Open Access Scientific Reports

Review Article Open Access

aqueous or lipid phases of liposomes with various sizes, compositions and other characteristics by di erent preparation techniques. An ideal method of liposome formulation is preparing liposome with high entrapment e ciency, narrow particle size distribution and long term stability. Numbers of techniques have been reported for preparation of liposomes such as Bangham method, the detergent depletion method, the ether/ethanol injection method, the reverse phase evaporation and the emulsion method [48]. e majority of liposome preparation methods require using organic solvents to dissolve lipids but these organic solvents are harmful to the

techniques is to obtain e cient drug entrapment and increase stability of the liposome products [52]. Most of drugs listed in Table 1 using liposomes as carrier to increase the drug solubility in aqueous solution and also decrease drug toxicity in human body. Ambisome, Doxil and Myocet are the examples for improving therapeutic index by reducing the toxicities associated with the free drugs.

Storage of Liposomes: l ophili ation

Liposomes dispersed in aqueous solution generally face physical and chemical instabilities a er long term storage [53]. Hydrolysis and oxidation of phospholipids and liposome aggregation are the common cause of liposome instabilities. According to the literature, many methods have been investigated for the stabilization of liposomes, such as lyophilization, freezing and spraying drying. In commercial liposome-based drugs (Table 1), AmBisome, Amphotec, Myocet, Visudyne and LEP-ETU are all lyophilized products. In general, freeze-drying increases the shelf-life of liposomal formulations and preserves it in dried form as a lyophilized cake to be reconstituted with water for injection prior to administration [54]. Furthermore, cryoprotectants need to be added to maintain particle size distribution of liposomes a er freeze-drying- rehydration cycle. Various types and concentrations of sugars have been investigated for their ability to protect liposomes against fusion and leakage during lyophilization processes [54]. In commercial liposome lyophilized products, lactose was used as a cryoprotectant in the formulation of Amphotec, Myocet and Visudyne and sucrose was added in the formulation of Ambisome and LEP-ETU to increase liposome stability during lyophilization. Interestingly, these commercial lyophilized products showed similar shelf-life in comparison with other liposome products (eg: suspension and emulsions) and hence lyophilization may not have expected e ect on liposome stability. In 1998, Clemons et al. [55] compared the potency and therapeutic e cacy among the di erent lipid-based formulations of amphotericin B (Amphotec, AmBisome and Abelcet) for the treatment of systemic and meningeal cryptococcal disease. eir work indicated that the therapeutic e cacy of Amphotec and AmBisome was superior to that of Abelcet by up to 10-fold in survival and in clearing infection from all organs. In these three commercially available lipid-based formulations of amphotericin B, Amphotec and AmBisome are both lyophilized products and Abelcet is formulated as a suspension form. erefore, lyophilization may not extend the shelf-life of products but may increase therapeutic e cacy *in i* . Similar results were also reported in our previous studies. We investigated the stability of the siRNA-loaded liposomes in suspension and lyophilized powder form up to 1 month post manufacture [56]. Following formulation, the siRNA-loaded liposomes were stored at either 4°C or room temperature. e particle size and zeta potential of siRNA-loaded liposomes remained unchanged for both storage conditions. However, siRNA entrapment efficiencies for both storage conditions were observed to have decreased slightly over time. Surprisingly, the gene-silencing efficiency of siRNA-loaded liposomes in aqueous solution was almost completely abolished following 1-month of storage at either 4°C or room temperature. is was in contrast to liposomes prepared in the lyophilized powder form where 100% of the gene-silencing e ciency was retained following storage at either 4°C or room

temperature for a month. Although therapeutic e ciency of liposome-based drug may vary depending on the choice of lipids, the preparation technique, the physico-chemical characteristics of the bioactive, and the overall charge of the liposome, lyophilisation is absolutely essential for the long term storage of liposome-based drugs.

Liposomal anti-cancer drug researches: Do orubicin

Liposome delivery systems o er the potential to enhance the therapeutic index of anticancer drugs, either by increasing the drug concentration in tumor cells and by decreasing the exposure in normal host tissues. Doxorubicin is an anthracycline widely used to treat solid and hematological tumors, but its major drawback is the onset of resistance. erefore, doxorubicin-loaded liposomes were developed to combat aggressive tumors, like breast and ovary metastatic cancers and Kaposi's sarcoma. Myocet and Doxil were rst approved liposome-based drugs for cancer treatment. Both products contain doxorubicin but di erent particularly in the presence of Poly Ethylene Glycol (PEG) coating (Figure 1). In pharmacokinetic studies of doxorubicin-loaded liposomes, free doxorubicin had an elimination half-life time of 0.2 hours and an AUC (area under the cure) of 4 μg h ml^{-1} in patients an as compared with 2.5 hour and 45 μg h ml^{-1} for Myocet and with 55 hours and 900 μg h ml^{-1} for Doxil, respectively [15]. Both liposome products showed longer circulating half-life as compared with free drug. In phase III head to head comparison of free doxorubicin vs Myocet in patients with metastatic breast cancer, similar results presented in response rates (26% for both) and progression-free survival times (4 4975e(4 4975e(4 975e(3(i)-3(bo(-1)TjEd)-48(hu.n.[(rates)-3((26%)-3(for)-3(both))nr8(th4

DPPC alone, the rate of release and the amount released are relatively small. By incorporating a small amount of lysolipids, such as MSPC or Monopalmitoylphosphatidylcholine (MPPC), into DPPC liposomes, Tc is shi ed down slightly and membrane instability and drug release rate is signi cantly enhanced at Tc. Banno et al. [59] demonstrated that the presence of MSPC, rather than DSPE-PEG2000, in DPPC liposomes would give rise to the rapid drug release pro le in vitro and that represents lysolipid is the more important component in determining TSL contents release. Indeed, Banno's *in i* data showed that the presence of 9.6 mol% MSPC in TSL could result in more rapid elimination of the encapsulated doxorubicin ($T_{1/2}$=1.29h), compared to the formulation without lysolipid ($T_{1/2}$=2.91h). In 2007, Dromi et al. [36] compared the accumulation of doxorubicin in mice tumors among free doxorubicin, Doxil and ermoDox. Results showed that over time, doxorubicin gradually increased in tumors when both Doxil and ermoDox were used but not with free doxorubicin. At 24 hours a er administration, doxorubicin concentrations in tumors were found to be signi cantly higher with Doxil than ermoDox. ermoDox is currently under evaluation in clinical trials and hence the therapeutic e cacy of ermoDox is still unknown.

Liposomal anti-cancer drug researches: daunorubicin and paclita el

Daunorubicin and paclitaxel have also incorporated into liposomes for the formulation of liposomal anti-cancer chemotherapy drugs. DaunoXome is a commercial liposomal formulation of daunorubicin in which the drug is entrapped into small unilamellar vesicles composed of Distearoyl phosphatidylcholine (DSPC) and cholesterol in 2:1 molar ratio. LEP-ETU and EndoTAG-1 (previously called MBT-0206) are the potential liposomal formulations of paclitaxel and both are in clinical trials (Table 1 and 2). In comparison with conventional daunorubicin, DaunoXome was 36-fold higher in AUC and in vivo experiments

Product Name	Route of injection	Drug	Lipid composition	Approved indication	Ref
LEP-ETU (Powder/12 months)	Intravenous	Paclitaxel	DOPC, cholesterol and cardiolipin in 90:5:5 molar ratio	ovarian, breast and lung cancers	[5,27]
LEM-ETU	Intravenous	Mitoxantrone	DOPC, cholesterol and cardiolipin in 90:5:5 molar ratio	Leukemia, breast, stomach, liver, ovarian cancers	[5,27]
EndoTAG-1 (Powder/ 24 months)	Intravenous	Paclitaxel	DOTAP, DOPC and paclitaxel in 50:47:3 molar ratio	Anti-angiogenic properties, breast cancer	[5,28-30]
EndoTAG-2	Intravenous	camptothecin	DOTAP	Metastatic cancer	[28,30]
Arikace	portable aerosol delivery	Amikacin	DPPC and cholesterol	Lung infection	[31,32]
Marqibo	Intravenous	Vincristine	cholesterol and egg sphingomyelin in 45:55 molar ratio	metastatic malignant uveal melanoma	[5,33,34]
ThermoDox	Intravenous	Doxorubicin	DPPC, MSPC and PEG 2000-DSPE in 90:10:4 molar ratio	non-resectable hepatocellular carcinoma	[35,36]
Antragen	Intravenous	Tretinoin	DMPC and soybean oil	advanced renal cell carcinoma	[5]
T4N5 liposome lotion	Topical	Bacteriophage T4 endonuclease 5	unknown	xeroderma pigmentosum.	[37]
Liposomal Grb-2	Intravenous	**Grb2** antisense oligodeoxynucleotide	unknown	Acute Myeloid Leukemia, Chronic Myelogenous Leukemia, Acute Lymphoblastic Leukemia	[38]
Nyotran	Intravenous	Nystatin	DMPC, DMPG and cholesterol	systemic fungal infections.	[5]
LE-SN38	Intravenous	SN-38, the active metabolite of irinotecan AMCID 606 BDC 13.9.17incristine			

indicated increased uptake of daunoXome in tumour tissue at 24 h. In phase III trial of DaunoXome versus vincristine in AIDS-related Kaposi's sarcoma, the e cacy of DaunoXome was comparable to that of vincristine. Response rates (25% vs 28%), time to treatment failure (115 vs 99 days), and overall survival (369 vs 342 days) were similar on both treatment arms and hence DaunoXome may provide another safe and e ective chemotherapy [60].

Taxol® (paclitaxel) is a marketed product for the treatment of ovarian, breast, non-small cell lung cancer and AIDS-related Kaposi's Sarcoma [27]. It is one of the most e ective anticancer drugs available on the market. However, paclitaxel is only sparingly soluble in water and therefore, intravenous administration depends on the use of the non-ionic surfactant Cremophor EL (polyethoxylated castor oil) to achieve a clinically relevant concentrated solution. Unfortunately, Cremophor EL increases toxicity and leads to hypersensitivity reactions in certain patients. LEP-ETU formulation of paclitaxel is being developed to potentially reduce toxicities associated with Taxol®, by eliminating the drug formulation component polyoxyethylated castor oil. LEP-ETU formulations composed of 1,2-Dioleoyl- *n*-glycero-3-phosphocholine *(DOPC),* cholesterol and cardiolipin in 90:5:5 molar ratio were prepared by the modi ed thin- lm hydration method. *DOPC*, a zwitter ionic natural phospholipid, is rst chosen as one of the lipid components in LEP-ETU formulation because of a low Tc (−22°C), and which forms more exible liposomes to entrap highly hydrophobic molecules. Moreover, cholesterol is included in LEP-ETU formulations to increase the liposome stability. In cardiotoxicity, positively charged doxorubicin's a nity for negatively charged cardiolipin, a lipid abundant in heart tissue, is thought to be involved in drug localization in the heart tissue [61]. Liposomes containing cardiolipin, reportedly reduced cardiotoxicity associated with doxorubicin by altering the pharmacokinetics and tissue distribution of the drug and hence cardiolipin may also exert similar results in LEP-ETU. Fetterly et al. [62,63] evaluated the Maximum Tolerated Dose (MTD), Dose-Limiting Toxicities (DLT), and pharmacokinetics of Liposome-Encapsulated Paclitaxel (LEP-ETU) in comparison to Taxol®. e MTD of LEP-ETU was 325 mg/m^2 in phase I study of patients with locally advanced or metastatic carcinoma. is dose is higher than that achieved with Taxol, which is typically delivered at a dose range of 135 to 200 mg/m^2. e major toxicity to administration of paclitaxel is neuropathy. In the phase I study, neurotoxicity occurred in 5 of 12 patients (42%) treated with LEP-ETU at ≥325 mg/m^2. Although a direct comparison with Taxol® is not possible, neutropenia was seen in 53% of metastatic breast ca035ijwEred which forms more -24(oCIDndMCI(isoCII(isoCII.0546 278.ajwe)-32(stability.)-32(In)-9wd]TJ)]Tjb99wdn /MCID 753 BDC954

(1987) Functional reconstitution of influenza virus envelopes. EMBO J 6: 2651-2659.

24. Glück R, Mischler R, Brantschen S, Just M, Althaus B, et al. (1992) Immunopotentiating reconstituted influenza virus virosome vaccine delivery system for immunization against hepatitis A. J Clin Invest 90: 2491-2495.

25. D'Acremont V, Herzog C, Genton B (2006) Immunogenicity and safety of a virosomal hepatitis A vaccine (Epaxal) in the elderly. J Travel Med 13: 78-83.

26. Herzog C, Hartmann K, Künzi V, Kürsteiner O, Mischler R, et al. (2009) Eleven years of Inflexal V-a virosomal adjuvanted influenza vaccine. Vaccine 27: 4381-4387.

27. Zhang JA, Anyarambhatla G, Ma L, Ugwu S, Xuan T, et al. (2005) Development and characterization of a novel Cremophor EL free liposome-based paclitaxel (LEP-ETU) formulation. Eur J Pharm Biopharm 59: 177-187.

28.

Antibody titres after primary and booster vaccination of infants and young children with a virosomal hepatitis A vaccine (Epaxal). Vaccine 21: 4588-4592.

67.