CLINICAL APPROVED LIPOSOMAL FORMULATIONS: A OVER VIEW

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ABSTRACT:

Liposomes are vesicular colloidal carrier system composed of phospholipids bilayer encapsulate aqueous compartments. Since 1960's liposomes used as therapeutic tools for the delivery of bioactives.Hence number of liposomal formulations available in the market as compare to other carrier system such as nanoparticles,dendrimers etc. The structure and composition of liposomes has a great impact on their properties like biocompatibility,bioactive payload, immuo neutralization,absence of cytotoxicity and hemolytic toxicity. As a result of their unique liposomes are suitable for a wide range of biomedical and industrial applications.The paper gives a concise review of liposomes formulation physico-chemical properties and their clinical application in vaccination, tumor targeting, immunomodulation, topical delivery of bioactives for skin care,gene and antisense therapy. The goal of this review is to provide a short outline of the properties of liposomes and market research analysis of liposomes clinical approved products.

Keywords: Drug delivery; Liposome; Target ligand

1. Introduction

Liposomes were discovered in 1961 by Alec D. Bangham who was studying phospholipids and blood clotting,and since then they became very versatile tools in bioactive delivery.In this time liposomes have optimized as a model of biomembranes to delivery drug for clinical utility.The area of pharmaceutical applications of liposomes includes chemotherapy of cancer,fungal infections,vaccines delivery and most recently to gene therapy.Liposomal formulations available in market delivery the anticancer drugs,antifungal drug and vaccines.[1] [2] [3]

Liposomes are nano size artificial vesicles of spherical shape that can be produced from natural phospholipids and cholesterol. Bangham discovered that phospholipids combined with water immediately form a bilayered sphere because one end of each molecule is water soluble,while the opposite end is water insoluble.[4] [1] [3] [5]

Liposomes vesicular diameter size ranges from 0.02µm to 15µm.Liposomes are classified on the basis of preparation methods and nature lipid bilayer as small unilamellar vesicle[SUV] size range is 0.02-0.05µm,large unilamellar vesicles[LUV] size range is greater than 0.06µm and the multilamellar vesicle[MLV] size range is 0.1–0.5µm. The size,lamellarity (unilamellar or multilamellar) and lipid composition of the bilayers influence many of the important properties like the fluidity,permeability,stability and structure these can be controlled and customized to serve specific needs.The properties are also influenced by external parameters like the temperature,ionic strength and the presence of certain molecules[6] [7] **.** Prepared liposomes engineered for safe and effective drug delivery by manipulation of particle size,lamellarity,surface charge,sensitivity of pH changes and bilayer rigidity.Liposomes have great impact in drug delivery but their applicability is limited to specific due to short blood circulation half-life. The blood circulation half life of liposomes is dramatically increased by engineering of liposomes bilayer with polyethylene glycol (PEG).[8] [9]

Liposomes have various structural and nonstructural components,major building block components are Phospholipid and Cholesterol.Phospholipid (phospatidylcholine most commonly used lipid) is the major component of vesicular bilayer and cholesterol incorporation in bilayer provide fluidity,stability and membrane permeability in very high concentration up to 1:1 or 2:1 molar ratios of cholesterol to phospatidylcholine[10] [11] [12]**.**

2. Liposomes Preparartion Techniques

Liposomes are phospholipid vesicles composed of one or more phospholipid bilayer membranes and they carry aqueous or lipid drugs. The lipids are both hydrophobic and lipophilic in aqueous media, and their

hydrophobic regions sequestrate into spherical bilayers. These layers are referred to as lamellae. Liposomes vary in charge and their size, depending on the method of preparation and the lipids used[13].

Lipids, when hydrated by water, spontaneously form lipid vesicles with a broad range of sizes from several hundred nanometers to micrometers and composed of many layers of lipid membranes (multilamellar vesicles, or MLVs).The majority of applications, however, requires smaller and well – defined liposomes. For systemic delivery, liposomes with sizes in the range of 50-150nm (large unilamellar vesicles, or LUVs, in contrast, liposomes smaller than 50nm is called small unilamellar vesicles or SUVs) are desired. There are numerous ways of making liposomes, including detergent dialysis, sonication, solvent injection, reversephase, microfluidization/homogenization and extrusion and so on. But the most commonly used methods are extrusion and homogenization, because of their scalability, reproducibility and quality of the products.

Liposomes preparartion method summarized by the **Fig.1.** and commercially liposomes mainly manufactured by hydration and emulsification method. Payload of bioactives in liposomes by achieved by three primary mechanisms shown in **Fig. 2.**

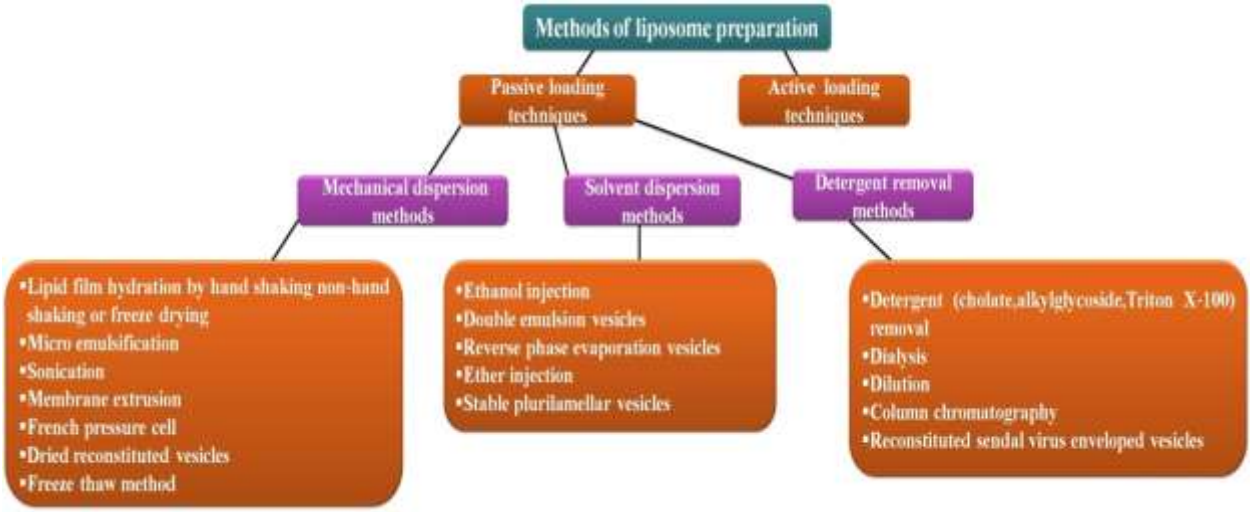

Fig.1. Method Of Preparation

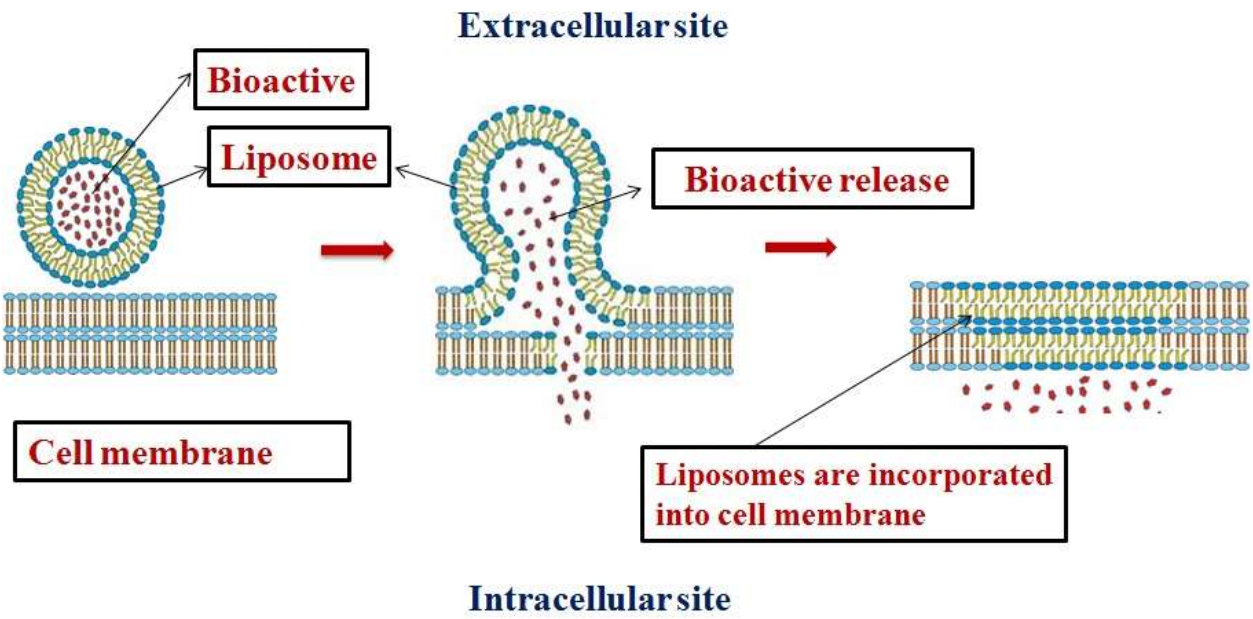

Fig.2. interaction

3. Characterization Of Liposomal Formulations

A company and research group engaged in the development of novel drug delivery carrier based on vesicular system, needs accurate characterization of liposomal morphology (size distribution and shape), lamellarity of lipid particles, drug encapsulations and drug release etc. However, common parameters of characterization sumaarized in **Table.1.** *In vitro* and *in vivo* disposition behaviors of liposomes depend upon the type of formulation techniques and physicochemical properties.

Liposomes characterized for the physical, chemical and biological parameters,physical characterization evaluates vesicular size and size distribution,surface topology,encapsulation efficiency,capture volume,lamellarity and *in vitro* drug release profile**.** Chemical characterization includes the determination of purity and potency. Biological characterization parameters are utilized to optimized the safety,efficay and suitability of finished formulation for therapeutic applications. Different important characterization parameters on liposomes enlisted in **Table.1** [14]

Table 1 Charecterization Of Liposomes

Physical Characterization		
Characterization parameters		***Analytical method/Instrument***
1	Vesicle shape and surface morphology	Transmission electron microscopy, Freeze-fracture electron microscopy
2	Mean vesicle size and size distribution (submicron and micron range)	Dynamic light scattering, zetasizer, Photon correlation spectroscopy, laser light scattering, gel permeation and gel exclusion
3	Surface charge	Free-flow electrophoresis
4.	Electrical surface potential and surface pH	Zetapotential measurements & pH sensitive probes
5	Lamellarity	Small angle X-ray scattering, ^{31}P-NMR, Freeze-fracture electron microscopy
6	Phase behavior	Freeze-fracture electron microscopy, Differential scanning colorimetery
7	Percent of free drug/ percent capture	Minicolumn centrifugation, ion-exchange chromatography, radiolabelling
8	Drug release	Diffusion cell/ dialysis
Chemical Characterization		
1	Phospholipid concentration	Barlett assay, stewart assay, HPLC
2	Cholesterol concentration	Cholesterol oxidase assay and HPLC
3	Phopholipid peroxidation	UV absorbance, Iodometric and GLC
4	Phospholipid hydrolysis, Cholesterol auto-oxidation.	HPLC and TLC
5	Osmolarity	Osmometer
Biological Characterization		
1	Sterility	Aerobic or anaerobic cultures
2	Pyrogenicity	Limulus Amebocyte Lysate (LAL) test
3	Animal toxicity	Monitoring survival rates, histology and pathology

4. Liposome Magic Properties And Challenge

Most important intresting properties of liposomes among other carriers (like Nanoparticles, Carbon nanotube, Dendrimer etc) are biocompatible, completely biodegradable, non-toxic, flexible, nonimmunogenic, suitable for delivery of hydrophobic,amphipathic and hydrophilic bioactives, sustained release properties, increased efficacy and therapeutic index of drug, reduced toxicity and increased stability of entrapped drug via encapsulation, Alter the pharmacokinetic and pharmacodynamic property of drugs (reduced elimination, increased circulation life time) and Flexibility to couple with site-specific ligands to achieve active targeting[15] Liposomes can be formulated as a suspension,as an aerosol,or in a semisolid form such as gel, cream and lotion,as a dry vesicular powder (proliposome) for reconstitution or they can be administered through most routes of administration including ocular, pulmonary, nasal, oral, intramuscular,subcutaneous,topical and intravenous. Liposomes also have some

draw backs such as production cost is high, leakage and fusion of encapsulated drug / molecules, Sometimes phospholipid undergoes oxidation and hydrolysis like reaction, Short half-life, low solubility, fewer stables[16 17] .

Mechanism Of Liposome-Cell Interaction

Understanding of liposome interactions with cells is key factor for the development of safe and effective liposomal formulation. Mechanism of Interaction of liposmomes with cells can be categorized in- exchange lipids cell membranes,adsorption,endocytosis or phagocytosis and fusion. Theses mechanism schematically described by the **Fig.2.,Fig.3.** and dependent on lipid composition, type of cell, presence of specific receptors and targeting vectors[18 19].

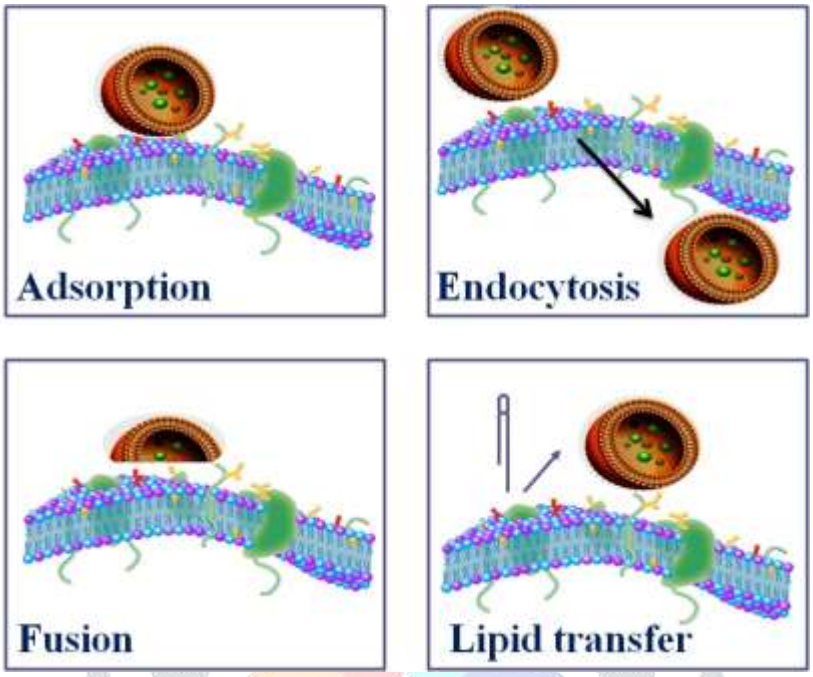

Fig.3. Cell Interactions

5. **Types Of Liposomes As Drug Delivery Carriers**

Different types of liposomes represented by **Fig.4.,**that are explored as drug delivery carriers includes conventional liposomes,stealth liposome coated with a polymeric conjugate such as PEG,Stealth liposome coupled with a functionalized ligand,Liposome with a single ligand and antibody[20] **.**

Fig.4. Types of liposomes

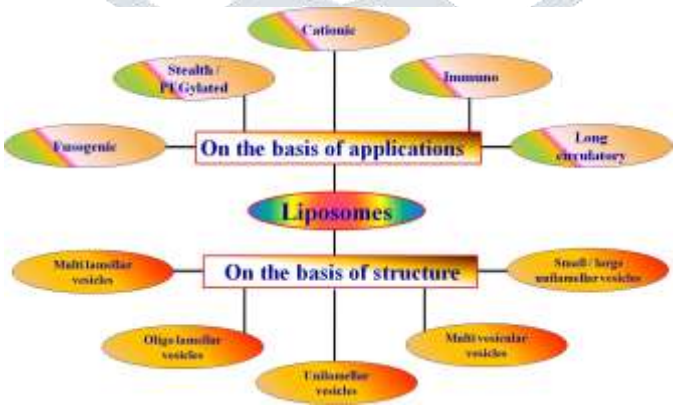

Fig.4. Types of liposomes

6.1 Conventional Liposomes

Conventional liposomes are first generation of liposome to be used in biomedical application and prepared by natural phospholipids or lipids such as 1,2-distearoryl-sn glycero-3-phosphatidyl choline (DSPC),sphingomyelin,egg phosphatidylcholine and monosialoganglioside[21]. Conventional liposomes have number of shortcomings,one of the major is short plasma half life due to instability in plasma,negatively or

positively charged surface[22] [23]. These shortcoming of to be masked by addition of cholesterol to conventional formulations, addition of "helper" lipids such as cholesterol and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) .Ambisone,Myocet,Daunoxome and Daunorubicin have clinical approval conventional liposome formulation[24] [25].

6.2 Stealth Liposomes

Number of challenges to liposomal drug delivery such as the inability,toxicity due to charged liposomes,low blood circulation half-life and steric stability can be overcome by the formulation of Stealth liposome[26] Stealth liposome developed by the surface modification of the liposome by engineering hydrophilic polymer polyethylene glycol(PEG),chitosan,silk-fibroin and polyvinyl alcohol(PVA) conjugates and synthesized liposome conjugates with number of additional properties such as high biocompatibility,nontoxicity,low immunogenicity and antigenicity[27] [28].PEGs are most widely explored in preparation of stealth liposomes[29]PEGylated liposomal doxorubicin (DOXIL/Caelyx) is the example of stealth liposome formulation to be approved by both the USA Food and Drug Administration (FDA) and Europe Federation[30].

6.3 Ligand conjugated Liposomes (Targeted liposomes)

Targeted liposomes synthesized by the anchoring surface with targeting moieties (such antibodies,peptide,glycoprotein,oligopeptide,polysaccharide,growth factors,folic acid and carbohydrate) through covalent and noncovalent interaction[30] [31].In covalent interaction and noncovalent interaction ligands are indirectly conjugated on the surface of liposome through a cross linkers and directly added to the mixture of phospholipids during the liposomal formulation, respectively[32].Doxorubicin loaded liposomes were surface engineered with monoclonal antibody and are now commercially available.Ligand conjugated liposomes increased the drug targeting potential by enhancing receptor mediated endocytosis[33]

6.4 Ligand spacer conjugated Liposomes

Recently the ligand-spacer conjugated liposome synthesized by the indirect conjugation of ligand via spacer(PEG,amino acids) to surface of liposomes and optimized for safety and efficacy of encapsulated bioactives[34] illustrated the application of folate-PEG conjugated liposomes(FPLs) for tumor targeting,FPLs showed significant higher uptake by MCF-7 cells as compared to folate conjugated and traditional liposomes,because of the folate-receptor mediated endocytosis.The data suggest that the folate-PEG coated polymeric liposomes (FPLs) may be a provide new platform for drug delivery[35].Folate-PEG coated cationic modified chitosan – Cholesterol liposomes for tumor-targeted drug delivery[35]**.**

6.5 New generation liposomes / Miscellaneous Liposomes

Currently maximum liposomal researcher concentrate on in the development of new generation liposome with higher safety and efficacy of bioactive.New generation liposomes includes:virosomes,stimuli-type liposomes and gene-based Liposomes.A virosome is specific type of liposome formulation developed by noncovalent coupling of a liposome and a fusogenic viral envelop. The stimulating agents in this case include pH,light,magnetism,temperature and ultrasonic waves.In stimuli-sensitive liposome triggered drug,protein,and gene delivery depend upon the environmental factors and designs with stimulating agents include pH,light,magnetism,temperature and ultrasonic waves[36].Gene-Based Liposomes have been utilized for efficient intracellular delivery of DNA and gene and prepared from amine(either quaternary ammonium,tertiary,secondary or primary)containing hydrophilic phospholipids and the liposomes prepared in this way are commonly referred to as cationic liposomes.Cationic liposomes have been used to promote the cellular uptake of DNA with resultant therapeutic protein expression by various organs in vivo[37]

6. Liposomal Formulations: Overview

Liposomes are an accepted,proven,commercially viable strategy to formulate bioactives for topical,oral,pulmonary or parenteral delivery contrast to other nanocarriers.Whether in the form of conventional liposomes,PEGylated liposomes formulations can be tailored to meet a wide range of product requirements dictated by disease indication,route of administration and considerations of cost, product stability, toxicity and efficacy[38] [39]

There are several liposome formulations that have been commercialized and there are many liposome formulations that are in various stages of clinical trials[40] **Fig.4, Table2.**

AmBisome® and Visudyne® are the product of liposome technology to improve drug solubility. Sophisticated "next generation" formulations(e.g.DOXIL®,Myocet®) focus on controlled delivery of small molecules,peptides,proteins or nucleic acids.Liposome formulation has also been commercially proven as a vaccine adjuvant.There are several marketed liposome products (Epaxal®,Inflexal®) and a growing number of

products under clinical development.The proven safety and efficacy of lipid based carriers make them attractive candidates for the formulation of pharmaceuticals as well as vaccines diagnostics and nutraceuticals[41].

Lipisome based formulation have been shown to reduce the toxicity of bioactives by altering pharmacokinetic profile of encapsulated bioactives and enhances the effective localization to desired site.Liposome controlled toxicity may allow the administration of various bioactives and platform for the current success of several marketed liposomal formulations of antifungal drug, amphotericin B(AmBisome®,ABELCET®) and anticancer drug,doxorubicin(Doxila,Myoceta). Many bioactives are selected for liposomal drug delivery on the basis of their ability to cross lipid membranes and show some tendency to dissolve in liposome formulations.The utility of this concept has recently validated by the introduction of a liposome formulation of a benzoporphyrin(Visudynea) for the treatment of age-related macular degeneration.Doxila and Myoceta are clinically approved liposome formulations can also protect fragile molecules from chemical degradation or transformation and significant increase in bioavailability[42].

In current scenario applications of liposome as for numerous bioactives delivery is now accelerated and **Fig.5.** illustrated the numerous clinically approved and phaseIII liposomal formulations available in market as a novel tool for health care systems[43].

Fig.5.Marketed Formulations

7.1 PEGylated Liposomal formulations:

Several PEGylated liposomal doxorubicin formulations are available in markets includes[44 45]: **Fig.6 Table. 3.**

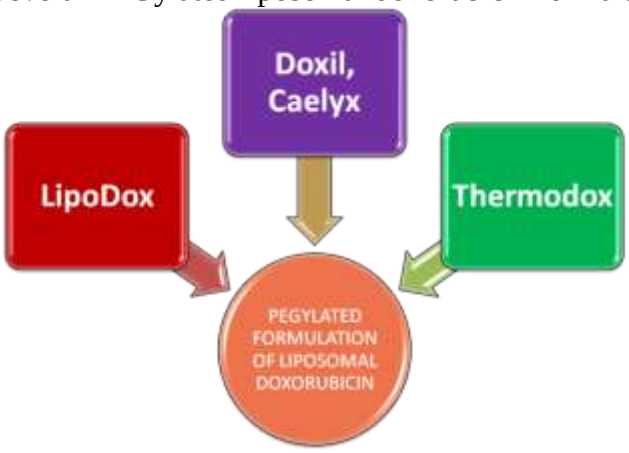

Fig.6.pegylated formulation

Fig.7. vaccine adjuvant

Table 3 List of Marketed Products

Marketed product	Drug used	Target diseases	Company	Ref.
Doxil™ or Caelyx™	Doxorubicin	Kaposi's sarcoma	SEQUUS, USA	Working P. K et al,1994
DaunoXome™	Daunorubicin	Kaposi's sarcoma, breast & lung cancer	NeXstar, USA	Forssen E. A.,etal,1996
Amphotec™	Amphotericin-B	fungal infections,	SEQUUS, USA	Hiemenz J. W- Walsh T.

		Leishmaniasis		J,1996
Fungizone®	Amphotericin-B	fungal infections, Leishmaniasis	Bristol-squibb, Netherland	Wasan M. W., Lopez-Berestein G.,1998
VENTUS™	Prostaglandin-E_1	Systemic inflammatory diseases	The liposome company, USA	Lasic D. D., Papahadjopoulos D.,1998
ALEC™	Dry protein free powder of DPPC-PG	Expanding lung diseases in babies	Britannia Pharm, UK	Bangham A. D etal,1965
Topex-Br	Terbutaline sulphate	Asthma	Ozone, USA	Chung K. F., Barens P. J.,1989
Depocyt	Cytarabine	Cancer therapy	Skye Pharm, USA	Patil S. G. et al,2005
Novasome®	Smallpox vaccine	Smallpox	Novavax, USA	Patil S. G. et al,2005
Avian retrovirus vaccine	Killed avian retrovirus	Chicken pox	Vineland lab, USA	Gregoriadis G.etal,1998
Epaxal –Berna Vaccine	Inactivated hepatitis-A Virions	Hepatitis A	Swiss serum & vaccine institute, Switzerland	Gluck R,1995
Doxil®	Doxorubicin Hcl	Refractory ovarian cancer	ALZA, USA	Forssen E. A., Ross M. E,1994
Evacet™	Doxorubicin	Metastatic breast cancer	The liposome company, USA	S. P. Vyas, R. K. Khar,2002
VincaXome	Vincristine	Solid Tumours	NeXstar, USA	S. P. Vyas, R. K. Khar,2002
Mikasome®	Amikacin	Bacterial infection	NeXstar, USA	S. P. Vyas, R. K. Khar,2002
Autragen™	Tretinoin	Kaposi's sarcoma	Aronex Pharm, USA	S. P. Vyas, R. K. Khar,2002
Shigella Flexneri 2A Vaccine	Shigella flexneri 2A	Shigella Flexneri 2A infections	Novavax, USA	S. P. Vyas, R. K. Khar,2002
Nyotran™	Nystatin	Systemic fungal infections	Aronex Pharm, USA	S. P. Vyas, R. K. Khar,2002

7.1.1 Doxil, Caelyx -

This is a PEGylated formulation of liposomal doxorubicin.The liposomes are composed of hydrogenated soya PC (HSPC):cholesterol:PEG 2000-DSPE(56:39:5molar ratio).It is used for treatment of refractory Kaposi's sarcoma,recurrent breast cancer and ovarian cancer[44]

7.1.2 LipoDox (Liposomal doxorubicin)-

This is a PEGylated formulation of liposomal doxorubicin.The liposomes are composed of DSPC:cholesterol:PEG2000-DSPE(56:39:5molar ratio).It is used for treatment of refractory Kaposi's sarcoma,recurrent breast cancer and ovarian cancer[46]

7.1.3 Thermodox (Liposomal doxorubicin)-

This is a PEGylated formulation of liposomal doxorubicin.Thermodox is a triggered release formulation.The liposomes will release their content upon heat.The tumor is heated up using radio frequency ablation(RFA).The liposomes release their content inside the tumor upon heat. The liposomes are composed of DPPC,mono steroyl PC(MSPC) and PEG2000-DSPE.It is used for treatment of primary liver cancer(Hepatocellular carcinoma) and also recurrent chest wall breast cancer.Thermodox is in phase III of clinical trial[45]**.**

7.1.4 Lipoplatin (Liposomal cisplatin)-

This is a PEGylated formulation of liposomal cisplatin.The liposomes are composed of DPPG, Soy PC,cholesterol and PEG2000-DSPE.It is used for treatment of epithelial malignancies such as lung,head and neck,ovarian,bladder and testicular cancers.

7.1.5 Vaccine adjuvant liposomal formulations-

Vaccine adjuvant Liposome formulations(VAL) have also been clinical approved for vaccine delivery.There are numerous VAL(Epaxal®,Inflexal®) are available in market and a rising number of VAL under clinical trials[47]

7.1.6 Epaxal (Hepatitis A vaccine)-

Liposomes have been used as a vaccine adjuvant in this formulation.Inactivated vaccines usually contain an adjuvant which potentiates the immune response to the antigen.During the last 70 years aluminium salts have been the only adjuvant licensed for human.The adjuvanting activity is based on their serving as an antigen depot and inducing a localized inflammatory response. These liposomes also known as immunopotentiating reconstituted influenza virosomes(IRIV) are composed of DOPC/DOPE in 75:25 molar ratio.The liposomes are sized to 150 nm[48]

7.1.7 Inflexal V (Influenza vaccine)-

Liposomes have been used as a vaccine adjuvant in this formulation.The liposomes are composed of DOPC/DOPE in 75:25 molar ratio[49]**.**

7.2 Conventional Liposomal formulations (Nonpegylated formulation)

7.2.1 Myocet (Liposomal doxorubicin)

This is a non PEGylated formulation of liposomal doxorubicin.The liposomes are composed of egg PC (EPC):cholesterol(55:45 molar ratio).It is used in combinational therapy for treatment of recurrent breast cancer[50]

7.2..2 DaunoXome (Liposomal Daunorubicin)-

This is a non PEGylated formulation of liposomal Daunorubicin.The liposomes are composed of DSPC and cholesterol(2:1) molar ratio and it is sized to 45nm.It is used for treatment of Kaposi's sarcoma[50]**.**

7.2.3 Ambisome (Liposomal Amphoteracin B)-

This is a non PEGylated formulation of liposomal Amphoteracin B.The liposomes are composed of HSPC,DSPG,cholesterol and amphoteracin B in 2:0.8:1:0.4 molar ratio.Used for treatment of fungal infection[50]**.**

7.2.4 Marqibo (Liposomal vincristine)-

This is a non PEGyated formulation of liposomal vincristine.The liposomes are composed of egg sphingomylin and cholesterol.It is used for the treatment of metastatic malignant uveal melanoma.Marqibo is in phaseIII of clinical trial**.**

7.2.5 Visudyne (Liposomal verteporfin)-

This is a non PEGylated formulation of liposomal verteporfin(BPD-MA).The liposomes are composed of BPD-MA:EPG:DMPC in 1:05:3:5 molar ratio.It is used for treatment of age-related macular degeneration,pathologic myopia and ocular histoplasmosis[50]**.**

7.2.6 DepoCyt (Liposomal cytarabine)-

This is a non PEGylated formulation of liposomal cytarabine.The Depo-Foam platform is used in DepoCyt.Depo-Foam is a spherical 20 micron multi-lamellar liposome matrix comprised of Cholesterol:Triolein:Dioleoylphosphatidylcholine(DOPC):Dipalmitoylphosphatidylglycerol (DPPG) in 11:1:7:1 molar ratio.The drug is used by intrathecal administration for treatment of neoplastic meningitis and lymphomatous meningitis[50]**.**

7.2.7 DepoDur (Liposomal morphine sulfate)-

This is a non PEGylated formulation of liposomal cytarabine.The Depo-Foam platform is used in DepoCyt.Depo-Foam is a spherical 20 micron multi-lamellar liposome matrix comprised of Cholesterol:Triolein:Dioleoylphosphatidylcholine(DOPC):Dipalmitoylphosphatidylglycerol (DPPG) in 11:1:7:1 molar ratio.The drug is used by epidural administration for treatment of postoperative pain following major surgery[50]**.**

7.2.8 Arikace (Liposomal amikacin)-

This is a non PEGylated formulation of liposomal amikacin. The liposomes are composed of Derol.The size of the liposomes is between 200-300nm.It is used for treatment of lung infections due to susceptible pathogens.Arikace is used in nebulized form and it is inhaled by the patients.The drug is in phaseIII of clinical trial[50]**.**

7.2.9 LEP-ETU (Liposomal Paclitaxel)-

This is a non PEGylated formulation of liposomal Paclitaxel.The liposomes are composed of DOPE,cholesterol and cardiolipin.Its is used for treatment of ovarian,breast and lung cancer. LEP-ETU is completing phase II of clinical trials.The liposomes are sized to 150 nm.Liposomes represent versatile and advanced nanodelivery systems for a wide range of biologically active compounds. These relatively non-toxic systems have a considerable potential to entrap both hydrophobic and hydrophilic drugs. The entrapment of the drug into the

liposomes is used to bypass the frequent generic toxicity associated with the drug.Thus, it represents a very effective route that enhances the drug therapeutic effect. The final amount of the encapsulated drug is affected by a selection of an appropriate preparation method providing a preparation of liposomes of various size, lamellarity and physico-chemical properties. The modification of liposomes permits a passive or active targeting of the tumor site. This effect enables an efficient drug payload into the malignant cells of tumor, while the non-malignant cells become minimally impacted[51] **.**

7. FDA APPROVED PRODUCTs

Doxil®, the first FDA-approved nano-drug (1995), is based on three unrelated principles: (i) prolonged drug circulation time and avoidance of the RES due to the use of PEGylated nano-liposomes, (ii)high and stable remote loading of doxorubicin driven by a transmembrane ammonium sulfate gradient, which also allows for drug release at the tumor; (iii) having the liposome lipid bilayer in a "liquid ordered" phase composed of the high-Tm(53°C) phosphatidylcholine and cholesterol. Due to the EPR effect,Doxil is "passively targeted" to tumors[52]**.**

DepoCyt® (cytarabine liposome injection) is a sterile,injectable suspension of the antimetabolite cytarabine, encapsulated into multivesicular lipid-based particles. Chemically, cytarabine is 4amino-1 -β-D-arabinofuranosyl-2(1H)-pyrimidinone, also known as cytosine arabinoside (C9H13N3O5, molecular weight 243.22)[53].

DaunoXome® (daunorubicin citrate liposome injection) is a prescription drug indicated as a first line cytotoxic therapy for advanced HIV-associated Kaposi's sarcoma. DaunoXome® is not recommended in patients with less than advanced HIV-related Kaposi's sarcoma. Cardiac function should be monitored regularly in patients receiving DaunoXome® (daunorubicin citrate liposome injection) because of the potential risk for cardiac toxicity and congestive heart failure. Cardiac monitoring is advised especially in those patients who have received prior anthracyclines or who have pre-existing cardiac disease or who have had prior radiotherapy encompassing the heart. AmBisome (NeXstarPharmaceuticals, San Dimas,CA) is a unilamellar liposomal formulation of amphotericin B that was recently approved for use as empirical treatment for presumed fungal infections in febrile neutropenic patients and for aspergillosis, candidiasis, and cryptococcosis infections refractory to amphotericin B.It is a small closed microscopic sphere (<100 nm in diameter) with an inner aqueous core (i.e. a true liposome).AmBisome remains as an intact sphere in vitro and for prolonged periods of time in vivo during the processes of systemic transport and pharmacologic action.

Epaxel vaccine play a vital role in protecting against disease and have contributed significantly to the improvement of global public health. Smallpox was eradicated through the use of vaccines. Significant advances include the introduction of combination vaccines and the development of new vaccine technologies.In 2010, a Crucell vaccine was given to 190 people every minute. Over the full year, more than 105 million doses of vaccines were distributed in more than 100 countries, thereby preventing more than 3.6 million cases of infectious diseases and over 809, 000 deaths that would otherwise have occurred.

Inflexal® V virosomal adjuvanted vaccine offers protection against influenza thanks to its virosome technology—one of Crucell's patented innovations.Virosomes are reconstituted influenza virus envelopes, constructed without the genetic information of the virus so that they are unable to replicate or cause infections.In the context of vaccines,virosomes serve as both a carrier system and an adjuvant. Inflexal® V is the only adjuvanted influenza vaccine licensed for all age groups (from 6 months upwards).Since its launch in 1997,Inflexal® V has been licensed in 38 countries with over 60 million doses distributed.Extensive experience in the market has confirmed its efficacy and favorable safety profile.The vaccine's unique design and manufacturing process eliminate the need for thiomersal(a vaccine preservative) or formaldehyde(commonly used to inactivate influenza viruses) and minimize residual traces of antibiotics,detergent and hen's egg protein compared with other influenza vaccines.The vaccine's antigen composition changes in accordance with annual recommendations issued by the World Health Organization (WHO) on the basis of data on the circulating influenza virus strains[54]**.**

Myocet non-pegylated liposomal doxorubicin made by Enzon Pharmaceuticals for Cephalon in Europe and for Sopherion Therapeutics in the United States and Canada. Myocet is approved in Europe and Canada for treatment of metastatic breast cancer in combination with cyclophosphamide, but is not yet approved by the FDA for use in the United States. It is currently being studied by Sopherion Therapeutics in a pivotal phase III global registrational trial in concurrent combination with Herceptin (trastuzumab) and Taxol (paclitaxel) for treatment of HER2-positive metastatic breast cancer[55].

VERTEPORFIN (VER te PORE fin) is used to treat macular degeneration.It is activated by light. This medication is given,then the eye(s) are treated with a laser light.This is called photodynamic therapy (PDT).This treatment results in a slowing of the disease and helps to maintain vision.

AMPHOTEC® is a sterile, pyrogen-free, lyophilized powder for reconstitution and intravenous (IV) administration. AMPHOTEC consists of a 1:1 (molar ratio) complex of amphotericin B and cholesteryl sulfate.Upon reconstitution,AMPHOTEC forms a colloidal dispersion of microscopic disc-shaped particles**.**

Lipoplatin (Liposomal cisplatin) is a nanoparticle of 110 nm average diameter composed of lipids and cisplatin . Liposome This new drug has successfully finished Phase I, Phase II and Phase III human clinical trials .It has shown superiority to cisplatin in combination with paclitaxel as a chemotherapy regimen in non-small cell lung cancer (NSCLC) adenocarcinomas.

DepoDur® (morphine sulfate extended-release liposome injection) is a sterile suspension of multivesicular liposomes using proprietary DepoFoam® formulation technology containing morphine sulfate, intended for epidural administration[53].

The FDA has approved vincristine sulfate liposome injection (Marqibo, Talon) for patients with Philadelphia chromosome–negative (Ph–) acute lymphoblastic leukemia (ALL). The drug is approved for patients who have relapsed or progressed following two or more treatment regimens[56]**.**

The new formulation encapsulates vincristine sulfate in liposomes. According to Talon Therapeutics, the liposome encasement allows for higher drug concentrations—as high as two to three times during individual doses and 10 times cumulatively—with a more predictable release **Table.4 & 5** [57] .

Table 4. Approved liposomal and lipid-based drug formulations.

Product	**Company**	**Drug Composition/size**	**Therapeutic indication**
Cancer DaunoXome®	NeXstar/Gilead, www.gilead.com	Daunorubicin DSPC/chol liposomes	~ 60 nm Advanced Kaposi Sarcoma
Doxil®/Caelyx®	Alza Corp. www.alza.com	Doxorubicin HSPC/chol/PEG-DSPE liposomes, 80 - 120 nm	Metastatic ovarian cancer and advanced Kaposi's sarcoma
Myocet™ The Liposome	Company/Elan, www.lipo.com	Doxorubicin EPC/chol liposomes,	~ 100 nm Metastatic breast cancer
Infectious diseases AmBisome®	NeXstar/Fujisawa www.gilead.com	AmpB HSPC/chol/DSPG liposomes, 55 - 75 nm	Systemic fungal infections, visceral leish-maniasis
Abelcet® The Liposome	Company/Elan, www.lipo.com	AmpB Complex with lipids (DMPC, DMPG)	Systemic fungal infection
Amphocil®/ Amphotec®	Alza Corp. www.alza.com	AmpB Complex with cholesteryl sulfate	Systemic fungal infections

Table 5. Approved liposomal vaccines

Product™	Company	Drug	Composition/size	Therapeutic indication
Epaxal Berna	Swiss Serum and Vaccine Institute, www.berna.org	Inactivated Hepatitis A virus	Virosomes (influenza virus envelope phospholipids incorporating influenza virus surface antigens supplemented with EPC and PE), ~150 nm	Hepatitis A
Inflexal Berna V	Swiss Serum and Vaccine Institute, www.berna.org	Surface antigens of influenza virus (hemagglutinin and neuraminidase from influenza A and B)	Virosomes (influenza virus envelope phospholipids and EPC), ~150 nm	Influenza

Conclusions

Liposomes are one of the unique drug delivery system, which can be of potential use in controlling and targeting drug delivery. Liposomes are administrated orally, parenterally and topically as well as used in cosmetic and hair technologies, sustained release formulations, diagnostic purpose and as good carriers in gene delivery. One major problem associated in the formulation of liposome due to physicochemical and biological instability. These stability problems can be alleviated by using various methods like lyophilization, proliposome, pH sensitive liposome, microencapsulation and steric stabilization. Nowadays liposomes are used as versatile carriers for targeted delivery of drug. The application of nanotechnology to medicine has clearly advanced beyond academic curiosity and research. Recently nanomedicines are on the market, the majority based on liposome drug delivery systems.

References:-

1. Bochot, A.; Fattal, E., Liposomes for intravitreal drug delivery: a state of the art. *J Control Release* **2012,** *161* (2), 628-34.
2. Elhissi, A., Liposomes for Pulmonary Drug Delivery: The Role of Formulation and Inhalation Device Design. *Current pharmaceutical design* **2017,** *23* (3), 362-372.
3. Lee, Y.; Thompson, D. H., Stimuli-responsive liposomes for drug delivery. *Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology* **2017,** *9* (5).
4. Rudokas, M.; Najlah, M.; Alhnan, M. A.; Elhissi, A., Liposome Delivery Systems for Inhalation: A Critical Review Highlighting Formulation Issues and Anticancer Applications. *Medical principles and practice : international journal of the Kuwait University, Health Science Centre* **2016,** *25 Suppl 2* (Suppl 2), 60-72.
5. Yang, F.; Jin, C.; Jiang, Y.; Li, J.; Di, Y.; Ni, Q.; Fu, D., Liposome based delivery systems in pancreatic cancer treatment: from bench to bedside. *Cancer treatment reviews* **2011,** *37* (8), 633-42.
6. El-Hammadi, M. M.; Arias, J. L., An update on liposomes in drug delivery: a patent review (2014-2018). *Expert opinion on therapeutic patents* **2019,** *29* (11), 891-907.
7. Pilch, E.; Musiał, W., Liposomes with an Ethanol Fraction as an Application for Drug Delivery. *International journal of molecular sciences* **2018,** *19* (12).
8. Luo, G.; Yang, Q.; Yao, B.; Tian, Y.; Hou, R.; Shao, A.; Li, M.; Feng, Z.; Wang, W., Slp-coated liposomes for drug delivery and biomedical applications: potential and challenges. *Int J Nanomedicine* **2019,** *14*, 1359-1383.
9. Carita, A. C.; Eloy, J. O.; Chorilli, M.; Lee, R. J.; Leonardi, G. R., Recent Advances and Perspectives in Liposomes for Cutaneous Drug Delivery. *Current medicinal chemistry* **2018,** *25* (5), 606-635.
10. Hussain, A.; Singh, S.; Sharma, D.; Webster, T. J.; Shafaat, K.; Faruk, A., Elastic liposomes as novel carriers: recent advances in drug delivery. *Int J Nanomedicine* **2017,** *12*, 5087-5108.
11. Ascenso, A.; Raposo, S.; Batista, C.; Cardoso, P.; Mendes, T.; Praça, F. G.; Bentley, M. V.; Simões, S., Development, characterization, and skin delivery studies of related ultradeformable vesicles: transfersomes, ethosomes, and transethosomes. *Int J Nanomedicine* **2015,** *10*, 5837-51.

12. Daeihamed, M.; Dadashzadeh, S.; Haeri, A.; Akhlaghi, M. F., Potential of Liposomes for Enhancement of Oral Drug Absorption. *Current drug delivery* **2017,** *14* (2), 289-303.
13. Mirab, F.; Wang, Y.; Farhadi, H.; Majd, S., Preparation of Gel-Liposome Nanoparticles for Drug Delivery Applications(). *Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual International Conference* **2019,** *2019*, 3935-3938.
14. Podlipec, R.; Koklic, T.; Strancar, J.; Mravljak, J.; Sentjurc, M., Influence of cancerostatic perifosine on membrane fluidity of liposomes and different cell lines as measured by electron paramagnetic resonance. *Croat Med J* **2012,** *53* (6), 558-567.
15. Allen, T. M.; Cullis, P. R., Liposomal drug delivery systems: from concept to clinical applications. *Adv Drug Deliv Rev* **2013,** *65* (1), 36-48.
16. van den Hoven, J. M.; Van Tomme, S. R.; Metselaar, J. M.; Nuijen, B.; Beijnen, J. H.; Storm, G., Liposomal drug formulations in the treatment of rheumatoid arthritis. *Molecular pharmaceutics* **2011,** *8* (4), 1002-15.
17. Bochot, A.; Fattal, E., Liposomes for intravitreal drug delivery: A state of the art. *Journal of controlled release : official journal of the Controlled Release Society* **2012,** *161*, 628-34.
18. Haslwanter, D.; Blaas, D.; Heinz, F. X.; Stiasny, K., A novel mechanism of antibody-mediated enhancement of flavivirus infection. **2017,** *13* (9), e1006643.
19. Corbo, C.; Molinaro, R.; Taraballi, F.; Toledano Furman, N. E.; Sherman, M. B.; Parodi, A.; Salvatore, F.; Tasciotti, E., Effects of the protein corona on liposome-liposome and liposome-cell interactions. *Int J Nanomedicine* **2016,** *11*, 3049-63.
20. Abd El-Alim, S. H.; Kassem, A. A.; Basha, M.; Salama, A., Comparative study of liposomes, ethosomes and transfersomes as carriers for enhancing the transdermal delivery of diflunisal: In vitro and in vivo evaluation. *Int J Pharm* **2019,** *563*, 293-303.
21. Nguyen, T. X.; Huang, L.; Gauthier, M.; Yang, G.; Wang, Q., Recent advances in liposome surface modification for oral drug delivery. *Nanomedicine (London, England)* **2016,** *11* (9), 1169-85.
22. Alavi, M.; Hamidi, M., Passive and active targeting in cancer therapy by liposomes and lipid nanoparticles. *Drug metabolism and personalized therapy* **2019,** *34* (1).
23. Patil, Y. P.; Jadhav, S., Novel methods for liposome preparation. *Chem Phys Lipids* **2014,** *177*, 8-18.
24. Wang, W. X.; Feng, S. S.; Zheng, C. H., A comparison between conventional liposome and drug-cyclodextrin complex in liposome system. *Int J Pharm* **2016,** *513* (1-2), 387-392.
25. Xi, Y.; Jiang, T.; Chaurasiya, B., Advances in nanomedicine for the treatment of ankylosing spondylitis. **2019,** *14*, 8521-8542.
26. Bulbake, U.; Doppalapudi, S.; Kommineni, N.; Khan, W., Liposomal Formulations in Clinical Use: An Updated Review. *Pharmaceutics* **2017,** *9* (2).
27. Mineart, K. P.; Venkataraman, S., Fabrication and Characterization of Hybrid Stealth Liposomes. **2018,** *51* (8), 3184-3192.
28. Capriotti, A. L.; Cavaliere, C.; Piovesana, S., Liposome protein corona characterization as a new approach in nanomedicine. *Analytical and bioanalytical chemistry* **2019,** *411* (19), 4313-4326.
29. Pireddu, R.; Pibiri, M.; Valenti, D.; Sinico, C.; Fadda, A. M.; Simbula, G.; Lai, F., A novel lactoferrin-modified stealth liposome for hepatoma-delivery of triiodothyronine. *Int J Pharm* **2018,** *537* (1-2), 257-267.
30. Wang, Q.; He, L.; Fan, D.; Liang, W.; Fang, J., Improving the anti-inflammatory efficacy of dexamethasone in the treatment of rheumatoid arthritis with polymerized stealth liposomes as a delivery vehicle. **2020,** *8* (9), 1841-1851.
31. Han, W.; Yin, G.; Pu, X.; Chen, X.; Liao, X.; Huang, Z., Glioma targeted delivery strategy of doxorubicin-loaded liposomes by dual-ligand modification. *Journal of biomaterials science. Polymer edition* **2017,** *28* (15), 1695-1712.
32. Belfiore, L.; Spenkelink, L. M.; Ranson, M.; van Oijen, A. M.; Vine, K. L., Quantification of ligand density and stoichiometry on the surface of liposomes using single-molecule fluorescence imaging. *J Control Release* **2018,** *278*, 80-86.
33. Nogueira, E.; Gomes, A. C.; Preto, A.; Cavaco-Paulo, A., Design of liposomal formulations for cell targeting. *Colloids Surf B Biointerfaces* **2015,** *136*, 514-26.
34. Meka, R. R.; Mukherjee, S.; Patra, C. R.; Chaudhuri, A., Shikimoyl-ligand decorated gold nanoparticles for use in ex vivo engineered dendritic cell based DNA vaccination. *Nanoscale* **2019,** *11* (16), 7931-7943.

35. Dunne, M.; Zheng, J.; Rosenblat, J.; Jaffray, D. A.; Allen, C., APN/CD13-targeting as a strategy to alter the tumor accumulation of liposomes. *J Control Release* **2011,** *154* (3), 298-305.
36. Bednar, K. J.; Hardy, L.; Smeekens, J.; Raghuwanshi, D.; Duan, S.; Kulis, M. D.; Macauley, M. S., Antigenic Liposomes for Generation of Disease-specific Antibodies. *Journal of visualized experiments : JoVE* **2018,** (140).
37. Hadinoto, K.; Sundaresan, A.; Cheow, W. S., Lipid-polymer hybrid nanoparticles as a new generation therapeutic delivery platform: a review. *Eur J Pharm Biopharm* **2013,** *85* (3 Pt A), 427-43.
38. Rivankar, S., An overview of doxorubicin formulations in cancer therapy. *Journal of cancer research and therapeutics* **2014,** *10* (4), 853-8.
39. Ghosh, S.; Carter, K. A.; Lovell, J. F., Liposomal formulations of photosensitizers. *Biomaterials* **2019,** *218*, 119341.
40. Azanza, J. R.; Sádada, B.; Reis, J., Liposomal formulations of amphotericin B: differences according to the scientific evidence. *Revista espanola de quimioterapia : publicacion oficial de la Sociedad Espanola de Quimioterapia* **2015,** *28* (6), 275-81.
41. Li, Y.; Wang, M.; Huang, B. W.; Ping, Y.; You, J.; Gao, J. Q., Transcriptome-wide elucidation of liposomal formulations for anticancer drug delivery. *Int J Nanomedicine* **2017,** *12*, 8557-8572.
42. Kumari, P.; Ghosh, B.; Biswas, S., Nanocarriers for cancer-targeted drug delivery. *Journal of drug targeting* **2016,** *24* (3), 179-91.
43. Sheoran, R.; Khokra, S. L.; Chawla, V.; Dureja, H., Recent Patents, Formulation Techniques, Classification and Characterization of Liposomes. *Recent patents on nanotechnology* **2019,** *13* (1), 17-27.
44. Zhen, S.; Li, X., Liposomal delivery of CRISPR/Cas9. *Cancer gene therapy* **2020,** *27* (7-8), 515-527.
45. Doi, Y.; Shimizu, T.; Ishima, Y.; Ishida, T., Long-term storage of PEGylated liposomal oxaliplatin with improved stability and long circulation times in vivo. *Int J Pharm* **2019,** *564*, 237-243.
46. Zhu, Y.; Wang, F.; Zhao, Y.; Zheng, X., Pegylated liposomal doxorubicin-related palmar-plantar erythrodysesthesia: a literature review of pharmaceutical and clinical aspects. *European journal of hospital pharmacy : science and practice* **2020**.
47. Li, S.; Goins, B.; Zhang, L.; Bao, A., Novel Multifunctional Theranostic Liposome Drug Delivery System: Construction, Characterization, and Multimodality MR, Near-Infrared Fluorescent, and Nuclear Imaging. *Bioconjugate Chemistry* **2012,** *23* (6), 1322-1332.
48. Suk, J. S.; Xu, Q.; Kim, N.; Hanes, J.; Ensign, L. M., PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Adv Drug Deliv Rev* **2016,** *99* (Pt A), 28-51.
49. Dhakal, S.; Cheng, X.; Salcido, J.; Renu, S.; Bondra, K.; Lakshmanappa, Y. S.; Misch, C.; Ghimire, S.; Feliciano-Ruiz, N.; Hogshead, B.; Krakowka, S.; Carson, K.; McDonough, J.; Lee, C. W.; Renukaradhya, G. J., Liposomal nanoparticle-based conserved peptide influenza vaccine and monosodium urate crystal adjuvant elicit protective immune response in pigs. *Int J Nanomedicine* **2018,** *13*, 6699-6715.
50. Bozzuto, G.; Molinari, A., Liposomes as nanomedical devices. *International journal of nanomedicine* **2015,** *10*, 975-999.
51. Gao, W.; Hu, C.-M. J.; Fang, R. H.; Zhang, L., Liposome-like Nanostructures for Drug Delivery. *Journal of materials chemistry. B* **2013,** *1* (48), 10.1039/C3TB21238F.
52. Balocco, A. L.; Van Zundert, P. G. E.; Gan, S. S.; Gan, T. J.; Hadzic, A., Extended release bupivacaine formulations for postoperative analgesia: an update. *Current opinion in anaesthesiology* **2018,** *31* (5), 636-642.
53. Zahednezhad, F.; Zakeri-Milani, P., The latest advances of cisplatin liposomal formulations: essentials for preparation and analysis. **2020,** *17* (4), 523-541.
54. Xiao, P.; Zhao, J.; Huang, Y.; Jin, R.; Tang, Z.; Wang, P.; Song, X.; Zhu, H.; Yang, Z.; Yu, N., A Novel Long-circulating DOX Liposome: Formulation and Pharmacokinetics Studies. *Pharmaceutical nanotechnology* **2020,** *8* (5), 391-398.
55. D'Mello, S. R.; Cruz, C. N.; Chen, M. L.; Kapoor, M.; Lee, S. L.; Tyner, K. M., The evolving landscape of drug products containing nanomaterials in the United States. *Nature nanotechnology* **2017,** *12* (6), 523-529.
56. Kang, S. J.; Park, S. J.; Mishig-Ochir, T.; Lee, B. J., Antimicrobial peptides: therapeutic potentials. *Expert review of anti-infective therapy* **2014,** *12* (12), 1477-86.
57. Cafaro, A.; Giannini, M. B.; Silimbani, P.; Cangini, D.; Masini, C.; Ghelli Luserna Di Rorà, A.; Simonetti, G.; Martinelli, G.; Cerchione, C., CPX-351 daunorubicin-cytarabine liposome: a novel formulation to treat patients with newly diagnosed secondary acute myeloid leukemia. *Minerva medica* **2020,** *111* (5), 455-466.