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Research Article

Liposomes: Current Approaches for Development and Evaluation

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ABSTRACT
Liposomes (50-1000nm) are the part of a specific type of drug delivery system which is non-toxic and biodegradable in nature. That having ability to reduce the toxicity also enhances the therapeutic efficiency and protects the drug which is encapsulated, from the degradation and immediate dilution. These can be prepared by using various techniques like lipid hydration method, sonication method and solvent injecting method etc. But the selection of technique is depended upon the size of liposome which we want. The main disadvantage of this dosage form is it is very much costly and also having time consuming process. But it has major applications in the form of extrusion for homogeneous size, long circulating liposomes, triggered release liposome, remote drug loading, ligand targeted liposomes and containing combination of drugs. These applications are helpful for advanced drug delivery of anticancer, antifungal and anti-inflammatory drug, the delivery of gene medicine, delivery of anaesthetic and antibiotic drug. The newer researches in this field include hybrid liposomes, phototrigerable liposomes which are fabricated to have the improved functionality. These serves as the upcoming novel nanomedicinal chemotherapy technique.

Keywords: Liposomes, biodegradable, encapsulated, ligand targeted phototrigerable, and nanomedicinal.

INTRODUCTION

To obtain perfect action of drug, functional molecules could be transported to the site of action by carrier and released to perform their task, for which the carrier itself should be non-toxic, biodegradable and of a suitable shape and size accommodate wide variety of substances[1]. Among the variety of targeted drug delivery system liposome science and technology is one of the fastest growing scientific fields contributing to areas such as drug delivery and cosmetics etc. These are very much used due to their several advantages like it have an ability to incorporate not only the water soluble but also the oil soluble agents, and it have also the versatility in terms of number of lamellae, fluidity, charge and size[2, 3].

Supramolecular aggregate that contain amphipathic molecules which generally called as vesicles. These generally contain synthetic or natural phospholipids formed in an aqueous phase. It was discovered by Dr. Alec D Baghman in 1961 working as british hematologist. Liposome word is derived from two Greek words '*Lipo'* means '*Fat'* and '*Soma'* means '*Body*[4]. These are small vesicles of spherical shape composed of aqueous compartment which is totally enclosed by a membranous lipid bilayer. The membranous lipid bilayer formed by phospholipids which have a non-polar tail and polar head (Fig. 1). A non-polar tail of this layer is formed by long chain of hydrocarbons which ultimately repels a water molecule as well as polar molecules. [4] It consists of an artificially prepared vesicle which contains a lipid bilayer. It can also be used as a vehicle for the administration of pharmaceutical drugs and nutrients. It may also contain the mixed lipid chain with surfactant properties such as egg or phosphatidylethanolamine. When we added the nonionic surfactants instead of the phospholipids that formulation is called as noisome. It contains the polar character which is responsible for encapsulation of the polar drugs while the phospholipids section/part is responsible for the encapsulation of the non-polar drugs. In this channel proteins can be encapsulated without losing their activity. The drugs which are biologically active also incorporated into the liposomes and prevents the degradation and dilution the drugs[5].

Components of liposomes

Phospholipids

The phospholipids contain glycerol moieties which are most commonly used for the preparation of liposomes. Phosphatidic acid is precursor from where these are derived. The examples which are used i.e.

Phosphatidyl choline
Phosphatidyl ethanolamine
Phosphatidyl serine
Phosphatidyl inositol
Phosphatidyl glycerol: Generally used for saturated fatty acids not for an unsaturated fatty acid.

Sphingolpids

The backbone of sphingolipid is sphingosine or their related bases. The most commonly used sphingolipid is sphingomyelin. The main purpose of adding this in the liposomes is to provide the layers of surface having charged group.

Sterols

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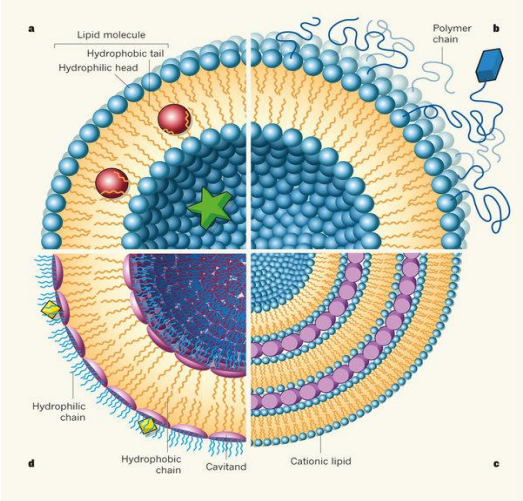

Figure 1: Structre of liposome[4].

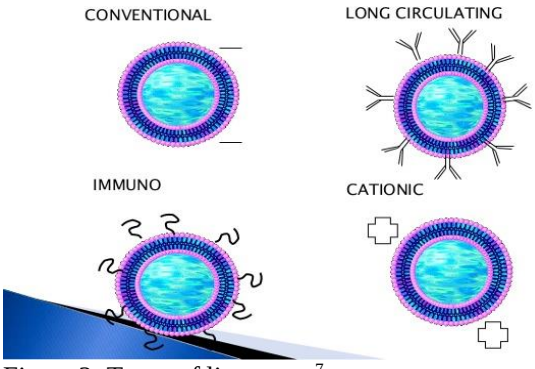

Figure 2: Types of liposomes[7].

In the liposomes preparation, the cholesterol and their derivatives are added for decreasing the fluidity of the bilayer and reduce the permeation of the hydrophilic drugs.

Synthetic Phospholipids

There are examples of synthetic phospholipids which are used for saturated phospholipids in liposome preparations like Dipalmotyl Phosphatidyl choline etc. and for unsaturated phospholipids like Dioleoyl Phosphatidyl glycerol etc.

Polymeric materials

When the diacetylenic group of the hydrocarbon chain is reacts with the synthetic phospholipids in the presence of U.V. radiations that results in the formation of the polymerized liposomes which have a significantly high permeability barrier to entrap the hydrophilic drugs.

Cationic lipids

Cationic derivatives of the cholesterol are used as cationic lipids. Except from cholesterol there are also lipids which is acts as cationic lipids in the formulation of liposomes. For e.g. Dioleoyl propyl trimethyl ammonium chloride (DOTAP).

Other substances

In the variety of others substances lipids surfactants are used for the formulation of the liposomes. A variety of polyethoxylated mono, dialkylamphiphiles and polyglycerol are mainly used in the cosmetic preparation. The single chain and double chain lipids containing the fluoro carbon chain results in the formation of the stable liposomes[6].

Classification of liposomes

The sizes of liposome vesicles are ranges from 0.025µm-2.5µm. Liposome either contain a single bilayer membrane or contain a multiple bilayer membrane.

Based upon the structural parameters these are classified into three types

Unilamellar Vesicles

Small Unilamellar Vesicles (SUV): - The sizes of these vesicles are in 20nm-40nm range.

Medium Unilamellar Vesicles (MUV): - The sizes of these vesicles are in 40nm-80nm range.

Large Unilamellar Vesicles (LUV): - The sizes of these vesicles are in 100nm-1000nm range.

Oligolamellar vesicles: - These types of liposomes consist of more than 2 and less than 10 bilayers of lipids.

Multilamellar vesicles: -These types of liposomes consist of more than 10 bilayers of lipids.

Based upon the composition

Liposomes consist of bilayer constituents like cholesterol and hydrophilic conjugated polymer lipids having natural or synthetic lipids. So, depend upon the material composition or substituent it divided into the five type's i.e. conventional liposomes**,** Immune liposomes**,** Cationic liposomes**,** pH sensitive liposomes**,** long circulating liposomes[7, 10]. (Fig. 2).

Advantages of liposome

There are various advantages on liposomes i.e. more stable via encapsulation process, increases the therapeutic index of the drugs like Actinomycin-D, increases the efficacy and reduces the toxicity of the final formulation, minimize adverse drug effect/No side effects, variety of drugs has been given in low dose via encapsulation, gives the passive targeting to tumor tissue, pharmacokinetics effects were improved, flexible and completely bio degradable in nature[2, 7, 11].

Disadvantages

Solubility problem may arise

Costly

Have a short half-life

Chemical reactions like oxidation and hydrolysis may take place due to the presence of phospholipid[7].

Allergic reactions may take place.

Leakage and fusion.

Techniques of Liposome Preparation

The properly selection of the liposome preparation is depending upon the physicochemical properties of the drug which got entrapped into it, nature of the dispersion medium in which the lipid vesicles are dispersed and shelf life of the vesicle, proper size batch-to-batch reproducibility. The rigidity of the bilayer also plays a very important role during the preparation of the liposomes[12].

Preparation of Multilamellar liposome

Lipid hydration stage

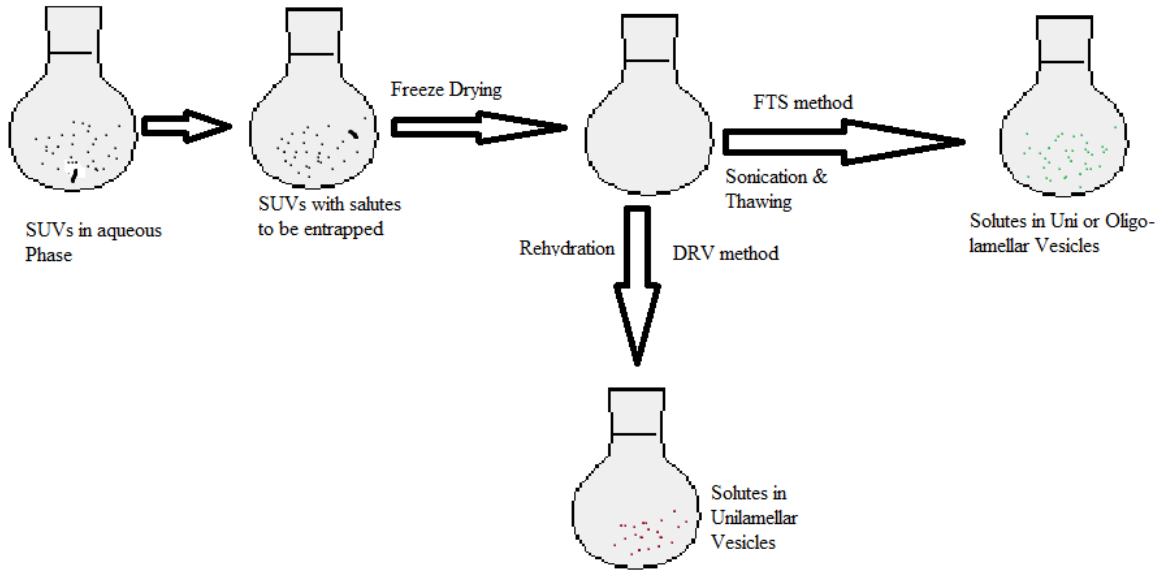

Figure 3: Preparation of liposome by Hand shake vesicle method[12].

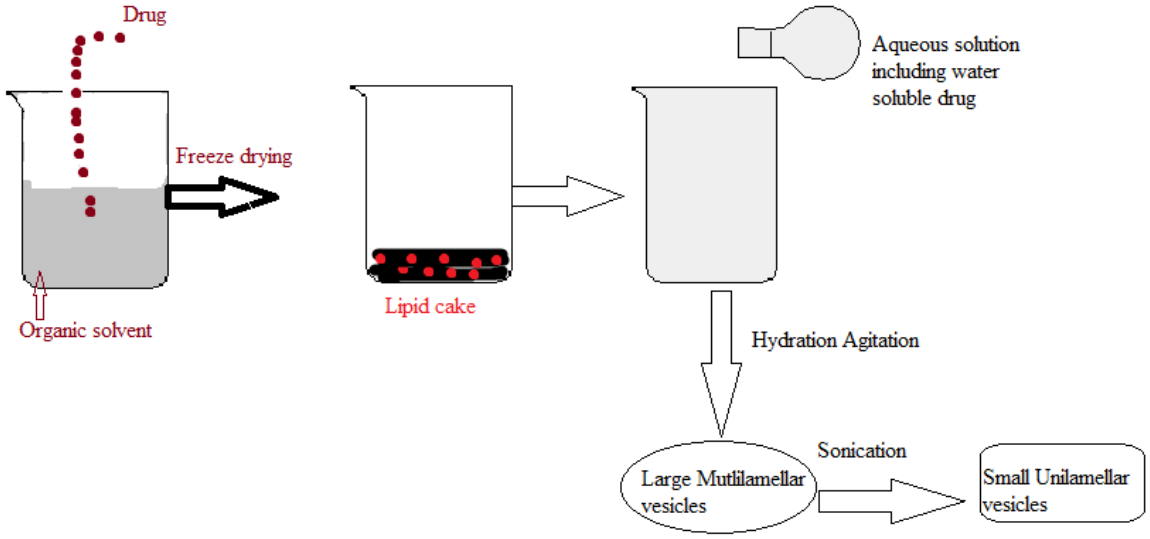

Figure 4: Liposomes preparation by Sonication method[15].

This method is also called as *hand shake vesicles method* and widely used now days. Firstly, at the bottom of the round bottom flask, the thin film is formed after the drying of the lipid solution. And by the addition of the aqueous buffer to the flask the formed layer is hydrated and properly shakes it for few mins. This step is occurring at a temperature which is above than the liquid-gel crystalline transition temperature. An encapsulation process has been done by adding the compound into the buffer solution or to the organic solvent depends upon the solubility of the lipids. The main advantage is size distribution is homogenous and encapsulation is high. The major disadvantage of this method is size distribution is heterogeneous and low internal volume[12].

Solvent spherule method: - This process involved dispersing in the aqueous solution small spherules of volatile hydrophobic solvent in which lipids had been dissolved. In a water bath, MLVs were formed when controlled evaporation of organic solvent occurred[14].

Preparation of Small Unilamellar liposomes

Sonication method

Sonication method is most commonly used method for the preparation of small unilamellar vesicles now days. In this method, the multilamellar vesicles are sonicated with the probe sonicator or the bath type sonicator under the

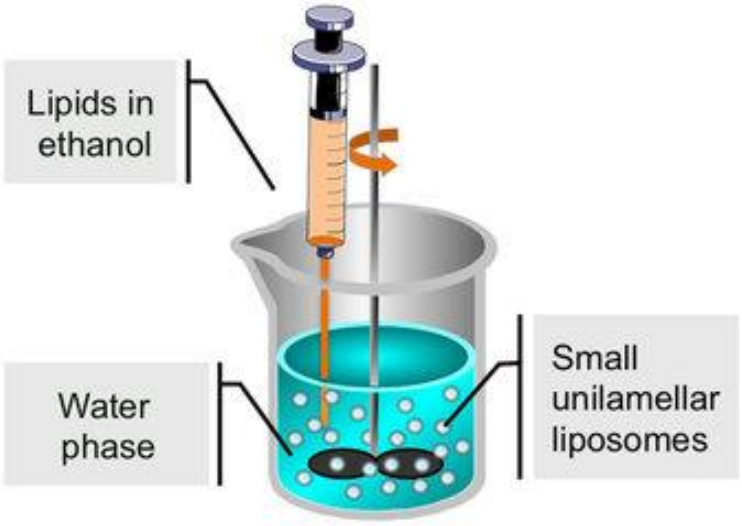

Figure 5: Preparation of liposomes by ethanol injecting method[18].

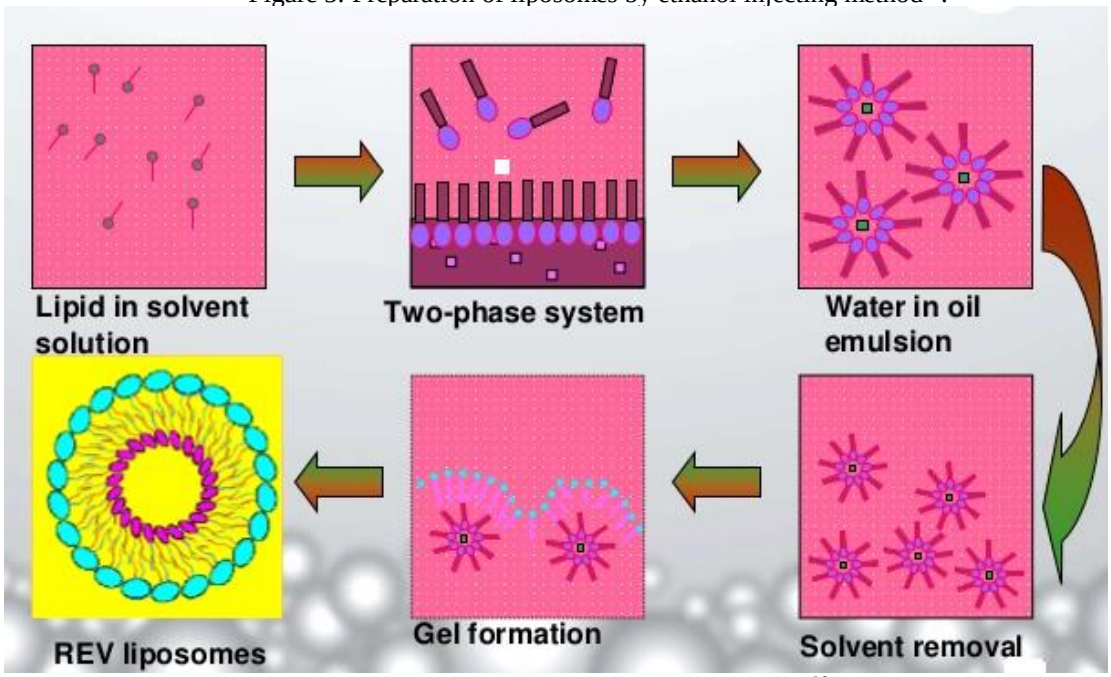

Figure 6: Reverse phase evaporation method[20].

control environmental conditions. In this method, the major disadvantage is metal contamination with the probe tip and low internal volume. The second main disadvantage is the multilamellar vesicles are also formed with the small unilamellar vesicles.

Probe sonication

In this type of sonication the tip of sonicator is directly engage into the liposome dispersion. In this method, an input of energy into the lipid dispersion is very high. Due to the coupling of energy at tip results in hotness; that's why the vessel is place in water or in an ice bath.

Bath sonication

In this type of method the lipid dispersion is placed into the measuring cylinder after that the cylinder is subjected to the bath sonicator. The material which is sonicated through this method is kept in the sterile vessel, which is not used in the probe sonication method[15].

The main disadvantage is lipid degradation occurs due to high energy and sonication tips release titanium particles into liposome dispersion.

French pressure cell methodIn this technique the vesicles are formed by the extrusion process. By using this process, the multilamellar vesicles are extruded at 20,000 psi at 4C temperature by the small orifice. It is good as compared to the sonication method. The main advantage is less leakage and more stable liposomes are formed compared to sonicated forms. The major disadvantage of this method is difficult to achieve the temperature condition[16].

Preparation of Large Unilamellar Vesicles

Solvent injection method

Ether infusion method

In this method the solution of lipid is properly mixed with the diethyl ether and very slowly injected through a fine

Table 1: Marketed formulations.

Trade name	Active Ingredients	Manufacturing Company	Biological Activity
AmBisome	Amphotericin B	Gilead sciences & Fujisawa health cares	Antifungal
Doxil	Doxorubicin	J&J Alza company	Antineoplastic agent
Daunoxome	Daunorubicin	Gilead sciences	Anticancer
Myocet	Doxorubicin	Elan company	Anticancer
Amphotec	Amphotericin B	SEQUSS	Leishmaniasis
Topex Br	Terbutaline Sulphate	Ozone	Asthma
Depocyt	Cytarabine	Skype Pharma	Anticancer
Novasome	Small pox vaccine	Novavax	Small pox
Vincasome	Vincristine	Nextar	Anticancer

needle into the aqueous solution of the material to be encapsulated at reduced pressure condition. By the removal of the ether under vacuum results in formation of the liposomes[17]. In this method lipids are treated very carefully and also there is less chances of an oxidative degradation in this method. The main drawback of this method is that it is a very time-consuming method for the preparation of the liposomes.

Ethanol injection method

This method is very much similar with the above method. In this method, firstly lipid solution of ethanol is prepared and then solution is rapidly injected into the excess of buffer through a fine needle results into the formation of liposomes[18]. When we prepare the liposomes by using this method there is chances of formation of small unilamellar vesicles is increases.

Calcium induced fusion method

In this method the liposomes are prepared by the addition of the EDTA. In this method, firstly the calcium is added to the Small Unilamellar Vesicles which results into the formation of the multilamellar vesicles due to the induction of the fusion. With the addition of an EDTA to the multilamellar vesicles results into a formation of the Large Unilamellar Vesicles. In this technique, liposomes are prepared by the acidic phospholipids. The main advantage of this is the liposomes are of heterogeneous size range. And the disadvantage is that in this the liposomes are only formed from the acidic phospholipids[19].

Detergent removal method (removal of non-encapsulated material)

Dialysis

All the detergents at their CMC (critical micelles concentration) solubilize the lipids. When the detergent is removed at this concentration the micelles are more in the phospholipids which results into the combining both of them and formation of liposomes takes place.

Gel permeation chromatography: With size, special chromatography detergent is removed by using this method. In this technique, a column is used through which liposomes can't permeates while the detergents molecules are easily pass through them so in this way we removed the detergent by using this technique.

Dilution

By doing the dilution with buffer we can easily remove the detergents from the prepared liposomes[20].

Reserves phase evaporation method

In this method firstly an emulsion is prepared by sonication. After that formation of viscous gel is takes place with the removal of the organic solvent. When we remove all the solvent by the rotary evaporation under reduced pressure results in formation of liposomes. The main drawback of this technique is that denaturation of proteins takes place due to the sonication[21].

Characterization of liposomes

The characterization parameters used for the evaluation of liposomes are mainly divided into three categories which include Physical, Chemical and Biological characterization of liposomes.

Physical evaluation includes the determination of size, surface features, shape and a drug release from the vesicles.

Chemical evaluation includes studies which prove the potency and purity of various lipophilic constituents.

Biological evaluation includes safety and suitability of the formulation for the therapeutic purposes.

From all the parameters which are mentioned above few are explaining from them:

Shape of the vesicle and lamellarity

The shape of the vesicles are determined by using the electronic microscopy and lamellarity means the presence of double layer in it and that is evaluated by using the freeze-fracture electron microscopy and P-31 Nuclear Magnetic Resonance(NMR) analysis.

Size of the vesicles and size distribution

There are various types of techniques are reported in the literature till now for the evaluation of the vesicle size and size distribution like Electron microscopy, Light microscopy, Fluorescent microscopy, field flow fraction, Laser light scattering photon correlation spectroscopy Light microscopy, Gel exclusion and Gel permeation. From these all techniques, electronic microscopy is a very best technique which is used for the evaluation of liposomes. But the main problem with this method is that the method is very time consuming method and numbers of apparatus is required for the evaluation of liposomes by this method. So, due to these reasons it is not used frequently now days. Instead of Electron microscopy light scattering microscopy method is used.

Drug release

By using the well-established and calibrated in vitro diffusion cell we can assess the mechanism of drug release from the prepared liposomes. In this method, the media is prepared which mimics like the human body

fluids. By using this media, we got an idea about the release of the drug through the liposomes. If we go for in vivo then it is very time consuming process and also a costly process so to prevent that we go for the in vitro testing.

Encapsulation efficiency and Trapped volume

The encapsulation efficiency tells us about the amount of hydrophilic part/polar part which is encapsulated inside the prepared liposome and it normally denoted as %entrap/ mg lipid. Encapsulation can be determined by using the two techniques i.e. Protamine aggregation method and the Minicolumn centrifugation method. From the above-mentioned methods, the protamine aggregation method is commonly used for the negatively charged and for neutral molecules. Trapped volume is the very important parameter which tells about the vesicles morphology. The amount of aqueous volume entrap per unit volume of lipid is called trapped volume. It generally ranges from 0.5-30 microliters/micro mol. The trapped volume is determined experimentally by dissolving the lipid into the polar media having non-permeable radioactive salute. By doing centrifugation the external radioactive salute was removed and the proportion of solute trapped is determined. The second-best method to determining the trapped volume is to replace the external layer of lipid by deuterium oxide which is an inert fluid. After replacing the external layer the sample is subjected to the NMR spectroscopy from which we get to know the amount of aqueous drug entrapped in the liposomes.

Phase response and Transitional Behaviour

The phase transition is determined by using Differential Scanning Colorimeter (DSC), Freeze fracture electronic microscopy [27,28].

Marketed formulations[9,28]

Applications

Liposomes are widely used now days in the field of food application. These are used as an alternative for preventing the proteolytic degradation as well as the interactions of antimicrobial peptides with the food components[8]. These are also used as an anticancer drug. Pegylated liposomal doxorubicin is used to treat the breast cancer as treatment as monotherapy as well as combination with other chemotherapeutics[9,10]. It also Increases the antimicrobial efficacy. In this application, the antimicrobial agents have been encapsulated in the liposomes because it prevents the enzymatic degradation of the antimicrobial agents, several antiretroviral nucleotide analogues are used for the treatment of Acquired Immunodeficiency Syndromes[22, 23]. It also increases the drug permeability through the skin surface. These are also used as targetable drugs carriers[24- 26].

RESULT AND DISCUSSION

In a broad range of pharmaceutical applications liposomes have been used. Liposomes having enhanced drug delivery to disease locations, by ability of long circulation residence times, are now achieving clinical acceptance. Within the disease site, liposomes promote targeting of particular diseased cells. Now days, liposomal drugs exhibit reduced toxicities and retain enhanced efficacy compared with their free complements. Now in the future it is observed that which of the above applications and speculations will prove to be successful. However, on the basis of pharmaceutical applications and available products, it can be assured that liposomes established their position in modern delivery systems. From the several described techniques only few have potential to manufacture liposomes at large scale. The main problem which is considered is the sterility control and stability. To overcome the sterility problem the prepared liposomes are passes through the 400nm pore sizes Millipore filters. Basically, these are formulated from the polymerizable phospholipids which are exposing the UV light. The liposome which is prepared by this method is more stable as compared to others. The newer researches in this field include hybrid liposomes, phototrigerable liposomes which are fabricated to have the improved functionality. These serve as the upcoming novel nanomedicinal chemotherapy technique. The use of liposomes in the delivery of drugs and genes are promising and undergo further developments in future.

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