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Injectable Nano-Liposome: Manufacturing Stratergies and Key Challenges

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ABSTRACT:
Nanoliposomes have emerged as a promising and versatile platform in the field of drug delivery. Injectable nanoliposomes offer a transformative approach to systemic drug delivery. These microscopic phospholipid bilayer vesicles encapsulate and deliver a variety of therapeutic agents directly into the bloodstream, unlocking several advantages like prolonged Circulation,enhanced Therapeutic Index, improved targeting, controlled Release and improved Bioavailability. Their biocompatibility, stability and ability to encapsulate both hydrophilic and hydrophobic drugs make nanoliposomes a versatile tool for enhancing drug efficacy and minimizing side effects. Nanoliposomes physicochemical characteristics which can be adjusted to suit the requirement of patients and drugs include size, charge and lipid composition. This review article focuses on nanoliposome, its preparation techniques, characterization, advantages, challenges, application and marketed formulation of nanoliposome in the injectable pharmaceutical industry.

Keywords: Injectable nanoliposomes, lyophilization, cholesterol, formulation methods, challenges.

I. INTRODUCTION

Conventional drug delivery systems [DDS] are used to deliver therapeutic molecules by various routes like oral, injection, or topical. These systems were extensively in use because of their ease in administration. However, there are many disadvantages governed mainly due to the lack of compatibility, poor biodistribution, burst release, toxicity and low amount of drug reaching the target sites. There is a need for highly effective and non-toxic alternatives to treat existing and emerging diseases.

Particular focus is being paid to the need to create safer, more effective, biocompatible, and patient-complied therapies in biomedical settings by employing nanotechnology as a possible platform for the creation of novel drug delivery systems [DDS].Nanocarriers are microscopic particles with a size of 1-1000 nanometers[1]. The nanocarriers available in drug delivery like liposome, micelles, dendrimer, carbon nanotube and polymeric nanoparticles are available either to develop new formulations or improve the existing ones but have certain disadvantages like low penetration, biocompatibility, low entrapment efficiency and toxicity issues. So, there is a need for more efficient nano-vesicular systems like nano-liposomes[2,3].

Nano-liposomes are lipid bilayer vesicles in nano-sized range. Due to their bilayer structure composed of lipid and aqueous layer they can encapsulate both hydrophilic and hydrophobic drug [4]. They have unique properties and various clinical trials have revealed that nanoliposomes are great candidates for varied delivery systems such as anti-cancer, anesthetics, anti-fungal, gene medicines and anti-inflammatory drugs

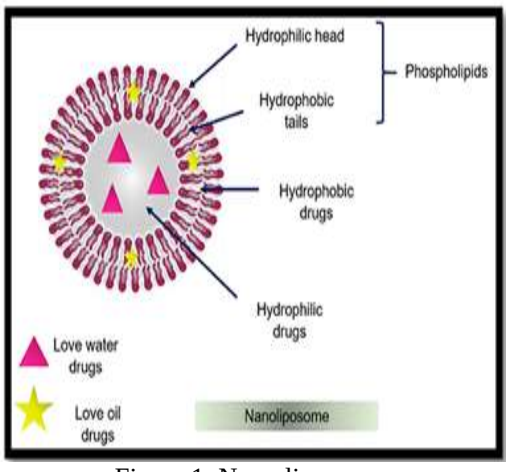

Figure 1: Nano-liposome

For many reasons, injectable nano-liposomes hold great promise as a drug delivery technology.

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1.1 **Advantages of nano-liposome which is helpful in drug delivery system:**

1. Increased stability: Nano-liposomes are more stable than liposomes due to their smaller size, which reduces the likelihood of aggregation.
2. Prolong the release of drugs, which can reduce the frequency of dosing.
3. Protect drugs from degradation[5].
4. Enhanced permeability: They have larger surface area to volume ratio than liposomes which results in easy access to cross cell membranes and deliver drugs to target tissue sites.
5. Improved targeting: Drug delivery efficiency can be increased and adverse effects can be reduced by tailoring nano-liposomes to target particular tissues or disease locations[6].
6. Encapsulate a wide variety of drugs, including small molecules, proteins, and nucleic acids[7].

1.2 Lyophilized nano-liposomes

Nimodipine [NMD]is used to treat brain damage and is a calcium channel blocker whichworks by dilating cerebral arterioles and enhances flow rate of blood in the cerebral area. There are marketed NMD tablets and NMD capsules available but its high first-pass metabolism [8]. NMD has issues like poor water-solubility and low bioavailability when administered orally. So injection of nimodipine was made by ethanol injection method but there are problems of patient compliance, causes phlebitis and due to its poor water-solubility of NMD there is crystallization of drug taking place. Then NMD-loaded nano-suspensions and nanospheres were prepared but the preparation takes a lot of time and the solid lipid gelatinized in the nanospheres. Nano-liposomes was made which proved to be the ideal formulation in which microscopic vesicles contain phospholipid bilayers entrapping aqueous compartments. NMD is lipophilic in nature and easy to incorporate in the bilayer of the liposomes. The stability of the formulation is 12 days. The stability problem was overcome by lyophilization. The nano-liposome product is freeze-dried with certain cryoprotectants and is reconstituted before administration. By lyophilization the shelf-life increases of the formulation.

Lyophilized nano-liposomes also known as freeze-dried nano-liposomes, are liposomes that have been dehydrated using a process called lyophilization. Lyophilization is a gentle drying method that removes water from the liposomes without damaging their structure. This process allows the liposomes to be stored in a dry state for extended periods of time without losing their activity[9, 10]

1.3 There are several advantages to using lyophilized nano-liposomes for drug delivery:

1. Improved stability: Lyophilization removes water from the nano-liposomes, which helps to prevent the degradation of the encapsulated drug and the liposome structure. This increases the shelf life of the formulation.
2. Enhanced bioavailability: Lyophilized nano-liposomes are more easily rehydrated than liquid nano-liposomes, which can improve their absorption and bioavailability when administered orally or parenteral[11].
3. Reduced risk of contamination: The lyophilized form of nano-liposomes is less susceptible to contamination by microorganisms, which can be a concern with liquid nano-liposome formulations.
4. Convenient storage and transportation: Lyophilized nano-liposomes are dry and lightweight, making them easier to store and transport than liquid nano-liposomes[12].

1.4 CLASSIFICATION OF NANO-LIPOSOME BASED ON SIZE

On the basis of size liposomes can be small, medium and large[13].

On the basis of layers liposomes can be unilamellar, oligolamellar and multilamellar[14].

Table 1: Type of nano-liposome

Type	vesicles	size	Lipid bilayer
MLV	Multilamellar vesicles	0.5-5	5-20 lipid bilayer
SUV	Small unilamellar vesicles	20-200 nm	1 lipid bilayer
LUV	Large unilamellar vesicles	> 200 nm	1 lipid bilayer
GUV	Giant unilamellar vesicles	> 1	1 lipid bilayer

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MVV	Multi vesicular vesicles	> 1	Multi lipid bilayer

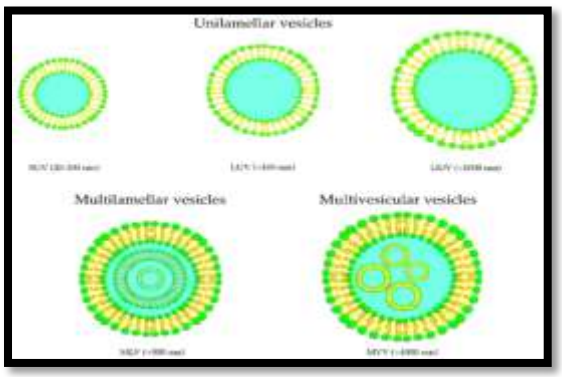

Figure 2: Type of vesicle system

II. NANO-LIPOSOME COMPOSITION

The main ingredients in preparing nano-liposomes is phospholipids; they are amphiphilic in nature which possess both hydrophilic and hydrophobic properties. The phospholipid head group is hydrophilic[water loving] and its tail is hydrophobic [water hating]. In bilayer structure the hydrophobic tails face inside, whereas the hydrophilic heads face outward[15]. The bilayer that is formed is impermeable to water-soluble molecules. Along with this nano-liposomes contain sterols, the most common sterol used in cholesterol. Addition of sterols in bilayers changes the properties of vesicles in the nano-liposomes and enhances stability by modifying fluidity in the lipid bilayer and preventing crystallization of the phospholipids[16].

The parameters that is considered in the method selection are:
1. The physicochemical nature of the drug which is entrapped or encapsulated
2. Liposomal ingredients
3. The vehicle in which liposomes is dispersed
4. Drug and other excipients effective concentration and toxicity
5. Characterization and shelf-life of nano-liposome[17]
6. Is the method reproducible in large-scale production?

There are four fundamental steps in all liposome preparation techniques:
1. Lipids are mixed in organic solvent.
2. The lipid is slowly added in aqueous media.
3. Purification of liposome.
4. Analyzing and characterizing the final formulation[18].

III. METHODS TO MANUFACTURE NANOLIPOSOME

1. Thin-film hydration: It involves dissolving lipids in an organic solvent and evaporating the organic solvent in rotatory evaporator to form a thin film and hydrating the film with an aqueous buffer[19].

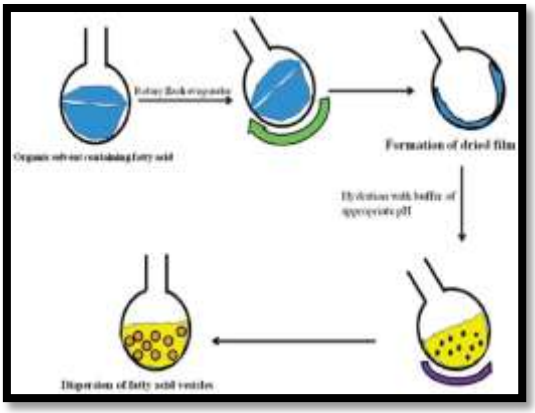

Figure 3: Thin film hydration method

2. Supercritical fluid method [SCFM]: In this method supercritical carbon dioxide [$SCCO_2$] is used as a solvent to dissolve lipids and drugs at high pressure and temperature causing them to reach a supercritical state. The supercritical solution is then expanded through a nozzle rapidly, which decreases pressure and temperature. This sudden expansion leads to the formation of small unilamellar liposomes. The liposomes formed are hydrated in an aqueous buffer to stabilize their structure. $SCCO_2$ is a non-toxic, non-flammable, and environmentally friendly solvent[20].

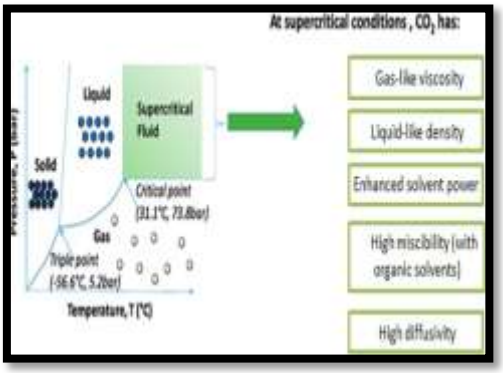

Figure 4: Supercritical fluid method

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3. Ethanol injection: It involves injecting phospholipid solution in ethanol into agitated aqueous media. The ethanol is diluted in the aqueous media causes the lipid molecules to separate, form bilayer which then form nano-liposome[21].

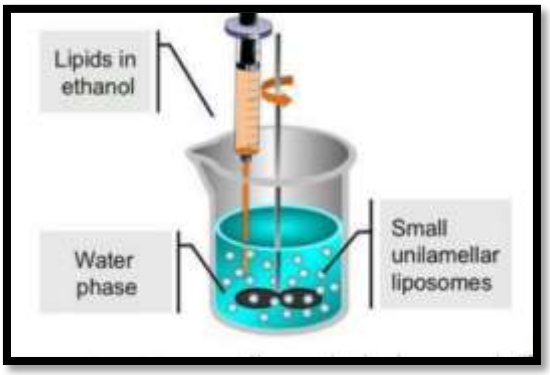

Figure 5: Ethanol injection method

4. Ether Infusion Method: An organic solvent is chosen based on the solubility of the lipids, mostly diethyl ether and chloroform is chosen. Aqueous solution of drug is prepared. In organic solvent drop wise aqueous solution is added. The ether will become saturated with water, forming a thin layer at the bottom of the vial or beaker. On stirring closed liposomal structures are made. The organic solvent is removed by rotary evaporation or by placing the mixture under vacuum. The resulting liposomal suspension can be used immediately or stored in the refrigerator or frozen for later use[22].

5. Solvent evaporation: It involves dissolving lipids and drugs in an organic solvent and then evaporating the solvent under reduced pressure. This causes the lipids to self-assemble into liposomes[23].

6. Reverse phase evaporation: Dissolve lipids and detergent [Triton X-100 and CHAPS] in an organic solvent [diethyl ether and chloroform]. Prepare the aqueous solution. Adding the aqueous solution to the organic solution. The ratio of organic to aqueous phase determines the size of the micelles. The organic solvent is evaporated using rotary evaporator or a lyophilized. The rate of evaporation should be slow and controlled to prevent the formation of emulsions. Above phase transition temperature the formulation is dried. This will allow the bilayer to reorganize and form stable liposomes[24].

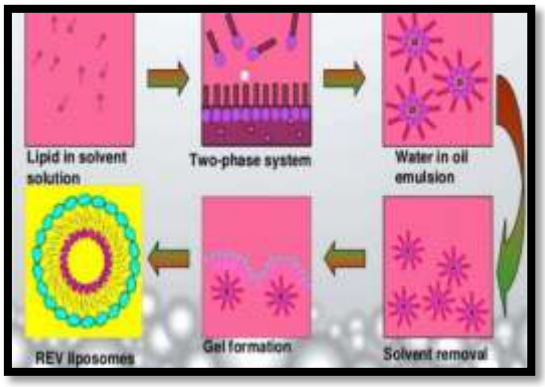

Figure 6: Reverse phase evaporation method

7. Micro-fluidization/ Micro-emulsion: The micro fluidizer instrument works by dividing high pressure into two portions and is passed through a very fine aperture inside the chamber[25].

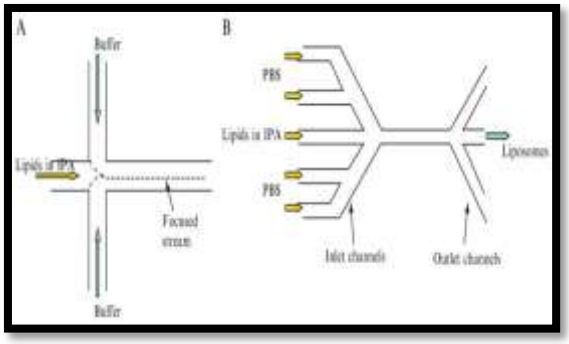

Figure 7: Microfluidization method

8. Membrane extrusion: This method involves forcing a solution of lipids and drugs through a porous membrane. The membrane causes the lipids to self-assemble into liposomes. Membrane extrusion is a simple technique to prepare various size and size distribution liposome[26].

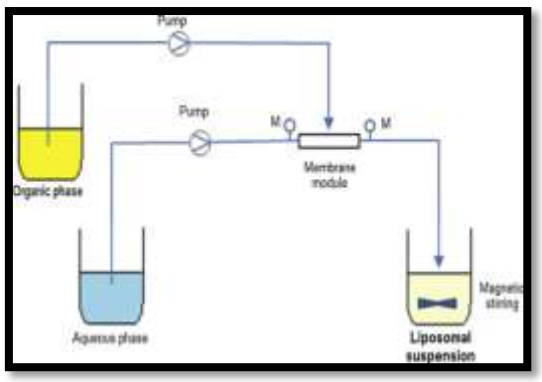

Figure 8: Membrane extrusion

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9. Mozafari Method: Mozafari designed a home-made glass vessel to develop nano-liposome. The vessel has several baffles and seven turbulences. The liposomal ingredients are added in a heated mixture which contains the active compound. Then the mixture is heated at elevated high temperature in nitrogen atmosphere before mixing the phospholipid components[27].

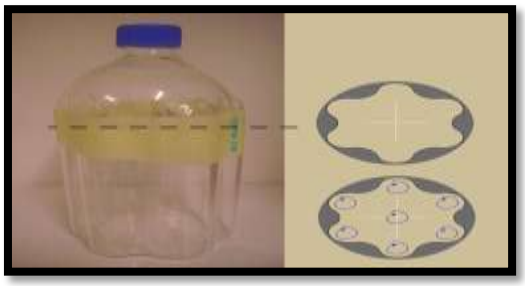

Figure 9: Mozafari Method

10**. Particles from Gas Saturated Solution:** It is a two-step procedure. In the first step in a mixing container, the solute is saturated with CO2. The second step involves the expansion of the gas-saturated solution with the help of a nozzle. As the temperature drop the material solidifies and causes particle formation[28].

Table 2: Advantages and disadvantages of nano-liposome method

Methods	Advantages	Disadvantages
Thin film hydration	● Easy and economical to use ● Scalable	● Use of organic solvents ● For water soluble drug the encapsulation efficiency is low
supercritical fluid method [SCFM]	● Solvent free process ● Control particle size	● Stability ● Difficult for large scale production
Ethanol injection	● Simple and rapid process ● High yield ● Good stability	● Low encapsulation efficiency ● Prone to aggregation
Ether injection	● Simple and easy process ● Produce unilamellar vesicles	● Solvents used is hazardous due to its flammability properties
Solvent evaporation	● Various size nano-liposome can be formulated	● Drug loading difficulty
Reverse phase evaporation	● Well-defined size distribution ● Versatile	● Use of large amount of organic solvent
Membrane extrusion	● Easy method	● The size of liposome is limited
Mozafari	● Easily scalable	● Use of inert nitrogen

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3.1 Factors that can affect the quality of injectable nano-liposomes:

1. The purity of the lipids: Lipid impurities have the potential to cause liposomes to become unstable and lose their ability to carry drugs.

2. The choice of solvent: The solvent should dissolve the lipids and the drug without compromising the stability of the liposomes.

3. The pH of the aqueous phase: The aqueous phase pH should not alter the size and stability of the liposomes[29].

4. The temperature of the process: The process temperature can affect the formation of liposome formation and its stability.

5. The sterilization method: The sterilization method should not damage the liposomes[30].

IV. CHARACTERIZATION METHOD

Nano-liposome can be characterized by:

1. **Visualization**: Optical microscope can detect particles size greater than 300 nm and any contamination with larger particles. Electron microscopy helps to measure size distribution of nano-liposomes[31].
2. **Zeta Potential:** It tells about the stability of nano-liposome. If the zeta potential is higher the repulsion between the particles are higher and more stable the formulation is. Zeta potential also helps to understand how to control the aggregation, fusion and precipitation of nano-liposomes[32].
3. **Lamellarity Determination:** P-31 Nuclear Magnetic Resonance [P NMR] and Freeze-Fracture Electron Microscopy analysis tells about the number of bilayer present in the vesicles[33].
4. **Entrapment Efficiency:** It tells the amount of drug that is loaded in a nano-liposome. The suspension is centrifuged at 5000 rpm for 60 min. The supernatant is separated and filtered. The filtrate was diluted using suitable solvent and measured using a suitable spectrophotometry method like UV and HPLC[34].
5. **Percentage yield:** Practical yield was calculated as the weight of nanoparticles that are formed from the method used in relation to the sum of weight of starting material. It helps in identifying which method gives the highest yield.

V. CHALLENGES

The major challenges while formulating injectable nano-liposomes are:

1. **Scalability:** It is challenging to produce injectable nano-liposomes in sufficient quantities at a reasonable price. To ensure the manufacture of consistent and top-notch liposomes, the manufacturing process needs to be closely monitored and controlled.
2. **Stability:** Drug can be released easily if they are unstable in the body. This decreases the effectiveness of the drug and enhances the risk of side effects. Another challenge is bilayer fusion and drug leakage which has impact on the physical stability, low shelf life of nano-liposomes and affects the reproducibility and stability of nano-liposomes. It is possible to stabilize liposomes by adding specific lipids or polymers to their structure[35].
3. **Immunogenicity:** Injectable nano-liposomes effectiveness can be decreased due to the trigger of an immune response. This happens because the body attacks the liposomes because it perceives them as foreign. Liposomes can be coated with hydrophilic polymers or manufactured from natural lipids to lessen their immunogenicity[36].
4. **Targeting:** It can be difficult to administer nano-liposomes to the desired location within the body at desired site. It is necessary for the liposomes to be able to move freely throughout the bloodstream and avoid taken up by the liver and spleen.
5. **Drug loading:** It can be challenging to load injectable nano-liposomes with drugs. The drug needs to be able to enter the liposome structure without causing any structural instability. To improve drug loading, liposomes can be made larger or have their lipid composition modified[37].
6. **Sterilization**: Sterilizing injectable nano-liposomes without damaging the drug and structure of lipid bilayer can be challenging. Common sterilization methods include heat and radiation which can denature the lipids in the nano-liposome structure and affect the functionality. Sterilizing nano liposome injections is important for the safety and efficacy of these drug. Therefore, alternative methods are preferred for nano liposome injections like:

- Filtration:

Filtration involves passing the liposome formulation [solution, suspension or emulsion] through a filter. The size of the filter is such that it is smaller than the size of microorganisms and can

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remove bacteria, viruses, and other contaminants. This method is easy and does not affects the properties of the liposomes.

- Aseptic Processing:

To avoid contamination, aseptic processing is used which involves manufacturing and handling the liposome injection in a sterile manner. Using sterile equipment in sterile conditions and adhering strictly to hygiene guidelines

The sterilization method is selected depending on a number of factors like the type of liposomes, size of liposome, sterility level and the size of the batch of the production process. Filtration is selected for small-scale production and takes less time whereas aseptic processing is preferred for large-scale production and where a high level of sterility is needed[38].

7. Using environment friendly excipients: By Avoiding the use of hazardous organic solvents, detergents and excipients like phospholipids in the final formulation. Increasing the use of environment friendly solvent should be prioritized.
8. Regulatory requirements: Nano-liposomes are complex in nature and they lack evaluation parameter. Several marketed liposomal formulation like doxorubicin and amphotericin have gone off-patent. So, there is a need for bioequivalence testing[39].

5.1 Challenges of Lyophilized Nano-liposomes

1. **Possibility of aggregation:** During lyophilization nano-liposomes may fuse or aggregate together which will affect the drug delivery properties.
2. **Loss of encapsulation efficiency**: The lyophilization process can release the drug from the nano-liposome which leads to a loss of encapsulation efficiency.
3. **Selection of right excipients**: The choice of right excipients is important during lyophilization process as it can either cause aggregation or prevent aggregation. It is important for the integrity and stability of nano-liposome[40].

VI. APPLICATIONS

Nano-liposomes are being used for a wide range of drug delivery applications like:
1. Cancer therapy: Nano-liposomes deliver anticancer drugs directly to targeted sites that are tumor cells and improve drug efficacy and reduce side effects.
2. Gene therapy: Gene therapy vectors can be delivered to target cells via nano-liposomes, offering opportunity for the treatment of numerous hereditary illnesses.
3. Antimicrobial therapy: Nano-liposomes can deliver antimicrobial drugs to the site of infection, which helps to combat antibiotic-resistant bacteria.
4. Vaccine delivery: Vaccines can be administered to the body via injectable nano-liposome technology. They minimize the possibility of adverse effects and increase the vaccine's efficacy.
5. Cosmetics: It is a new and growing technologies for skin care and cosmetic products[41,42].

VII. MARKETED NANOLIPOSOME FORMULATION

1. Ambisome: It is an FDA-approved amphotericin B liposome for injection [45-80 nm], an antifungal drug used to treat a variety of serious fungal infections like aspergillosis, coccidioidomycosis, blastomycosis and leishmaniasis. Ambisome is more effective than amphotericin B when treating leishmaniasis and aspergillosis.
2. Zeposia: It is an FDA-approved injectable liposomal formulation containing vincristine sulfate. It is a drug used to treat cancer, Hodgkin's lymphoma and acute lymphoblastic leukemia. Zeposia is more effective than vincristine sulfate in the treatment of cancer and Hodgkin's lymphoma.
3. Onivyde: It is a nano-liposome injection of irinotecan [88~95 nm].
4. Vyxeos: It is an FDA-approved liposomal formulation [100 nm] of daunorubicin and cytarabine. It is a chemotherapy drugs used to treat myeloid leukemia. When treating adult AML, vyxeos is superior to cytarabine and daunorubicin taken separately[43,44].

VIII. CONCLUSION

One of the most promising encapsulation techniques in the quickly developing field of nanotechnology is the usage of nano-liposomes.Their unique properties and ability to address several limitations of conventional drug delivery system create new opportunities for advancing disease treatment and improving patient outcomes.Nano-liposomes are one of the only drug delivery system which can be of potential in controlling and targeting drug. Nano-liposomes faces several challenges in terms of large-scale production, cost, and targeting specificity. Future

research should focus on addressing these challenges and exploring novel targeting strategies to unlock the full potential of this technology.There are number of active pharmaceutical ingredient that can be made into nano-liposomes ranging from pharmaceuticals, cosmetics and nutraceuticals.

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