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## Preparation and characterization of tramadol PEG-coated multivesicular liposomes for sustained release

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The purpose of the present study was to prepare multivesicular liposomes (MVL) with a high drug loading capacity for intramuscular sustained release and to investigate their potential applicability towards tramadol, and to improve the stability of liposomes by coating PEG. The basic physiochemical properties of tramadol MVLs and PEG-coated MVLs were studied. The average particle sizes of optimum preparation were 18.2  $\mu\text{m}$  and 31.3  $\mu\text{m}$ . The entrapment efficiency was up to 80%. The encapsulation efficiency of tramadol MVLs and PEG-coated MVLs was measured. The results confirmed the possibility of multivesicular liposomes as a sustained-release delivery system. Tramadol was continuously released from MVL formulations in PBS (pH 6.8) *in vitro*, and reached a maximum of 80% within 72 h. The results show that tramadol PEG-coated MVLs could provide sustained release according to the first order kinetic equation.

### 1. Introduction

Tramadol is a weak  $\mu$ -receptor agonist that also inhibits the reuptake of serotonin and norepinephrine. It is considered as a general analgesic, which may be effective in many chronic pain conditions, including bone and visceral pain, and neuropathic pain (Tiwari et al. 2003). A controlled and sustained delivery system comprising a biocompatible and biodegradable matrix would offer clinically important therapeutic advantages, such as significantly reduced dose frequency and improved efficacy. Tramadol is readily soluble in water and has a pKa of 9.41; the *n*-octanol/water log partition coefficient (logP) is 1.35 at pH 7.0. Multivesicular liposomes (MVLs) compared with conventional liposomes provided high encapsulation capacity for water-soluble drugs (Mantripragada 2002). It was reported that MVLs can be used to develop sustained release formulations of water-soluble drugs with a high drug loading capacity (Zhong et al. 2005; Langston et al. 2003). The multivesicular liposomes contain a neutral lipid, which is an integral structural component, allowing a unique multivesicular liposomal structure (Kim et al. 1983; Glantz et al. 1999). Polyethylene glycol (PEG) is advantageous as a modifying agent because it is inert, water-soluble, nontoxic, and modular in size (Ye et al. 2006). Pegylation can offer several advantages, which include longer circulation times of the MVLs (Li et al. 2003; Vyas et al. 2006).

In this paper, we report the encapsulation of tramadol in PEG-coated MVLs and demonstrate its sustained delivery and the results of tramadol may be a suitable model for understanding the release behaviors of water-soluble drugs of the PEG-coated MVLs.

### 2. Investigations and results

#### 2.1. Characterization of PEG-coated MVLs encapsulated tramadol formulations

The structure determined by microscope confirmed the existence of a polymer layer on the surface of liposome particles. By com-

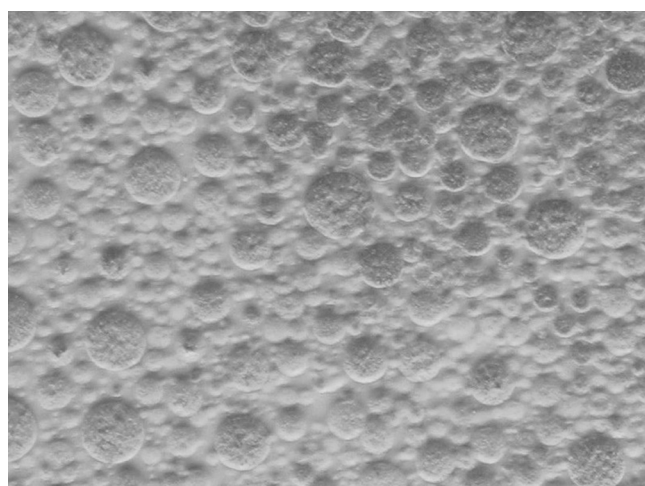
parison with the non-coated liposome, the polymer coated one showed larger particle size and better encapsulation.

Fig. 1 shows the morphology of tramadol MVLs particles from a representative formulation at 400 $\times$  magnifications with a light microscope. The particles were from the two tramadol MVLs, showing a smooth and spherical morphology. Two tramadol MVLs showed similar median sizes ranging from 1 to 50  $\mu\text{m}$  in diameter, with  $\sim 95\%$  of the particles in the size range 20–40  $\mu\text{m}$  (Fig. 1a,b). The microscopic photographs of the PEG-coated MVLs tramadol showed that the shape of the liposome was round and integrated. PEG-coated MVLs had some big particles and hydrated layer was observed (Fig. 1b).

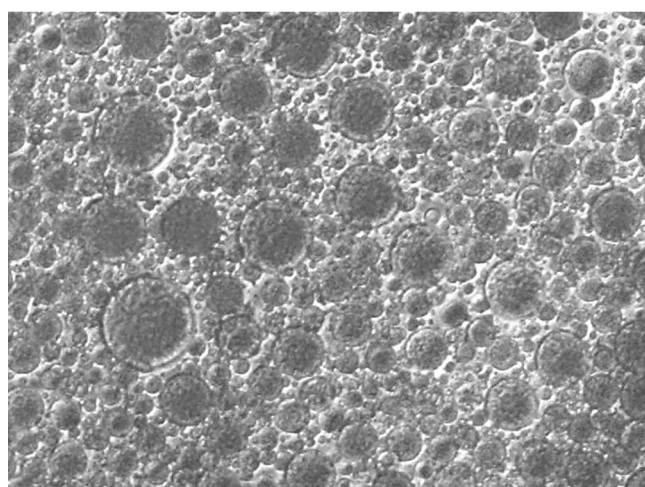
Tramadol MVLs were coated with PEG by simply mixing the liposomal suspension with the PEG solution. PEG-coated MVLs structures determined by light microscopy confirmed the existence of polymer layer on the surface of liposome particles. By comparison with the non-coated liposome, the polymer coated one showed larger particle size and a low encapsulation efficiency (Table). Grain size variation of MVLs and PEG-coated MVLs were investigated (Fig. 2). It was seen that the PEG-coated MVLs showed better stability and prolonged release.

#### 2.2. Encapsulation efficiency of tramadol PEG-coated MVLs

The loading efficiency was determined by the ratio of the amount of drug in the final liposome suspension to the total amount of drug used in the first aqueous solution. The Table presents a summary of some major characteristics of large unilamellar vesicles (LUV), MVLs and PEG-coated MVLs of tramadol showed different efficiency (30.7%, 82.4% and 80.1% respectively). A relatively high percentage of encapsulation of MVLs and PEG-coated MVLs of tramadol ( $\approx 80\%$ ) was achieved. The particle size of MVLs was 18.2  $\mu\text{m}$ , and that of the PEG-coated MVLs was about 31.3  $\mu\text{m}$ . The drug entrapment efficiencies of



(a)



(b)

Fig. 1: Light micrograph of (a) tramadol MVLS and (b) PEG-coated MVLS

the PEG-coated MVLS were higher (80.1%) than that of LUV (30.7%).

### 2.3. Release of tramadol from PEG-coated MVLS

The release duration from the tramadol PEG-coated MVLS and MVLS was extended up to 72 h (Fig. 3), while the tramadol large unilamellar vesicles (LUV) released 85% of drug within 12 h. In process stability studies of tramadol PEG-coated MVLS exhibited better stability when exposed to PBS as compared to LUV.

Profile of tramadol containing PEG-coated MVLS in the PBS showed a lower initial burst release than on-coated MVLS with a higher initial burst release of nearly 50% followed by almost complete release. The results confirmed the possibility of PEG-coated MVLS as a long-acting sustained release delivery system for intramuscular application.

**Table: General characteristics of tramadol MVLS and PEG-coated MVLS**

	LUV	MVLS	PEG-coated MVLS
Encapsulation efficiency (%)	30.7	82.4	80.1
Particle size ( $\mu\text{m}$ )	1.9	18.2	31.3
Zeta Potential (mV)	-10.8	-22.7	-28.7

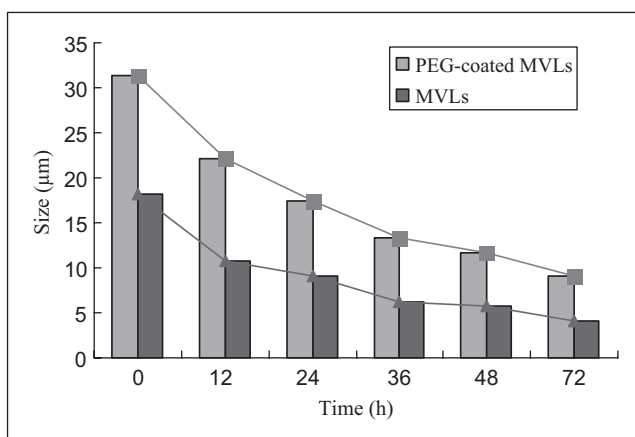


Fig. 2: Three days storage stability particle size change for tramadol MVLS and PEG-coated MVLS

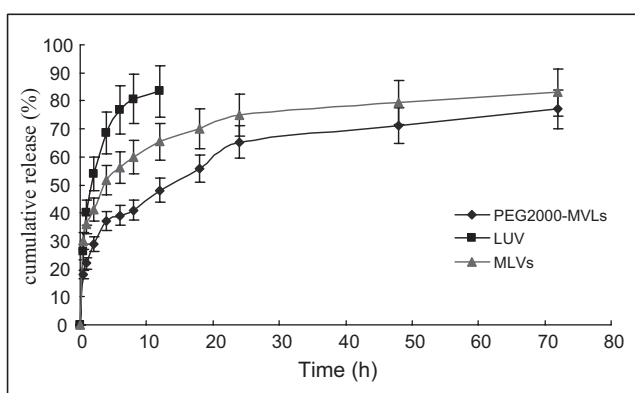


Fig. 3: *In vitro* release in PBS, pH 6.8, at 37 °C from tramadol LUV (■), MVLS (▲) and PEG-coated MVLS (◆). The experiments were repeated three times. The SD. was found to be less than 3% of the mean values

The release of tramadol from the MVLS and PEG-coated MVLS were all sustained to 72 h with a long period of first-order release although the release profiles of liposomes were influenced by the properties of liposomes. For instance, the release of tramadol PEG-coated MVLS was lower than that of MVLS and LUV. The system of PEG-coated MVLS could effectively reduce burst effects of tramadol compared to non-coated ones. The results indicated that stabilizing the PEG-coated MVLS would highly affect the release behavior of tramadol from the liposomes. When tramadol PEG-coated MVLS were incubated for 72 h at 37 °C, the release duration from the MVLS extended as long as 72 h for 90% of drug (Fig. 4). Approximately 77% and 85%

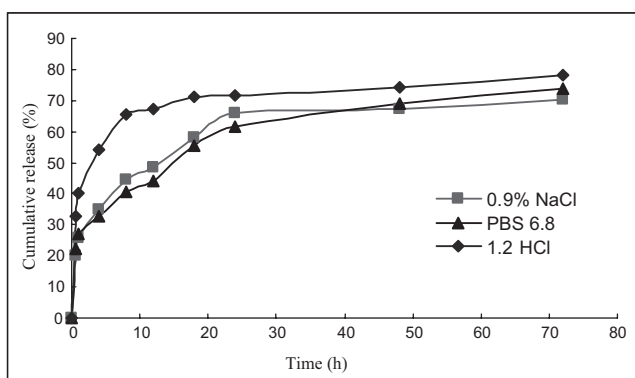


Fig. 4: *In vitro* release profiles of tramadol from PEG-coated MVLS in 0.9% NaCl (■), PBS, pH 6.8 (▲), pH 1.2 HCl (◆) at 37 °C from tramadol PEG-coated MVLS. The experiments were repeated three times. The SD. was found to be less than 3% of the mean values

of tramadol were released from the PEG-coated MVLs within 72 h in 0.9% NaCl, PBS pH 6.8 and pH 1.2 hydrochloric acid, respectively.

The surface coating on the liposome provided by the hydrophilic polymer chains provides colloidal stability and a prolonged release. The local concentration gradient of PEG chains from the liposome surface leads to an osmotic imbalance, changes in thermodynamic properties, and hydration of the lipids and influenced by different media.

### 3. Discussion

An *in vitro* release assay showed sustained release of the drug from the MVLs (Depofoam) particles (Ramprasad et al. 2002, 2003). The PEG-coated MVLs remained stable, and therapeutic levels of drug were delivered without an initial rapid release. PEG-coated MVLs have been developed which can sustain the release of water-soluble drugs such as tramadol in 2~3 days that are influenced by the surface properties of liposomes, suggesting that the stability of PEG2000 plays an important role in release. Release behaviors in different solutions from PEG-coated MVLs are highly similar to each other, indicating that the results of tramadol may be a suitable model for understanding the release behaviors of water-soluble drugs of the PEG-coated MVLs. The duration of the release effect of tramadol can be controlled for 2 to 3 days, in order to accommodate various dosing schedules to match the therapeutic need.

The light microscopy pictures showed the difference in morphology of the tramadol PEG-coated MVLs particles and changed over time in PBS at 37 °C. The mechanism of drug release from MVLs possibly included diffusion and surface erosion. The PEG2000 in PEG-coated MVLs is postulated to stabilize the membrane, which is supported by the fact that PEG-coated MVLs release differently in different media. Tramadol PEG-coated MVLs in the PBS showed lower initial burst release with sustained and incomplete release over a period of 72 h. In contrast, LUV showed a higher initial burst release, i.e., nearly 85% followed by almost complete release.

### 4. Experimental

#### 4.1. Reagents and chemistry

Light microscopy for checking the liposomes particles was performed with Axiovert 40 CFL microscope (Carl Zeiss). The zeta potential and the size of MVLs were measured using a Nicomp 370 instrument (Santa Barbara, CA, USA). Reverse phase-HPLC (RP-HPLC) was performed with a Hewlett-Packard Model 1100 HPLC system and a Waters C18 symmetric column (Millipore, MA USA). Tramadol was purchased from Duoduo Pharmaceutical Group (Qiqihaer, PR China). High-purity soybean phosphatidylcholine (PC) for injection was purchased from Shanghai Aikang Fine Chemical Co., Ltd. (Shanghai, PR China). 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dipalmitoyl-sn-3-phosphoglycerol (DPPG) were purchased from Lipoid GmbH (Ludwigshafen, Germany). All reagents were of analytical grade.

#### 4.2. Preparation of tramadol multivesicular liposomes (MVLs) and PEG-coated MVLs

The multivesicular liposomes containing tramadol were prepared by a double-emulsification process (Jain et al. 2005). This process involves the formation of a w/o/w emulsion. The first step is the formulation of a w/o emulsion. Four mL of the aqueous solution containing 20 mg/mL tramadol, 20 mg/mL L-arginine (adjusted to a pH of 4.6 by hydrochloric acid), and 4% sucrose, were emulsified with 4 mL of a lipid solution containing phospholipids (PC, 80 mM DOPC, 80 mM DPPG), 20 mM cholesterol, and 40 mM triglyceride in chloroform. The first emulsion was then mixed with a second aqueous solution containing 4% sucrose and 20 mg/mL L-arginine (adjusted to a pH of 4.6 by hydrochloric acid). To form the w/o/w emulsion (second emulsion), chloroform was removed by flushing nitrogen gas over the surface of the second emulsion at 50 °C, to form the MVLs. The preformed MVLs were added to a solution contain-

ing a concentrated dispersion of 10% PEG2000 solutions and incubated 30 min to achieve insertion of the micellar lipid conjugates into the preformed liposomes. Finally, PEG-coated MVLs were washed by centrifugation at  $600 \times g$  for 5 min and re-dispersed in isotonic sorbitol, diluted with 20 mg/mL L-Arginine; adjusted pH to 5.6 with hydrochloric acid.

#### 4.3. Tramadol PEG-coated MVLs particle characterization

The tramadol MVLs and PEG-coated MVLs were confirmed by light microscopy, and the diameter and zeta potential of the liposomes were determined by Nicomp 370. The measurement was conducted in duplicate and the mean values were used. The tramadol MVLs or PEG-coated MVLs suspensions were diluted 4-fold into PBS, pH 6.8, a 0.5 mL. Samples were incubated under gentle rotating conditions (10 cycles/min) at 37 °C for 72 h. Particle size of the liposomes was determined by Nicomp 370.

#### 4.4. Trapping efficiency of tramadol in PEG-coated MVLs

The amount of encapsulated tramadol in the tramadol formulations was determined by RP-HPLC-UV at 270 nm after solubilization with methanol. Tramadol was separated at a flow rate of 1.0 mL/min, using a linear gradient of acetonitrile (30~80%) in water. The percent encapsulated tramadol, and the amount of free drug in the suspension was determined as described previously. The morphology of the MVLs particles was examined using a light microscope at 400 $\times$  magnification.

Free and tramadol multivesicular liposomes (entrapped and adsorbed) were separated by ultracentrifugation for at  $600 \times g$  at a temperature of 15 °C for 0.5 h. The supernatant was collected, diluted to about 10  $\mu$ g/mL. The total amount of tramadol in multivesicular liposomes was measured by the following procedure: 100  $\mu$ L of MVLs was completely dissolved by the addition of 100  $\mu$ L of 3% Triton X-100, incubated at 15 °C for 30 min, and then assayed by RP-HPLC. The trapping efficiency was calculated by the following equation:

$$\text{Trapping efficiency \%} = 100$$

$$\times (\text{total amount of drug} - \text{amount of free drug}) / \text{total amount of drug}$$

#### 4.5. Release of tramadol from PEG-coated MVLs

*in vitro* release experiments were performed as follows methods: The tramadol MVLs or PEG-coated MVLs suspensions were diluted 5-fold into PBS, pH 6.8, a 0.5 mL sample of the diluted suspension was used for each time point; samples were incubated under gentle rotating conditions (10 cycles/min) at 37 °C for 72 h. Samples were taken for analyses according to the planned schedule to which 0.1 mL of normal saline was added, and particle pellets were then obtained by centrifugation in a centrifuge at  $600 \times g$  for 30 min and determined by HPLC, using a RP-C18 column, a mobile phase of pH 5.0 phosphate buffer: water-methanol (70:30) at a flow rate of 1 mL/min.

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