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Hesperidin Loaded Liposomes for the Treatment of Diabetes and Hypertension

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Abstract

Antimicrobial agents, cancer treatments, diabetes drugs, hypertension drugs, antifungal drugs, peptide hormones, enzymes, vaccines, and genetic materials are just some of the many drugs that Hesperidin loaded Liposomes (HLLs) have been shown to improve the delivery of in recent years. Liposomes can be broken down further into subgroups based on lamellarity, size, charge, and function due to variations in preparation processes and lipid compositions. They can be used for drug delivery via several routes of administration thanks to their adaptable behaviour, which is not dependent on their solubility. Liposomes loaded with hesperidin have the ability to target a chemical to specific tissues, potentially improving the therapeutic efficacy of several drugs. Medications' in vitro and in vivo efficacy can both be boosted by a drug delivery technology called hesperidin-loaded liposomes it can also decrease its toxicity, and increase its efficacy by delivering the molecule in a more regulated fashion. This article discusses analytical methods for managing physical, chemical, and biological characteristics in the production of various drugs, as well as ways for creating hesperidin-loaded liposomes. The main characteristics of the formation and manufacturing processes of liposome nanocarriers are covered in this article, with a focus on the structural characteristics and crucial factors that govern the development of acceptable and stable formulations. We detail the primary benefits (and drawbacks) of each method, as well as their suitability for mass industrial manufacturing.

Keywords: *HLLs, liposome nanocarriers, microviscosity, Lyophilization, thermolabile ect.*

1. Introduction

For the drug to have the desired impact, the ideal delivery method would introduce it gradually throughout the body over a certain amount of time. Cancer and fungal disease treatments, for example, often involve the use of drugs that are extremely hazardous to healthy tissue.[1] Reducing the amount of medication that reaches healthy cells and tissues could help reduce its toxicity. It has long been believed that liposomes provide a highly accurate representation of cell membranes. Liposomes are small vesicles with a spherical shape and an aqueous centre that are formed of one or more lipid bilayers. Population vesicles, which can range in size, are produced when lipids are disseminated in an aqueous medium and the mixture is then stirred.[2]. The head is hydrophilic, meaning it prefers the presence of water, while the tail is hydrophobic due to its composition as a lengthy hydrocarbon chain [3-4]. The main part of liposomes, phospholipids, easily combine with the lipids in the skin. This makes it easier for drugs to get into and target specific areas of the skin. Liposomes are made more stable and less permeable thanks to the cholesterol integrated into the lipid membrane. Cholesterol's characteristics cause the bilayer's microviscosity to increase and its fluidity to decrease. [5] .

Liposomes can encapsulate medications of widely varied lipophilicity once they have transitioned between the aqueous and lipid phases [6]. Positive effects of using liposomes as drug delivery systems include their biodegradability and non-toxicity, adaptability (they can deliver both hydrophilic and lipophilic drugs), increased efficacy and therapeutic index, improved medication stability due to encapsulation (it can't be broken down by enzymes in circulation), and decreased side effects [7]. Many other methods, including intravenous, oral, nasal, intramuscular, pulmonary, topical, and ophthalmic, can be used to administer the medications contained in liposomes [8]. Creams, ointments, capsules, solutions, sprays, etc. are only some of the vesicles that can be used to transport these substances. Many different types of cancer can be treated with liposomes, including bacterial, fungal, and ocular cancer; arthritis; asthma; diabetes; immune system illnesses; herpes; pain relief drugs and topical anaesthesia. Liposomes can be broken down into several different groups according to their composition, process, structural characteristics, and potential medical, cosmetic, or biological uses. Both naturally occurring and artificial phospholipids can be used to create liposomes [9]. There are several different types of liposomes, including those that are pH-sensitive, cationic, immune, temperature- or heat-sensitive, magnetic, and sterically stabilised, also known as "stealth" liposomes. SUVs typically measure less than 50 nanometer in length, while LUVs are typically longer than 50 nanometer. Liposome targeting can be either passive or aggressive. As a result of this phagocytosis, the captured medication is passively incorporated into the target organ. Active targeting calls for immunoliposome synthesis prior to tissue-specific interaction [10]. Fusion, changes in pH and temperature, and other mechanisms can be used to release the medicines from the capsules [11]. Liposomal formulations have a variable rate of release, depending on the medication. Drug retention in the liposomes for several hours after injection is necessary to realise the therapeutic effects of the formulation [12].

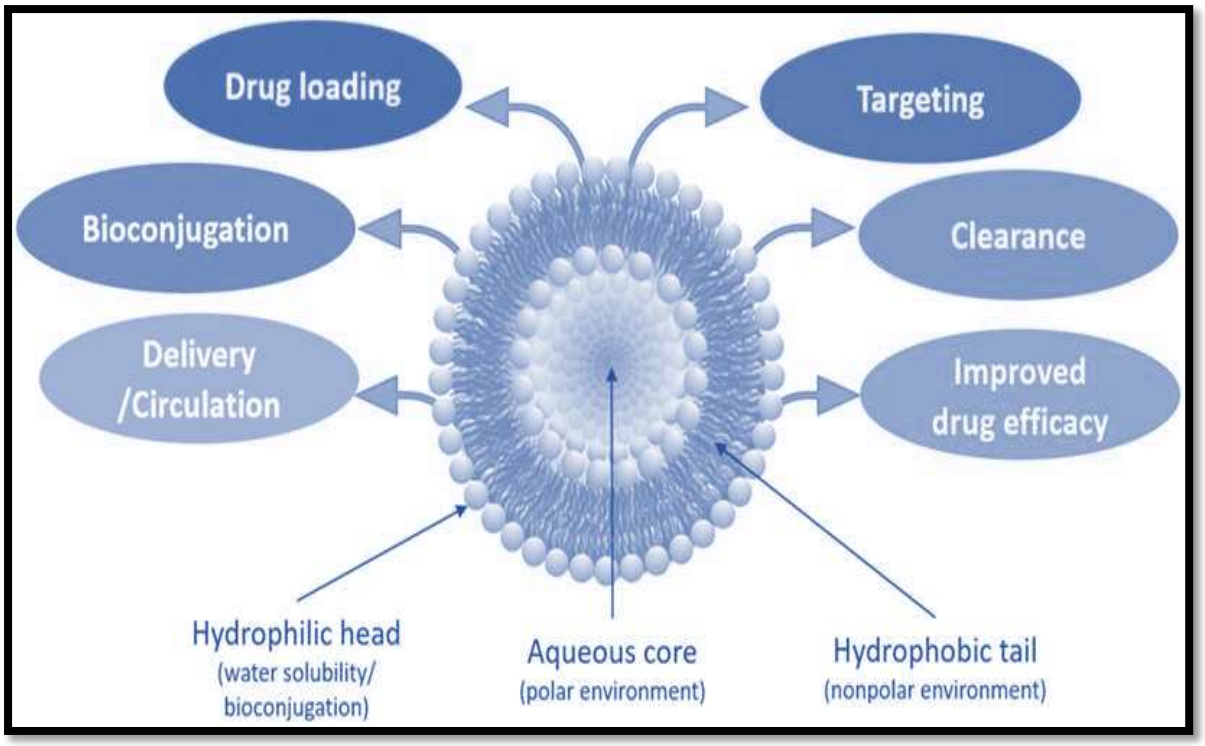

Fig.1 Flow diagram showing the formation of Liposomes

Liposomes can become chemically unstable due to free radical reactions involving the Ester bond hydrolysis or the peroxidation of unsaturated acyl chains of lipids [13]. It is possible to stabilise liposome formulations by adjusting their size distribution, pH, and ionic strength. Phosphatidylcholine liposomes are most stable at a pH of 6.5, according to the study. When storing liposomes, lyophilization is recommended to prevent the encapsulated medicine from leaking out due to fusion. This technique is used to dehydrate frozen goods under very low pressure. Products that are too delicate to withstand heat drying undergo this method instead. Liposomes' safety during clinical application can be greatly enhanced through sterilisation [14]. Liposomes' high thermolability and sensitivity to heat, radiation, and/or chemical sterilisation agents are major obstacles in the sterilisation process. Liposomal composition, lipid concentration, production process, and medication employed all play significant roles in determining encapsulation efficiency. When liposomes are prepared in a variety of ways, they take on new shapes, sizes, and properties [15].

The bioflavonoid hesperidin is often isolated from citrus fruits. Hesperidin's antioxidant qualities, together with its anticancer, antibacterial, anti-inflammatory, antidiabetic, and hepatoprotective activities, have been the subject of much study [16]. Hesperidin has been shown to significantly speed up wound healing in both animal models of excision wounds and human clinical trials. Hesperidin has been shown to lessen the severity of post-healing scarring and fibrosis in animal experiments. When medications are used to treat wounds, they are often absorbed into the body and can have negative effects on healthy cells. As a result, a number of studies have investigated the effectiveness of medicine delivery to the wound site via the dressing [17]. This method has the potential to deliver medications precisely where they are needed without causing harm to surrounding tissue. Therefore, it is crucial to enhance the distribution of hesperidin withoutincreasing its toxicity or decreasing its bioavailability in topicalpreparations.

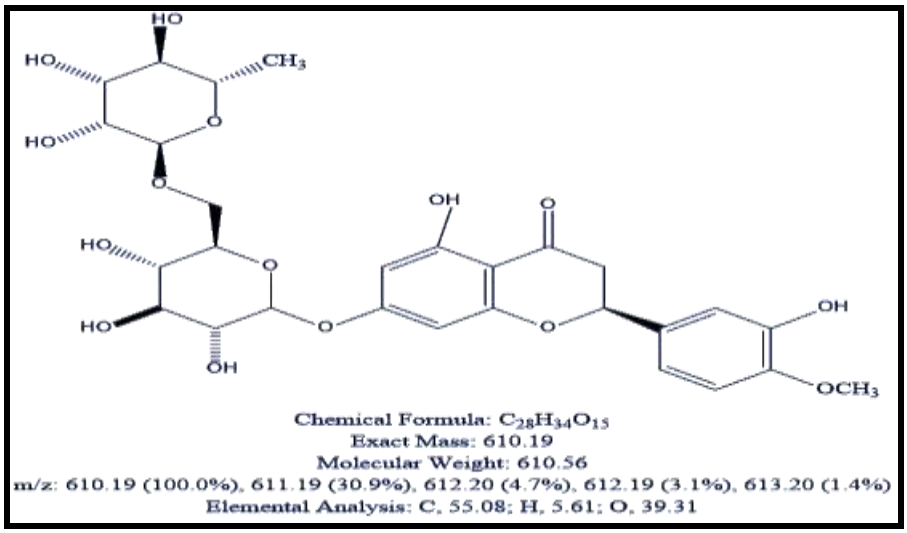

Fig.2 Chemical identification of the bioflavonoid compound Hesperidin

2. Hesperidin-loaded liposome (HLL) preparation methods [18-19]

Effective drug entrapment, a limited particle size distribution, and a high degree of stability over time are the hallmarks of a successful liposome formulation strategy. Liposomes are prepared in a similar fashion regardless of the technique used: first the lipid is hydrated, then the particles are sized, and lastly, the non-encapsulated medication is removed [20]. Liposomes can be made using either passive mechanical dispersion techniques or active loading techniques. By first introducing the medicine to the liposomes in either the organic phase of a lipid-soluble chemical or the aqueous phase of a water-soluble medicine, the drug is encapsulated in the passive loading strategy[21]. Classifying liposomes by their preparation methods involves considering the organic solvent used, the number of lamellae produced, the liposome size, and the intended use showing in the (Table 1). Liposomes owe a great deal of their construction and stability to phospholipids.

Table 1: Preparation strategies for administering liposomes for medicines

Liposomal drug delivery	**An approach**	**Type of liposome***
Drugs against cancer		
5-fluorouracil	Reverse-phase evaporation, ethanol injection, extrusion, and the lipid-film hydration technique.	SUV SUV MLV MLV MLV MLV
Vinblastine sulphate	Sonication and thin-film hydration	MLV SUV
Doxorubicin	Extrusion and hydration of a lipid film	MLV
Bis-demethoxy Curcumin analogue	Sonication and thin-film hydration	MLV SUV
Tamoxifen	The Hydration of Thin Films	MLV
Antibiotic drugs		
Microencapsulation and vaporization of solvents	Solvent evaporation and microencapsulation	Microencapsulation and vaporization of solvents
Reverse phase evaporation method	Method of reverse phase evaporation	Method of reverse phase evaporation
Antifungal drugs		
Fluconazole	The Hydration of Thin Films	MLV

Amphotericin B	The Hydration of Thin Films	MLV
Clotrimazole	Rotary evaporation method	MLV
Mafenide acetate		
Amikacin		
Immunosuppressive drugs		
Tacrolimus (Fk-506)	The Hydration of Thin Films	MLV
Sirolimus	The Hydration of Thin Films	MLV

3. Method of Thin-Film Hydration (TFH) (Bangham Technique)[22]

The manufacture of hesperidin-loaded liposomes is often done using the thin-film hydration approach, Similarly to the Bangham Technique. The organic solvent is then removed through a series of evaporations at 45–60 °C while under vacuum. A dry stream of nitrogen or argon can be used to evaporate the organic solvent from volumes as small as 1 mL in a fume hood, while rotary evaporation is often used for higher volumes. A uniform, dry lipid film (of stacked bilayers) is created once the organic solvent is evaporated. Finally, the lipid film employed in the pharmaceutical formulation is hydrated using a buffer solution, such as distilled water or a normal (phosphate) saline buffer at pH 7.4. The 1-2 hour hydration procedure is typically carried out between 60 and 70 degrees Celsius, much above the phase transition temperature of the constituent lipids. At this point, the lamellae of the swelling lipids may become detached from the inner vessel surface thanks to the agitation (stirring). Overnight storage at T = 4 °C allows the hesperidin-loaded liposome suspension to fully hydrate the lipids. Hesperidin-loaded Liposomes of extremely varied size and lamellarity are formed at the time of hydration, when the lipid is swelled and hydrated. Hesperidin-loaded liposomes can incorporate (passively) lipophilic pharmaceuticals by dissolving them with the phospholipid mixture prior to thin film production, whereas hydrophilic payloads can be introduced inside the hydration media to incorporate (actively) into the liposome. Next, the liposomes' size and lamellarity are decreased, which completes the production process. Low entrapment efficiency, low yields, and difficulties extracting the organic solvent are the key issues of the Bangham process.

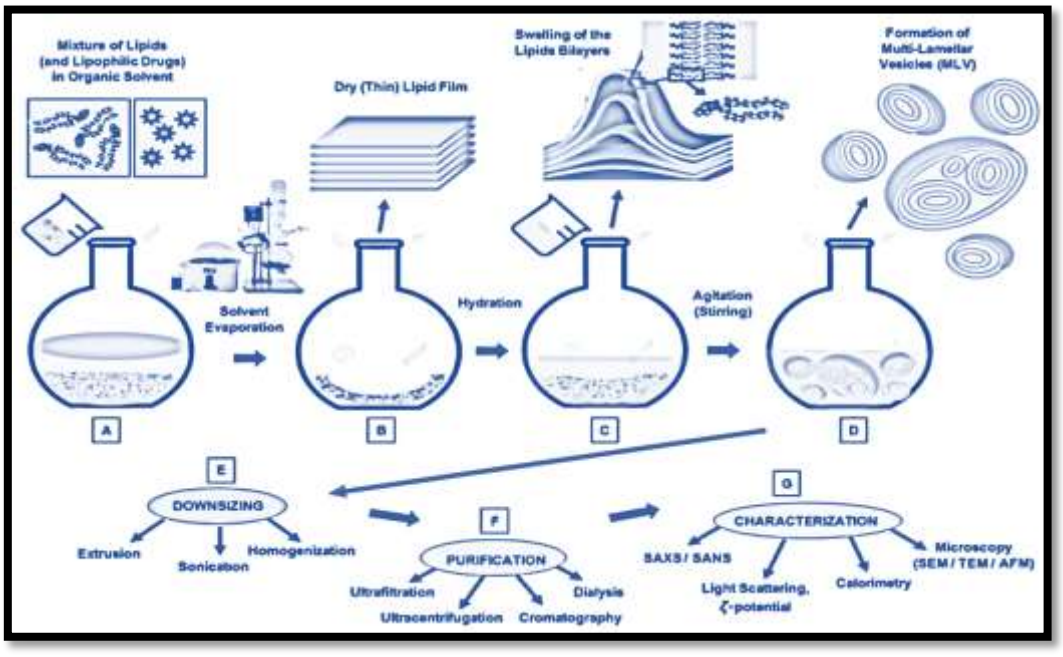

Fig.3 flowchart depicts the various steps in hesperidin loaded liposomes via the thin-film hydration technique.[23]

4. Preparation Strategy of HLLs by Ethanol Injection [24]

A buffer solution, such as warm distilled water or TRIS-HCl, is injected rapidly with ethanol containing phospholipids. The aqueous phase lipids are more likely to self-assemble when ethanol is diluted in water below a critical concentration. The fast dilution of ethanol (in the water phase) helps the lipid molecules to form crystals and the building of bilayer planar pieces (stacks) that surround the water phase. Lastly, when ethanol evaporates, it helps lipids recombine into whole molecules, which then leads to the formation of closed, single-layer vesicles. A significant component in liposome production is the amount of ethanol that is added. A simplified diagram of the ethanol injection method and its essential steps are shown. This automated process shortens the time and money needed to produce liposomes while simultaneously screening for a wide variety of properties.

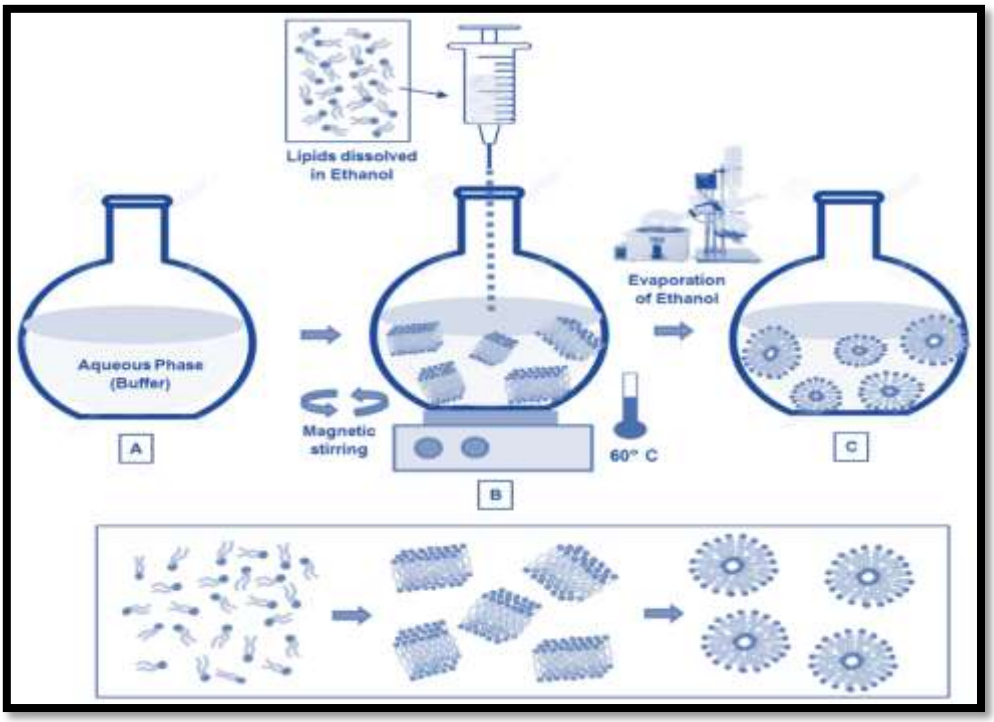

Fig.4 This diagram shows the fundamental steps of the ethanol injection procedure

5. Drug Loading in Hesperidin containing Liposome (HLLs)[24]

Liposomes are tiny particles that are made to carry and distribute drugs that are either lipophilic (can dissolve in the lipid bilayer), hydrophilic (can dissolve in the aqueous core), or amphiphilic (can dissolve in both the lipid bilayer and the aqueous core). In addition to the production technique, the chemical properties of pharmaceuticals and the liposome's composition and physicochemical qualities (such as the concentrations and charge characteristics of the lipids and drug/lipid ratios) all influence the quantity of drug loading. Drugs are added to the liposomes at the time of synthesis in the passive loading approach. Solvent dispersion, mechanical dispersion, and detergent removal are all viable options for implementing this procedure. During the early steps of liposome self-assembly, lipophilic medicines like those used in the thin TFH technique are integrated (in significant concentrations) within the lipid bilayers.. Different methods of liposome production allow for the development of strategies to enhance the aqueous phase within the core volume. After liposomes have been prepared, active loading techniques can be used to incorporate pharmaceuticals into the particles. In most cases, active-loading strategies have a greater EE than passive ones. Here, the liposomes' initial buffer (the aqueous core) is able to convert the

charge of any uncharged pharmaceuticals entering the liposomes so that they can be retained inside the liposomes.

6. Post-Formation Processing of Hesperidin loaded Liposomes[25]

I. Purification of Hesperidin loaded liposome

Before the final nanoformulation can be employed, non-encapsulated substances that are present in the produced liposomes' exterior (liquid) environment must be eliminated by a purification procedure. Examples of these substances include pharmaceuticals, small molecules, and contaminants. As a result, there is an additional phase in the creation of liposomes—purification. Ultra-filtration, ultra-centrifugation, dialysis, and (size exclusion, gel-permeation, or ion-exchange) chromatography are the principal methods for extracting the non-encapsulated components. Because of this, the manufacturer needs to make it very obvious (on the nanoformulation standards) how much solvent is still present in the finished product.

II. Sterilization of Liposomes (HLLs)[26]

It is essential that liposome nanoformulations be free of any living creatures (such as bacteria, fungi, spores, etc.) that are capable of changing their properties because the parenteral route is so commonly utilized. Therefore, it is crucial to ensure that the area is totally clear of microbes. Numerous techniques, including as steam heating (autoclaving), ultraviolet and gamma-ionizing radiation, chemicals, and filtration procedures, can be used to sterilize objects. However, making effective use of sterilisation processes is still a difficulty due to liposomes' extreme fragility and susceptibility to physico-chemical changes. In this article, we'll take a quick look at the key features of sterilisation processes and emphasise the most important ones. Multiple studies have shown that this technique leads many changes in liposomes, including phase changes and aggregation, lipid hydrolysis and oxidation and drug breakdown (or leakage). Because of this, only certain nanoformulations of liposomes can benefit from this method.

7. Controlling Hesperidin-loaded liposomal composition by analytical technique [27,28]

Various analytical techniques are used to regulate the liposomal formulations' physicochemical and biological properties, stability, and drug entrapment efficiency. The most important characteristics of liposomes include size distribution, lamellarity, surface charge, drug entrapment effectiveness, phospholipid concentration, cholesterol concentration, osmolarity, sterility, and pyrogenicity. [29]

I. Physical Parameters

Among the physical factors that can be modified are vesicle size, mean vesicle size, vesicle size distribution, surface charge, electrical potential, surface pH, lamellarity, phase behavior, free drug/capture ratio, and drug release. [30]. Dynamic light scattering is commonly used to determine the size distribution of liposomes, while electron microscopy or a spectroscopic approach is used to determine their lamellarity. Liposome size distribution is maintained in physically stable liposomal compositions. Leakage of the medication from the vesicles, as well as vesicle aggregation or fusion to generate larger particles, can contribute to the particles' physical instability.

Table.2 Physical parameter of hesperidin loaded liposome

S.No.	Physical Parameter	Values of HLLs
01	Tem (Vesicle count)	1342
02	Mean AR	1.34

03	Mean Vesicle size SD(nm)	66±14
04	Median vesicle size(nm)	65.8
05	Zeta potential (Mv)	8.34±3.20
06	Mean size SEM(nm)	75.8±0.5
07	Polydispersity index	0.036

II. Fluorescence and confocal microscopy use light to examine samples. [31]

With light microscopy, which uses visible light and a very simple set of lenses, an image of the sample is magnified and has a highest resolution of about 250 nm (the smallest spot size that can be achieved without diffraction). It is helpful in determining the size, shape (homogeneity), and aggregation level of a liposome system or of hundreds-micrometre-long gigantic unilamellar vesicles (SUVs), but it cannot provide information on the lamellarity of the vesicles or the structures of the SUVs themselves. By incorporating minute concentrations (1 mol%) of fluorescent dyes into the aqueous phase (or lipid bilayers), it is possible to visualize the structure (and dynamics) of liposomes with just a little impact on the lipid membrane's structural properties. with conclusion, no one technology exists that can be utilized to evaluate the many structural characteristics of the nanoformulations of the liposomes at various stages of the drug-delivery process with high resolution.

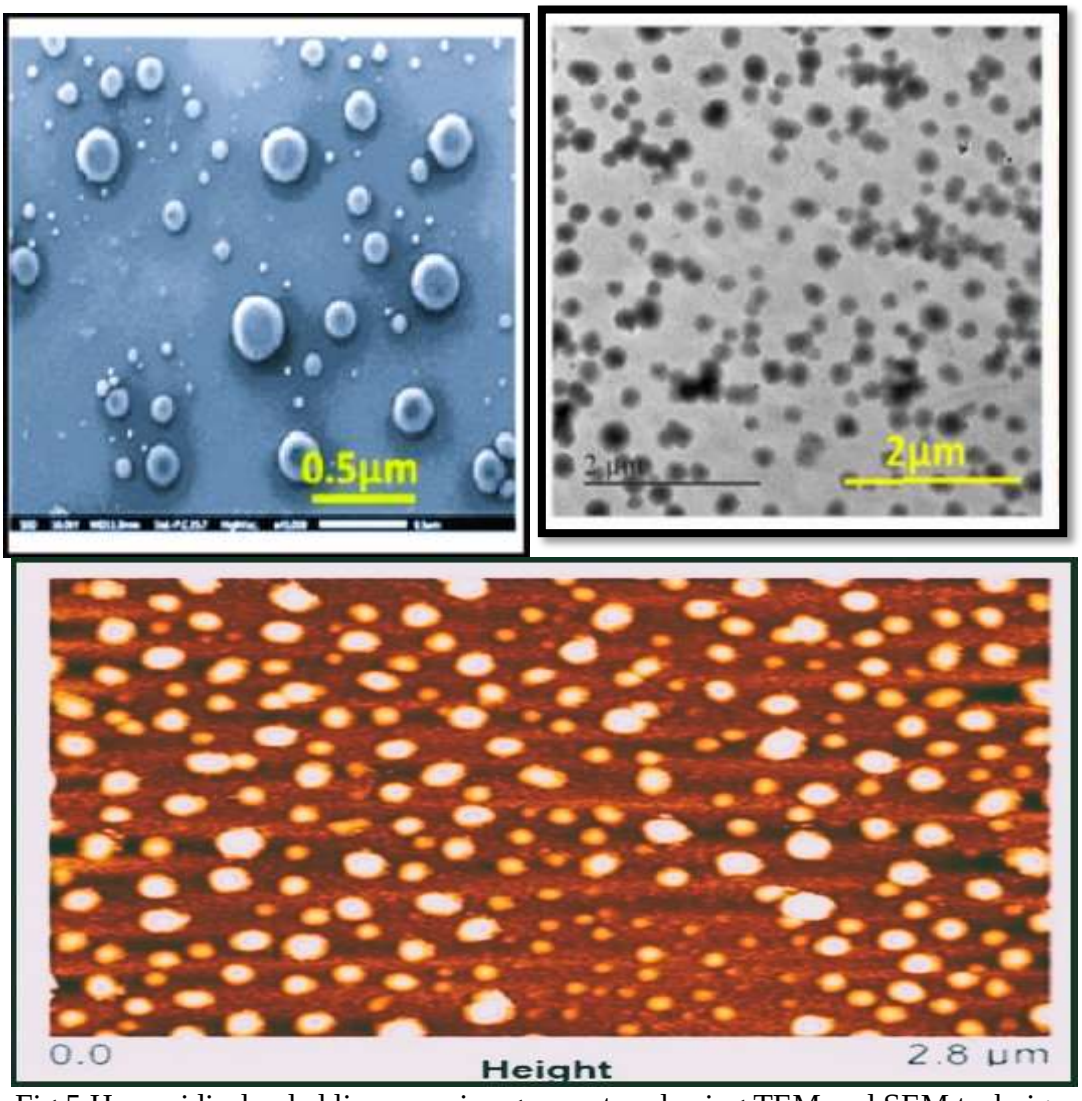

Fig.5 Hesperidin-loaded liposome images captured using TEM and SEM techniques.

III. Technique for Dynamic Light Scattering [32]

The structural and dynamic characteristics of nanoparticles in solution, such as bio-macromolecules, colloids, nanoemulsions, liposomes, and gels, can be studied using dynamic light scattering (DLS). The Stokes-Einstein relation D = kBT/6RH, which describes the (translational) diffusion coefficient D as a function of the hydrodynamic radius RH, the absolute temperature T, and the solvent viscosity (where kB is the Boltzmann constant), explains the Brownian motion of the nanoparticles. Scattering intensity measurements can also reveal the nanoparticles' size distributions within the liposomes.

IV. Potential Technique Using Zeta (ζ) [33]

IV. Zeta (ζ) Potential Technique[33]

Electrostatic effects in charged nanocarriers can be mitigated with the help of zeta potential measurements. The biodistribution, pharmacokinetics, cellular affinity, drug uptake, and overall stability of liposomes are all significantly influenced by the zeta potential. A liposome nanocarrier dispersed in water acquires its surface charge through the ionization of its surface end-groups (or the adsorption of charged species on its surface). Zeta potential is a representation of the electrical potential at the nanoparticle interface, whereas distributed counter-ions will create a shell (Stern layer) around the particle's surface. Because it measures the electrostatic attraction between nanoparticles in a dispersion, the zeta potential is a crucial sign of the colloidal stability of liposome dispersions. Small zeta potential nanoparticles have a propensity to aggregate or flocculate, whereas big (negative or positive) zeta potential nanoparticles are electrically stabilized and have very high colloidal stability.

V. Characterization Techniques of HLLs[34-36]

Different types of spectroscopy, such as ultraviolet (UV), Fourier transform infrared (FT-IR), electron paramagnetic resonance (EPR), electron spin resonance (ESR), and circular dichroism, can be used to find out how well active drugs and large molecules fit into liposomes[37]. Two complementary biophysical methods, differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC), can be used to study how medicines bind to and interact with lipids. Nuclear magnetic resonance (NMR) is a quantitative spectroscopy method for the non-destructive and non-invasive investigation of drug-liposome interactions. At a sub-ngstrom resolution, it offers critical information on changes in the bilayer structure or dynamics of lipid molecules and lipid-based nanostructures.

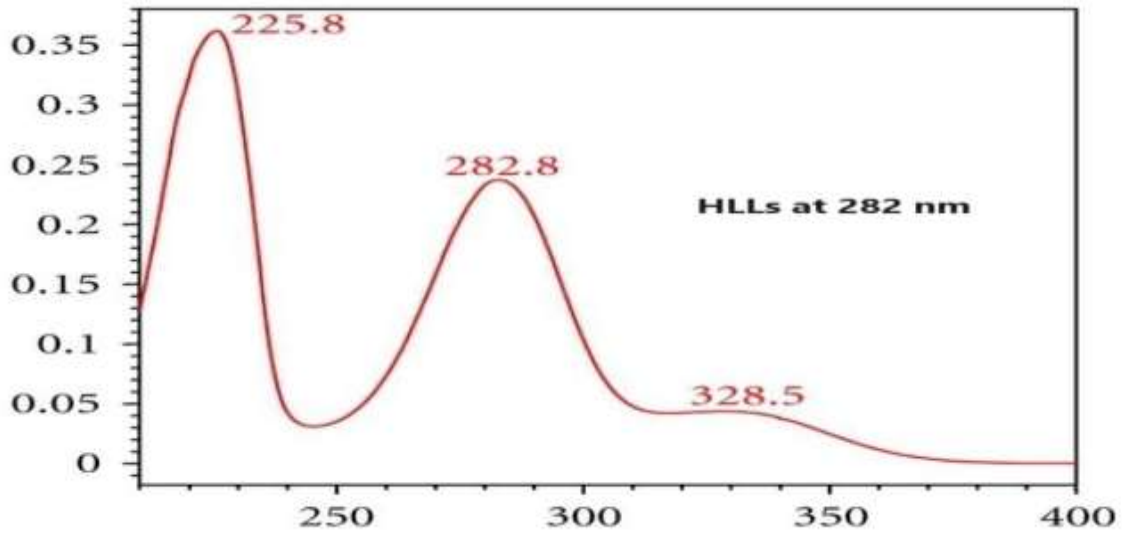

Fig.6 UV graph of hesperidin loaded liposome at 300nm

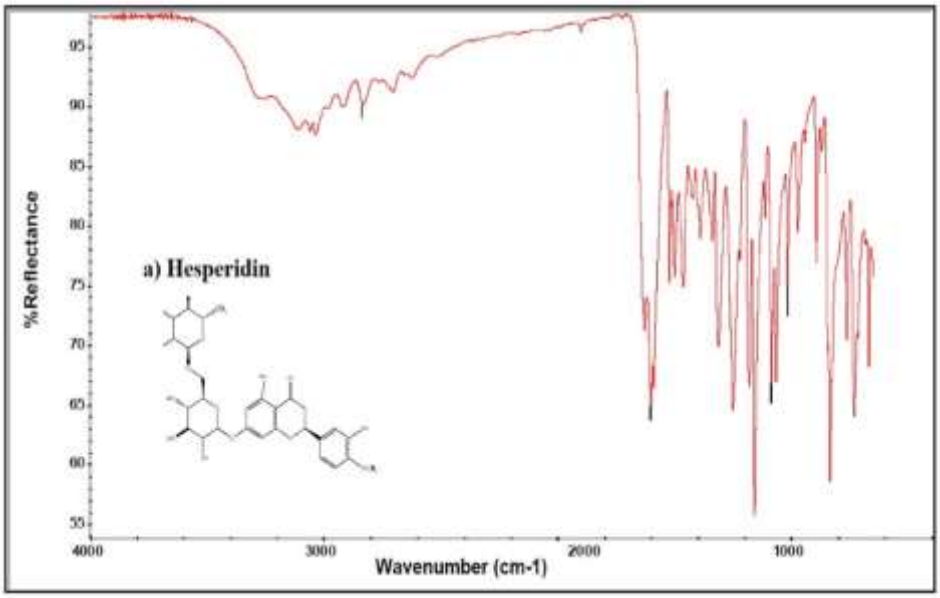

Fig.7 IR graph of hesperidin loaded liposome (HLLs)

VI. In Vitro Drug Release Study[38,39]

Studies of drug release in vitro often employ a dialysis technique. Validated diffusion cells with either a cellophane or dialysis membrane are used for these kinds of investigations. At 37 degrees Celsius, an aliquot of the finished formulation is tested for drug realisation using a UV spectrophotometer after being deposited in a dialysis membrane. The donor medium is a liposomal formulation, while the receptor medium is made up of an acetate buffer at pH 5, a citrate-phosphate buffer, and a phosphate buffer at pH 7.4. When a dialysis membrane is used, the amount of drug discharged is tracked for either 30 minutes or 8 hours. Franz diffusion cells are used in the diffusion experiments.

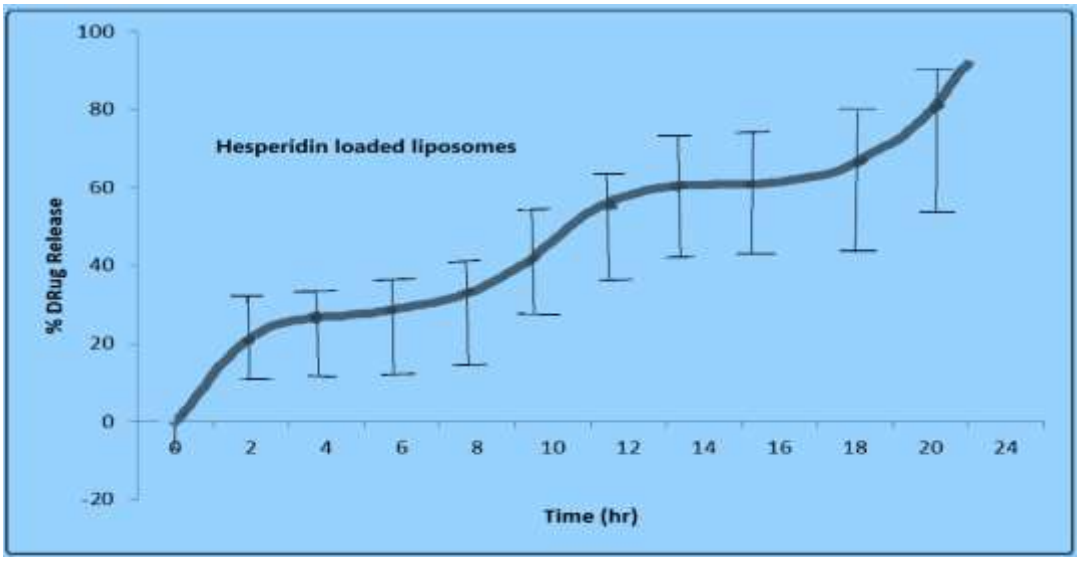

Fig.8 % drug release at 288 nm from hesperidin-loaded liposome

VII. Chemical Parameters of HLLs[40,41]

You can employ chemical analysis to assess the potency and purity of liposomes. The phospholipid concentration, cholesterol concentration, phospholipid peroxidation, phospholipid hydrolysis, cholesterol auto-oxidation, and osmolarity are the chemical

parameters that are most frequently measured. Phospholipid concentration is critical for method control and optimisation. Stewart's approach, which relies on the development of a coloured complex between phospholipids and an ammonium ferrothiocyanate reagent, was used to quantify the phospholipid content in liposomes. Cholesterol concentrations are measured via a colorimetric test of a coloured complex produced after the interaction of cholesterol and freshly prepared hesperidin content liposome**.**

VIII. Biological Parameters of HLLs[42]

The viability of a medical formulation can be established with the use of biological parameter determination. Liposomes loaded with hesperidin are tested for their sterility, pyrogenicity, and toxicity to animals in order to complete the biological characterisation process.

Antihypertensive activity of hesperidin loaded liposomes usingTail Cuff Method[43]

Grouping Hypertension Inducers

The animals were separated into seven groups of six the day before the experiment was to begin. One of the seven groups was the control group, which had no special treatment. In the other 6 groups, hypertension was produced by the subcutaneous (s.c.) administration of DOCA salt (20 mg/kg) twice weekly for 4 weeks. Rodents were monitored weekly to report BP changes using a noninvasive tail cuff technique. In 4 weeks, blood pressure was artificially raised to >140 mmHg, and at the start of week 5, each group received their designated treatment for 7 days. The hypertensive control group received a placebo (CMC). At the end of the fifth week, non-invasive blood pressure measures were performed once more to monitor any changes in blood pressure brought on by the experimental medication. After giving each set of rats a dose of the HLLs, readings of non-invasive blood pressure (NIBP) were taken from the rats' tails. After placing each rat in the NIBP restrainer, a cuff equipped with a temperature sensor was attached to the end of its tail and heated to 33–35 °C. Diastolic blood pressure (DBP) was computed from systolic blood pressure (SBP) and mean blood pressure (MBP) using the equation below. Systolic blood pressure (SBP), mean blood pressure (MBP), and heart rate were obtained directly using pulse tracing.

$$DBP = \frac{3MBP - SBP}{2}$$

Table 3 The animals were arranged in a precise way.

Group name	Treatment
I Group	Normal control
II Group	Hypertensive control
III Group	Standard (Prazosin 1mg/kg)
IV Group	Sample-I Drug loaded liposomes (HLLs)

Groups	0 week	1 week	2 week	3 week	4 week	5 week
Normal control	104.6±8.2	123.7±8.1	120.5±6.3	115.3±8.6	113.2±5.9	115.9±2.9
Hyper tensive control	119.2±4.2	122.9±2.6	150.8±7.0	150.6±2.8	172.2±4	160.6±6.6

Standard (Prazosin1mg/kg)	120.1±6.2	123.4±2.2	142.7±2.5	150.4±2.7	171.6±1.5	114.5±6.3
Sample-Hesperidinloaded liposomes (HLLs)	119.1±1.3	131.4±4.2	120.6±2.9	150.3±3.2	160.8±5.3	132.5±8.8

Table 4:HLLs impact on the systolic blood pressure (mmHg) in hypertensive rats induced by DOCA

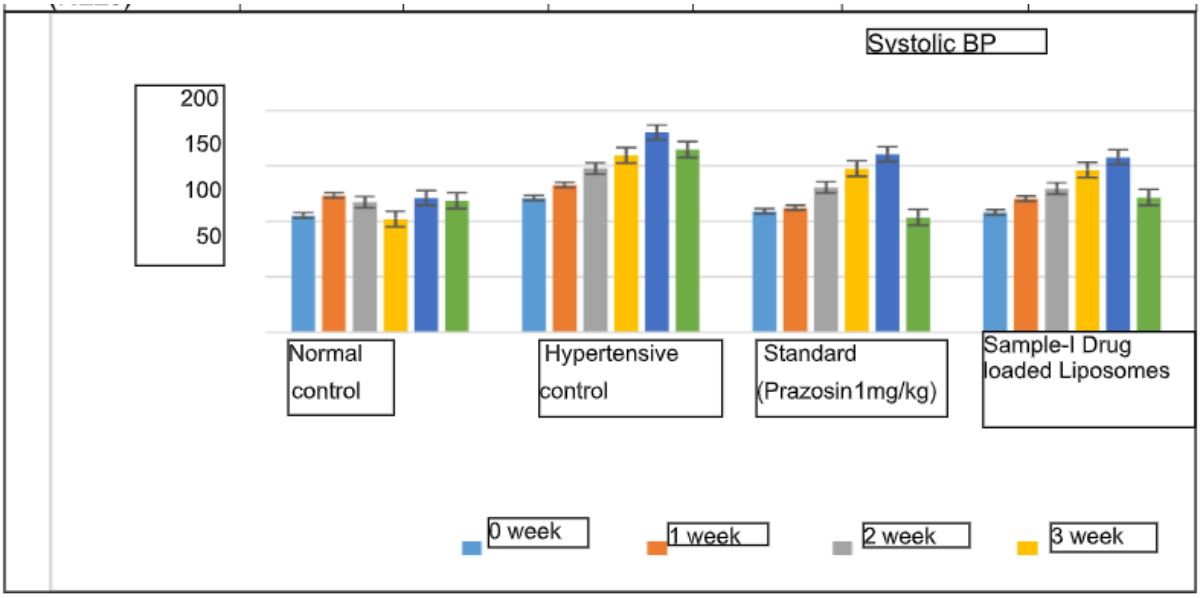

Fig.9 Compound-induced changes in systolic blood pressure (mmHg) in DOCA hypertensive rats

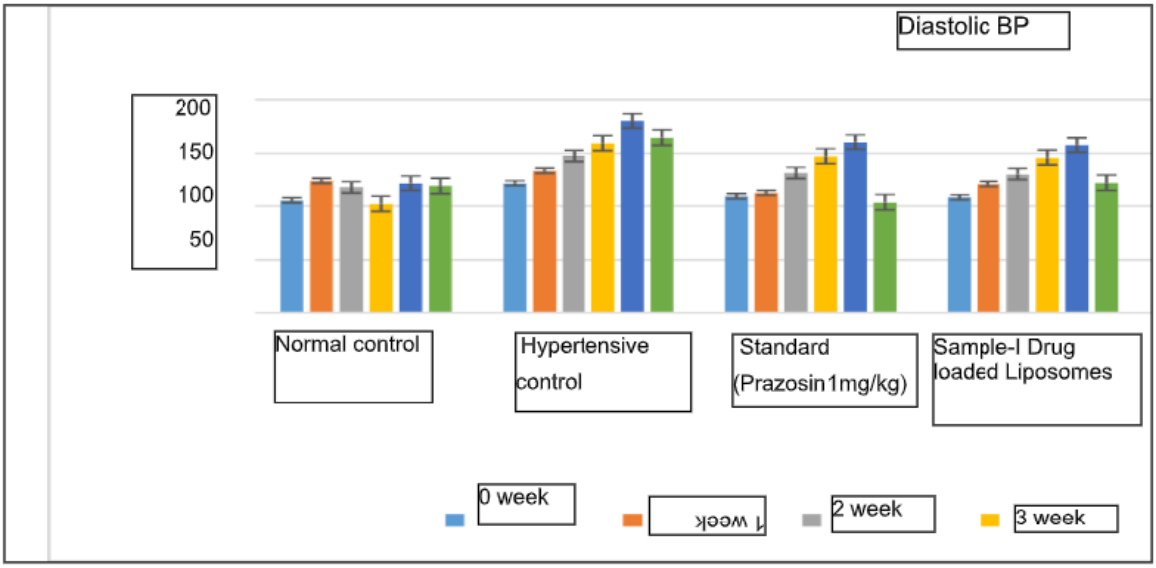

Fig.10 Compound-specific impact on diastolic blood pressure (mmHg) in DOCA hypertensive rats

8. Conclusions

In this paper, we analyse the primary characteristics of hesperidin loaded liposome nanocarrier formation methods, paying particular attention to the structural parameters and important aspects that affect the creation of a reliable nanoformulation. Both conventional

and cutting-edge approaches to hesperidin loaded liposome preparation are covered in an effort to bring the reader up-to-date on the latest findings and point the way for future studies. Traditional methods for hesperidin loaded liposome preparation are still widely used since they are straightforward to apply and require nothing in the way of specialised instruments for instrumental improvement. However, because these methods might affect the structure of a molecule (and hence its function), they may not be applicable to the processing of a wide range of biomolecules. In view of recent advances in nanotechnology and biomedicine, simple production methods for encapsulating both hydrophobic and hydrophilic molecules are increasingly sought after. These methods should ideally not involve the use of organic solvents or other sophisticated machinery. For liposomes to be able to deliver hydrophilic cargo to a specific site of interest, new approaches are needed to address the pressing problem of system instability. The chronic lung condition, pulmonary arterial hypertension (PAH), is severe and often fatal. There has been an improvement in patient quality of life thanks to current pharmacotherapies, although PAH medications have drawbacks such as short-term pharmacokinetics, instability, and low organ specificity. Historically, nanotechnology-based delivery techniques have been useful in extending the half-lives of chemotherapeutics in circulation and boosting their accumulation in tumours by enhancing their permeability through fenestrated vasculature. Higher accumulation of HLLs in diseased tissues has been demonstrated in preclinical investigations to be related with myocardial infarction and heart failure, which are characterized by endothelial dysfunction and altered vasculature. As a result, NP-based drug delivery is being investigated as a treatment approach for PAH. This behaviour has recently been reported in preclinical models of PAH as well. The benefits of NPs for effective treatment of PAH were explored, including enhanced therapeutic delivery to sick lungs for greater drug bioavailability, and novel nanotherapeutic methods for PAH were emphasised. Traditional methods of liposome manufacture still have some limitations, such as a lack of scalability for large-scale production and low encapsulation efficiencies. This is why it is important to investigate new methods of formation and implement unique technologies; doing so may spur the development of cutting-edge liposome nanoformulations in the lab, making them amenable to large-scale manufacturing and more efficient clinical applications.

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