Formulation and Evaluation of Quercetin crowned N-acetylcysteine liposomes.

A Dissertation Submitted To
THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
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In partial fulfillment of the requirements for the award of degree of
MASTER OF PHARMACY
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Submitted by
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Under the guidance of
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EVALUATION CERTIFICATE

This is to certify that the dissertation entitled **"Formulation and Evaluation of Quercetin crowned N-Acetylcysteine liposomes"** submitted to The Tamilnadu Dr.M.G.R Medical University, Chennai, is a bonafide project work of **Mr. M.Vigneshwar (Reg. No: 261711357)**, carried out in the Department of Pharmaceutics, Jaya College Of Paramedical Sciences College Of Pharmacy Thiruninravur,Chennai-24. in partial fulfillment for the degree of MASTER OF PHARMACY under the guidance of Prof **Dr.masilamani M.Pharm,Phd.** HOD, Department of Pharmaceutics,Jaya College Of Paramedical Sciences College Of Pharmacy, Thiruninravur, Chennai-602024.

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DECLARATION BY THE CANDIDATE

I hereby declare that the matter embodied in the dissertation entitled ***"Formulation and Evaluation of Quercetin Crowned N-acetylcysteine liposomes"*** *is a bonafide and genuine research work carried out by me under the guidance of* Prof **Dr.Masilamani M.Pharm,Phd.** HOD, *Department of Pharmaceutics, Jaya College Of Paramedical Sciences College Of Pharmacy. The work embodied in this thesis is original and has not been submitted the basis for the award of degree, diploma, associate ship (or) fellowship of any other university (or) institution.*

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CERTIFICATE

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Date: *Signature*

Place: Chennai.

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TABLE OF CONTENTS

FORMULATION AND EVALUATION OF QUERCETIN CROWNED N-ACETYLCYSTEINE LIPOSOMES.

1.INTRODUCTION:

In today's modern world there is a need for the development of newer formulation which are more effective and more beneficial to the ailment of the disease, hence there is always in some part of the world research works are going to make the world better and healthier. The novel drug delivery system is one of the place where eyes of the researchers are keen due to their superior benefits over the conventional dosage forms. The conventional dosage forms suffer various limitations like higher dose required lower effectiveness, toxicity and adverse effects.

The method by which the drug is delivered can give a significant effect on its efficacy and safety. The new ideas on controlling the pharmacokinetics, pharmacodynamics,non specific toxicity, Immunogenicity, Biorecognition and efficacy of drugs are generated. These new strategies, often called drug delivery system (DDS) which are based on the interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry and molecular biology.

The drug delivery system and drug targeting are now widely employed to minimize the drug degradation and loss, to prevent harmful side-effects and to increase the bioavailability of the drug hence the various drug delivery and drug targeting system are currently under development.[1]

Therapeutic benefits[2] of newer systems includes

Increased efficacy

Site specific targeting

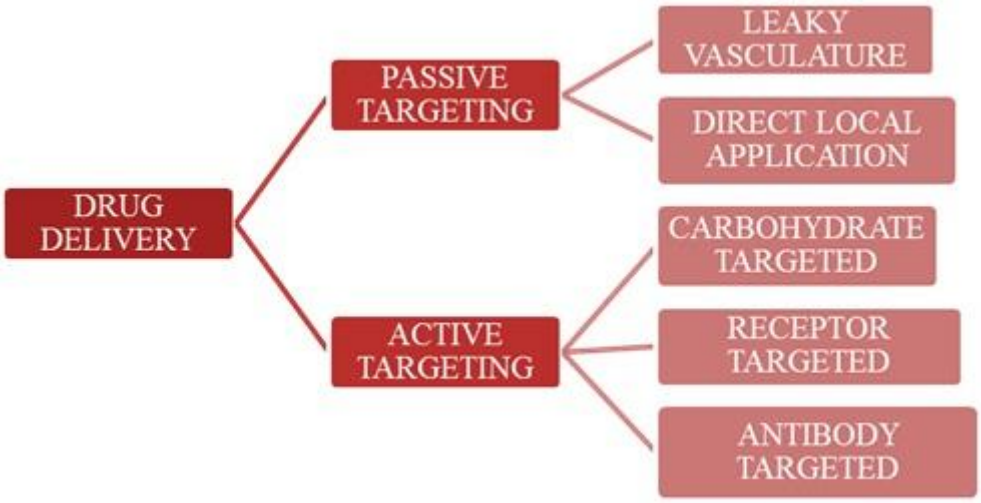
Decreased toxicity/side effects

Increased convenience

Viable treatment for previously incurable disease

Potential for prophylactic applications

Better patient compliance.



1.1NOVEL DRUG DELIVERY SYSTEM

Evolution of existing drug molecule from a conventional form to a novel delivery system can drastically improve its patient's compliance, safety and efficacy also in novel drug delivery an existing drug can get a new life into the pharmaceutical market. The novel drug is efficient to present the maximum fraction of administered dose at the target site. Various carriers like nanoparticles, micro particles, lectins polysaccharides and Liposome's can be used to target the drug to a specific site.

The need for the development of the newer drug delivery system with better efficacy and fewer side effects to the patients has prompted pharmaceutical companies to involve in the development to newer delivery system. The novel drug delivery system is gaining importance nowadays and it will be the future of the drug formulations in the near future.

Various drug delivery systems which are gaining interest

1. Liposomes

2. Nanoparticles

- Nanotubes
- Nanowires
- Nanocantilever
- Nanoshells
- Quantum dots
- Nanopores
- Gold nanoparticles
- Bucky balls

- Carbon Nanotubes

3. Microspheres

4. Monoclonal antibodies

5. Niosomes

6. Released erythrocytes and as drug carriers.

Liposomes:

Liposomes are a form of vesicles that consist either of many or just one phospholipids bilayers. The polar nature of the liposomal center enables polar drug molecules to be easily encapsulated. Both Amphiphilic and lipophilic molecules are solubilised inside the phospholipid bilayers according to their affinity towards the phospholipids. Participation of non ionic surfactants instead of phospholipids in the bilayers formation results in niosomes. Channel proteins can be incorporated without loss of their activity within the hydrophobic domain of vesicle membranes, acting as a size-selective filter, only allowing passive diffusion of small solutes such as ions, nutrients and antibiotics.

Thus, drugs that are encapsulated in a nanocage-functionalized with channel proteins are effectively protected from premature degradation by proteolytic enzymes.The drug molecule, however, can diffuse through the channel, driven by the concentration difference between the interior and the exterior of the nanocage.[3]

Dendrimers are nanometer-sized, highly branched and monodisperse macromolecules with symmetrical architecture. They consist of a central core, branching units and terminal functional groups. The core together with the internal units, determine the environment of the nanocavities and consequently their solubilizing properties, whereas the external groups the solubility and chemical behavior of these polymers. Targeting effectiveness is affected by attaching targeting ligands at the external surface of dendrimers, while their stability and protection from the Mononuclear Phagocyte System (MPS) is being achieved by fictionalization of the dendrimers with polyethylene glycol chains (PEG). Liquid

Crystals combine the properties of both liquid and solid states. They can be made to form different geometries, with alternative polar and non-polar layers (i.e., a lamellar phase) where aqueous drug solutions can be included.

Nanoparticles: Nanoparticles (including nanospheres and nanocapsules of size 10-200nm) are in the solid state and are either amorphous or crystalline. They are able to adsorb and/or encapsulate a drug, thus protecting it against chemical and enzymatic degradation. In recent years, biodegradable polymeric nanoparticles have attracted considerable attention as potential drug delivery devices in view of their applications in the controlled release of drugs, in targeting particular organ/or tissue, as carriers of DNA in gene therapy, and in their abilities to deliver proteins peptides and genes through per oral route[4].

classification of nanomaterials:

A) Nanotubes- They are hallow cylinders made of carbon atoms. They can also be filled and sealed, forming test tubes or potential drug delivery devices.

B) Nanowires- Glowing silica nano wire is wraped around a single stand of human hair. It looks delicate. It is about five times smaller than virus applications for nano wires include the early sensing of breast and ovarian malignancies.

C) Nanocantilever- The honey comb mesh behind this tiny carbon cantilever is surface of fly's eye. Cantilevers are beams anchored at only one end. In nano world they function as sensors ideal for detecting the presence of extremely small molecules in biological fluid.

D) Nanoshells- Nanoshells are hollow silica spheres covered with gold. Scientists can attach antibodies to their surfaces enabling the shells to target certain shells such as cancer cells. Nano shells one day also are filled with drug containing polymers.

E) Quantum dots- Quantum dots are miniscule semiconductor particles that can serve as sign pots of certain type of cells or molecules in the body. They can do this because they emit different wavelengths of radiations depending upon the type of cadmium used in their cores.

F) Nan pores- Nanopores have cancer research and treatment applications. Engineered into particles, they are holes that are so tiny that DNA molecules can pass through them one strand at a time allowing for highly precise and efficient DNA sequencing. By engineering nanopores into surface of drug capsule that are only slightly larger than medicines molecular structure, drug manufacturers can also use nanopores to control rate of drug's diffusion in body.

G) Gold nanoparticles- These nanoparticles seen in transmission electron micrograph image, they have solid core. Researchs at north western university are using gold particles to develop ultra sensitive detection systems for DNA and protein markers associated with many forms of cancer including breast, prostate cancer.

H) Bucky balls- Bucky ball is common for a molecule called buckminsterfullerene, which is made of 60 carbon atoms formed in shape of hollow ball discovered in 1985. Bucky balls and other fullerenes because of their chemistry and their unusual hollow cage like shape extremely stable and can withstand high temperatures.

Applications-

Bucky balls may see widespread use in future products and applications, from drug delivery vehicles for cancer therapy to ultra hard coating and military harmor.
Bucky ball- Antibody combination delivers antitumor drugs.
Bucky balls to fight allergy.
Bucky balls as powerful antioxidants and also inhibitor of HIV.

Demerits:

Bucky balls hurt cells.
Bucky balls have high potential to accumulate in living tissue.

Carbon nanotubes:

Carbon Nanotubes can be modified to circulate well within the body. Such modifications can be accomplished with covalent or non covalent bonding. Modifications can increase or decrease circulation time within the body. Carbon nanotubes no significant toxicity when they have modified so as to be soluble in aqueous body type fluids. They enter readily into the cells.

Cancer cells in tumor are larger than normal cells and also exhibit leakage. Large molecules which circulate slowly can leak into and accumulate in cancer cell. Carbon Nanotubes carrying active agents have been demonstrated in animal studies to do this. Researchers have also used carbon tubes to deliver the precursors of active drug which they call a prodrug[5] eg: Cisplatin

Microspheres

Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200μm. Materials used for preparing Microspheres are polymers.

They are classified into two types:
Synthetic Polymers
Natural polymers
Synthetic polymers are divided into two types.
Non-biodegradable polymers
Poly methyl methacrylate (PMMA)
Glycidyl methacrylate
Epoxy polymers
Biodegradable polymers
Lactides, Glycolides & their co polymers
Poly alkyl cyano acrylates
Poly anhydrides

Synthetic polymers:
Poly alkyl cyano acrylates is a potential drug carrier for parenteral as well as other ophthalmic, oral preparations. Poly lactic acid is a suitable carrier for sustained release of narcotic antagonist, anti cancer agents such as cisplatin, cyclophosphamide, and doxorubicin.

Sustained release preparations for anti malarial drug as well as for many other drugs have been formulated by using of co-polymer of poly lactic acid and poly glycolic acid. Poly anhydride microspheres (40μm) have been investigated to extend the precorneal residence time for ocular delivery.

Polyadipic anhydride is used to encapsulate timolol maleate for ocular delivery. Poly acrolein microspheres are functional type of microspheres. They do not require any activation step since the surfacial free CHO groups over the poly acrolein can react with NH2 group of protein to form Schiff's base.

In case of non-biodegradable drug carriers, when administered parenterally, the carrier remaining in the body after the drug is completely released poses possibility of carrier toxicity over a long period of time. Biodegradable carriers which degrade in the body to non-toxic degradation products do not pose the problem of carrier toxicity and are more suited for parenteral applications

Natural polymers obtained from different sources like proteins, carbohydrates and chemically modified carbohydrates.
Proteins: Albumin, Gelatin, and Collagen

Carbohydrates: Agarose, Carrageenan, Chitosan, Starch

Chemically modified carbohydrates: Polydextran, Poly starch.

Natural polymers: Albumin is a widely distributed natural protein. It is considered as a potential carrier of drug or proteins (for either their site specific localization or their local application into anatomical discrete sites). It is being widely used for the targeted drug for the targeted drug delivery to the tumor cells.

Gelatin microspheres can be used as efficient carrier system capable of delivering the drug or biological response modifiers such as interferon to phagocytes. Starch belongs to carbohydrate class. It consists of principle glucopyranose unit, which on hydrolysis yields D-glucose. It being a poly saccharide consists of a large number of free OH groups. By means of these free OH groups a large number of active ingredients can be incorporated within as well as active on surface of microspheres. Chitosan is a deacylated product of chitin. The effect of chitosan has been considered because of its charge. It is insoluble at neutral and alkaline pH values, but forms salts with inorganic and organic salts. Upon dissolution, the

amino groups of chitosan get protonated, and the resultant polymer becomes positively charged[6].

Resealed Erythrocytes as Drug Carriers:

Erythrocytes, the most abundant cells in the human body, have potential carrier capabilities for the delivery of drugs. Erythrocytes are biocompatible, biodegradable, possess very long circulation half lives and can be loaded with a variety of chemically and biologically active compounds using various chemical and physical methods.

Erythro = red and cytes = cell

Erythrocyte is red cell. Erythrocyte is biconcave discs, a nucleate Filled with hemoglobin (Hb), a protein that functions in gas transport. It contains the plasma protein spectrin.

Healthy adult male=4.5millions/µml

Healthy adult female=4.8million/ µml

Immature RBC are called "RETICULOCYTES."[7]

Properties of resealed erythrocyte of novel drug delivery carriers:

1) The drug should be released at target site in a controlled manner.

2) It should be appropriate size, shape and should permit the passage through capillaries. And Minimum leakage of drug should take place.

3) It should be biocompatible and should have minimum toxic effect.

4) It should possess the ability to carry a broad spectrum of drug.

5) It should possess specific physicochemical properties by which desired target size could be recognized.

6) The degradation product of the carriers system , after release of the drug at the selected site should be biocompatible. It should be physico -chemically compatible with drug.

7) The carrier system should have an appreciable stability during storage.

Advantage:

1) They are natural part of body, so they are biodegradable in nature.

2) The entrapment of drug does not require the chemical modification of drugs

3) The entrapment of drug also does not require the chemical modification of the substance to be entrapped.

4) They are non immunogenic in action and can be targeted to disease tissue/organ..

5) They prolong the systemic activity of drug.

6) Isolation of erythrocyte is easy and larger amount of drug can be encapsulated in small volume of cells

7) They can target the drug within reticuloendothelial system.

8) They facilitate incorporation of protein and nucleic acid in eukaryotic cells by cell infusion with RBC.

Disadvantage:-

1) They have a limited potential as carrier to non-phagocyte target tissue.

2) Possibility of clumping of cells and dose dumping may be there[8].

Drug loaded Erythrocytes:

This is one of the growing and potential systems for delivery of drugs and enzymes. Erythrocytes are biocompatible, bio-degradable, posses long circulation half life and can be loaded with variety of biologically active substances. Carrier erythrocytes are preapared by collecting blood sample from the organism of interest and separating erythrocytes from the plasma. By using various physical and chemical methods cells are broken and drug is entrapped into erythrocytes, finally they are resealed and resultant carriers are then called as "resealed erythrocytes". Upon reinjection the drug loaded erythrocytes serve as slow circulation depots targets the drug to reticulo-endothelial system[9].

Niosomes:

In niosomes, the vesicles forming amphiphile is a non-ionic surfactant such as Span – 60 which is usually stabilized by addition of cholesterol and small amount of anionic surfactant such as dicetyl phosphate. Niosomes and liposomes are equiactive in drug delivery potential and both increase drug efficacy as compared with that of free drug. Niosomes are preferred over liposomes because the former exhibit high chemical stability and economy. Surfactant forming niosomes are biodegradable, non-immunogenic and biocompatible. Incorporating them into niosomes enhances the efficacy of drug, such as nimesulide, flurbiprofen, piroxicam, ketoconazole and bleomycin exhibit more bioavailability than the free drug. [10]

Comparison of Niosomes and Liposome's:

Niosomes are now widely studied as an alternative to liposome, which exhibit certain disadvantages such as –they are expensive, their ingredients like phospholipids are chemically unstable because of their predisposition to oxidative degradation, they require special storage and handling and purity of natural phospholipids is variable. Niosomes are prepared from uncharged single-chain surfactant and cholesterol whereas liposomes are prepared from double chain phospholipids (neutral or charged).

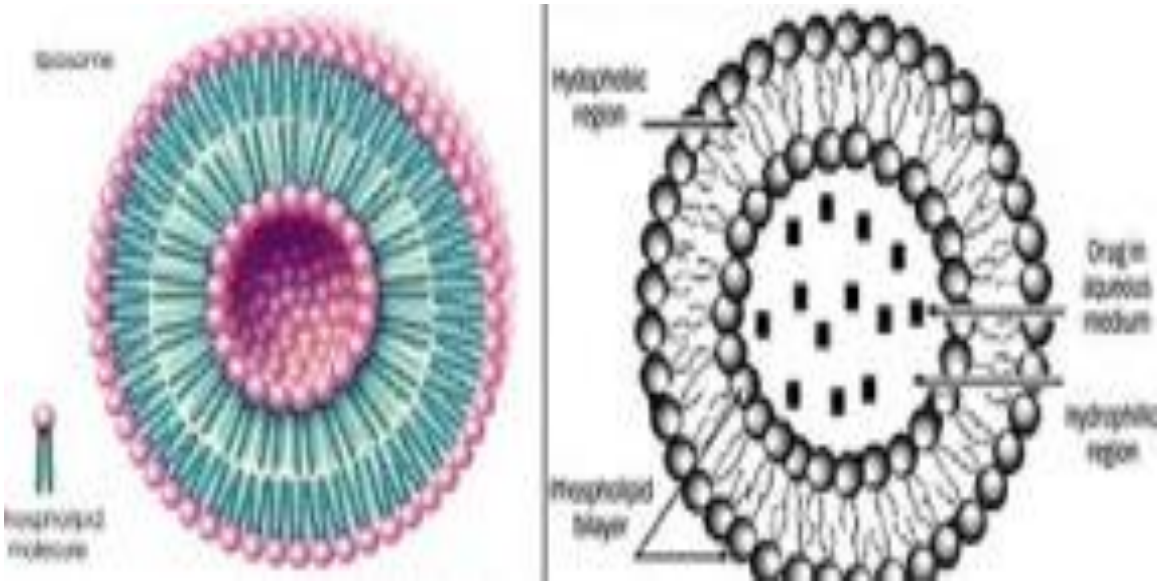
Niosomes behave in-vivo like liposome, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability.

Encapsulation of various anti neoplastic agents in these carrier vesicles has been shown to decrease drug induced toxic side effects, while maintaining, or in some instances, increasing the anti-tumor efficacy. Such vesicular drug carrier systems alter the plasma clearance kinetics, tissue distribution, metabolism and cellular interaction of the drug. They can be expected to target the drug to its desired site of action and/or to control its release.Various type of drug deliveries can be possible using niosomes like targeting, ophthalmic, topical and parenteral.

1.2 LIPOSOMES AND ITS CLASSIFICATION

The emergence of bilayered vesicles as a efficient carriers for drugs, vaccines and diagnostic agents has made the liposomes a most prominent and rapid developing novel formulation moreover the site avoidance and site specific can be acheived by the liposomes and also it can reduce the Christian toxicity in potential drugs.

Liposomes are colloidal carriers which have size ranges from 0.01microm - 0.5microm in diameter.liposomes can encapsulate both hydrophilic and lipophilic drugs.



MECHANISM OF LIPOSOME FORMATION:

The basic part of liposome is formed by phospholipids, which are amphiphilic molecules (having a hydrophilic head and hydrophobic tail). The hydrophilic part is mainly phosphoric acid bound to a water soluble molecule, whereas, the hydrophobic part consists of two fatty acid chains with 10 – 24 carbon atoms and 0 – 6 double bonds in each chain.

.

When these phospholipids are dispersed in aqueous medium, they form lamellar sheets by organizing in such a way that, the polar head group faces outwards to the aqueous region while the fatty acid groups face each other and finally form spherical/ vesicle like structures called as liposomes.

The polar portion remains in contact with aqueous region along with shielding of the non-polar part (which is oriented at an angle to the membrane surface) When phospholipids are hydrated in water, along with the input of energy like sonication, shaking, heating,homogenization, etc. it is the hydrophilic/ hydrophobic interactions between lipid – lipid, lipid – water molecules that lead to the formation of bilayered vesicles in order to achieve a thermodynamic equilibrium in the aqueous phase. The reasons for bilayered formation include:

• The unfavorable interactions created between hydrophilic and hydrophobic phase can be minimized by folding into closed concentric vesicles.

• Large bilayered vesicle formation promotes the reduction of large free energy difference present between the hydrophilic and hydrophobic environment.

• Maximum stability to supramolecular self assembled structure can be attained by forming into vesicles.

CLASSIFICATION OF LIPOSOMES:

Various classes of liposomes have been reported in literature. They are classified based on their size, number of bilayers, composition and method of preparation. Based on the size and number of bilayers, liposomes are classified as

multilamellar vesicles (MLV),
large unilamellar vesicles (LUV)
and small unilamellar vesicles (SUV).

Based on composition,they are classified as
conventional liposomes (CL),
pH-sensitive liposomes,
cationic liposomes,
long circulating liposomes (LCL) and immuno-liposomes.

Based on the method of preparation, they are classified as
reverse phase evaporation vesicles (REV),
French press vesicles (FPV) and
ether injection vesicles (EIV).

MULTILAMELLAR VESICLES (MLV)

MLV have a size greater than 0.1 μm and consist of two or more bilayers. Their method of preparation is simple, which includes thin – film hydration method or hydration of lipids in excess of organic solvent. They are mechanically stable on long storage. Due to the large size, they are cleared rapidly by the reticulo-endothelial system (RES) cells and hence can be useful for targeting the organs of RES. MLV have a moderate trapped volume, i.e., amount of aqueous volume to lipid ratio. The drug entrapment into the vesicles can be enhanced by slower rate of hydration and gentle mixing. Hydrating thin films of dry lipids can also enhance encapsulation efficiency. Subsequent lyophilization and rehydration after mixing with the aqueous phase (containing the drug) can yield MLV with 40% encapsulation efficiency.

LARGE UNILAMELLAR VESICLES (LUV)

This class of liposomes consists of a single bilayer and has a size greater than 0.1 μm. They have higher encapsulation efficiency, since they can hold a large volume of solution in their cavity. They have high trapped volume and can be useful for encapsulating hydrophilic drugs. Advantage of LUV is that less amount of lipid is required for encapsulating large quantity of drug. Similar to MLV, they are rapidly cleared by RES cells, due to their larger size. LUV can be prepared by various methods like ether injection, detergent dialysis and reverse phase evaporation techniques.

Apart from these methods, freeze thawing of liposomes, dehydration/rehydration of SUV and slow swelling of lipids in non-electrolyte solution can also be used to prepare LUV.

SMALL UNILAMELLAR VESICLES (SUV)

SUV are smaller in size (less than 0.1 μm) when compared to MLV and LUV, and have a single bilayer. They have a low entrapped aqueous volume to lipid ratio and characterized by having long circulation half life. SUV can be prepared by using solvent injection method (ethanol or ether injection methods) or alternatively by reducing the size of MLV or LUV using sonication or extrusion process under an inert atmosphere like nitrogen or Argon. The sonication can be performed using either a bath or probe type sonicator. SUV can also be achieved by passing MLV through a narrow orifice under high pressure. These SUV are susceptible to aggregation and fusion at lower or negligible/ no charge.

METHODS OF PREPARATION:

The conventional methods for preparing liposomes include solubilizing the lipids in organic solvent, drying down the lipids from organic solution, dispersion of lipids in aqueous media, purification of resultant liposomes and analysis of the final product.Of all the methods used for preparing liposomes, thin-film hydration method is the most simple and widely used one. MLV are produced by this method within a size range of 1 – 5 μm. If the drug is hydrophilic it is included in the aqueous buffer and if the drug is hydrophobic, it can be included in the lipid film. But the drawback of this method is poor encapsulation efficiency (5 – 15% only) for hydrophobic drugs. By hydrating the lipids in presence of organic solvent, the encapsulation efficiency of the MLV can be increased. LUV can be prepared by solvent injection, detergent dialysis, calcium induced fusion and revese phase evaporation techniques. SUV can be prepared by the extrusion or sonication of MLV or LUV.

All these preparation methods involve the usage of organic solvents or detergents whose presence even in minute quantities can lead to toxicity. In order to avoid this, other methods like polyol dilution, bubble method and heating method have been developed without using any organic solvents or detergent.

1.3 LIPOSOMAL MARKET FORMULATIONS

Various Liposome technologies specifically developed for the preparation of clinically used liposome-based products.

1. Stealth Liposome Technology

Stealth technology has been explored in developing a drug delivery system that makes their detection by the mononuclear phagocyte system difficult. In this technology, strands of the polymer(s) are attached to drug molecules or a system that can improve the safety and efficacy of the therapeutic agents. Generally, polyethylene glycol (PEG) is used as a polymer and the process is called PEGylation. In general, PEGylation is attained by the incubation of a reactive derivative of PEG with the target moiety. Covalent linkage of liposome to a PEG protects the active moiety from the recipient's immune system, which results in reduced immunogenicity and antigenicity. It also produces alterations in the physiochemical properties of the active moiety, including changes in the hydrodynamic size, which further reduce its renal clearance and thereby prolongs its circulatory time. Also, it provides hydrophilicity to hydrophobic drugs and reduces dosage frequency. These changes do not diminish efficacy and show reduced toxicity.[11] Furthermore, due to the leaky nature of the tumor vasculature, nano-sized formulations with prolonged circulatory time show enhanced permeation and retention (EPR) and slowly accumulate in the tumor bed. This technology provided a very successful liposome-based product namely Doxil® as an intravenous injection for the management of advanced ovarian cancer, multiple myeloma and HIV-associated Kaposi's sarcoma. This technology helps to achieve a customised dosage profile.

2. Non-PEGylated Liposome Technology

Non-PEGylated liposome (NPL) is a unique drug-delivery system that came as a breakthrough in cancer therapy by offering the benefits of PEGylated-liposome while eliminating the side effects associated with PEG such as hand-foot syndrome (HFS). NPL Doxorubicin (NPLD) injection providesa better safety profile over

conventional DOX and Doxil®. NPLD not only reduces the cardiac toxicity associated with DOX, but also the dose-limiting toxicity linked with the use of Doxil®, such as HFS.This is achieved by a combination of specific composition and a unique manufacturing process of the NPLD liposome, which gives it the desired physicochemical properties. The NPLDs have an increased circulation time and less cardiotoxicity as compared with conventional DOX. Since NPLD do not have a PEG coating, they are not associated with the painful HFS, which is a dose-limiting adverseevent with PEG-DOX [12]. Myocet® is a NPLD manufactured by Elan Pharmaceuticals, Princeton NJ, approved in Europe and Canada for the management of metastatic breast cancer in combination with cyclophosphamide.

3. DepoFoam™ Liposome Technology

DepoFoam™ is a proprietary, extended-release drug delivery technology introduced by Pacira Pharmaceuticals, Inc., Parsippany, NJ, USA. DepoFoam™ is the core technology behindseveral marketed products such as Depocyt®, DepoDur™ and Exparel®. DepoFoam™ technologyencapsulates drugs in its multivesicular liposomal platform without modification of their molecular structure. The multivesicular liposomes releases drug(s) over a required period of time ranging from 1 to 30 days. DepoFoam™ consists of microscopic spheroids (3–30 μm) with granular structureand single-layered lipid particles composed of a honeycomb of numerous nonconcentric internal aqueous chambers containing the bounded drug. Each particle contains numerous non-concentric aqueous chambers bounded by a single bilayer lipid membrane. Each chamber ispartitioned from the adjacent chambers by bilayer lipid membranes composed of synthetic analogs of naturally existing lipids (DOPC, DPPG, cholesterol, triolein etc) [13]. Upon administration, DepoFoam™ particles release the drug over a period of hours to weeks following erosion and/or reorganization of the lipid membranes. DepoFoam™ technology improved the properties of both small and large molecules. This technology considerably improved patient care by providing a remarkable solution for medications that require frequent multiple injections and have a short period of action or side effects.

4. Lysolipid Thermally Sensitive Liposome (LTSL) Technology

Thermo sensitive liposomes have been studied for drug release at sites of elevated temperature. Generally, lipids, e.g., DPPC, MSPC, with a transition temperature between 40 and 45 ◦C have been used in the preparation of these liposomes. These novel liposomes are being developed toexhibit temperature-dependent release of encapsulated drug(s). Local tissue temperature is generally elevated to 42 ◦C by radiofrequency ablation, a technique based on the application of radiofrequency. Lipid components present in the liposome undergo a gel to liquid transition at elevated temperatures, making it more permeable, and thus releasing the drug. Moreover, application of local hyperthermia causes leakage of blood vessels within tumors, thereby increasing accumulation of liposomes in the tumor. ThermoDox® of Celsion Corporation is being tested in phase III clinical trial uses LTSL(lysolipid thermally sensitive liposome) technology to encapsulate DOX for the treatment of various solid tumors. For ThermoDox®, this technology allows a 25 times greater concentration of the drug in the treatment area than intravenous (i.v.) DOX. Also, DOX concentration increases significantly in the circulation when compared to other liposomally encapsulated DOX.[14]

2. LITERATURE REVIEW

2.1 N-ACETYLCYSTEINE

N-acetylcysteine (NAC) is the acetylated derivative of the amino acid L-cysteine. NAC is precursor of L-cysteine.It is a drug as well as nutritional supplement. Historically NAC has been used as a mucolytic agent in chronic respiratory illnesses, as well as an antidote for hepatotoxicity due to acetaminophen overdose.

PHYSICO-CHEMICAL PROPERTIES

Description
White crystalline powder with slight acetic odour

Molecular formula
C5H9NO3S

Molecular mass
163.195 g mol-1

Melting point
109c to 110 c

Specific rotation
+5 (c=3% in water)

Optical rotation
Between +21 to +27

pH
2.0 to 2.8

Solubility
It is soluble in water and ethanol
It is practically insoluble in ether and chloroform.

Loss on Drying
Not more than 1%

Residue on ignition
Not more than 0.5%

Assay
NLT 98.0% and NMT 102%.

USES
The various uses of NAC are as follows
As Mucolytic agent,
As Antioxidant[15,16],
In PCOS[17,18],
In Preterm birth[19],
In Acetaminophen toxicity[20],
In Ulcerative colitis,
In Male infertility,
In Chronic bronchitis,
In liver cancer,
In muscle Fatigue[21],
In Hemodialysis,
In Parkinson's disease[22],
In asthma and
In Alzheimer's disease.

Biochemistry and Pharmacokinetics
NAC is a sulfhydryl-containing compound rapidly absorbed into various tissues following an oral dose, deacetylated and metabolized in the intestines and liver, and its metabolites incorporated into proteins and peptides. Peak plasma levels of NAC occur approximately one hour after an oral dose; at 12 hours post-dose it is undetectable in plasma. Despite a relatively low bioavailability of only 4-10 percent, oral administration of NAC appears to be clinically effective[23].

The biological activity of NAC is attributed to its sulfhydryl group, while its acetyl substituted amino group affords it protection against oxidative and metabolic processes[24-25].NAC administration is an effective method of increasing plasma glutathione (GSH) levels, as incorporation of cysteine into GSH appears to be the rate-limiting step in GSH synthesis.

MECHANISMS OF ACTION

NAC's effectiveness is primarily attributed to its ability to reduce extracellular cystine to cysteine, and as a source of sulfhydryl groups. NAC stimulates glutathione synthesis, enhances glutathione-S-transferase activity, promotes liver detoxification by inhibiting xenobiotic biotransformation, and is a powerful nucleophile capable of scavenging free radicals[26-27.] NAC's effectiveness as a mucolytic agent results from its sulfhydryl group interacting with disulfide bonds in mucoproteins, with mucus subsequently being broken into smaller, less viscous units.NAC may also act as an expectorant by stimulating ciliary action and the gastro-pulmonary vagal reflex, thereby clearing mucus from the airways[28].
Studies have also shown NAC to be of benefit in heart disease by lowering homocysteine and lipoprotein (a) levels via dissociation of disulfide bonds[29-30], protecting against ischemia and reperfusion damage via replenishment of the glutathione redox system31, as well as potentiating the activity of nitroglycerin.[32]

Side Effects and Toxicity

NAC is generally safe and well tolerated even at high doses. The most common side effects associated with high oral doses are nausea, vomiting, and other gastrointestinal disturbances; therefore, oral administration is contraindicated in persons with active peptic ulcer. Infrequently, anaphylactic reactions due to histamine release occur and can consist of rash, pruritis, angioedema, bronchospasm, tachycardia, and changes in blood pressure. Intravenous administration has, in rare instances, caused allergic reactions generally in the form of rash or angioedema[33].NAC is "Ames test" negative, but animal studies on embryotoxicity are equivocal. In addition, studies in pregnant women are inadequate; therefore, NAC should be used with caution during pregnancy, and only if clearly indicated.[34] Oral administration of NAC and charcoal at the same time is not recommended, as charcoal may cause a reduction in the absorption of NAC.[35]

In addition, as with any single antioxidant nutrient, NAC at therapeutic doses (even as low as 1.2 grams daily) has the potential to have pro-oxidant activity and is not recommended at these doses in the absence of significant oxidative stress.

Dosage

The typical oral dose for NAC as a mucolytic agent and for most other clinical indications is 600-1,500 mg daily in three divided doses. In patients with cancer or heart disease the therapeutic dosage is higher, usually in the range of 2-4 grams daily. For acetaminophen poisoning, NAC is administered orally with a loading dose of 140 mg/kg and 17 subsequent doses of 70 mg/kg every four hours. In acetaminophen poisoning it is important to begin administering NAC within 8-10 hours of overdose to ensure effectiveness.

Warnings and Contraindications

NAC may have a protective effect on normal tissue in individuals utilizing many cancer chemotherapeutic agents however, two studies noted that NAC inhibits cytotoxicity of the cancer chemotherapy drug cisplatin and an animal study suggests NAC might reduce the anti-neoplastic action of doxorubicin. These combinations should be avoided unless further information recommends otherwise.

2.2 BIOENHANCERS

Bioavailability is the rate and extent to which a therapeutically active substance enters systemic circulation and becomes available at the required site of action. Intravenous drugs attain maximum bioavailability, while oral administration yields a reduced percentage due to incomplete drug absorption and first-pass metabolism. Methods of increasing bioavailability of a drug, correspondingly increase levels in the bloodstream,and thus the efficacy, which in turn reduces the drug dosage required to achieve a given therapeutic effect. Until now, methods of increasing drug bioavailability have operated within a narrow manipulative framework, mainly based on physical processes including micronization, deaggregation of micronized molecules, timed/site release preparations, solubilization of active drug and polymorphic/crystal form selection and nanotechnology (nanotechnology is at the experimental stage so it is a promising future method).A bioenhancer is an agent capable of enhancing bioavailability and bioefficacy of a particular drug with which it is combined, without any typical pharmacological activity of its own at the dose used.

Concept of Bioavailability Enhancers

The concept of bioavailability enhancer is derived from traditional old age Ayurveda black pepper; long pepper and ginger are collectively called as Trikatu.

In Sanskrit
Trikatu means Three acrids.

The action of bioavailability enhancer was first discovered by Bose in 1929 who described the action of long pepper to adhatoda vasaka leaves which increased activity of vasaka. The term bioavailability enhancer was first coined by Indian scientist at Regional Research lab. Jammu, who discovered and named piperine as world's first bioavailability enhancer in 1979.36It offers comfortable, convenient, and noninvasive way to administer drugs due to following advantages are

a) Dose reduction
b) Minimization of drug resistance.
c) Minimization of drug (especially true in case of anticancer drug like taxol).

d)Ecological benefit.
e) Safety of the environment.

Ideal Properties of the Bioenhancers

The contribution of bioenhancers have been reviewed which states that the ideal bioenhancers.

a) Should be nontoxic, non-allergenic and non-irritating.
b) Should not produce own pharmacological effects.
c) Should be rapid-acting with predictable and reproducible activity
d) Should be unidirectional in action.
e) Should be compatible with other active pharmaceutical ingredients.
f) Should be stable with time and environment.
g) Should be easily formulated into a various dosage form.
h) Should be easily available and cost effective.

2.2.1 QUERCETIN

Chemical and physical data

Nomenclature:

Chemical Name: 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one

IUPAC Systematic Name:

3,3′,4′,5,7-Pentahydroxyflavone

Synonyms:

CI 75670; CI Natural Yellow 10; 3,3′,4′,5,7-pentahydroxyflavone; 3,4′,5,5′,7-pentahydroxyflavone; 3,5,7,3′,4′-pentahydroxyflavone; quercetine

Structural and molecular formula:

$C_{15}H_{10}O_7$

Relative molecular mass:

302.24

Chemical and physical properties

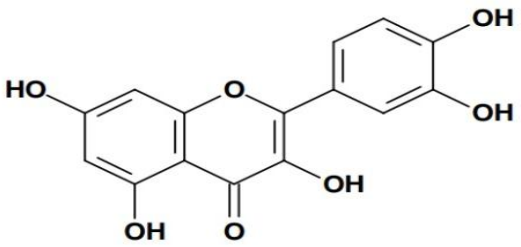
(a) Description: Pale-yellow needles (dihydrate)

(b) Boiling-point: Sublimes

(c) Melting-point: 316.5°C

(d) Solubility: Slightly soluble in water and diethyl ether; soluble in ethanol and acetone

STRUCTURE



Quercetin is a plant pigment,abundantly occurs in many ethnic plants,especially onion and tea, therefore, a sufficient amount may be consumed daily. [37]Quercetin has importance in terms of ethnopharmacology such as its use as antioxidant, anticancer and neuroprotective. [38]It has been reported as an efficient free radical scavenger (antioxidant).[39]

In clinical trials (phase-I), quercetin has been reported to exhibit inhibitory effect on tyrosine kinase which suggests that it has antitumor therapeutic potentials.[40]

Quercetin has increased bioavailability of so many compounds hence it can be used as a bioenhancer.Since it is a good per oral agent and cost effective compound it is selected for the study.

2.3 EXCIPIENTS

1.LECITHIN

Description

Yellowish powder or granule in oil free form and Brown viscous liquid in oily form.Lecithin was first isolated in 1845 by the French chemist and pharmacist Theodore Gobley.

IUPAC
[(2~{R})-3-hexadecanoyloxy-2-[(9~{Z},12~{Z})-octadeca-9,12-dienoyl]oxypropyl] 2-(trimethylazaniumyl)ethyl phosphate

chemical name

soybean lecithin

PLPC

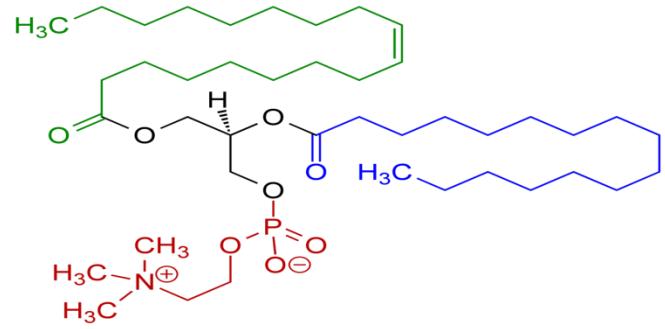
Molecular Formula

c42 H80no8p

Molecular weight

758.075g/mol

STRUCTURE:



Soybean lecithin is a complex mixture containing 65-75% phospholipids together with triglycerides and smaller amounts of other substances. The major phospholipids include Phosphatidylcholine, Phosphatidylethanolamine and inositol-containing phosphatides. Other substances reported include carbohydrates, pigments, sterols and sterol glycosides.

The major components of soybean-derived lecithin are:
33–35% Soybean oil
20–21% Phosphatidylinositols
19–21% Phosphatidylcholine
8–20% Phosphatidylethanolamine
5–11% Other phosphatides
5% Free carbohydrates
2–5% Sterols
1% Moisture

It has low solubility in water, but is an excellent emulsifier. In aqueous solution, its phospholipids can form liposomes, bilayers sheets, micelles, or lamellar structures, depending on hydration and temperature. This results in a type of surfactant that usually is classified as amphipathic.

2. CHOLESTEROL

Description:

White or faintly yellow pearly granules or crystals

Molecular formula:

C27h46o

Molecular weight:

386.664g/mol

Chemical names:

Cholesterol, cholesterin, cholesteryl alcohol.

IUPAC:

(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol.

Boiling point:

360 c

Melting point:

148.5 c

Solubility:

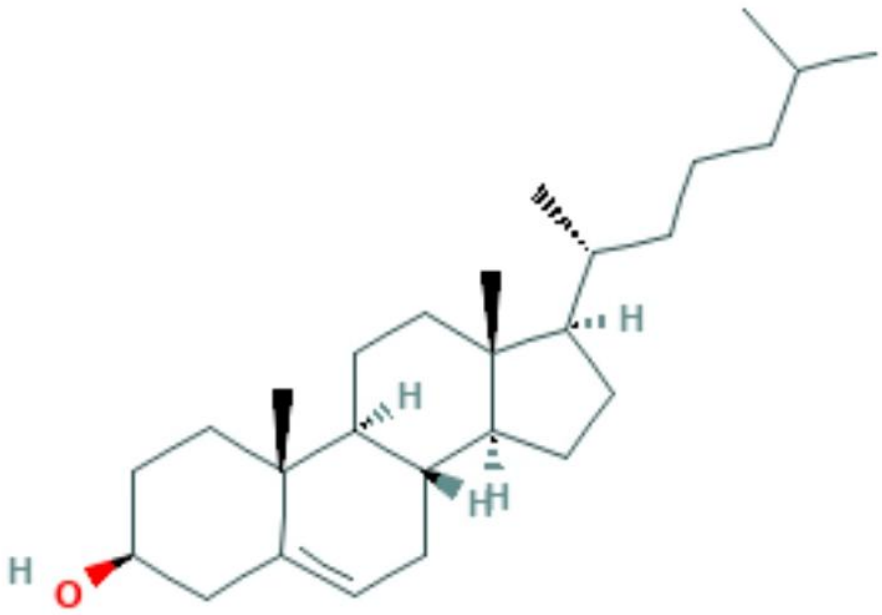
Moderately soluble in hot alcohol,

Soluble in benzene,oils, fats and aqueous solution of bile salts

Optical rotation:

-34 to 38 deg

STRUCTURE



3. RESEARCH ENVISAGED AND PLAN OF WORK

3.1 RATIONALE OF THE WORK

By the complete understanding of the literature's it is clear that the N-acetyl cysteine is an excellent drug with many therapeutic advantage but it has very poor bioavailability which makes its dose higher as much as 600mg to 1200mg Hence there arises a need for the development of a novel drug delivery with enhanced bioavailability and cost effective by reducing the dose required.

The novel formulation can be used to increase the bioavailability one such method is liposomal drug delivery system, since liposomes are cost effective and well established in market it has been chosen for the study

The Bioenhancers are the one which can be used to reduce the dose of the drug since they will increase the biosorption of the drug thereby ensures effective therapeutic effect at a reduced dose. Quercetin is one such bio enhancing agent which is readily available and very cost effective and it has no interaction with the drug it is chosen for the study

Thus the rationale of the study is to

Liposomal drug delivery has been reported to increase the half life and improve the biodistribution of N-acetylcysteine.[51-53]

Increase the bioavailability and reduce the dose of the drug N-acetylcysteine using bioenhancers Quercetin in the novel liposomal formulation.

3.2 AIM AND OBJECTIVE OF STUDY

The formulation and development of liposomal formulation which increases the bioavailability and reduces the dose of the drug N-acetylcysteinecysteine.
This research works formulation aims at providing a better bioavailability to the drug which has poor bioavailability-10% in oral route. Hence formulation technique with bio enhancing method is used.

Liposomal formulation of N-acetylcysteine is made with prior studying of few literature's which states there is increase in the bioavailability of the drug to some extent (15% - 20% increase) Hence this work aims at increasing more the bioavailability and also to reduce the dose using the Bioenhancers, which are proven to improve the absorption of the drug is chose one such compound is Quercetin which is an excellent bioavailability enhancer.

Thus the aim and objective of the study is to prove that

Drug +Liposomal formulation + Bioenhancers=Increased bioavailability of the drug

(i.e)

N-acetylcysteine + Quercetin in liposomal formulation will have increased bioavailability of the drug N-acetylcysteine.

3.3 PLAN OF WORK

The plan of the work is to ensure that the liposomal formulation of Drug crowned with Quercetin with stability and it will improve the bioavailability of the Drug.

At first the drug with low bioavailability is searched with various literature and found N-acetylcysteine has low bioavailability and very wide range of therapeutic effect hence it is chosen for the study

Then form the literature it is found that Quercetin is good and safe bioenhancers and cost effective also hence it is chosen.
Then with the help of various articles the liposomal formulation which has increased the bioavailability of various low bioavailable drugs is chosen for the formulation, then the formulation is processed.

After the Formulation, evaluation of Drug N-acetylcysteine with Quercetin the bioenhancers is made to ensure the formulation stability and also its compatibity.

4.EXPERIMENTAL WORK

4.1 PREFORMULATION WORK

A complete Preformulation study is essential for better understanding of the various physico-chemical properties of the N-Acetyl Cysteine and its interaction with various excipients used. The choice of excipients is key in development of a dosage form. It forms the first and foremost step in the development of a robust dosage form that can outweigh the rigors of processing and shelf life. Preformulation proves to be a cost-reducing process in the long run, by reducing most of the challenges during formulation development and evaluation.

Preformulation studies include:

1. Authentication of drug
2. Evaluation of physico-chemical properties
3. Evaluation of micromeritic properties
4. Drug-excipient compatibility study

1. Authentication of drug:

a) UV spectrum of drug:

At first Weigh accurately 10 mg of N-Acetylcysteine and transfer it a to 100 ml volumetric flask. The drug was then dissolved in small volume of water and the volume was made upto 100 ml to obtain a stock solution of 100 µg/ml. 1 ml of the above stock solution was again diluted with water upto 10 ml to obtain a stock solution of 10 µg/ml. The resulting solution was then scanned between 200 nm to 400 nm in a double beam UV/ Visible spectrophotometer.

b) Melting point:

The melting points of the drug sample were determined in open capillary method. Drug was filled up to 4-5 mm in glass capillaries and the one end was sealed by flame. The drug filled capillary was then dipped in liquid paraffin inside the melting point apparatus equipped with magnetic stirer for uniform heat transfer. Melting point temperature range was noted from when the drug just starts to melt till it completely melts. The experiment was done in triplicate and the average was noted.

2. Evaluation of physico-chemical properties:

a) pH:

The pH of 1% solution of N-Acetyl Cysteine was determined by digital pH meter.

b) Loss on drying (LOD):

Drug Sample was weighed 1 gm and kept for checking the loss on drying on LOD apparatus Sartorius-MA45 at 105°C for 3 min. Percentage loss of moisture is determined.

3. Evaluation of micromeritic properties:

The flow properties of the drug was examined the rheological studies are performed under normal Room temperature.

The Hausner's ratio is used to find the flow property of the drug.

4. Drug-excipient compatibility study:

Excipients are vital part of any formulation and it is necessary to ensure its suitability with the drug of choice. Drug sample and excipients were subjected together in a definite proportion and in predetermined conditions, show physical and chemical changes in case of incompatibility. There are different tests to evaluate this incompatibility. N-Acetyl Cysteine with the excipients is taken in the different ratio and is kept in two different conditions (wet and dry) for the study.

The FT-IR and DSC studies are performed to check the incongruity between the drug and the excipients to be used in the formulation.

Differential Scanning Calorimetry:

Drug Samples (2-5 mg) were weighed and transferred to hermitically sealed in flat-bottomed aluminum pans. Samples of individual substances as well as 1 : 1 physical mixtures of acetylcysteine and excipients, prepared by grinding in a mortar with a pestle, were analyzed. DSC analyses were carried out with a Perkin Elmer DSC 4 Thermal Analyser.Thermograms were obtained by heating over the temperature range between 25- 200°C in nitrogen atmosphere (40 mL min-I) at a constant heating rate of 10 K min-I.

4.2 FORMULATION OF LIPOSOMES

After the Preformulation studies the formulation work is initiated.

MATERIAL AND METHODS:

Methanol, Chloroform, deionized water, Potassium dihydrogen Phosphate, Disodium hydrogen Phosphate, N-acetylcysteine, polysorbate 80, Quercetin,Cholesterol and soya lecithin(phospholipids).

PREPARATION OF PHOSPHATE BUFFER SOLUTION pH 7.4:

First Dissolve 28.80gm disodium hydrogen phosphate & 11.45gm of potassium dihydrogen phosphate in distilled water in the 1000 ml volumetric flask, make up the volume up to 1000 ml & maintain to the pH 7.4 (Indian Pharmacopoeia 1996).

4.2.1 PREPARATION OF N-ACETYLCYSTEINE LOADED LIPOSOMES

Reverse Phase Evaporation method:

In this method the phospholipid 1.2 g and cholesterol 0.06 g were solubilized in 40 ml of chloroform with help of ultrasonication for 5 min. Then aliquot of 4ml of aqueous solution of 1 mg/ml of N-acetylcysteine and 0.8% (w/v) of polysorbate 80 are mixed in phosphate buffer 7.4 which results in water in oil emulsion.
Then this water in oil emulsion is sonicated for 5 min which resulted in reverse Phase micelles. Then the organic solvent was evaporated at 25 c which results in organogel.The organogel then was reversed to vesicles by addition of 96 ml of aqueous solution containing 1 mg/ml of N acetylcysteine and 0.8%(w/v)of polysorbate 80 under agitation 280 RPM for 30 min in rotary evaporator at normal atmospheric pressure. Then the vesicles are homogenized and passed through series of membrane filters and the N acetylcysteine loaded liposomes are formulated.

A blank is also prepared as same as above procedure without the addition of N-acetylcysteine.

4.2.2 FORMULATION OF QUERCETIN CROWNED WITH N-ACETYLCYSTEINE LIPOSOMES:

In this method the phospholipid 1.2 g and cholesterol 0.06 g were solubilized in 40 ml of chloroform with aid of ultrasonication for 5 min.Then aliquot of 4ml of aqueous solution of 1 mg/ml of N-acetylcysteine and 0.8%(w/v) of polysorbate 80 are mixed in phosphate buffer 7.4 containing 0.3 mg of Quercetin which results in water in oil emulsion.
This water in oil emulsion is sonicated for 5 min which resulted in reverse Phase micelles. Then the organic solvent was evaporated at 25 c which results in organogel.The organogel then was reversed to vesicles by addition of 96 ml of aqueous solution containing 1 mg/ml of N acetylcysteine and 0.8%(w/v)of polysorbate 80 under agitation 280 RPM for 30 min in rotary evaporator at normal atmospheric pressure. Then the vesicles are homogenized and passed through membrane filters and the N acetylcysteine loaded liposomes crowned with Quercetin are formulated.

4.3 EVALUATION STUDIES

The formulations are evaluated using the following methods,

PARTICLE SIZE:

The particle size of the prepared Quercetin crowned N-Acetyl cysteine loaded liposomes (QNAC-Lip) and blank liposomes (B-lip) were analyzed for particle size distributions by LD and DLS method, considering the numberweight-mean (D3.0). And TEM also used to clearly know about the particles nature.

PARTICLE CHARGE:

Particle zeta potential (surface charge, mV) were measured using dynamic light scattering (DLS) technique on a Zetasizer (Nano-ZS) at the room temperature.

Method to Determine the drug loading and Entrapment efficiency:

The Entrapment efficacy of NAC in liposomes was determined by HPLC method. The HPLC apparatus was equipped with a Smart line HPLC Pump 1000, a PDA Detector 2800 (set at 210 nm). Each sample (20 µL) was injected through a Smart line auto sampler and data were obtained and processed with ChromGate software. Then the Chromatographic separation was performed on a C18 reverse-phase column, 4.6 mm × 25 cm, using an isocratic mobile phase of methanol/water at gradient ratios of 10/90 to 50/50 in 15 min (pH was adjusted to 3.4 with glacial acetic acid). The retention time of NAC (at a flow rate of 1 mL.min-1) was 12 min. Standard curve of NAC was determined from NAC solutions in the range of 5–500µg/ml and concentration of unknown samples was calculated from their standard curves.Then, 5 mg of the prepared formulations was dissolved in 1 mL of methylene chloride and 2 mL of deionized water was added and stirred for 5 min to extract NAC from the preparation. The aqueous phase containing NAC was diluted suitably and analyzed using HPLC.

DETERMINATION OF ENCAPSULATION EFFICIENCY

Encapsulation efficiency of the liposomes was measured from the percentage of the amount of NAC encapsulated to the total amount of NAC initially added. The encapsulated drug amount was calculated by subtracting the NAC amount quantified by HPLC from the amount of drug initially added.

DETERMINATION OF DRUG LOADING

Drug loading was determined from the ratio of the encapsulated NAC amount to the whole weight of NAC-loaded liposomes.

STABILITY TEST

A) Differential scanning calorimetric (DSC) studies

Accurately weighed sample of drug (2 mg) were transferred to aluminum pans and sealed. All samples were run at a heating rate of 10o C/min over a temperature range 40-43oC using Shimadzu DSC-60 Thermal Analyzer.

B) Fourier transmission infrared (FTIR) spectroscopy of drug:

The Infrared spectroscopy of the sample was carried out to ascertain identity of the drugs. A pellet of approximately 1 mm diameter of each drug was prepared by compressing 3-5 mg of the drug with 100-150 mg of potassium bromide in KBr press. The pellet was mounted in IR compartment and IR spectrum of N-Acetyl Cysteine Were recorded at a resolution of 4cm-1 over a range of 4000-400 cm -1 using a Shimadzu Model 8400 FTIR. Nitrogen gas was purged at the rate of 50 ml/min to maintain inert atmosphere.

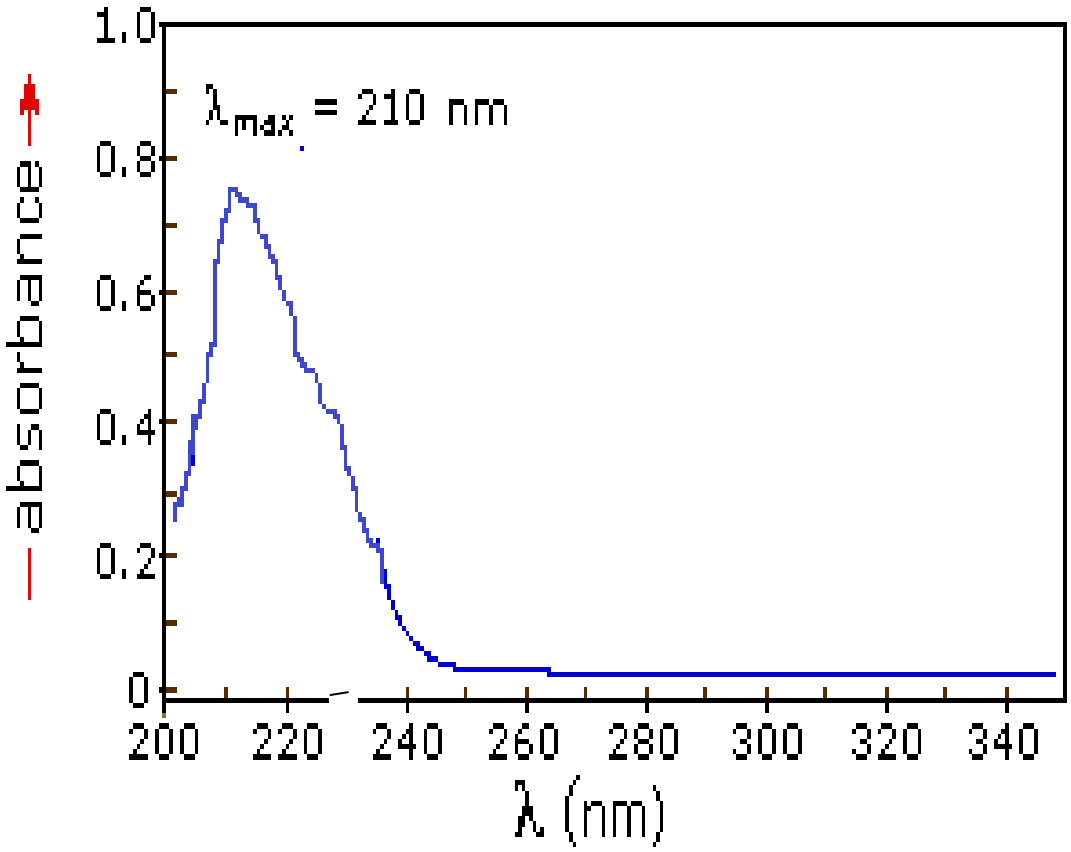
5. RESULT AND DISCUSSION

5.1 PREFORMULATION WORK RESULTS:

Authentication of drug:-

A) UV spectrum of drug:

The solution of N-Acetyl Cysteine in water was found to have a λ max of 205nm. In the literature, λ max is reported as 205-210 nm. So the given sample complies with standard.



B)Melting point

The melting point of the sample was found 109^0 cto be and from the literature's it is found to be same as standard .

2. Evaluation of physico-chemical properties:

A) pH:

The pH of 1% solution of N-Acetyl Cysteine was found to be 2.19 and it is accordance with the standard.

B)Loss on Drying

The LOD of the sample was found to be 0.14% and it also confirms with that of the standard.

3.Evaluation of micrometric properties

The flow property of N-acetylcysteine was founded and it was Poor flowing according to Hausner's ratio.

4.Drug excipient study

Observations were made after 7, 15, 21 and 30 days. No changes were observed in samples kept at 25 ± 3°C / 60 ± 5% RH and 40 ± 2 °C /75 ± 5% RH.
This shows all the excipients were stable and no incompatibility were seen.

5.2 FORMULATION RESULTS :

5.2.1 Formation of N-acetylcysteine loaded liposomes:

The reverse Phase Evaporation technique produced N-acetylcysteine loaded liposomes and blank liposomes. The liposomes formed are stable and doesn't had any aggregation or lumps on storing at less than 20^0 c.

5.2.2Formation of Quercetin crowned N-acetylcysteine loaded liposomes:
The reverse Phase Evaporation technique was used and it produced Quercetin crowned N-acetylcysteine loaded liposomes. The liposomes formed are stable and doesn't had any aggregation or lumps on storing at less than 20^0 c.

5.3 EVALUATION RESULTS:

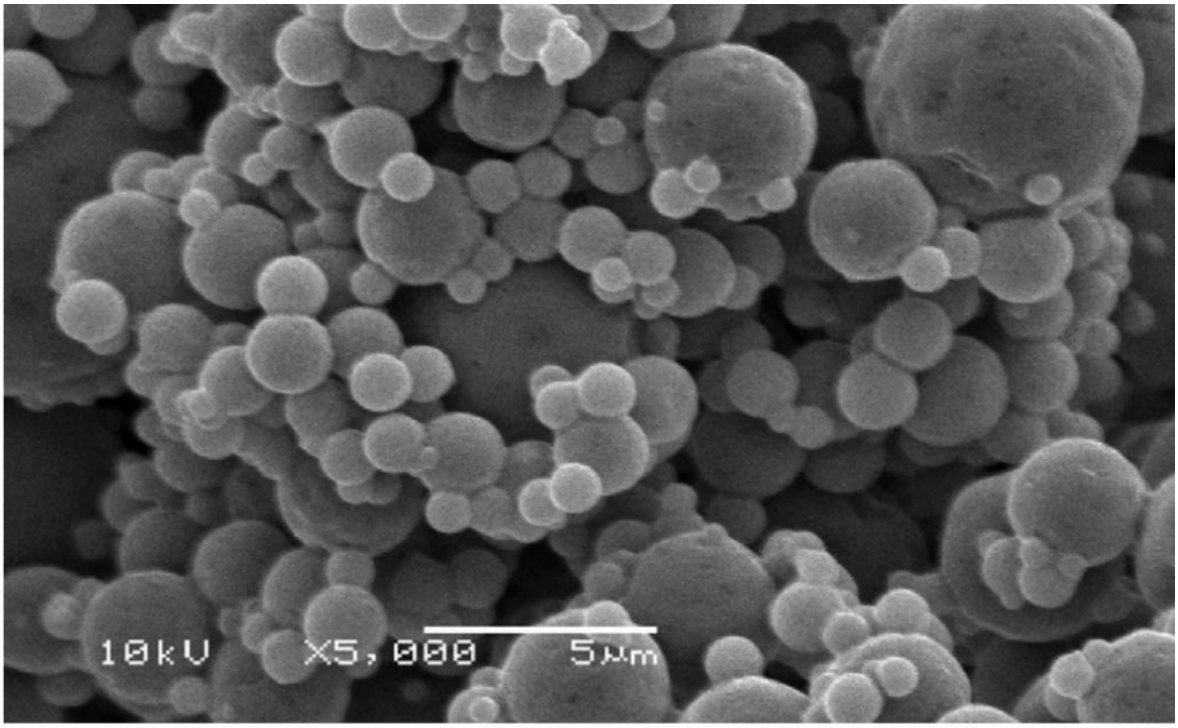
Particle size:
The particle size was determined using LD and DLS and the results are as follows.

Technique	Formulation	Results
Laser Diffraction	QNAC-lip B-lip	117+_05 111+_07
DLS	QNAC-lip B-lip	79+_22 72+_06

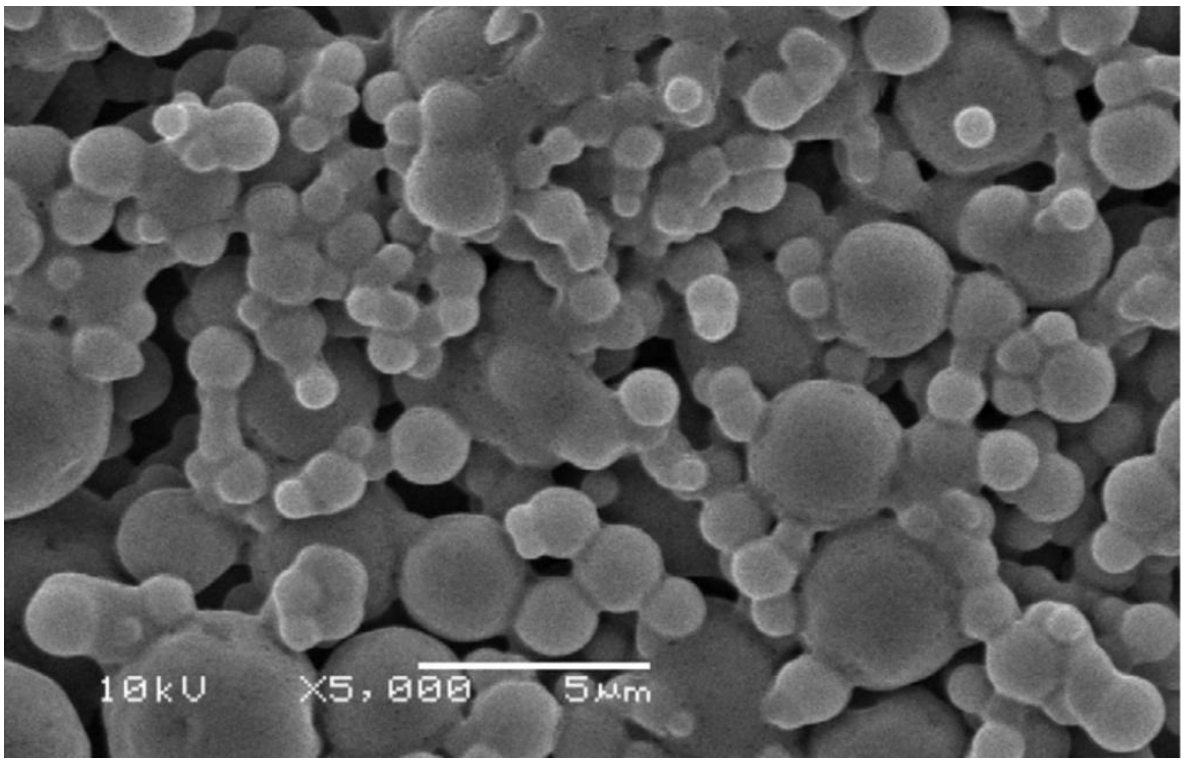
SPAN values calculated by laser diffraction were lower than 1.0 for both formulations (0.89 ± 0.12 for QNAC-Lip and 0.82 ± 0.01 for B-Lip), indicating good homogeneity in terms of particle size distribution of these systems.

TRANSMISSION ELECTRON MICROSCOPY:
TEM micrograph of liposomes shows lamellae. TEM show that the combination of Phosphatidylcholine and a non-ionic surfactant, like polysorbate 80, leads to formation of unilamellar phospholipids vesicles. Furthermore, in order to check if it was possible to visualize the nanometric structures after aqueous redispersion, morphological analysis of redispersion were performed by TEM. The results demonstrate the presence of nanometric structures, probably primary liposomes in B-Lip and Q-NAC-Lip.

B-LIPOSOMES



Q-NAC LIPOMES



Particle charge

The zetasizer used to find the particle surface charges and it was found to be

Entrapment efficiency

The entrapment efficiency of the formulated Q-NAC liposomes was found to be 40%±6

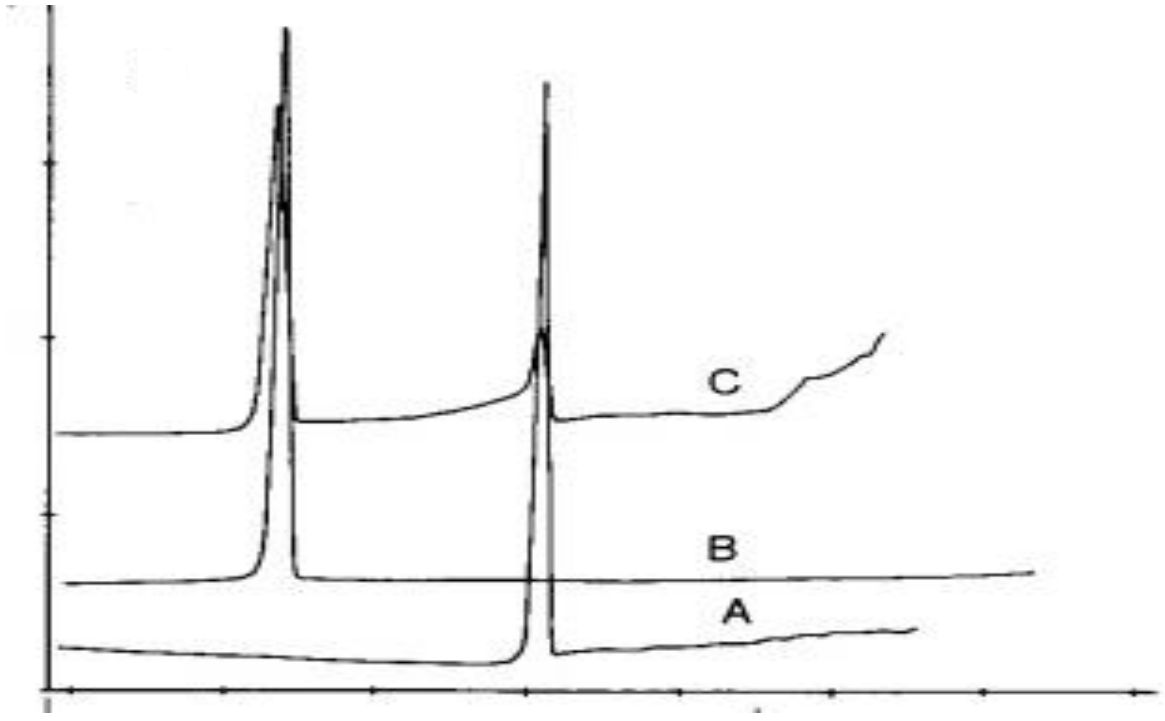
Drug content

The Drug content of the prepared formulation was found to be 0.95 ±0.03 mg mL1 of NAC was found in QNAC-Lip.

STABILITY TEST AND AST:

A)DSC

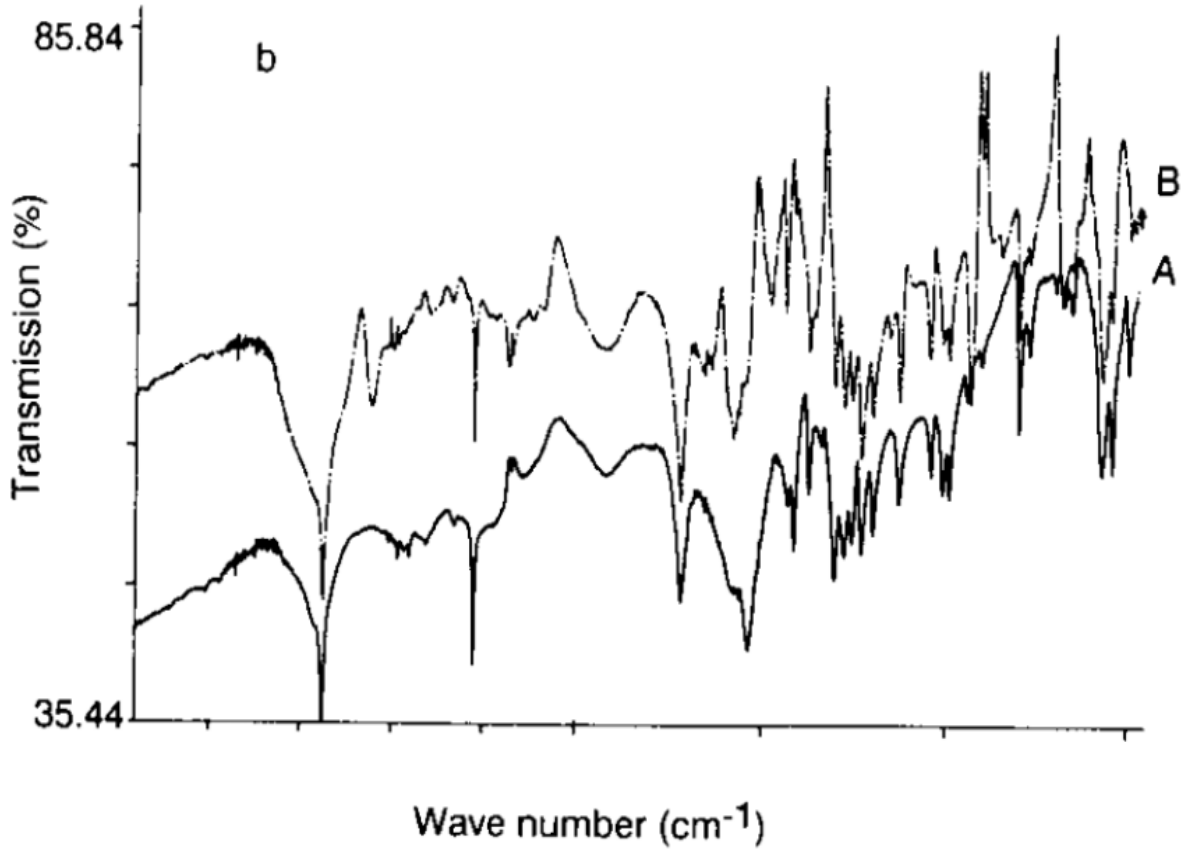
In differential scanning calorimetry no changes in the temperature was seen hence the formulation appears to be stable and ingredients are compatible.



DSC showing the A) N-acetylcysteine, B) Excipient,C)Drug excipients mixture

B)FT-IR

The FT-IR analysis clearly shows that the prepared formulation is highly stable and also confirms the purity and authentication of the drug and excipients.



Ft-Ir Spectrum Of N-Acetylcysteine(A) And Differential Spectrum Of Drug With Excipient (B).

ACCELERATED STABILITY TEST:

After 3 months of accelerated stability studies, optimized formulation did not show any substantial change in physical characteristics, Drug content and drug release. Thus the developed formulation was found to be stable.

6.SUMMARY AND CONCLUSION

The formulation and evaluation of the prepared Quercetin crowned N-acetyl Cysteine liposomes shows that there is no incongruity between drug and other ingredients used in the preparation of the formulation.
The study also the reverse phase evaporation method is very suitable for liposomal formulation which is more effective in our study compared to the literature.
The evaluation shows that the formed liposomes are unilamellar structures.

The entrapment and loading of the drug was better in the method compared to the previous study. The AST results also suggested that the formulation is highly stable.

The evaluation studies clearly prove that the formed liposomes are well formulated with crowned by Quercetin on the N-acetylcysteine liposome. Further the study has to be conducted for in-vivo studies to find the bioavailability, pharmacokinetic, Drug interactions (if any) and its therapeutic efficacy.

CONCLUSION

The formulation shows a newer alternative option in the novel drug delivery combined with the bioenhancing technique which will more effective then the single technique of either bioenhancing or novel formulation
This type of techniques will be very useful for low bioavailable drugs which are having larger dose for their therapeutic effect and it also proves to be a very dose effective in terms of N-acetylcysteine.

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