



## LIPOSOMAL DRUG DELIVERY SYSTEMS FOR PROSTATE CANCER THERAPY: AN UPDATED REVIEW

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### Abstract

The liposome-based drug delivery system has emerged as a revolutionization of less toxic, biodegradable and biocompatible nano-medicine to overcome the adverse side effects of conventional approaches for cancer chemotherapeutics. The review provides a brief description of liposome composition, classification, benefits, drawbacks, clinical applications, and manufacturing techniques. This review specifically summarized wide-ranging research on the liposome-based drugs delivery for prostate cancer chemotherapeutics. Liposome-based marketed products for cancer chemotherapy are also included in this review. Nonetheless, despite the several liposome-related advantages over traditional systems, there are currently only a few liposome-based products on the market. It was therefore concluded that progressive research in this area will be challenging until the pharmaceutical industry does not fully embrace liposome technology.

**Keywords:** Liposome, manufacturing techniques, drug delivery, prostate cancer, chemotherapeutics.

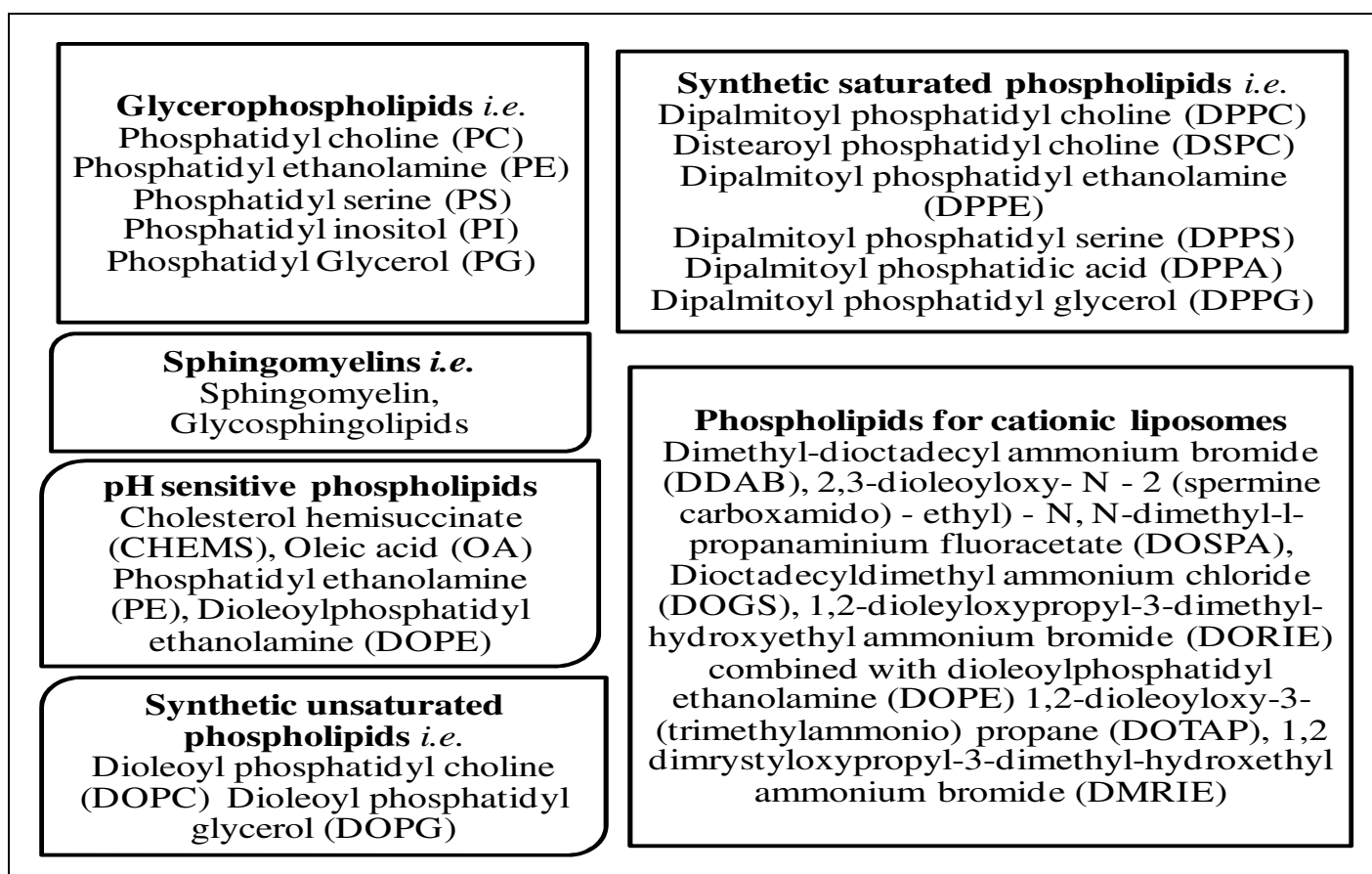
### Introduction

Cancer is one of the world's major causes of death that can affect every organ of the body. Conventional drug delivery strategies may target tumors as well as healthy tissues that previously cause adverse side effects for patients with cancer. Consequently, liposome-based drug delivery has emerged as a revolutionizing less toxic, biodegradable and biocompatible nanocarrier with an effective drug delivery to tumour cells (Bardania *et al.*, 2017; Bulbake *et al.*, 2017; Zahednezhad *et al.*, 2019). Liposomes are closed spherical bilayer phospholipids vesicles consisting predominantly of phospholipids and membrane permeability cholesterol and characterized by a lipid area containing hydrophobic medicines and an inner aqueous cavity for hydrophilic drug trapping (Daraee *et al.*, 2016; Ding *et al.*, 2006; Koning *et al.*, 2003; Marsh *et al.*, 2012). A thorough research into the liposomal drug delivery process has therefore been conducted in recent years, leading to several liposome-based drug formulations being developed for clinical use in cancer therapy. In this context, this review article discusses the composition and classification of liposomes and outlines the benefits and disadvantages of liposomes as well as explores liposomal production techniques, the clinical use and liposomal-based marketed products (Alavi *et al.*, 2017; Sercombe *et al.*, 2015; Valizadeh *et al.*, 2015). The objectives of this study were to conduct a comprehensive review of the literature on recent developments in liposome-based chemotherapeutics of prostate cancer. For this purpose, an extensive search of the literature published between the periods from 2000 to 2019 was conducted using the PubMed,

Google Scholar, and ScienceDirect databases. The keywords used in the search strategy were 'liposome', 'composition and classification', 'benefits and drawbacks', 'manufacturing techniques', 'clinical application', 'application of liposomes in prostate cancer', 'marketed products', and 'patents' in various combinations. A total of 122 documents were considered for this study after the deletion of duplicates found in the search and evaluation of the significance of the research articles. This would increase awareness of the gaps in liposomal research and thus provide benefits and opportunities for students and research scientists to better understand future prospective for research and development in the field of liposome-based chemotherapeutics for prostate cancer.

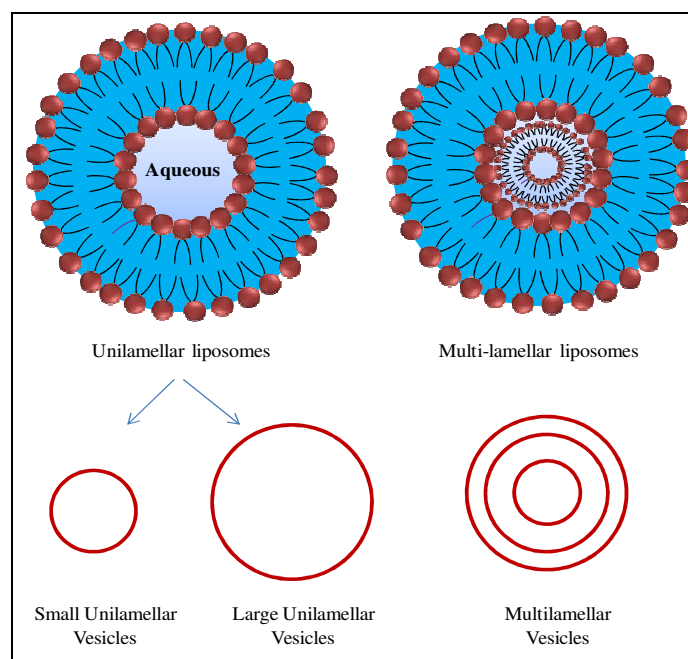
### Composition and classification of liposomes

Phospholipids used to make liposomes include glycerophospholipids, sphingolipids, and synthetic phospholipids that are saturated or unsaturated. Glycerophospholipids consist of hydrophobic acyl hydrocarbon and hydrophilic phosphate which aggregate characteristically to create a planar bilayer structure. Sphingolipids consist of fatty acid, choline and carbohydrates (Ahmed *et al.*, 2019; Eldin *et al.*, 2016). Cholesterol is the primary constituent lipid bilayer that fills the fissure of asymmetric phospholipid packing to prevent flip-flop, prevents phase transition to reduce leakage, provides rigidity in the membrane and protects against hydrolytic degradation (Liu *et al.*, 2016; Luo *et al.*, 2016; Varypataki *et al.*, 2015). Figure 1 shows various types of lipids used in liposome development.



**Fig. 1 :** Types of phospholipids utilized for manufacturing of liposomes.

Liposomes are classified by size and shape as multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs) and large unilamellar vesicles (LUVs) (Figure 2) (Daraee *et al.*, 2016; Hope *et al.*, 1986). Based on intracellular drug delivery mechanisms, liposomes are categorized as traditional liposomes (Daraee *et al.*, 2016; Immordino *et al.*, 2006), pH sensitive liposomes (Chu *et al.*, 1990; Litzinger *et al.*, 1992; Wang *et al.*, 1987), cationic liposomes (Felgner *et al.*, 1987; Gustafsson *et al.*, 1995), conjugated liposomes (Wang *et al.*, 2014), magnetic liposomes (Kawai *et al.*, 2005), immune liposomes with different surface binding antibodies (Alving *et al.*, 1991; Gregoriadis *et al.*, 1987), long circulating forms containing neutral lipids with high transition temperatures (Allen *et al.*, 1987), and PEGylated liposomes (Mohamed *et al.*, 2019). PEGylation has been reported to increase liposome clearance in the liver and spleen by mononuclear phagocytic cells (Harris *et al.*, 2003; Papahadjopoulos *et al.*, 1991). Because of PEGylation's stealth property PEGylated liposomes are not taken prematurely by MPS cells with better chances of reaching and providing diseased organs with higher levels of therapy compared to non-PEGylated cells (Pisal *et al.*, 2010; Matthews *et al.*, 2004). In the field of drug delivery, a number of regulatory approvals are issued to PEGylated and non-PEGylated liposomes. PEGylated liposome based formulation Doxil ® (encapsulating doxorubicin) has been approved for treatment of several organ cancers such as ovary, breast, Kaposi's sarcoma, and multiple myeloma (Barenholz *et al.*, 2012; James *et al.*, 1994; Markman *et al.*, 2006). Nevertheless, many lipid-related side effects have been caused by PEGylated liposomes. Doxil ® triggered immediate reactions to hypersensitivity after first injection in some patients (Chanan-Khan *et al.*, 2003; Szebeni *et al.*, 2014).



**Fig. 2 :** Classification of liposomes on the basis of size and shape

### Liposomal Benefits and Drawbacks

Numerous advantages of liposomes over traditional medicines include increased effectiveness, drug therapeutics index, extended blood circulation residence, and improved stability by encapsulation. For systemic or non-systemic treatment, liposomes are non-toxic, versatile, bio-compatible, completely biodegradable as well as non-immunogenic. Liposomes help to reduce harmful drug toxicity to sensitive tissues. Liposomes are site-specific and can be combined with site-specific ligands to accomplish active targeting. In

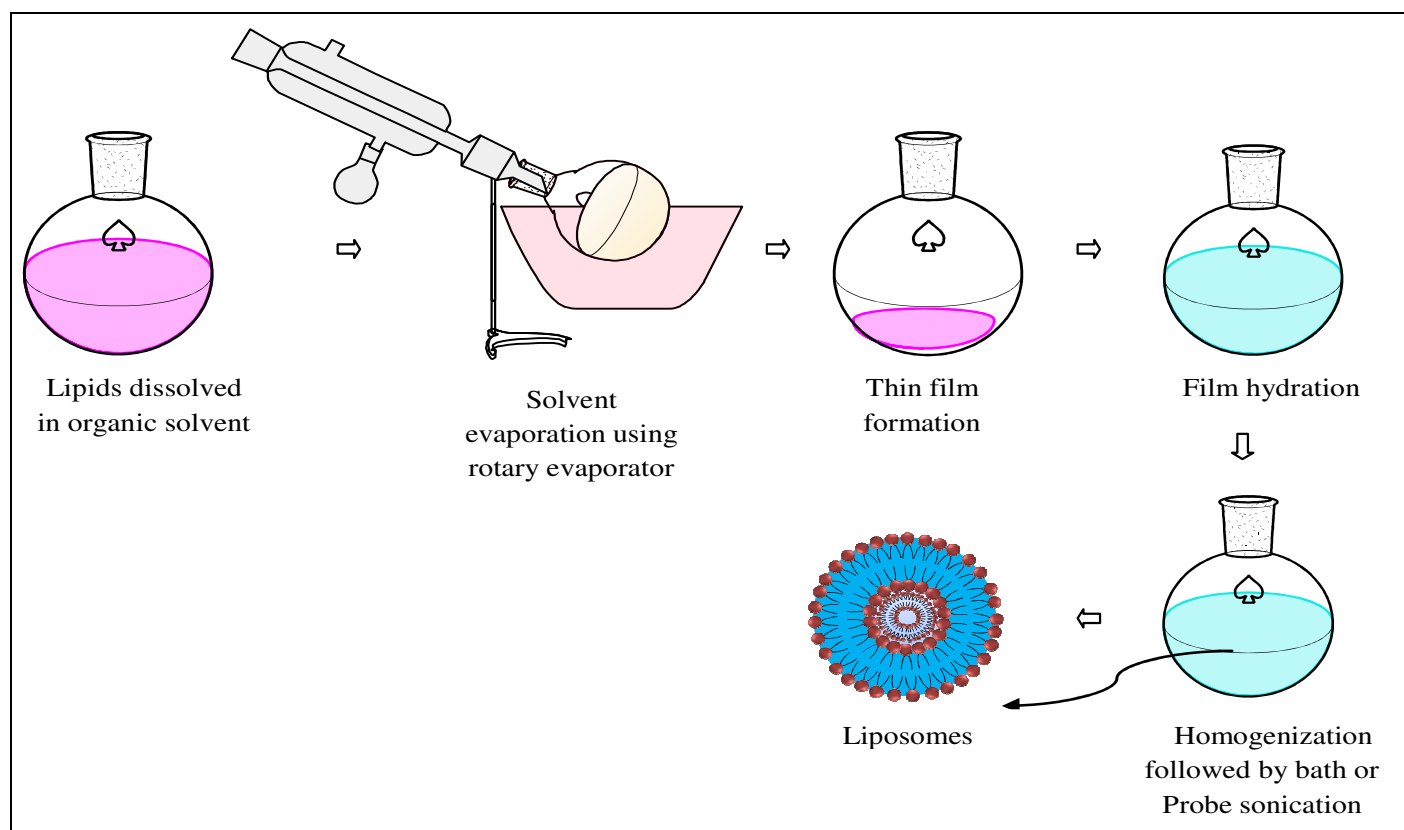
addition to the advantages, liposomes also have several disadvantages that include less solubility, small half-life, leakage of embedded drug, high fabrication costs, and less stability (Akbarzadeh *et al.*, 2013; Gabizon *et al.*, 1998; Lamichhane *et al.*, 2018).

### Liposome production techniques

All liposome preparation methods involve four basic steps which are drying lipids out of organic solvent; the lipid is spread in aqueous media; the resulting liposome is removed, and the final product will be evaluated (Akbarzadeh *et al.*, 2013).

### Mechanical dispersion methods

Schematic representation of lipid film hydration method has been shown in Figure 3. In sonication method, MLV are sonicated with either bath or probe sonicator. In probe sonication, a sonicator's tip is specifically located in dispersion. In bath sonication, the dispersion of the liposome in a flask is mounted into a bath sonicator. MLVs are extruded through a narrow orifice in French pressure cell extrusion (Akbarzadeh *et al.*, 2013; Kataria *et al.*, 2011; Riaz *et al.*, 1996).



**Fig. 3 :** Schematic representation of lipid film hydration method

### Solvent dispersion methods

In ether injection technique, the lipid solution dissolved in a diethyl ether or ether-methanol mixture is slowly introduced into an aqueous substance solution for encapsulation at 55-65 °C or at reduced pressure. The subsequent vacuum extraction of ether contributes to the formation of liposomes. The key disadvantages of the technique are the heterogeneous population (70-200 nm) and the high temperature treatment of compounds. The lipid ethanol solution is rapidly injected into excess buffer in the ethanol injection technique, resulting in the development of MLVs. The drawback of the process is that the population is heterogeneous (30-110 nm), the liposomes are highly diluted, the removal of all ethanol is difficult due to the fact that it forms azeotrope with water and the possibility of inactivation in the presence of ethanol of the various biologically active macromolecules (Akbarzadeh *et al.*, 2013).

### Detergent removal method

In dialysis technique, the detergents used for lipid solubilization forms the micelles at their critical micelles concentrations which eventually combine to form LUVs. The dialysis can be carried out in dialysis bags immersed in

massive detergent-free reservoirs (equilibrium dialysis). In detergent removal (absorption) technique, Organic polystyrene adsorbers such as XAD-2 beads and Bio-beads SM2 assist in detergent adsorption (cholate, alkyl glycoside, Triton X-100) from a mixed micellar solution. Gel-permeation chromatography involves the extraction of detergent by using special size pre-saturation chromatographic columns such as Sephadex G-50, Sephadex G-1 00, Sepharose 2B-6B and Sephacryl S200-S1000 which has been pre-saturated with lipids. The liposomes do not penetrate into these columns, although they can percolate through the inter-bead spaces (Akbarzadeh *et al.*, 2013).

### Clinical applications of liposomes

Liposome based drug carrier systems have wide applications under several medical conditions such as cancer, inflammation, and fungal diseases. Liposomes are also utilized in gene and vaccine delivery as well as for diagnostic purposes. However, other applications include encapsulation of food and cosmetic ingredients are attributable to stability and reduced toxicity (Abu *et al.*, 2017; Allen *et al.*, 2013; Bozzuto *et al.*, 2015; Bulbake *et al.*, 2017; Fahr *et al.*, 2005; Fan *et al.*, 2013; Gabizon *et al.*, 1998; Tseng *et al.*, 1998).

Liposomal formulations have low immunogenicity along with ability to transmit drug to target tissues and improved bio-distribution (Park *et al.*, 2002; Samad *et al.*, 2007). Many liposome based drug products gets approval for intramuscular, intravenous and oral drug delivery (Koshkina *et al.*, 1999; Rogers *et al.*, 1998). Many liposomal-based pharmaceutical products e.g. DepoDur™, Ambisome®, Doxil® are known as drug delivery systems for the significant contribution in the healthcare sector (Bulbake *et al.*, 2017; Sharma *et al.*, 1997).

### Recent research studies on liposomes for prostate cancer

Cancer is one of the world's leading causes of death that can affect any body organ. Conventional approaches to drug delivery may target tumours and healthy tissues that cause adverse side effects in patients with cancer beforehand. The liposome-based drug delivery system has been found highly effective in cancer therapy due to tumour targeting achievement, decreased systemic toxicity, and increased blood circulation residence time. A systematic literature review on several types of liposome e.g. PEGylated, magnetic, conjugated, cationic or immune-liposomes for chemotherapeutics of prostate cancer has therefore been performed. Recent studies have demonstrated the potential use of PEGylated liposomes of celastrol which is water-insoluble small molecule having short blood circulation time. PEGylated distearoyl phosphatidylcholine celastrol liposomes were found highly appropriate for clinical applications in comparison to dioleoyl phosphatidylcholine liposomes since distearoyl phosphatidylcholine liposomes exhibit superior serum stability, greater encapsulation efficiency and slower drug release. The enhanced intracellular accumulation of celastrol liposomes was evaluated over vertebral-cancer of the prostate cells (Wolfram *et al.*, 2014). Another group of researchers explored curcumin packed liposomes coated with prostate membrane-specific antigen-specific antibodies for targeted drug delivery with improved therapeutic index efficacy of curcumin (Thangapazham *et al.*, 2008). In a recent study, modified liposomes filled with lipopolymer (P3) consisting of prostate-specific membrane antigen ligand (PSMAL), polyethylene glycol 2000, and palmitate for theranostic delivery for prostate specific membrane antigen (PSMA+) expressing prostate cancer LNCaP cells. Doxorubicin-loaded P3-liposomes are significantly more toxic to LNCaP cells ( $p < 0.05$ ) along with an IC<sub>50</sub> reduction of ~5-fold and exhibits accelerated blood clearance accredited to the development of anti-PEG antibodies which influences bioavailability, drug targeting and efficacy of encapsulated drug (Yari *et al.*, 2019). In the study of Mahira *et al.* cationic coated hyaluronic acid (targeting moiety) liposomes loaded with cabazitaxel (cancer cell inhibitor) and silibinin (cancer stem cells inhibitor) were investigated to provide targeted drug delivery through CD44 receptors over expressed on cancer stem cell (Mahira *et al.*, 2019). Approximately  $14.87 \pm 0.41$  percent and  $33.95 \pm 0.68$  percent cytotoxicity were found against PC-3 and DU-145 human prostate cancer cell lines that indicated proficient cytotoxicity. In another study, curcumin and resveratrol loaded liposomes were synthesized to overcome low absorption and bioavailability problems which limits their clinical use. In-vitro studies in male B6C3F1/J mice revealed that combination therapy of curcumin and resveratrol successfully restrain cell growth, induce apoptosis and may decrease prostate cancer incidence

caused by loss of tumour suppressor gene phosphatase and tensin homolog (Narayanan *et al.*, 2009). Another study reported increased biocompatibility, enhanced cell membrane permeability and cytotoxic ability of sugar incorporated ruthenium (III) complex loaded egg phosphatidylcholine, cholesterol and distearoylphosphatidylethanolamine-methyl-polyethyleneglycol conjugate liposomes (D'Amora *et al.*, 2019). Zhang *et al* synthesized docetaxel loaded hybrid elastin-like polypeptide/liposome nanoparticles surface coated with gastrin-releasing peptide ligand which gets self-assembled on alteration in temperature conditions, thereby exhibited slow release of docetaxel and produced improved anti-cancer activity through gastrin-releasing peptide receptor overexpressing in PC-3 cells (Zhang *et al.*, 2018). A combination therapy consisting of imatinib-mitoxantrone liposomes with improved *in-vivo* therapeutic activity was discovered by Pinto *et al* for treatment of hormone-refractory prostate cancer. It has been found that owing to co-loading with imatinib, mitoxantrone exhibits equivalent growth inhibition effect with four time's lower dose in nude mice bearing subcutaneous PC-3 xenograft mode by co-loading with imatinib which established this as an excellent strategy which improved the therapeutic index of mitoxantrone for clinical development in prostate cancer therapy (Pinto *et al.*, 2010). A comparative study of doxorubicin saline suspension and sterically stabilized doxorubicin liposomes for tissue distribution and therapeutic effect on human prostate carcinoma xenografts was performed (Vaage *et al.*, 1994). Laser scan microscope and microfluoro-meter studies revealed that liposomes produced 25-fold increase in doxorubicin concentration in human prostate carcinoma PC-3 in Swiss mice and significantly highly effective for treatment of prostate cancer with minimized systemic side effects. Kroon *et al* investigated that liposomal delivery of dexamethasone inhibits the growth of malignant bone lesions in mouse model of prostate cancer bone metastases through enhanced permeability and retention effect at well-tolerated clinically-relevant dosages and therefore, offers potential treatment alternative for advanced, metastatic prostate cancer (Kroon *et al.*, 2015). Another study evaluated the therapeutic activity of secretory phospholipase A-2 responsive liposomes in DU-145/LNCaP/PC-3 cells lines and found 1.5 to 2- fold increases in intracellular drug levels in comparison to sterically stabilized liposomes (Mock *et al.*, 2013). In a comparative study, Jantscheff *et al.* formulated gemcitabine liposomes to protect drug from enzymatic degradation with augmented deposit within tumour tissues (Jantscheff *et al.*, 2009). Another study investigated  $\alpha 5\beta 1$ -targeted stealth liposomes functionalized with fibronectin-mimetic peptide (PR\_b) for efficient intracellular drug delivery to LNCaPs human prostate cancer cell lines and observed superior functionalization, enhanced efficacy of internalization and cytotoxicity of LNCaPs prostate cancer cells. Therefore, PR\_b-targeted liposomes has great prospective for efficient and specific drug delivery to prostate cancer cells (Demirgöz *et al.*, 2008). Another study investigated that anisamide-conjugated doxorubicin stealth liposomes exhibited significant targeted delivery of liposomal doxorubicin via sigma receptor overexpressed in prostate cancer DU-145 cells. *In-vitro* studies illustrated that anisamide efficiently mediated doxorubicin targeting, improved cellular uptake and cytotoxicity DU-145 cells (Banerjee *et al.*, 2004). For the treatment of prostate cancer, doxorubicin and simvastatin loaded herceptin antibody-conjugated liposomes are

synthesized by Li *et al* with reduced side effects and targeted drug delivery via human epidermal growth factor-2 receptors overexpressed in prostate cancer PC-3 cell line (Li *et al.*, 2019). The enhanced cytotoxic activity of doxorubicin-loaded folate-targeted liposomes has been reported in androgen-sensitive human prostate adenocarcinoma (LNCaP) cells with folate-targeted liposomes which provide targeted prostate cancer cell therapy via prostate-specific antigen receptor over-expressed in prostate cancer tissue (Patil *et al.*, 2018). In another comparative study, zoledronic acid containing self-assembly PEGylated nanoparticle were compared with zoledronic acid encapsulating PEGylated liposomes. It has been reported that nanoparticle but not liposomes caused significant reduction of tumour associated macrophages and therefore, suggested prospective preclinical development of nanoparticle of zoledronic acid for treatment of human cancer (Marra *et al.*, 2012). Ikemoto *et al* developed doxorubicin loaded Bauhinia purpurea agglutinin-PEG-modified liposomes bound specifically to human prostate cancer cells but not to normal cells and demonstrated enhanced anti-tumour activity of drug in comparison to only pegylated liposomes (Ikemoto *et al.*, 2016). Another study by Sauvage *et al* demonstrated that liposomal 6BrCaQ illustrated superior apoptosis induction and anti-cancer activity on PC-3 cell lines (Sauvage *et al.*, 2016). Work from a research group has shown higher cellular uptake and higher cytotoxicity of conjugated liposomes with cyclic arginine/glycine/aspartic acid/tyrosine/lysine peptide (cRGDyk) (Wang *et al.*, 2014). Bandekar *et al* has shown that targeted  $\alpha$ -particle generator  $^{225}\text{Ac}$  pegylated, PSMA J591 antibody or A10 PSMA aptamer labelled liposomes selectively bind, become internalized, and kills prostate-specific membrane antigen-expressing cells for targeted anti-vascular radiotherapy (Bandekar *et al.*, 2014). Cao *et al* reported achievement of active targeting of paclitaxel through RGD peptide based liposomes which specifically bind to integrins receptors over-expressed on prostatic cancer cells (Cao *et al.*, 2015). Moreover, uptake efficiency of RGD-liposomes was 5.2 and 3.2-fold in comparison to plain liposomes in PC-3 and DU145 cells, respectively. Another group of researchers studied that peptide SP204-conjugated liposomes exhibited enhanced intracellular drug delivery and cytotoxicity. Moreover, conjugation of these liposomes to imaging agents e.g. quantum dots and super paramagnetic iron oxide nanoparticle resulted in highly precise delivery of doxorubicin and vinorelbine to prostate cancer cells and therefore, SP204 targeting peptide has significant prospective for drug targeting and molecular imaging in prostate cancer (Yeh *et al.*, 2016). Another group of researchers discovered hyperthermic effect of magnetic cationic liposomes of colloidal magnetite in rat prostate cancer. The tumour temperature increased to 45°C while body temperature remained at around 38°C (Kawai *et al.*, 2005). Furthermore, it was investigated that repeated hyperthermia using magnetic cationic liposomes may be a promising new therapy for hormone-refractory human prostate cancer in the future (Kawai *et al.*, 2006). In addition it was found that magnetic cationic liposomes along with alternating magnetic field heat therapy suppressed tumour proliferation in bone micro-environment for bone metastatic lesions treatment (Kawai *et*

*al.*, 2008). The research group of Yano *et al* investigated anti-tumor activity of small interfering RNA/cationic liposome complex. The difficulty in administration of siRNA has been overcome through combining siRNA with cationic liposome to provide a novel siRNA therapy for cancer patients (Yano *et al.*, 2004). Another research group of Bode *et al* investigated that paclitaxel loaded cationic liposomes are effectively absorbed by immature vascular endothelial cells having negative electric cell surface charge to produce neovascular targeting effect for treatment of prostate cancer as demonstrated through in-vivo study in male ad concluded this as promising technique for angiogenesis suppression in comparison to conventional treatment (Bode *et al.*, 2009). A study by Xiang *et al* demonstrated that prostate-specific antigen (PSA) and prostate-specific membrane antigen modified liposomes are particularly uptaken by tumour cells (Xiang *et al.*, 2013). In-vivo studies demonstrated that dual-modified liposomes revealed maximized upset of polo-like kinase-1 in tumour cells and induction of tumour cell apoptosis. Targeted delivery of topotecan, vinorelbine, and doxorubicin using anti-CD166 single chain variable fragment immune-liposomes has been investigated with enhanced killing of prostate cancer cells and found that immunoliposomal topotecan was the most effective in cytotoxicity assays on tumour cell lines (Roth *et al.*, 2007). Furthermore, a different antibody-liposome complex (immunoglobulin M (IgM) typed anti-PSMA monoclonal antibody (PSMA IgM; 732-20) coupled to poly-L-lysine and mixed with cationic liposomes containing plasmid DNA) was into prostate cancer cells (LNCaP) for targeting gene therapy of prostate cancer (Ikegami *et al.*, 2005). Ikegami *et al* coupled anti-PSMA monoclonal antibody (mAb) MLN591 with poly-L-lysine and incubated with plasmids and transfected these complexes with cationic liposomes into prostate cancer cells (LNCaP) to achieve targeting gene therapy (Ikegami *et al.*, 2006). Furthermore, significant inhibitory effect of LNCaP xenografts mouse model was discovered through suicide gene therapy. Doxorubicin loaded immunoliposomes modified with prostate cell-specific monoclonal antibody (mAb 5D4) for selective targeting of prostate cancer cell lines expressing mAb 5D4 antigen with enhanced cytotoxicity in comparison to non-targeted doxorubicin liposomes without increase in systemic toxicity was investigated by research group (Sawant *et al.*, 2008). To minimize the systemic toxicity of doxorubicin, Baek *et al* synthesized RNA aptamer-conjugated liposome of doxorubicin specific to PSMA with considerably enhanced in-vitro cellular binding and uptake in LNCaP xenograft mice which enable their use in clinical practice with minimized side effects (Baek *et al.*, 2013). Another group of researcher of Sung *et al* demonstrated that targeted delivery of L1 cell adhesion molecule (L1CAM) using liposome-encapsulated siRNA could effectively inhibit prostate cancer growth in mouse bone as well as cell proliferation in prostate cancer cells though knockdown of L1CAM expression in prostate cancer cells by RNA interference and therefore, provides evidence that siRNA-based L1CAM could be major contributor for prostate cancer metastasis (Sung *et al.*, 2014). Table 1 demonstrates the processing method and composition of various liposomes used to treat prostate cancer.

**Table 1 :** Recent studies in development of liposome-based formulation for prostate cancer.

Drug (Technique)	Type of liposomes	Lipids/solvents	Cell lines/assay for in-vitro anticancer activity	Reference
Celastrol (Membrane extrusion)	Pegylated Liposomes	Di-oleoyl phosphatidylcholine, Cholesterol, Distearoyl Phosphatidylcholine, Distearoylphosphatidyl-ethanolamine-methylpolyethylene glycolconjugate-2000/ Dimethyl sulfoxide	Vertebral-cancer of the prostate cells (VCaP)/Cell counting kit-8 (CCK-8)	Wolfram <i>et al.</i> , 2014
Curcumin, prostate membrane-specific antigen-specific antibodies (Probe sonication)	Immunoliposomes	Dimyristoyl phosphatidylcholine, Dipalmitoyl phosphatidylcholine, Cholesterol/Chloroform	Human prostate cancer cell lines (LNCaP and C42B)/ MTT* assay	Thangapazham <i>et al.</i> , 2008
Doxorubicin HCl (Thin film hydration)	Pegylated immunoliposomes	Dipalmitoyl phosphatidylcholine, Dimyristoylphosphatidyl-glycerol, Cholesterol/ Chloroform, Methanol	LNCaP and PC3 cells/ <sup>18</sup> F-PSMAL uptake assay	Yari <i>et al.</i> , 2019
Cabazitaxel, Silibinin (Ethanol injection)	Cationic liposomes	1, 2-dioleoyloxy-3-(trimethylammonio) propane/Ethanol	PC-3 and DU-145 cells/MTT*and clonogenic assay	Mahira <i>et al.</i> , 2019
Curcumin and resveratrol	Liposomes	Dimyristoylphosphatidyl-glycerol	Murine phosphatase and tensin homolog (PTEN)-CaP8 prostate cancer cells/MTT* assay	Narayanan <i>et al.</i> , 2009
Pyridine Ruthenium (Thin film hydration)	Conjugated pegylated liposomes	Phosphatidylcholine, Cholesterol/Chloroform, Methanol	PC-3 cells/ crystal violet assay	D'Amora <i>et al.</i> , 2019
Docetaxel , gastrin-releasing peptide ligand (Thin film hydration)	Conjugated liposomes	Egg yolk lecithin, Cholesterol/Methanol, Chloroform	PC-3 and DU-145 cells/MTT* assay	Zhang <i>et al.</i> , 2018
Imatinib/Mitoxantrone/ lipid thin-film hydration technique	Liposomes	Distearoylphosphatidylcholine, Cholesterol/Chloroform	PC-3 tumor xenograft-bearing mice model	Pinto <i>et al.</i> , 2010
Paclitaxel	Pegylated liposomes	Hydrogenated soy phosphatidylcholine/cholesterol/polyethylene glycol-distearoyl-phosphatidyl-ethanolamine	Prostate adenocarcinoma PC-3	Vaage <i>et al.</i> , 1994.
Dexamethasone/Ethanol injection method	Pegylated liposomes	Poly (ethylene glycol) 2000-distearoylphosphatidylethanolamine, dipalmitoyl-phosphatidylcholine and cholesterol/Ethanol	Human osteotropic prostate cancer cell line PC-3M-Pro4luc2/rat model	Kroon <i>et al.</i> , 2015
Doxorubicin encapsulated in sterically-stabilized liposomes and Secretory phospholipase A2 (sPLA2) responsive liposomes/lipid film hydration	Pegylated liposomes	Palmitoyl-oleoylphosphatidylcholine, cholesterol, and methoxypoly(ethylene glycol) 2000)-distearoyl phosphatidylethanolamine/ Chloroform	LNCaP, DU-145 and PC-3 cells lines/ 6-Carboxyfluorescein assay and MTT* assay	Mock <i>et al.</i> , 2013
Gemcitabine/ high-pressure homogenizer	Liposomes	Hydrogenated egg phosphatidylcholine/cholesterol, lipid AG,	Human hormone-refractory Du145 (ATCC HTB-81), and PC-3 (ATCC CRL-1453) cells/ Evans blue in-vivo vascular leakage assay/ In-vitro luciferase assay	Jantscheff <i>et al.</i> , 2009
Fibronectin (Membrane extrusion)	PEGylated liposomes	Dipalmitoyl phosphatidylcholine, Cholesterol, Dipalmitoylphospho-ethanolamine-N-(methoxy (polyethylene glycol)-2000)/ Chloroform, Methanol, Water	Human prostate cancer cell lines (LNCaP)/Quantikine Human TNF- $\alpha$ /TNFSF1A immunoassay kit	Demirgöz <i>et al.</i> , 2008
Doxorubicin (Bath sonication)	PEGylated liposomes	Cholesterol, Palmitoleyl-phosphatidyl choline, Distearoyl phosphatidyl-ethanolamine-[methoxy-polyethylene glycol (2000)]/Chloroform, dimethylsulfoxide	DU-145 cells/ MTT* colorimetric assay	Banerjee <i>et al.</i> , 2004

Doxorubicin, Simvastatin (Thin film hydration)	PEGylated liposomes	Soybean phosphatidylcholine, Cholesterol, Distearoylphospho-ethanolamine-N-[carboxy (polyethylene glycol)-2000]/ Chloroform	PC3 and human umbilical vein endothelial cells (HUVEC)/ Cell counting kit-8	Li <i>et al.</i> , 2019
Doxorubicin (Membrane extrusion)	PEGylated liposomes	Hydrogenated soybean phosphatidyl-choline, N-(carbamoyl-methoxy-polyethylene glycol 2000)-distearoyl phospho-ethanolamine/Ethanol, t-Butanol	LNCaP cells/ In-vitro cytotoxicity assay	Patil <i>et al.</i> , 2018
Zoledronic Acid/modified reverse-phase evaporation technique	PEGylated liposomes	Egg phosphatidylcholine, Dioleoyl-3-trimethyl-ammonium-propane Chloride, Diacyl glycerophosphoethanolamine-N-[methoxy(polyethylene glycol)2000], Cholesterol/ Chloroform, methanol	Human prostate carcinoma PC3 cell line/mouse model	Marra <i>et al.</i> , 2012
Bauhinia purpurea agglutinin/thin film hydration	PEGylated liposomes	Dipalmitoylphosphatidylcholine, cholesterol and methoxy-polyethyleneglycol (2000)-conjugated distearoyl phosphatidylethanolamine/ Chloroform	Human prostate cancer cells lines DU145 and LNCaP/ liposome binding and in-vitro anti-proliferative assay	Ikemoto <i>et al.</i> , 2016
6BrCaQ	PEGylated liposomes	Egg phosphatidylcholine, Cholesterol and distearoyl-phosphatidyl-ethanolamine-N-[methoxy(polyethyleneglycol)-2000]/Chloroform	Prostate adenocarcinoma PC-3 cells	Sauvage <i>et al.</i> , 2016
Cisplatin, cyclic arginine-glycine-aspartic acid-tyrosine-lysine peptide (cRGDyk) as $\alpha\beta3$ integrin ligand/Thin film evaporation-sonication method	Conjugated PEGylated liposomes	Egg Phosphatidylcholine, cholesterol, Distearoyl phosphatidyl-ethanolamine-N-[maleimide (polyethylene glycol)-2000]/Chloroform, methanol	RM-1 cells/ MTT* assay	Wang <i>et al.</i> , 2014
$\alpha$ -particle generator $^{225}\text{Ac}$ , labelled with PSMA** J591 antibody or with the A10 PSMA aptamer / lipid hydration method	Conjugated PEGylated liposomes	Dinonadecanoyl phosphatidylcholine, cholesterol, Distearoyl phosphatidyl-ethanolamine-N-[methoxy (polyethyleneglycol)-2000] (ammonium salt), Dipalmitoyl- phosphatidyl-ethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt)	LnCaP, Mat-Lu, HUVEC, BT474, and MDA-MB231/MTT* assay	Bandekar <i>et al.</i> , 2014
Paclitaxel, peptide(RGD)/ thin film hydration method	Conjugated PEGylated liposomes	Phosphatidylcholine and mPEG2000-distearoyl phosphatidylethanolamine and Mal-PEG 2000- distearoyl phosphatidyl-ethanolamine, cholesterol/ Chloroform	PC-3 cells and DU145 cells	Cao <i>et al.</i> , 2015
Doxorubicin, vinorelbine/Film hydration technique	Conjugated PEGylated liposomes	Distearoylphosphatidylcholine, cholesterol, and MPEG-2000-distearoyl phosphatidylethanolamine/ Chloroform	PC3, DU145, and LNCaP human prostate cancer cell lines/MTT* cell proliferation assay	Yeh <i>et al.</i> , 2016
Colloidal magnetite/ Sonication	Magnetic cationic liposomes	N-( $\alpha$ -trimethylammonioacetyl) didodecyl-D-glutamate chloride and dilauroylphosphatidylcholine and dioleoylphosphatidyl-ethanolamine	Rat prostate cancer cell line PLS 10/ In-vitro cytotoxicity assay	Kawai <i>et al.</i> , 2005
Colloidal magnetite/ Sonication	Magnetic liposomes	N-( $\alpha$ -trimethylammonioacetyl) didodecyl-D-glutamate chloride and dilauroylphosphatidylcholine and dioleoylphosphatidyl-ethanolamine	Human prostate cancer cell line PC-3 derived from bone metastatic lesions and LNCap derived from lymph node metastatic lesions)	Kawai <i>et al.</i> , 2006
Colloidal magnetite/ Sonication	Magnetic liposomes	N-( $\alpha$ -trimethylammonioacetyl) didodecyl-D-glutamate chloride and dilauroylphosphatidylcholine and dioleoylphosphatidyl-ethanolamine	Rat prostate carcinoma tissues (PLS-P)/ TRAP assays	Kawai <i>et al.</i> , 2008
Small interfering RNA, B717	Cationic liposome	2-O-(2-diethylaminoethyl)-carbamoyl-1,3-O-dioleoylglycerol and egg phosphatidylcholine	PC-3 (prostate carcinoma) cells/MTT* assay	Yano <i>et al.</i> , 2004

Paclitaxel	Cationic liposomes	-	Dunning R3327H rat prostatic adenocarcinoma subline MatLu/in-vitro animal study	Bode <i>et al.</i> , 2009
Small interfering RNA, PSA*** responsive and PSMA** mediated multifunctional liposomes/Film hydration followed by postinsertion method	Immuno-liposomes	Distearoyl phosphatidyl-ethanolamine-N-methoxy(polyethylene glycol) (ammonium salt), Distearoyl phosphatidyl-ethanolamine-N-maleimide(polyethylene glycol), Distearoyl phosphatidyl-ethanolamine-N-amino(polyethylene glycol), Soybean phosphatidylcholine, Cholesterol/Chloroform, methanol	Human prostate tumor cell line (PC-3 cells) and 22Rv1 xenograft prostate tumor cell line/Cellular uptake and cell apoptosis assay	Xiang <i>et al.</i> , 2013
Drug molecules (Topotecan, vinorelbine, doxorubicin) and anti-***CD166 scFv immunoliposomes /freeze-thawing method	Immuno-liposomes	Distearoylphosphatidylcholine, cholesterol, Poly(ethylene)glycol (PEG-2000)-derivatized distearoyl-phosphatidylethanolamine/ Ethanol	Prostate cancer cells (PC3, Du-145, and LNCaP)/ MTT* assay	Roth <i>et al.</i> , 2007
Immunoglobulin M (IgM) typed anti-PSMA** monoclonal antibody (PSMA IgM; 732-20)	Immuno-liposomes	-	Human adenocarcinoma cell lines of prostate, LNCaP, PC-3, and DU145/galactosidase reporter gene expression assay	Ikegami <i>et al.</i> , 2005
Anti-PSMA** monoclonal antibody (mAb) MLN591	Immuno-liposomes	-	Human prostate adenocarcinoma cell lines, LNCaP and PC-3	Ikegami <i>et al.</i> , 2006
Doxorubicin and monoclonal antibody 5D4 (mAb 5D4)	Immuno-liposomes	Hydrogenated soy phosphatidylcholine, cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt)/Citrate buffer saline	Human prostate cancer cell lines LNCaP, PC3, DU145/CellTiter Blue Kit assay	Sawant <i>et al.</i> , 2008
Doxorubicin and RNA aptamer-conjugated/Film hydration technique	Immuno-liposomes	Palmitoyloleoyl phosphatidylcholine, 1,2-Dioleoylphosphatidyl ethanolamine, distearoyl-phosphatidylethanolamine-N-[methoxy (polyethylene glycol)-2000], and cholesterol/ Chloroform, methanol	LNCaP and PC3 cells/cell viability assay	Baek <i>et al.</i> , 2013
L1 cell adhesion molecule (L1CAM) and small interfering RNA	Immuno-liposomes	-	Prostate cancer PC3 cells/Cell-migration and -invasion assays, cell-aggregation assay. anchorage-independent growth assay, luciferase assay	Sung <i>et al.</i> , 2014

\*MTT assay: 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide assay; \*\* PSMA: Prostate-specific membrane antigen; \*\*\*CD166: target antigen, and scFv: internalizing single chain variable fragment, \*\*\*\*PSA: prostate-specific antigen (PSA).

### Liposome-based existing marketed products

Due to extensive research in the field of liposomes, various molecules have recently been put on the market. Doxil® is a liposome injection of doxorubicin hydrochloride (an inhibitor of anthracycline topoisomerase II) that is encapsulated in intravenous stealth® liposomes (Barenholz *et al.*, 2012; Duggan *et al.*, 2011). Lipodox is doxorubicin hydrochloride incorporated in long-circulating pegylated liposomes formed with surface-bound methoxypolyethylene glycol (MPEG) to protect liposomes from mononuclear phagocyte system (MPS) detection and to increase blood circulation time (Berger *et al.*, 2014; Chou *et al.*, 2015). DaunoXome contains daunorubicin citrate salt solution encapsulated in lipid bilayer of cholesterol and

distearoylphosphatidylcholine (Cooley *et al.*, 2007; Petre *et al.*, 2007). Marqibo contains intravenous vincristine sulphate encapsulated in liposomes of sphingomyelin/cholesterol (Rodriguez *et al.*, 2009; Sarris *et al.*, 2000). CPX-351 is a liposomal dual-drug encapsulation of cytarabine and daunorubicin containing 5:1 synergistic drug ratio (Cortes *et al.*, 2015; Riviere *et al.*, 2011). MM-398 is an innovative nano-liposome of irinotecan having reduced systemic toxicity and augmented anti-cancer activity (Ko *et al.*, 2013; Geretti *et al.*, 2015; Roy *et al.*, 2013; Saif *et al.*, 2014). MM-302 is a novel PEGylated antibody-liposomal doxorubicin-conjugated injection targeting human epidermal growth factor-2 (HER2) (Suzuki *et al.*, 2008). MBP-426 is a novel oxaliplatin-encapsulated transferrin-conjugated N-glutaryl phosphatidylethanolamine (NGPE)-liposomal injection



suspension produced to enhance oxaliplatin's safety and efficacy by prolonged circulation period of the drug and targeting transferrin receptors on tumor cells (Goldberg *et al.*,

2013; Webb *et al.*, 2007). A brief overview of products marketed for cancer therapy based on liposomes is given in Table 2.

**Table 2 :** Liposome-based marketed products for cancer therapeutics.

Marketed product (Therapeutic agent)	Company name	Cancer type	Reference
Doxil® (Doxorubicin hydrochloride)	Johnson & Johnson	Kaposi's sarcoma in AIDS	Barenholz <i>et al.</i> , 2012; Duggan <i>et al.</i> , 2011
LipoDox (Doxorubicin)	Sun Pharma	Refractory ovarian cancer	Berger <i>et al.</i> , 2014; Chou <i>et al.</i> , 2015
DaunoXome (Daunorubicin citrate)	Galen	Kaposi's sarcoma in AIDS	Cooley <i>et al.</i> , 2007; Petre <i>et al.</i> , 2007
Marqibo® (Vincristine)	Talon	Acute lymphoblastic leukemia	Rodriguez <i>et al.</i> , 2009; Sarris <i>et al.</i> , 2000
CPX351 (Cytarabine and daunorubicin)	Celator Pharmaceuticals	Secondary acute myeloid leukemia (AML)	Cortes <i>et al.</i> , 2015; Riviere <i>et al.</i> , 2011
Onivyde (MM-398/Irinotecan)	Merrimack pharma	Metastatic pancreatic cancer	Ko <i>et al.</i> , 2013; Geretti <i>et al.</i> , 2015; Roy <i>et al.</i> , 2013; Saif <i>et al.</i> , 2014
MM-302 (Doxorubicin)	Merrimack pharma	Advanced metastatic HER2-positive breast cancer	Suzuki <i>et al.</i> , 2008
MBP-426 (Oxaliplatin)	Mebiopharm	Gastric cancer	Goldberg <i>et al.</i> , 2013; Webb <i>et al.</i> , 2007

## Conclusion

Over the past few years, numerous researchers have recognized the feasibility of using liposomes as effective drug carriers for chemotherapy for prostate cancer with targeted features of drug release, and extensively studied both in vitro and in vivo. Liposomes are an attractive system of drug carriers. Liposome-based pharmaceutical products are less toxic, biodegradable and biocompatible with the ability to load both hydrophilic and hydrophobic drug molecules. Drug carriers based on liposomes have been reported to increase the efficacy of the drug, therapeutic index, stability and pharmacokinetic effects; drug targeting of tumor tissue, reduced systemic toxicity, prolonged blood circulation time, improved safety, therapeutic efficacy and patient compliance over conventional medicines. Despite the several advantages associated with liposomes over traditional systems, however, only a few liposome-based products are currently available in the market. Until the pharmaceutical industry does not fully embrace liposome technology, progressing research in this area will be challenging.

## Acknowledgements

The authors express gratitude to Chitkara College of Pharmacy, Chitkara University, Punjab, India for motivation to compile this review.

## Conflict of interest

The authors declare no conflict of interest.

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