The melting point of Mesembrine was found to be in range 205-206°C which is of the pure drug. Hence drug sample was free from any type of impurities.

6.1.3 UV Spectroscopy:

Determination of Absorption Maxima (λ_{max}) Of Drug (Mesembrine) in Water, Methanol and Phosphate Buffer (pH- 6.8):

Table 6.3: Absorption Maxima (λ_{max}) in different solvents

S. No.	Solvent	Absorption Maxima (λ_{max})
1.	Water	275 nm
2.	Methanol	273nm
3.	Phosphate Buffer(pH- 6.8)	276nm

6.1.3.1 Standard Calibration Curve in Water:

With the help of observed value of absorbance at different concentrations a standard curve was plotted in MS-Excel.

Results:

Table 6.4: Absorbance of different dilutions of drug at 275 nm in Water

S.No.	Concentration (µg/ml)	Absorbance at 275 nm
1.	50	0.104 ± 0.005
2.	100	0.189 ± 0.002
3.	150	0.306 ± 0.007
4.	200	0.383 ±0.003
5.	250	0.476 ± 0.005
6.	300	0.534 ± 0.004
7.	350	0.680 ± 0.003
8.	400	0.747 ± 0.002
9.	450	0.871 ± 0.006
10.	500	0.950 ± 0.004

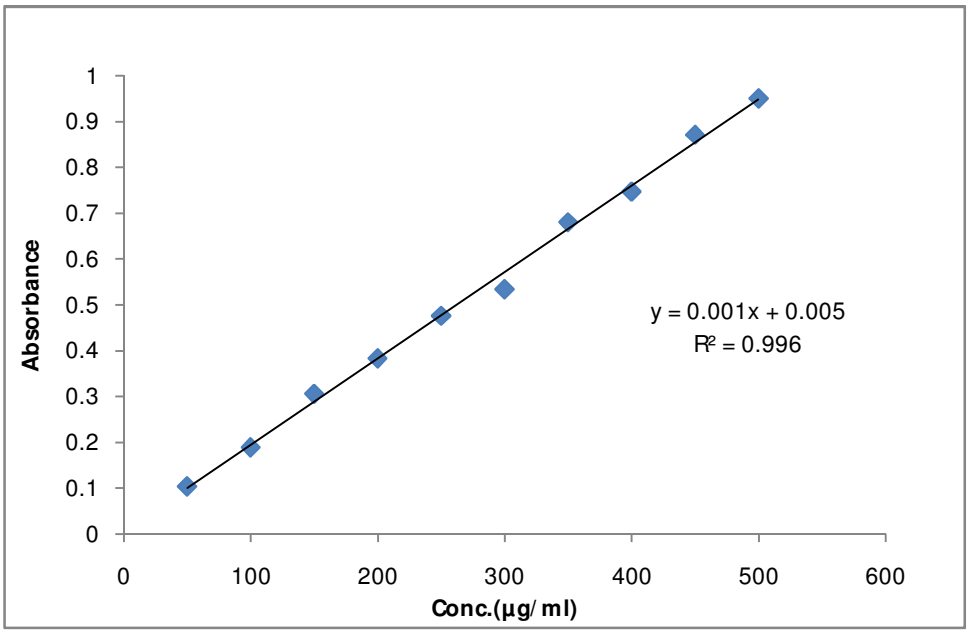

Figure 6.1: Standard calibration curve of Mesembrine in Water

The calibration curve for Mesembrine was obtained by using 50 to 500µg/ml solution of drug in Water. The absorbance was measured at 275 nm. The standard curve of Mesembrine as shown in graph indicated the regression equation Y = 0.001x + 0.005 and R^2 value is 0.996, which shows good linearity.

6.1.3.2 Standard Calibration Curve in Methanol:

With the help of observed value of absorbance at different concentrations a standard curve was plotted in MS-Excel.

Result:

Table 6.5: Absorbance of different dilutions of drug at in Methanol

Con. (µg/ml)	Abs
50	0.039±0.015
100	0.081±0.02
150	0.156±0.016
200	0.196±0.007

250	0.273±0.004
300	0.336±0.015
350	0.417±0.017
400	0.465±0.012
450	0.525±0.001
500	0.609±0.003

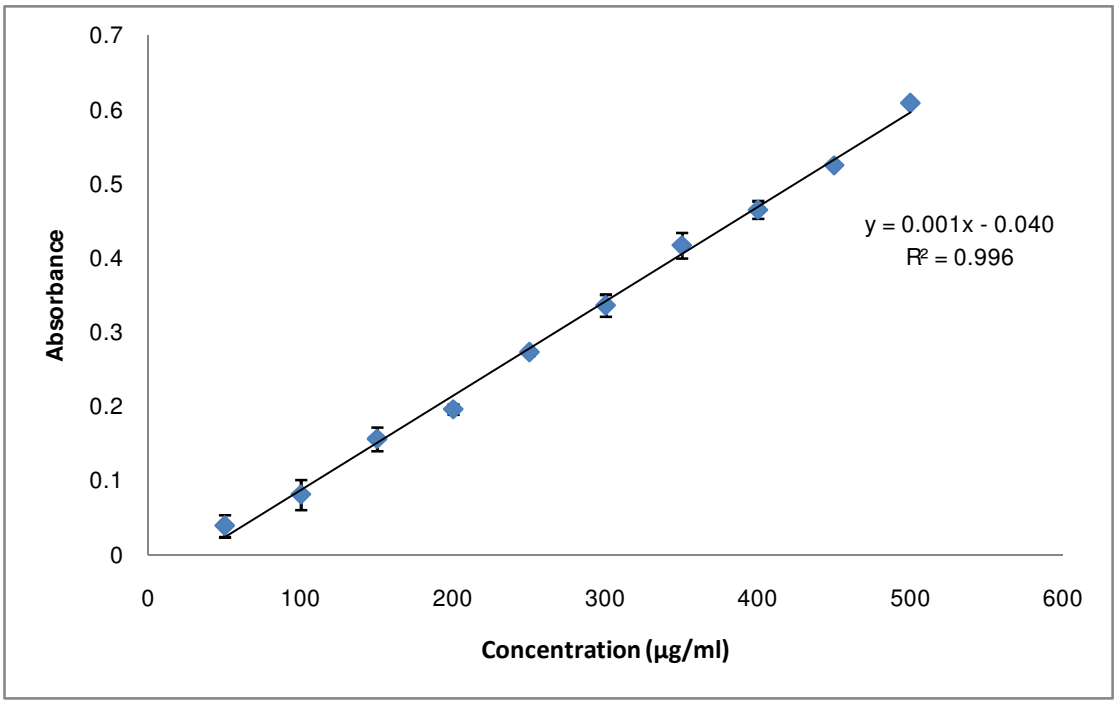

Figure 6.2: Standard calibration curve of Mesembrine in Methanol

The calibration curve for Mesembrine was obtained by using the 50 to 500µg/ml solution of drug in Methanol. The absorbance was measured at 273 nm. The standard curve of Mesembrine as shows in graph indicated the regression equation Y = 0.011x – 0.016 and R^2 value is 0.996, which shows good linearity.

6.1.3.3 Standard Calibration Curve in Phosphate Buffer (pH-6.8):

With the help of observed value of absorbance at different concentrations a standard curve was plotted in MS-Excel.

Results:

Table 6.6: Absorbance of different dilutions of drug at 276 nm in Phosphate Buffer (pH-6.8)

S.No.	Concentration (µg/ml)	Absorbance at 276 nm
1.	50	0.095 ± 0.005
2.	100	0.188 ± 0.004
3.	150	0.285 ± 0.006
4.	200	0.401 ± 0.003
5.	250	0.519 ± 0.004
6.	300	0.567 ± 0.007
7.	350	0.669 ± 0.009
8.	400	0.816 ± 0.003
9.	450	0.848 ± 0.005
10.	500	0.970 ± 0.004

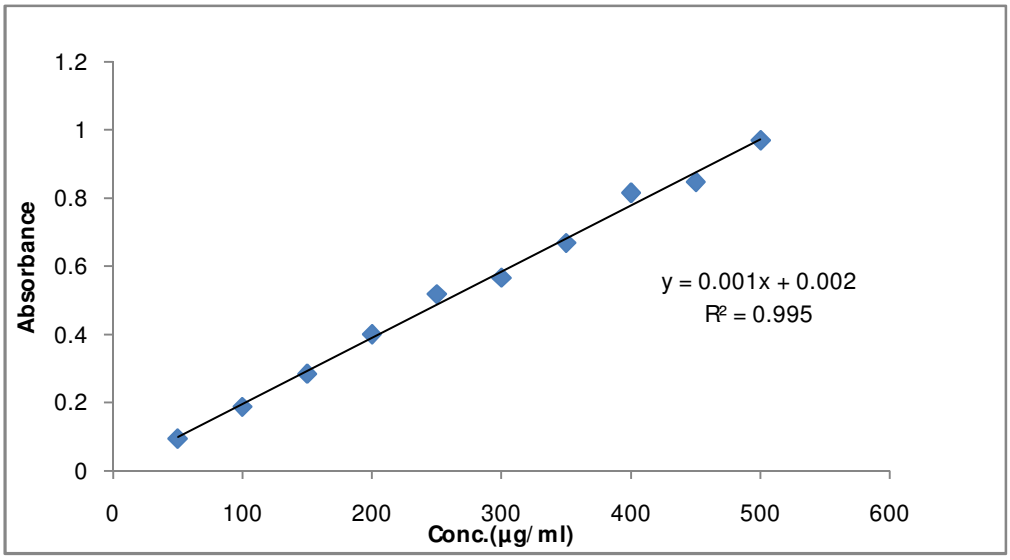

Figure 6.3: Standard calibration curve of Mesembrine in Phosphate Buffer (pH-6.8)

The calibration curve for Mesembrine was obtained by using 50 to 500µg/ml solution of drug in Phosphate Buffer (pH-6.8). The absorbance was measured at 276 nm. The standard curve of Mesembrine as shown in graph indicated the regression equation Y = 0.001x + 0.002 and R^2 value is 0.995, which shows good linearity.

6.1.4 Solubility studies:

The solubility studies conducted have the following results.

Table 6.7: Solubility profile of Mesembrine in different solvents

S.No.	Solvents	Inferences
1.	Ethanol	Slightly soluble
2.	Methanol	Sparingly soluble
3.	Dichloromethane	Freely soluble
4.	Water	Very soluble
5.	Chloroform	Very soluble

6.1.5 Partition Coefficient:

The partition coefficient of Mesembrine was observed as 1.56 and it shows that the Mesembrine was hydrophillic in nature.

6.2 Formulation development and Evaluation of Liposomes

6.2.1 Infra red spectroscopy:

6.2.1.1 Drug sample (Mesembrine)

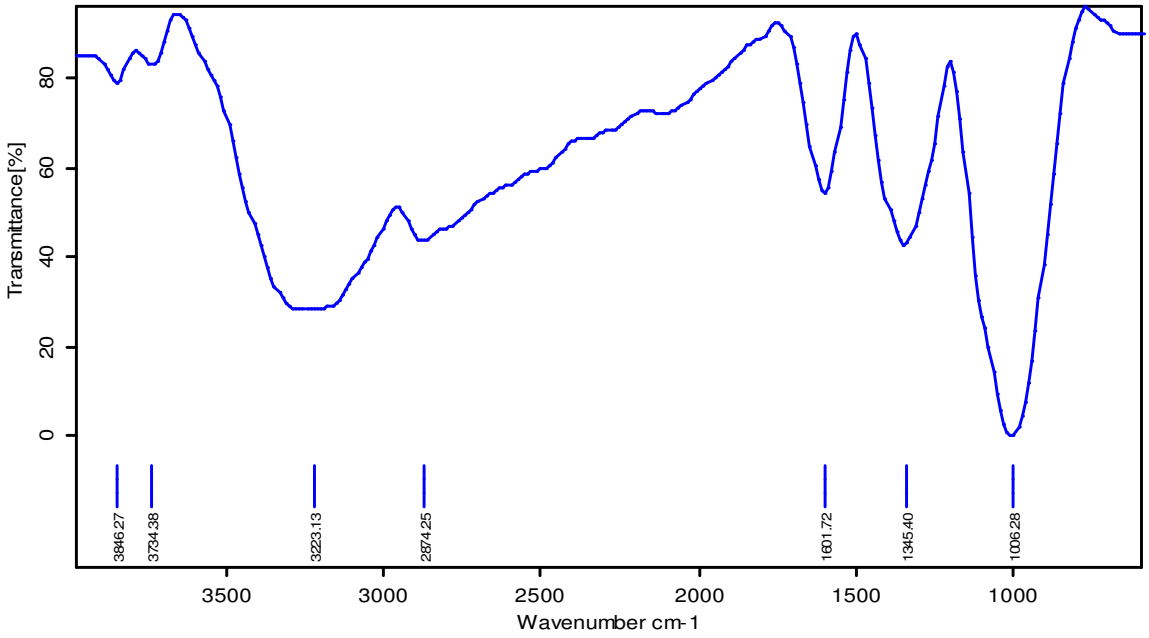

Figure 6.4: IR spectra of drug substance (mesembrine)

6.2.1.2 Drug formulation

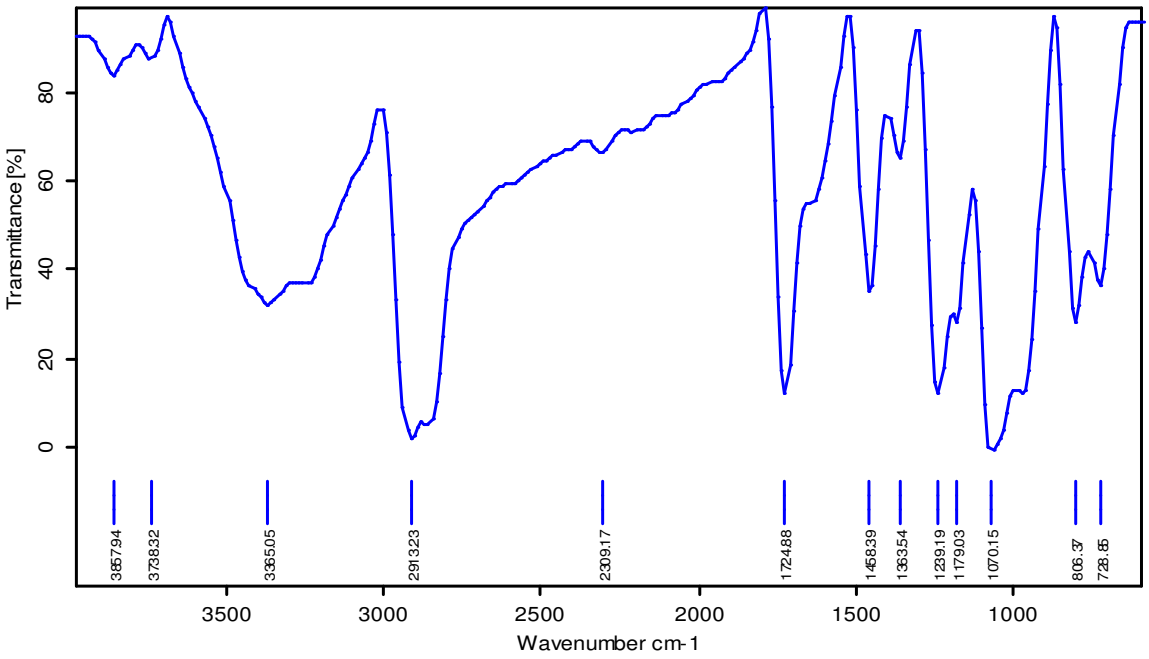

Figure 6.5: IR spectra of drug formulation (liposome containing mesembrine)

As observed from above spectra there is no change or shift in the drug peaks, hence confirming that the liposome encapsulation did not influenced the basic chemical properties of drug.

6.2.2 Particle size:

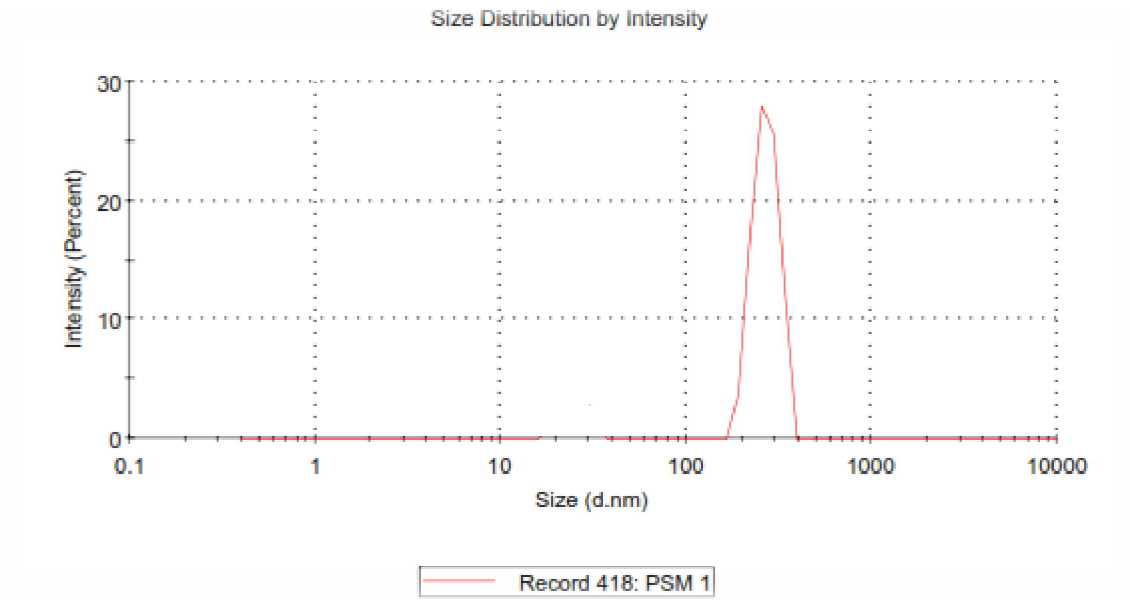

Figure 6.6: Graph showing partical size distribution

Table 6.8: Result of particle size distribution

z-average (d.nm): 427.9
PdI: 0.703
Intercept : 1.03

The developed liposomal system has average particle size in nano range (i.e. 427.9 nm). The PDI value was found to be below 1 which indicates that the developed liposomal formulation is close to monodispersed system. Thus, confirming the suitability of employed technique for their preparation.

6.2.3 Zeta potential:

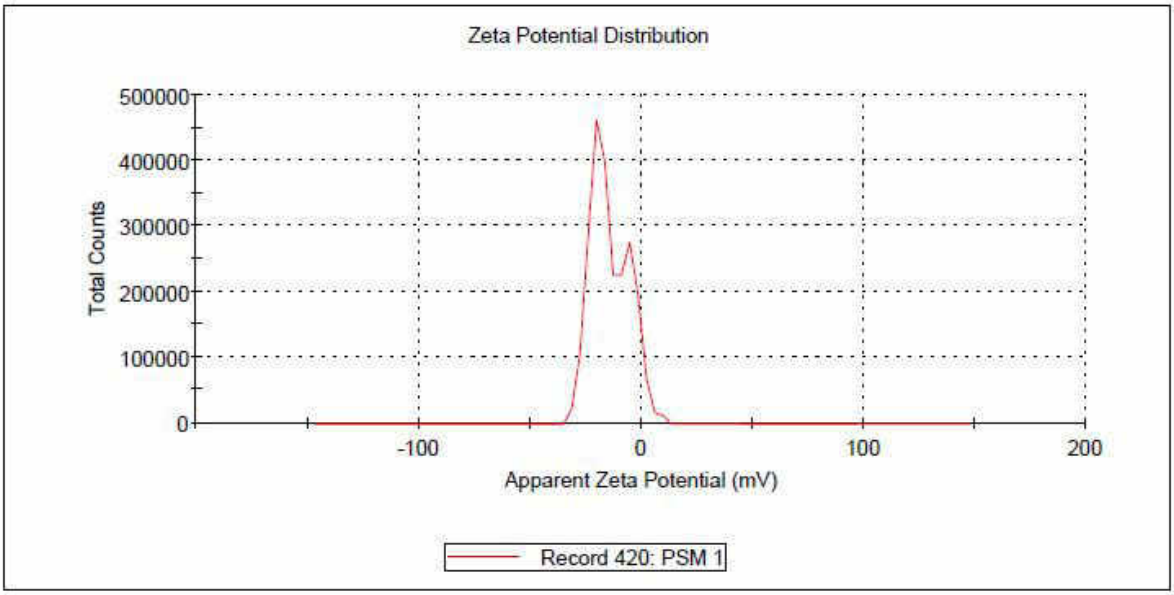

Figure 6.7: Graph showing zeta potential

Table 6.9: Result of zeta potential

Zeta potential(mV): -14.0
Zeta deviation (mV): 8.48
Conductivity (ms/cm): 0.366

The zeta potential was found to be -14.0, confirming the stability of the dispersed liposomes.

6.2.4 Drug Entrapment

Table 6.10: Formulation Composition (Trial 1)

Formulation Code	Phospholipon 90H(mg)	Cholesterol(mg)	% Drug Entrapment	pH of Formulation
L1	200	10	54	4.5
L2	**150**	**10**	**85**	**8.1**
L3	100	10	65.34	3.8
L4	50	10	49.35	5.8

Amount of Drug taken: 10mg

The maximum drug entrapment was found to be 85 % in **L2 Formulation**. The results shows that with increase in lipid content there is increase in % drug entrapment. However, after

certain level it decreases. This portrays that liposomes can hold a certain level of drug and above which the drug tend to come out of the vesicles, even if high lipid concentration is given.

Table 6.11: Formulation Composition (Trial 2)

F.Code	Phospholipon 90H(mg)	Cholesterol (mg)	Tween 80 (gm)	% Drug Entrapment	pH of Formulation
L5	150	10	0.2	69	7.0
L6	**150**	**10**	**0.15**	**92**	**8.0**
L7	150	10	0.10	84	8.1

Amount of Drug taken: 10 mg

The maximum drug entrapment was found to be 92% in case of L6 formulation containing Tween 80. The results obtained portrays that the incorporation of tween tend to increase entrapment efficiency of liposomes. This may be attributed to the fact that "tween" is a elasticizer for lipid membrane/ layer, which tend to decrease the drug leakage from vesicles. Hence, increasing the drug holding and drug entrapment efficacy.

Moreover it has been observed that more entrapment was obtained at basic pH than at acidic pH. This supports that alkaloidal drug are better stable in the vesicles in basic conditions.

6.2.5 In-vitro drug release studies:

Liposome Formulations containing 0.1% of Tween 80 of Total Formulation (Using Phosphate Buffer-pH 6.8):

Table 6.12: Final Formulation

F.Code	P.90H(mg)	Cholesterol(mg)	Tween 80(mg)	%Drug Release	Ph
L6	150	10	0.15	**88.62**	6.7

The drug entrapment was found to be 88.62%.

Table 6.13: Percentage Drug Release of L6 Formulation

Time(in hr)	% Drug Release
30(min.)	15.34 ±0.21
1	29.45 ±0.29
2	38.43 ±1.25
3	44.32 ±1.37
4	51.34 ±2.42
5	59.9 ±2.46
6	65.78 ±2.39
7	71.43 ±3.47
8	76.09 ±3.49
9	83.54 ±3.49
10	85.34 ±4.03
11	88.54 ±4.01
12	88.62 ±4.12

The *in-vitro* drug release of the formulated liposomes of mesembrine was found to be more than 80% in 9 hours which shows prolonged release as compared to the conventional dosage forms such as tablets and capsules. This may be attributed to the role of entrapment of mesembrine in lipid vesicles leading to the prolonged drug release.

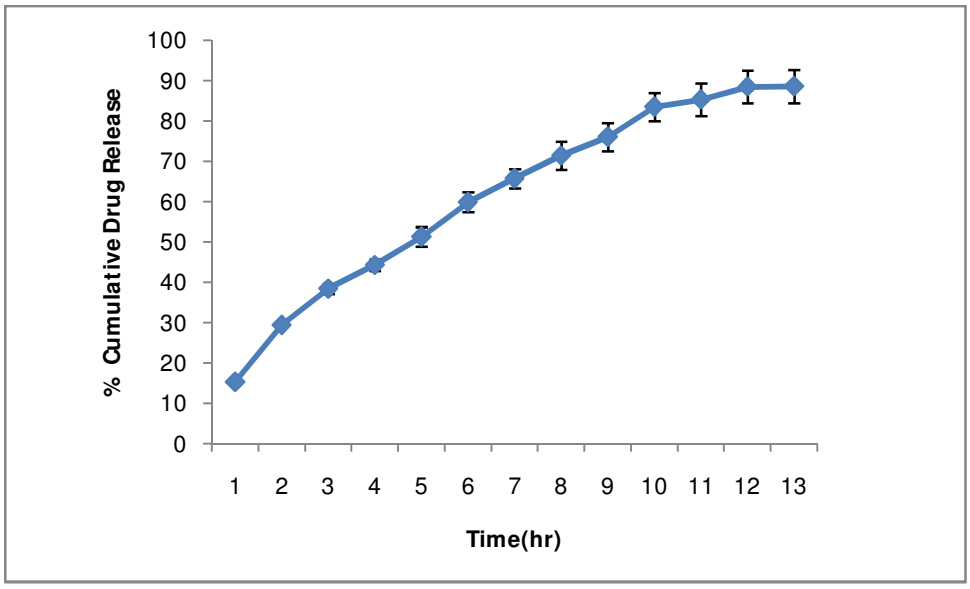

Figure 6.8: Percentage Drug Release of L6 Formulation

6.2.6 Transmission electron microscopy:

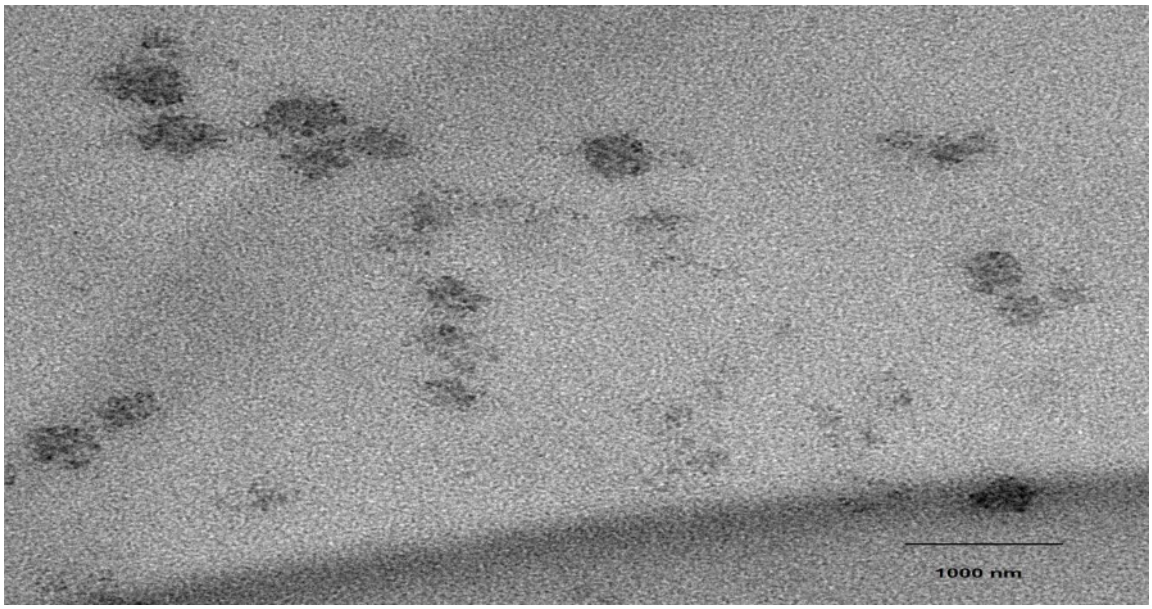

Figure 6.9: Transmission electron microscopic image of prepared liposomes.

The Transmission electron microscopy portrays that the developed liposomes are spherical in shape and no aggregation was observed. Moreover, no free drug crystals were observed in the microscopy.

Thus, the developed liposomes were found to be of spherical shape with average size below 500 nm and drug entrapment efficiency was 90%.

CHAPTER 7: CONCLUSION AND SUMMARY

Nanotechnology has proven to be potential approach for brain targeted drug delivery system. Liposomes has become successful carrier for drug to target brain with less cost effective , minimal side effects and less amount of expensive drugs used. Today various advancements and research has been carried out for ideal nano drug delivery system. So that patient can take smaller dose and yet have same benefits, deliver the drug to right place in living system. So the nano-liposomes tend to withstand all these demanding properties.

Thus, based on the aforementioned fact the project was initiated. To initiate the formulation development, certain preformulation studies like solubility, partition coefficient, stability and incompatibility etc. were carried out. The crucial formulation compounds *viz.* phospholipids, cholesterol and tween-80 were tested and selected at their requisite concentration levels.

The developed liposomes were characterized and tested for entrapment efficiency, micromeretics microscopy and surface charge. Finally, the developed liposomes were evaluated for *in-vitro* drug release and revealed that more than 80% drug was released in 9 hours.

Finally, it can be concluded that the liposome of mesembrine were developed and characterized suitably. Further, these developed system needs to be evaluate for *in-vitro* and *in-vivo* performance employing appropriate methods/ techniques to confirm their suitability for brain targeting.

CHAPTER 9: REFERENCES

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