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A Concise Review of Liposomal Vaccine Tailoring Methods for Enhancing Vaccination Efficacy

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Abstract:

Considering liposomes are just one of numerous drug delivery systems that can be used to deliver medication to a specific area, we explored them in this review article. Liposomes are small synthetic vesicles with a spherical form that can be made using cholesterol and organic phospholipids. Vaccination is the most effective method of preventing infectious diseases and saving lives. The development of lipid nanoparticles and liposomes as subunit vaccines for infectious diseases has undergone substantial research and development. Positively charged lipid-bilayer vesicles known as cationic liposomes are a brand-new and fascinating adjuvant approach that has just lately come back into fashion. The potential adjuvants for vaccinations include cationic liposomes.

Key word:

Liposomes, cationic liposomes, phospholipid, vaccination.

Introduction:

During the diffusion of phospholipids in water, they develop a closed structure naturally, with phospholipid bilayer membranes enclosing an external aquatic environment. The term "liposome" refers to this vesicular system ^[1]. The microscopic, spherical vesicles known as liposomes can be made from a variety of substances, containing membrane proteins, lengthy saturated fats, phosphatidylcholine, sphingolipids, cholesterol, and non-toxic surfactant ^[2]. liposomes are basically used to improve solubility and permeability of hydrophobic and hydrophilic drug. When particular lipids are hydrated in aqueous conditions, liposomes spontaneously form as Colloidal or microparticulate carriers are typically 0.05 to 5.0 μ m in diameter ^[3]. The material that composes liposomes is generally both biocompatible or biodegradable and They include an aqueous material that is surrounded by more than one lipid bilayers made of natural or synthetic materials ^[4]. Immunotherapy and vaccination work to combat diseases by utilising the host immune system, which is triggered by the antigenic components (also known as antigens) of disease-causing organisms (also known as pathogens). This immunity can then be used to eradicate infections with the same antigens (Ages) ^[5].

The key information about pandemic threats as well as their negative socioeconomic consequences for individual states and the global economy demonstrate. The importance of creating safe and efficient methods for treating and preventing viral infections ^[6]. Traditional vaccines contain live organisms that which is dead or diminished. When developing a vaccination using liposomes, it is important to consider the particle's net charge, and these particles are frequently either negative (anionic liposomes) or positive (cationic liposomes) ^[7]. As a vaccine adjuvant, liposomes were tested on humans for the first time, A malaria protein that had been synthesised was encased in multilamellar liposomes that also included cholesterol, neutral and anionic saturated phospholipids, and monophosphorylate (MPLA) as a lipid A adjuvant ^[8].

❖ Classification of liposomes:

Liposomes are categorised according to

1. Structure
2. preparation technique

3. Composition and application
4. Standard liposomes
5. Special liposomes

1. Classification based on the type of Structure: [9]

Type	Size	No. of layer	abbreviation
Large unilamellar vesicle	< 100 nm	One	LUV
Small unilamellar vesicle	20 -100 nm	One	SUV
Unilamellar vesicle	All size range	One	UV
Giant unilamellar vesicle	< 1 mm	One	GUV
Oligolamellar vesicle	0.1-1 mm	Approx. 5	OLV
Multi vesicular vesicle	More then1 mm	Multiple	MV
Multilamellar vesicle	More then 0.5	5-25	MLV

Table no 1: Classification based on structure

2. Classification based on the type of Preparation Technique: [10-14]

- Vesicle prepared by RPE method
- Multi lamellar vesicle made by RPE method
- Dried reconstituted vesicle
- Frozen multi lamellar vesicle
- Vesicle prepared by extrusion technique
- Dehydration- Rehydration method

3. Classification based on the type of composition and application:

Sr no	Types of liposomes	Composition	Ref no
1	Standard liposomes	Neutral and negatively charged phospholipid & cholesterol	9
2	Fusogenic liposomes	Reconstituted sendai virus envelops	16
3	PH complex liposomes	Phospholipid such as PER & DOPE	17

4	Positively charged liposomes	Cationic lipid with DOPE	18
5	Extended circulatory liposomes	CL or CLC attached with monoclonal antibody	9

Table no 2: Classification based on composition & application

4. Classification based on the type of Standard liposomes: [19]

- Stabilize natural lecithin (PC) formulations
- Glycolipids containing liposome
- chain phospholipids

5. Classification based on the type of special liposomes: [9]

- Bipolar fatty acid
- Antibody directed liposome.
- Methyl/ Methylene x- linked liposome.
- Lipoprotein coated liposome.
- Carbohydrate coated liposome.
- Multiple encapsulated liposome

❖ Advantages of liposomal DDS: [11,12,19]

1. Can be produced in various sizes.
2. Increased therapeutic effectiveness.
3. Cancers can be targeted with specific passive targeting.
4. Enhanced pharmacokinetic action.
5. It lowers the toxicity of encapsulated drug.
6. There are several different ways to administer it.
7. Both positively and negatively charged compounds have interaction capacity with liposomes.
8. The DNA is sometimes protected from damaging processes by liposomes.
9. Targeting specific tissues or cells with liposomes is possible.

❖ **Disadvantages of liposomal DDS:** [11,12,19]

1. It is possible to use solar energy during the day.
2. The production costs are high.
3. Leaking drug encapsulation while being stored.
4. It is unable to be used for rare diseases.
5. Phospholipid sometimes undergoes reactions like oxidation and hydrolysis.
6. Liposomes having a short half-life.
7. Liposomes also having low solubility.

Method of preparation:

❖ General method of preparation of liposomes [20]

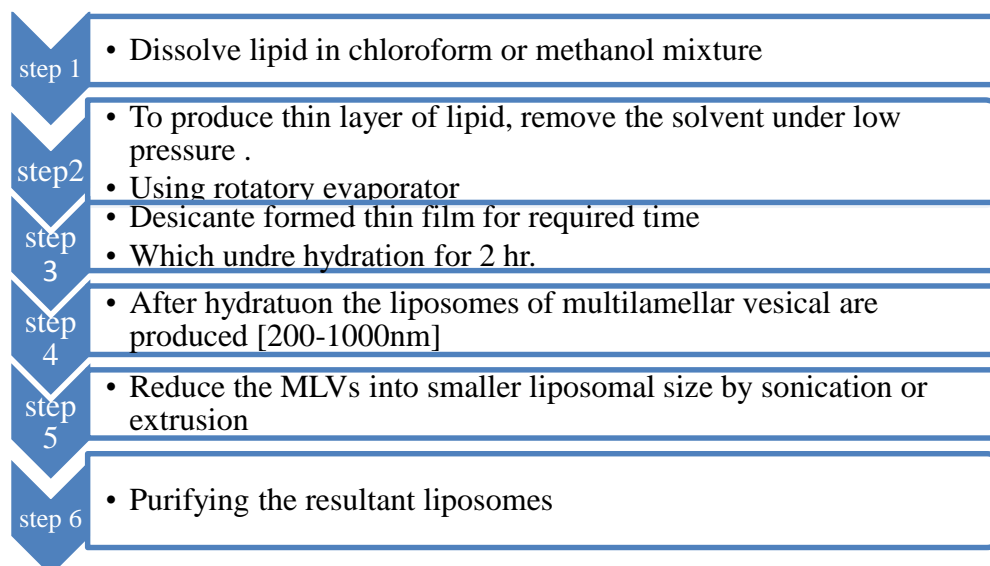


Fig no 1 : General method of preparation

❖ Specific method of preparation: [21,22,23]

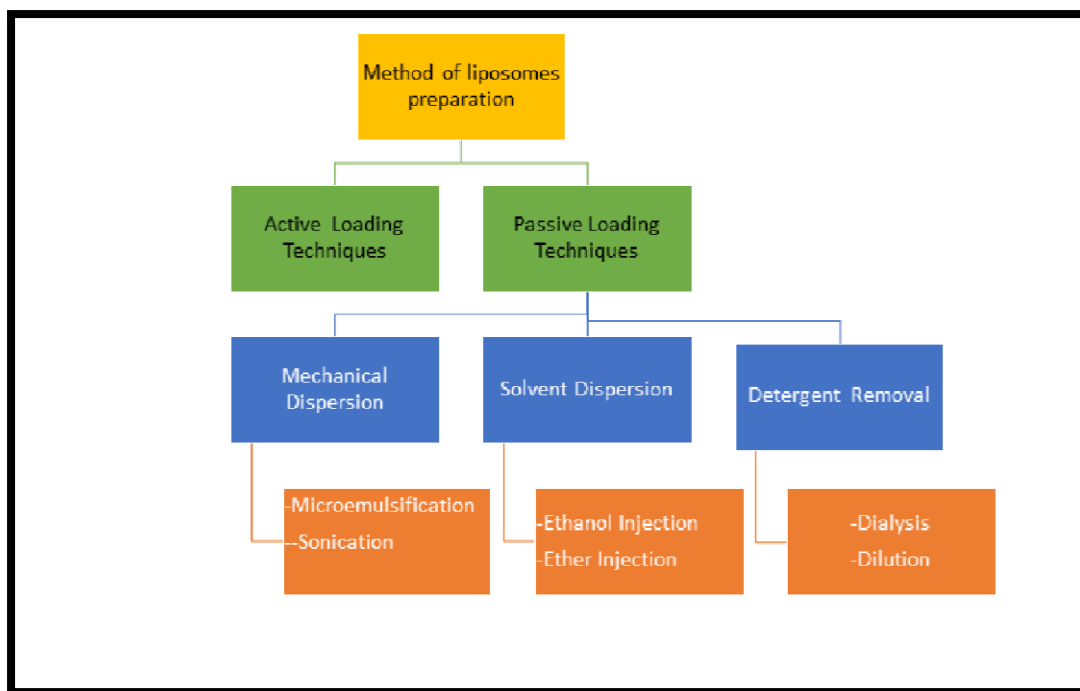


Fig no 2: Specific Method of Preparation

❖ **Passive loading technique:**

Different methods for producing liposomes result in different physicochemical properties, which affects how well they perform in vitro (for sterilisation and shelf life) and in vivo (for disposition) [22]. Passive loading technique are classified into 3 main Type which based on dispersion technique

1. Mechanical Dispersion Method
2. Solvent Dispersion Method
3. Detergent Removal Method

1. Mechanical Dispersion:

A. Sonication:

The SUVs manufacturing process with a diameter of between 15 and 25 μm is done using this method. Multi lamellar vesicle are sonicated using maybe a probe sonicators or a bath sonicators in inert environment. While bath sonicators are used for large volumes, when small amounts of high energy are needed, probe sonicators are used [23]. The main issues with this

technique include its extremely low internal volume/encapsulation efficacy, potential phospholipid and chemical degradation [24].

2. Solvent Dispersion:

A. Ether Injection:

This method is also known as solvent vaporization. between 55 and 65 degrees or when there is low pressure. Lipids in a mixture of any such methanol or ethanol and diethyl-ether solution is progressively infused into a liquid form of the substance that needs to be contained. As a result, liposomes are produced as a result of removing the ether under vacuum. The population's heterogeneity represents the technique's main problem (70 to 200 nm) and the chemicals that must be sealed off exposure to organic chemical at high temperatures [25].

B. Ethanol Injection:

Immediately injected into a large buffer excess is an ethanol-lipid solution. MLVs instantly begin to develop. The method has a number of drawbacks, including a population that they are extremely diluted liposomes that have a size range of 30 to 110 nm., ethanol that forms into an azeotrope with water that makes it difficult to remove completely, and a high likelihood that different macromolecules with biological activity will inactivate when even small quantity of ethanol [26].

3. Detergent Removal:

A. Dialysis:

At the necessary concentrations of microspheres, detergents have successfully solubilized lipids (CMC). The micelles get better and better at phospholipid when the detergent is withdrawn, eventually combining to form LUVs. Dialysis was used to eliminate the detergents. For the elimination of detergents, a commercial product known as Lipopreparation a dialysis system variant, is available. Equilibrium dialysis can be carried out using dialysis bags filled with sizable, detergent-free buffers [27].

❖ Liposomal based drug available in market:

Sr no	Product	Drug	Administration	Composition	Indication	Year approved	Ref no
1	Ambisome	Amphotericin B	Intravenous	HPSC, DSPG, cholesterol & amphotericin B	Fungal infection	1990	28
2	Abelcet	Amphotericin B	Intravenous	DMPC & DMPG in 7:3 molar ratio	Fungal infection	1996	29
3	DepoCyt	Morphine sulfate	Epidural	DOPC, DPPG, cholesterol	Lymphomatous	1995	30
4	Doxil	Doxorubicin	Intravenous	HPSC: cholesterol: PEG 2000-DSPE {56:39:5 RATIO}	Breast cancer	1990	31,32
5	depoDur	Morphine sulfate	Epidural	DOPC, DPPG, cholesterol and triolein	Pain	2004	33
6	Marqibo	Vincristine sulfate	Intravenous	Liposome Egg sphingomyelin and cholesterol	Acute lymphoblastic leukemia	2012	34
7	Visudyne	Verteporfin	intravenous	EPG and DMPC	Age-related molecular degeneration	2000	35,36

Table no 3: Liposomal based drug available in market

❖ Vaccines:

Many vaccinations are created using weakened or dead viruses, which allow antibodies to attach and kill live viruses [37]. Most important medical treatment is when battling infectious infections is vaccination [38]. The invention by Edward Jenner in 1796, vaccinations have eradicated infectious diseases like hepatitis, malaria, smallpox, diphtheria, and influenza on a global scale. This was accomplished at the end of the 18th century by using the smallpox prevention properties of clinically mild cowpox infections [39]. The person who has received the vaccination becomes immune to the disease on subsequent exposure, and the pathogen-induced sickness's undesirable side effects are avoided. Effective immunizations have largely controlled many infectious illnesses that previously caused major young children's morbidity and mortality [40].

❖ **Vaccine adjuvant:**

The word "adjuvant" originates from the Latin word "adjuvaer" which means "to help" or "to enhance." [41]. Recent developments in vaccine development have made it possible to create vaccinations that are extremely pure, safe, and straightforward. Adjuvants are components that can improve and/or modify immune responses that are specific to an antigen related to vaccines [42]. Modern vaccines can now be built on recombinant antigens with better safety profiles that have been logically designed and contain highly purified components. Accidentally, the immune-boosting abilities of an adjuvant added to a vaccination were discovered much like many other medical discoveries [43]. Adjuvants are necessary in vaccinations with poor immunogenicity antigens even if Vaccines made using intact (or dead) viruses or bacteria have a built-in immunity to disease (e.g., peptides, small haptens) [37]. For around 70 years, the only adjuvant permitted for use in certified vaccinations was aluminium, which was first used in human immunizations in 1932 [44]. Aluminium adjuvants are excellent for vaccinations targeting diseases destroyed mostly by antibodies since they work primarily to boost antibody formation. Intracellular pathogen-related infections have not been prevented by aluminium-adjuvanted vaccines [45]. The selection of adjuvant and formulation it may depend on a variety of variables, such as the physicochemical characteristics of the vaccination antigen, the desired type of immune response, the target's age population, and the method of vaccination [46]. Typically, immunisation with pure protein antigens produces a minimal T cell response and a minor antigen activity. To prevent wasting potentially effective vaccination antigen candidates, Adjuvant choice must be considered while choosing the vaccine antigen. Immunotherapy and vaccination work to combat diseases by utilising the host immune system, which is triggered

by the antigenic components (also known as antigens) of disease-causing organisms (also known as pathogens) [47].

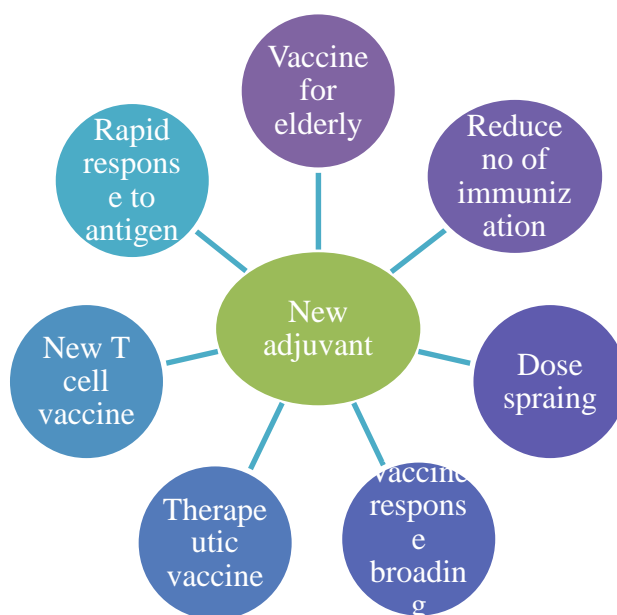


Fig no 3: benefits of adjuvant [48]

❖ **Classification of adjuvant:** [49]

Adjuvant are basically categorised based on their method of action, source, physiochemical properties and administration route.

1. Mineral salt
2. Tensioactive compound
3. Microorganism derivative compound
4. Emulsion
5. Nanoparticles antigen delivery methods
 - I. Liposome
 - II. Polycrystalline microsphere
 - III. Nano beds
 - IV. Virus like particle
6. Cytokines polysaccharides

7. Nuclei acid-based vaccine adjuvant

❖ Liposomes as vaccine adjuvant:

Out of all the other nanoparticle delivery techniques, liposomes, lipid vesicular structures, have shown the most promise as a vaccine delivery method ^[50]. The effective adjuvant and delivery technology known as liposomes is versatile and widely used. Despite the fact that most of their uses as agents for the administration of immunostimulatory molecules have been in the field of cancer immunotherapy, they can be thought of as a prime possibility for vaccine carriers. In addition to improving drug delivery, Employing liposomes as delivery methods and adjuvant for vaccines ^[51]. Targeted antigen distribution via a variety of administration routes is made possible by their versatility sizes, charges, and bilayer rigidity, and composition. Alec Bangham, a British haematologist, the first to discuss liposome technology ^[52]. He described liposomes as swollen phospholipid molecules with a complete variety in terms of size and structure systems for delivering drugs in small particles have the potential to serve as adjuvants ^[53]. To shield antigens from deterioration and to make antigen administration to antigen-presenting cells easier, they provide the capacity to insert subunit antigens within pathogen-sized particles ^[54]. Among the available technologies for delivering nanoparticle drugs, A result of its immunological function and adjuvant activity, the first system to be described was liposomes, Allison and Gregoriadis identified adjuvant characteristics (1974) ^[55]. The physicochemical properties of Adjuvants with Liposomes have a connection to the type of immunological response they promote, the biodistribution of liposomes, their exposure to lymph nodes, and the activation of the Innate defence mechanisms are all affected by the sizes and charge of the liposomes ^[56]. The encapsulated antigenic material is protected from the environment and released for an extessnded span of time. thanks to the liposomal adjuvant action, which improves Antigen-specific immune response and dendritic cell uptake. Inflexal (against influenza) and Epaxal (against hepatitis) These are the only two virosome-based vaccine systems that currently use liposomes as adjuvants ^[57].

Adjuvant class	Response	Vaccine detailed	Ref
Cationic liposomes	1]strong antigen-specific antibody and Th1/Th2 cell response 2]	vaccination against COVID-19 mRNA-1273, which is composed of lipid nanoparticles and nucleoside-modified mRNA.The	58

	Immunostimulatory depot effect	SARS-CoV-2 spike is encoded (s)Glycoprotein	
Reformed liposomes	1]strong antigen-specific antibody and Th1/Th2 cell response 2] Immunostimulatory depot effect	Tuberculosis Hybrid 1(H1) fusion protein combined with CAF01 liposome (NCT00922363) component of the recombinant protein from Chlamydia trachomatis CTH 522 combined with CAF01 liposome (NCT02787109)	59

Table no 3: types of liposomes adjuvant

A promising non-viral method of delivering human genes is cationic liposomes ^[60]. These liposomes typically consist of neutral phospholipids like dioleoylphosphatidyl ethanolamine and cationic lipid derivatives (DOPE). Widely Cationic liposome compositions include three different compound Transfectace, Transfectam, and DC-Cho 3BN-(N,N-dimethylaminoethane) carbamoyl-cholesterol. Cationic Dioleyloxypropyl Trimethylammonium Steroid liposomes (DOTMA) ^[61]. The liposomes' physico-chemical characteristics have a major effect on how well they perform. The adjuvant impact is significantly influenced by the surface charge in particular, and Most in vivo investigations indicate that positive liposomes are preferable to normal and nonpolar liposomes ^[62]. The adjuvant effects of cationic liposomes are probably highly affected by their potential to choose antigens for APC endocytosis. According to Vangasseri et al, charged liposome made of ethylphosphocholine, such as 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (DOEPC), are more effective than those made of trimethylammonium propane lipids, like 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), at increasing DC maturation ^[63]. There is the wide range of cationic liposomes which are used as vaccine adjuvant.

❖ **Adjuvant based on DOTPA:** ^[64-67]

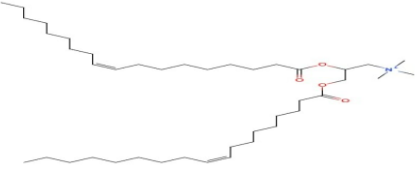
The quaternary ammonium compound DOTPA is positively charged and has two 18-carbon unsaturated hydrophobic fatty acid chains. Leventis and Silvius created it for the first time in 1990. Its structure consists of a 2-oleoyl chain-coated glycerol backbone joined to a quaternary amine head group. The difference between DOTMA and DOTAP is that ester bonds, not ether bonds, connect the chains to the backbone. A lipid's sensitivity ranges from 25% to 30%. However, DOTAP is totally protonated at pH (7.4), necessitating the use of additional energy to successfully separate the DNA from the lipoplex. Therefore, DOTAP should be paired with a helper lipid, as seen in the majority of cationic lipid formulations, to be more successful in gene delivery. In this, lipoplex that exhibits resistance to serum interaction has been created using high temperature and a lengthy incubation period. At room temperature (T0°C), In aquatic conditions, the lipids self-organize into bilayers. The phase of these bilayers is fluid crystallization. According to Simberg et al. (1141), DOTAP-based liposomes are effective transfection agents, Additionally, it has been demonstrated that DOTAP liposomes improve immune responses to protein antigens 116–18 as well as DNA-encoded antigens. Clathrin is required for the endocytic pathway by which DOTAP mediates the endocytosis of antigen by APCs. According to certain research, DOTAP may also have immunomodulatory effects. Increased cytolytic T-cell activity after immunisation using artificial enzymes or molecules demonstrates that how CD8 T-cell activation is brought on by DOTAP vesicle.

❖ **Adjuvant based on DC-CHOL:** [68-71]

It was first created in 1991 by "GAO and HUANG." It has a cholesterol moiety that is joined to a hydrolysable dimethyl ethylene diamine by an ester link. Cholesterol is chosen because it contributes to lipid membranes and is biocompatible and stable. Similar to DOTAP, the cationic cholesterol derivative DC-Chol generates liposomes that significantly improve transfection and produce a balanced Th1/Th2 response in a variety of disease types. In addition, Guy et al. demonstrate that administration of DC-Chol lipid nanoparticles to mice by intranasal or subcutaneous injection can enhance antigen-specific antibody responses to the complexation of adult divided inhibited influenza vaccine. Furthermore, macaques produced substantial reactions against the DC-Chol adjuvanted vaccine that were antigen-specific, but the vaccine itself had little effect, not even after getting a supplemental vaccination. Additionally, it has been shown that DC-Chol liposomes improve the response of a plasmid DNA model vaccination an in-vitro investigation was conducted using epithelial cell lines that mimic the uterus and vagina.

❖ **Adjuvant based on DDA lipid:** [72-74]

Gall first discovered DDA as a potent adjuvant in 1966 after he showed that it could raise antibody titres against DT in guinea pigs. The quaternary ammonium DDA has a positive charge compound with two hydrophobic side chains that are 18-C long and saturated. DDA fatty acids organize themselves into liposomes in a aquatic environment. when heated over the altering stages point of their gel to liquid, which is 47°C. Hilgers and Snippe have evaluated the adjuvant qualities of DDA in great detail and think that DDA is safe due to trials showing that it has no negative effects on people. As per Larsen et al., Non-specific cell injury does not result in the adjuvant effect observed in mice, The study's quaternary ammonium salts have an adjuvant effect without being toxic. The nostril epithelium is also in mouse that had received DDA Using the nasal route and been inoculated with BBG2Na, an antigen for the respiratory virus, was unaffected.

Lipid	Structure	Ref no
DOTPA		65

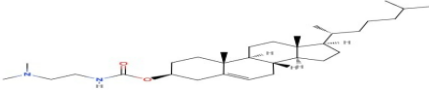

DC-Chol		68
DDA		73

Table no 4: chemical structure of cationic liposomes

❖ **Conclusion:**

Liposomes have been applied in a variety of pharmaceutical techniques. The effectiveness of the liposomal composition relies on its ability to continually transport the therapeutic agent to the desired location while minimising its (the medication's) side effects. Their versatility allows them to be applied to any pharmacological element and any mode of administration for the delivery of drugs, which independent of their characteristics. New vaccines are necessary to provide better healthcare throughout the world. The world's population should have access to these vaccines, and they should be secure, efficient, and economical. Adjuvants for vaccines are essential for enhancing vaccination efficiency and durability. Because they are flexible and have a proven track record as delivery methods, liposomes provide an effective adjuvant platform.

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