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Review Article

Preparation and Evaluation of Curcumin Loaded Liposomes

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ABSTRACT

Liposomes are versatile and widely used nanocarrier systems in modern pharmaceutical research due to their ability to improve drug delivery and therapeutic efficacy. These vesicular systems, composed of phospholipid bilayers, can encapsulate both hydrophilic and lipophilic drugs, thereby enhancing drug stability, bioavailability, and controlled release. Various preparation methods such as thin-film hydration, reverse-phase evaporation, and supercritical fluid techniques are employed depending on the desired characteristics and scale of production. Liposomes have shown significant applications in cancer therapy, vaccine delivery, gene transfer, and targeted drug delivery systems. In recent years, curcumin-loaded liposomes have gained considerable attention. Curcumin, a natural polyphenolic compound with potent anti-inflammatory, antioxidant, and anticancer properties, suffers from poor aqueous solubility and low bioavailability. Liposomal encapsulation effectively overcomes these limitations by improving solubility, protecting the drug from degradation, and enhancing cellular uptake. This results in improved therapeutic outcomes, particularly in cancer, inflammatory disorders, and liver diseases. Overall, liposomes, especially curcumin-loaded liposomes, represent a promising strategy for developing safer and more effective drug delivery systems.

INTRODUCTION

In the modern era of medicine, the main goal of drug delivery systems is not only to deliver drugs to the body but also to make sure that the drug reaches the right place, at the right time and in the right amount [1,2,3]. Traditional drug delivery often suffers from several problems like poor absorption, fast clearance from the body, side effects, and low effectiveness [4,5]. To overcome these limitations, scientists have developed various Novel Drug Delivery Systems, and one of the most successful among them is the Liposomal Drug Delivery System [6-8] .

A liposome is a small, spherical vesicle made mainly from phospholipids, which are natural molecules that form the outer layer of cell

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membranes. When phospholipids are placed in water, they naturally form a bilayer structure with an inner water-filled core and an outer lipid layer. This unique property allows liposomes to carry both water-soluble (hydrophilic) and fat-soluble (lipophilic) drugs at the same time. Because of this, liposomes are called versatile carriers in drug delivery science [9,10].

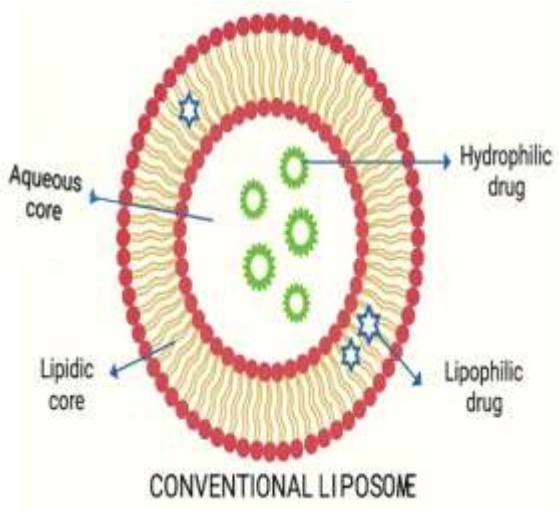

Figure 1 Conventional Liposome

Liposomal drug delivery systems have many advantages. They can protect the drug from being broken down in the body, increase the half-life of the drug in the bloodstream, reduce toxicity, and target specific tissues or organs. For example, anticancer drugs like doxorubicin cause severe side effects when given alone, but when delivered through liposomes (such as in Doxil®), the side effects are greatly reduced, and the drug works more effectively at the tumor site[6,9,8].

Liposomes can be classified based on their structure and size:

- Small Unilamellar Vesicles (SUVs) – have one lipid bilayer and small size (20–100 nm).
- Large Unilamellar Vesicles (LUVs) – have one bilayer but larger in size (100–250nm).
- Multilamellar Vesicles (MLVs) – contain multiple lipid bilayers [12,13] .

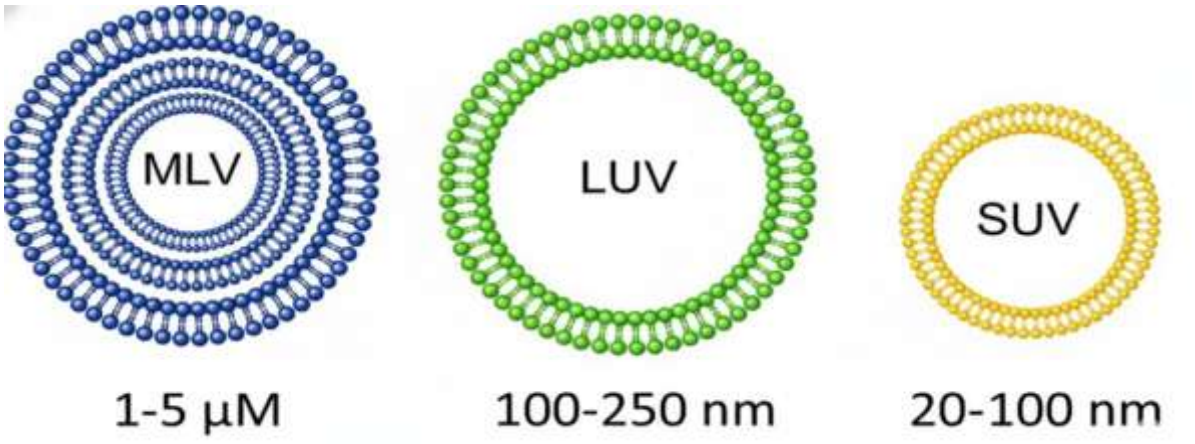

Figure 1 Different size of liposome

They can also be classified based on composition, such as conventional liposomes, PEGylated liposomes (stealth liposomes), targeted liposomes, and stimuli-responsive liposomes.

- Conventional liposomes are simple lipid vesicles.
- PEGylated liposomes are coated with polyethylene glycol (PEG), which helps them escape the immune system and circulate longer in the blood.
- Targeted liposomes have ligands or antibodies attached that guide them to a specific type of cell (for example, tumor cells).

- Stimuli-responsive liposomes are designed to release the drug in response to changes like pH, temperature, or enzymes at the disease site[14,15,16] .

The main goals of using liposomes in drug delivery are:

- To increase the bioavailability of drugs.
- To achieve controlled and sustained release.
- To reduce toxicity and side effects
- To deliver the drug specifically to the target organ or cell.
- To improve patient compliance by reducing dosing frequency.

Liposome Constituents

Liposome constituents are lipid and aqueous component that form a bilayer vesicle system responsible for drug encapsulation, stability, and controlled release.

1. Phospholipids

Phospholipids are the real backbone of any liposome.

They have two parts: a water-loving head and water-hating tails.

Because of this dual nature, they automatically arrange themselves into a bilayer, forming the outer shell of the liposome[17,18].

Common phospholipids used:

Phosphatidylcholine (PC) – most widely used, very safe.

Phosphatidylethanolamine (PE) – helps give flexibility.

These phospholipids decide how fluid, rigid, charged or stable is the liposome [19,20] .

Figure 3 Liposomes 3D structure

2. Cholesterol

Cholesterol works like a “support rod” inside the liposome membrane.

It sits between phospholipid tails and prevents the membrane from becoming too soft or too leaky[17,18].

Importance

Reduces drug leakage

Improves membrane strength

Gives better stability inside blood or body fluids.

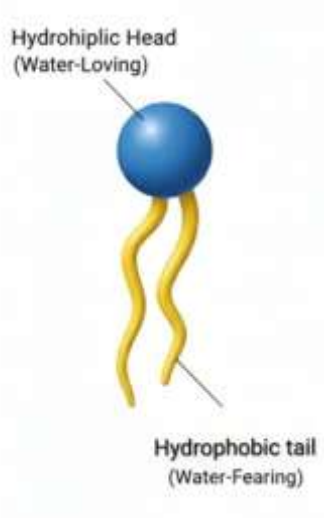

Figure 4 :Phospholipid

3. Aqueous phase

The aqueous phase is usually water or a buffer solution in which lipid by layer swells and the self assembles into liposomes.

Examples: Purified water, Phosphate buffer saline, Normal saline

Importance

It helps in swelling of lipids and formation of lipid vesicles.

It helps in drug intrapment.

Methods of Preparation

Over the years, several methods have been developed for liposome preparation. The choice of method depends on the drug's solubility, desired size, and scale of production.

1. **Thin-Film Hydration Method (Bangham Method):**

Thin Film Hydration Method (Hand-Shake Method)

This is the most classical and widely used method. Lipids such as lecithin and cholesterol are first dissolved in an organic solvent (usually chloroform, methanol, or a mix). This solution is placed in a round-bottom flask and evaporated using a rotavap or a warm water bath with hand-rotation.

As the solvent evaporates, it leaves behind a thin, even lipid layer on the inner wall of the flask. When an aqueous solution (like PBS, distilled water, or drug solution) is poured in, the dry lipid film absorbs the water and starts swelling. After a few minutes, the lipids naturally peel off and curl into multilamellar vesicles. A little shaking helps break them into smaller liposomes[23-25].

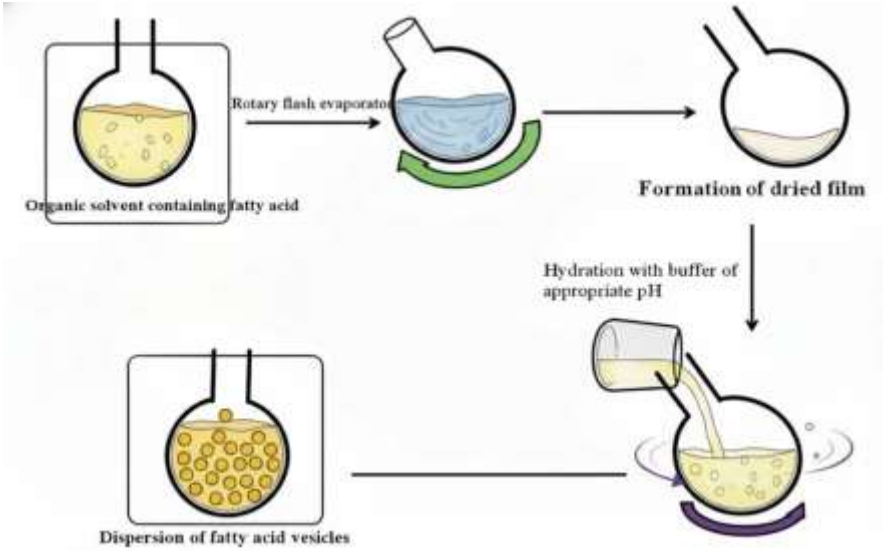

Figure 2 Thin film hydration Method

2. Reverse-Phase Evaporation Method:

Lipids are first dissolved in an organic solvent like diethyl ether or isopropyl ether. Then an aqueous solution containing drug is added to form a water-in-oil emulsion. Sonication is used to reduce the droplet size so the emulsion becomes stable. When the solvent is slowly evaporated under reduced pressure, the water droplets get squeezed together. Lipids coat these droplets and form large vesicles with high internal volume. This method is excellent for water-soluble drugs because it traps more aqueous content[26,27].

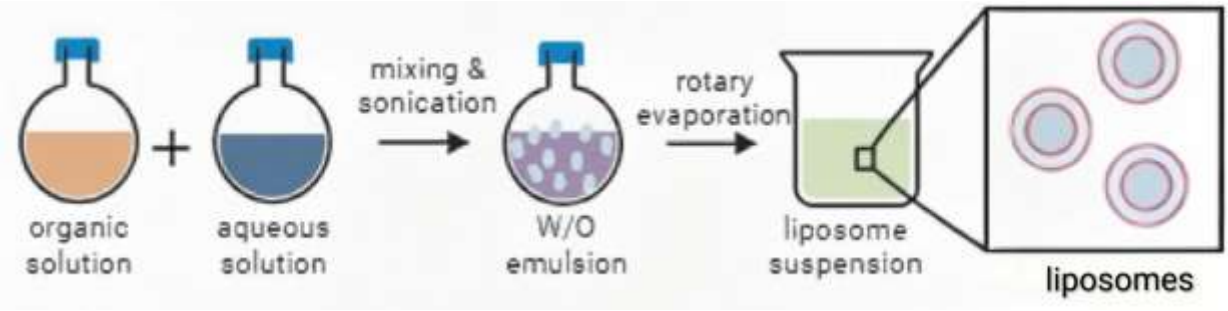

Figure 3 Reverse Phase Evaporation Method

3. Injection Method

- Ethanol Injection Method:

Lipids are dissolved completely in ethanol, forming a clear solution. Using a syringe, this lipid–ethanol solution is slowly injected into warm water or buffer under gentle stirring. As soon as the ethanol touches water, it rapidly diffuses away, and lipids spontaneously arrange themselves into small liposomes. The method is clean, fast, and avoids toxic solvents. Ethanol is safe and evaporates easily with mild heating if needed[28].

- Ether Injection Method**:**

Similar to ethanol injection, but here lipids are dissolved in ether. This lipid–ether solution is injected into warm water. Ether vaporises instantly due to its low boiling point, leaving lipids behind to form liposomes. The rapid evaporation helps form very small vesicles[29].

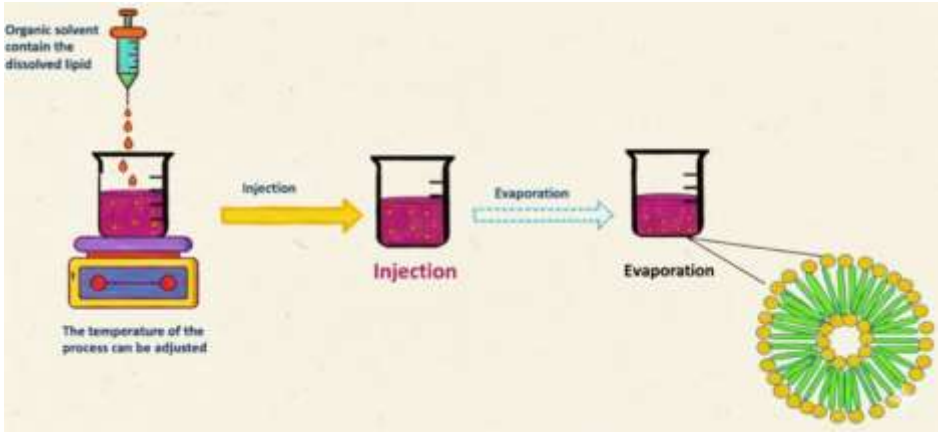

Figure 4 Injection Method

4. Supercritical Fluid Techniques :

In this method, the lipid phase and aqueous phase are first gently heated so they mix comfortably.

After warming, both phases are combined to form an emulsion, where tiny droplets of lipid are dispersed inside water. This emulsion is then homogenized(reduced particle size) using a high-speed mixer so that the droplets become very fine and uniform. Once the emulsion is ready, it is taken into a special chamber where supercritical CO_2 is passed through it. Supercritical CO_2 has the unique ability to behave like both a liquid and a gas, so it can easily extract and remove the organic solvent from the emulsion. As the solvent is removed, the lipids instantly solidify or arrange themselves to form liposomes or lipid nanoparticles. Finally, the CO_2 escapes harmlessly, leaving behind clean, uniform particles without any toxic solvent traces. This method is considered modern, clean, and environment-friendly because CO_2 does all the extraction work without leaving residues[30,31].

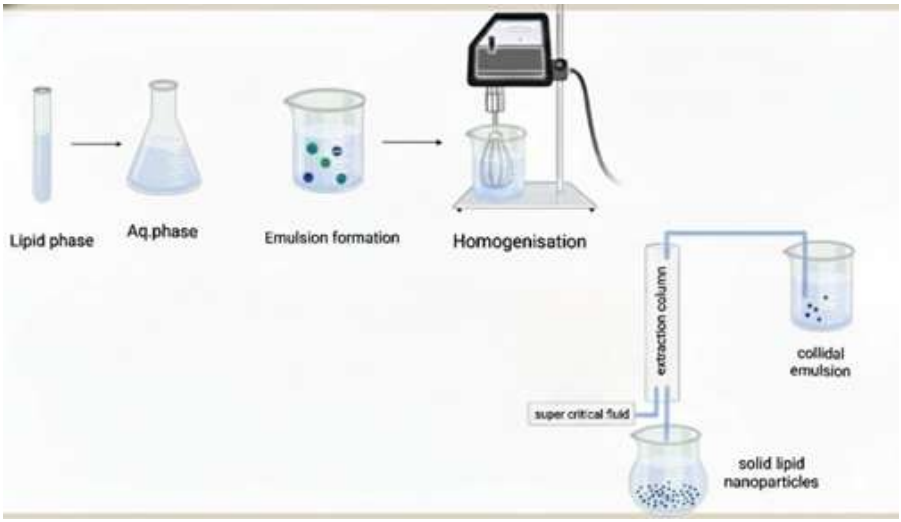

Figure 5 Super Critical Fluid Technique

5. Detergent Removal Method :

The detergent removal method is a gentle and clever way to prepare liposomes. In this technique, lipids are first mixed with a detergent. The detergent acts like a helper that breaks the lipids into tiny pieces and keeps them dissolved in water. At this stage, the mixture doesn't look like liposomes but it looks more like a clear solution. When the detergent is slowly removed, the lipids start coming closer again. Because they naturally want to form a bilayer, the lipids self-assemble and gently curve into small, round liposomes.

The detergent can be removed in different ways such as dialysis, gel filtration, or adsorption on special resins. Dialysis is the most common, where the mixture is kept in a dialysis bag and the detergent slowly travels out into the surrounding water. As the detergent leaves, liposomes begin to appear on their own and form a liposome.[32,33]

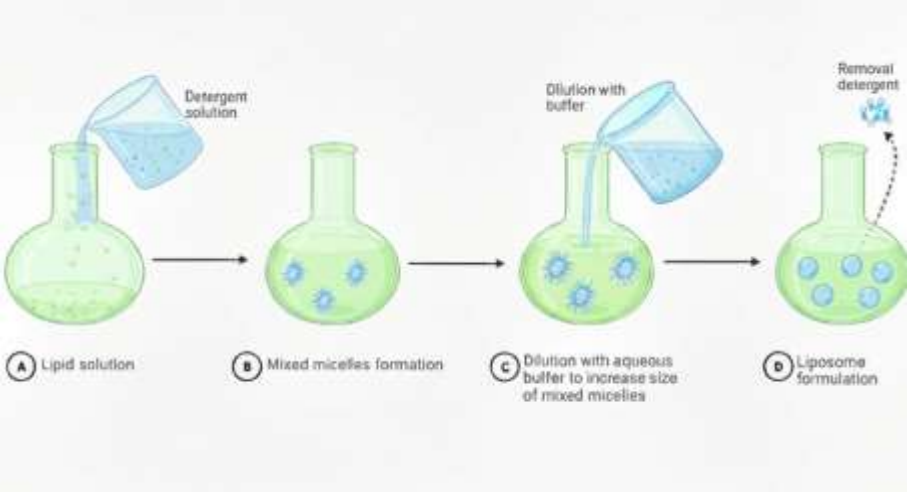

Figure 6 Detergent Removal Method

Steps in Liposomal Drug Delivery Mechanism. [34,37]

Step 1: Drug Encapsulation

Hydrophilic drugs are trapped inside the aqueous core.

Hydrophobic drugs are embedded within the lipid bilayer.

Depending on formulation methods (e.g., thin-film hydration, reverse-phase evaporation), different drugs can be loaded efficiently.

Step 2: Administration

Liposomes can be administered via intravenous, oral, topical, or inhalation routes, depending on the formulation.

Step 3: Circulation in the Body

Conventional liposomes are rapidly cleared by the mononuclear phagocyte system (MPS), especially by the liver and spleen.

PEGylation (attachment of polyethylene glycol) can be used to make "stealth liposomes," which evade immune detection and circulate longer.

Step 4: Targeting the Site of Action

There are two main targeting strategies:

1. Passive Targeting:

Utilizes the Enhanced Permeability and Retention (EPR) effect found in tumor tissues and inflamed areas.

Leaky vasculature in these tissues allows liposomes to accumulate more easily.

2. Active Targeting:

Surface of liposome is modified with ligands, antibodies, or peptides that recognize specific receptors on target cells.

Enhances uptake by target cells via receptor-mediated endocytosis.

Step 5: Cellular Uptake

Liposomes interact with the cell membrane through:

Fusion: Liposome merges with the cell membrane, releasing the drug into the cytoplasm.

Endocytosis: The cell engulfs the liposome, forming an endosome; the liposome then releases its contents inside the cell.

Step 6: Drug Release

Drug release can occur by:

- Fusion with cell membrane.
- Degradation of liposome inside lysosomes.
- Environmental triggers (e.g., pH, temperature, enzymes) .

Curcumin-Loaded Liposomes

Introduction

Curcumin is a natural polyphenolic compound obtained from Curcuma longa with anti-inflammatory, antioxidant, and anticancer properties. Its therapeutic use is limited due to poor solubility, low stability, rapid metabolism, and low bioavailability. Curcumin-loaded liposomes are designed to enhance solubility, stability, and targeted drug delivery [38].

Function

- Curcumin is entrapped within the lipid bilayer, protecting it from degradation [38].
- Liposomes improve solubility and absorption, leading to better bioavailability [39].
- Provide controlled and sustained release of curcumin [38].
- Enhance cellular uptake and drug accumulation in tissues [39].
- Enable targeted delivery (e.g., tumor or liver), reducing side effects [38].

Pharmacological Work

- Anticancer activity: Liposomal curcumin shows enhanced cytotoxicity and inhibits tumor growth by increasing cellular uptake [39].
- Anti-inflammatory activity: Reduces inflammatory mediators like cytokines and enzymes [40].
- Antioxidant activity: Scavenges free radicals and decreases oxidative stress [38].
- Hepatoprotective effect: Improves liver function and reduces oxidative damage in liver diseases [40].

Therapeutic applications of liposomes

1. Cancer therapy

Liposomes are widely used to deliver anticancer drugs because these drugs are highly toxic to normal cells. Liposomal drug delivery helps concentrate the drug at the tumor site and reduces damage to healthy tissues. This improves treatment effectiveness and reduces side effects such as heart and kidney toxicity. Example: Liposomal doxorubicin (Doxil®) used in breast cancer and ovarian cancer[41,42]

2. Treatment of infectious diseases

Liposomes improve the delivery of antibiotics and antifungal drugs to infected tissues. They allow

higher drug concentration at the infection site while reducing toxicity caused by high doses. This is especially useful in severe infections. Example: Liposomal amphotericin B (AmBisome) used for serious fungal infections.[43,44]

3. Vaccine delivery

Liposomes act as carriers and immune stimulators (adjuvants) in vaccines. They protect antigens from degradation and enhance immune response. This results in improved vaccine efficacy and reduced dose requirement.

Example: Lipid-based vaccine systems used in modern mRNA vaccines.[45,46]

4. Gene therapy and nucleic acid delivery

Liposomes are effective carriers for DNA, mRNA, and siRNA, which are otherwise unstable in the bloodstream. They protect genetic material and help deliver it into target cells for therapeutic action.

Example: Lipid nanoparticles used in mRNA-based COVID-19 vaccines .[45,46]

5. Brain-targeted drug delivery

Liposomes help drugs cross the blood–brain barrier, which normally blocks many medicines. They improve drug delivery to brain tissues, especially when modified with targeting ligands or administered intranasally.

Example: Liposomal formulations of dopamine for Parkinson's disease research[47].

6. Dermatological and transdermal therapy

Liposomes improve drug penetration through the skin and reduce irritation. They are commonly used in treating skin disorders and cosmetic formulations.

Example: Liposomal corticosteroids for eczema and psoriasis[48].

7. Controlled and targeted drug delivery

Liposomes can release drugs slowly or specifically at the disease site. This helps maintain constant drug levels in the body and reduces dosing frequency.

Example: PEGylated liposomal formulations for prolonged circulation(45).

Limitations of liposomes (with examples) [49,50]

1. Stability problems

Liposomes may undergo oxidation, hydrolysis, aggregation, or drug leakage during storage, reducing shelf life and effectiveness.

Example: Phospholipid oxidation leading to drug leakage.

2. High production cost

Liposome preparation requires costly materials, equipment, and quality control methods, making the final product expensive.

Example: High cost of liposomal anticancer drugs.

3. Short shelf life

Liposomes are sensitive to temperature and light and often require refrigeration, complicating storage and transport.

Example: Cold-storage requirement for liposomal injections.

4. Rapid clearance by immune system

Unmodified liposomes may be quickly removed by the body's immune system before reaching the target site.

Example: Conventional liposomes cleared by macrophages.

5. Limited drug-loading capacity

Some drugs cannot be efficiently encapsulated in liposomes, reducing therapeutic effectiveness. Example: Poor encapsulation of highly water-insoluble drugs.

6. Complex regulatory approval

Liposomal products require extensive testing for safety, stability, and reproducibility, making regulatory approval difficult.

Example: Long approval process for new liposomal formulations.

7. Scale-up difficulties

Maintaining uniform size and drug content during large-scale manufacturing is challenging.

Example: Batch-to-batch variation in industrial liposome production.

Evaluation Tests for Liposomes

1. Physical Appearance and Shape (Morphology)

In this test is done to visually confirm whether liposomes are properly formed or not. In simple words, we check how liposomes look. Ideally, liposomes should be round, smooth, and well-defined. We also check whether they have a single lipid layer (unilamellar) or multiple layers (multilamellar). Any broken, irregular, or collapsed vesicles indicate poor formulation. [51,52]

Instruments used: Optical microscope, Transmission Electron Microscope (TEM), or Scanning Electron Microscope (SEM).

Specification: Liposomes should be spherical with intact bilayer membranes and free from aggregation or rupture.

2. Particle Size and Size Distribution

In this test it measures the average size of liposomes and checks whether all particles are nearly the same size.

Size distribution is commonly expressed using the polydispersity index (PDI) , it indicates whether liposome are of nearly the same size or different . A uniform size distribution ensure better stability and reproducibility of formulation, whereas a wide size distribution may lead aggregation , rapid clearance, or inconsistent drug delivery .[52,53]

Instrument used: Dynamic Light Scattering (DLS) particle size analyzer. Specifications**:**

• Particle size: 50-500 nm (ideal for most liposomal drug delivery systems)

• Polydispersity Index value: ≤ 0.3 (indicates uniform and stable formulation)

3. Surface Charge (Zeta Potential)

The surface charge test, also known as zeta potential measurement, is performed to determine the electrical charge present on the surface of liposomes. This charge plays a very important role in maintaining the physical stability of the liposomal formulation. Liposomes with sufficient positive or negative surface charge repel each

other, which prevents them from sticking together, clumping, or settling during storage[53'54].

Instrument used: Zeta potential analyzer. Specification:

• Zeta potential ≥ +30 mV or ≤ –30 mV indicates good physical stability

• Values close to zero indicate poor stability and aggregation risk

4. Entrapment Efficiency (EE%)

In this method we determine the drug percentage is encapsulated inside the liposome by the help of entrapment efficiency.

A higher entrapment efficiency means less drug wastage and better therapeutic effect.

Liposomes are separated from free drug using centrifugation or dialysis. The amount of drug inside liposomes is then analyzed using UV spectrophotometer or HPLC .[51,54]

The calculation of Entrapment Efficiency :

$$EE\% = \frac{Drug\ inside\ Liposome}{Total\ drug\ added} \times 100$$

Specification:

• Acceptable EE%: ≥ 50%

• Good formulation: ≥ 70% entrapment efficiency

5. In-Vitro Drug Release Study

This test checks how fast or how slowly the drug comes out of the liposomes. Liposomes are placed in a liquid that mimics body fluids (like blood or gastric fluid), and drug release is measured over time. Ideally, liposomes should release the drug slowly and in a controlled manner, not all at once .[54,,55]

Method used: Dialysis bag method or diffusion method

Specification:

• Controlled release pattern without burst release

• 50–80% drug release within 12–24 hours (for sustained release liposomes)

6. Stability Studies (Shelf Life)

Stability testing is done to check whether liposomes remain stable during storage. We observe changes in size, entrapment efficiency, surface charge, and appearance over time when stored at different temperatures.[53,56]

Storage conditions:

• Refrigerator temperature: 4 °C

• Room temperature: 25 °C

Specification:

• No significant change in size, PDI, zeta potential, or EE%

• No aggregation, precipitation, or phase separation

7. Lamellarity Analysis

This test determines how many lipid bilayers are present in the liposome. Multilamellar liposomes can hold more drug, while unilamellar liposomes provide better controlled release. The number of layers directly affects drug loading and release behaviour.[49,52]

Instruments used: NMR spectroscopy, X-ray diffraction, or Transmission Electon Microscope

Specification:

• Clear identification of unilamellar or multilamellar structure

• Consistent lamellarity throughout the formulation

CONCLUSION

Liposomes have established themselves as an effective and reliable drug delivery system in modern pharmaceutical science due to their biocompatibility and structural similarity to biological membranes. They enhance drug stability, improve bioavailability, and allow controlled as well as targeted drug release. These properties make liposomes highly useful in treating diseases where conventional dosage forms show poor efficacy or cause significant side effects.

Curcumin-loaded liposomes represent an important advancement in this field. Curcumin, a natural compound with strong anti-inflammatory, antioxidant, and anticancer properties, suffers from poor solubility, rapid metabolism, and low bioavailability. Liposomal encapsulation overcomes these limitations by improving its solubility, protecting it from degradation, and enhancing its absorption. As a result, curcumin-loaded liposomes show improved therapeutic efficacy, especially in cancer treatment, inflammatory conditions, and liver disorders. They also enable better cellular uptake and targeted delivery, reducing toxicity to normal tissues.

In conclusion, liposomes provide a versatile platform for drug delivery, and curcumin-loaded liposomes significantly enhance the clinical potential of curcumin. With ongoing research and technological advancements, these systems are expected to play a key role in future pharmaceutical development, offering safer and more effective therapies.

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