Preparation, stability and pharmacokinetics evaluation of lipid microspheres loading a promising antitumor candidate, Timataxel

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ABSTRACT
Timataxel (13-(N-Boc-3-i-butylisoserinoyl-4,10-β-diacetoxy-2-α-benzoyloxy-5-β-20-epoxy-1,13-α-dihydroxy-9-oxo-19-norcyclopropa[g]tax-11-ene), used to be called TM-2, is a novel semi-synthetic promising candidate for cancer treatment. However the preformulation study showed that TM-2 was insoluble and chemically unstable in water, which would limit its application. This study aimed at the preparation of Timataxel lipid microspheres (TM-2 LMs) and investigated the difference between TM-2 LMs and TM-2 solution in pharmacokinetics. In this work, the final formulation was as follows: 0.10% (w/v) TM-2; 10.00% (w/v) oil phase (long chain triglyceride:media chain triglyceride = 2.50%:7.50%); 1.40% (w/v) phospholipid; 0.02% (w/v) NaH₂PO₄; 2.25% (w/v) glycerin and water to a total volume of 100 ml. The particle size distribution, content and entrapment efficacy were 205.0 ± 43.3 nm, 101.00%, and 99.12%, respectively. TM-2 LMs were stable during storage at 25 °C for 3 months, even under the condition of 60 °C and 4500 lx for 10 d. Phosphatidylethanolamine (PE) in phospholipid may contribute to the stability of TM-2 LMs. The pharmacokinetic parameters for TM-2 LMs were as follows: AUC₀⁻∞ 3663.71 μg/l h and the clearance 2.26 l/h/kg. As for solution, these parameters were 1712.52 μg/l h and 4.77 l/h/kg, respectively. The t½ of TM-2 LMs was similar to TM-2 solution. The pharmacokinetic results indicated that the AUC of TM-2 LMs was larger, the clearance was smaller than that of TM-2 solution. In a word, lipid microspheres were a promising drug delivery system for TM-2.

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1. Introduction

Paclitaxel, which was first extracted from the stem bark of Taxus brevifolia, exhibited excellent anti-tumor effect. The antitumor mechanism of paclitaxel is to combine with β-tubulin subunit, inhibit microtubule depolymerization, and finally lead to apoptosis [1,2]. Considering its superiority in the treatment of cancer, taxanes had attracted interests all over the world. Currently, taxane derivatives like paclitaxel, docetaxel and cabazitaxel have been commercialized as intravenous injections for years [3]. Multidrug resistance, mainly caused by overexpression of P-glycoprotein (P-gp), has limited the application of docetaxel and paclitaxel [4]. P-gp acts as an energy dependent drug efflux pump which could transport drugs to the extracellular medium [5], and meanwhile, paclitaxel and docetaxel are substrates of P-gp. Thus, it is essential to modify the structure of taxanes to improve activity especially against multidrug resistant tumor.

Timataxel (TM-2, C_{43}H_{57}NO_{12}), one of the novel semi-synthetic taxanes, has lower affinity to P-gp than docetaxel, and its structure was listed in Fig. 1. In vitro study has revealed that TM-2 exhibits more promising efficacy on a variety of human tumor lines than docetaxel or larotaxel, especially on multidrug resistant cancer cell lines involving KB/VCR and MCF-7/ADR [6,7]. The inhibition rate of A549 human lung xenografts was up to 82.24% [6,8]. Based on these encouraging results, TM-2 was selected for further preclinical study.

For many drugs, insufficient solubility and less stability in water limit their clinical use. Usually, the commercial taxane formulations are made up of Cremophor EL or Polysorbate 80, ethanol and water. Cremophor EL and Polysorbate 80 are non-ionic surfactants that are responsible for the occurrence of side effects, including acute hypersensitivity reactions and peripheral neurotoxicity [3]. What’s more, these solvents were supposed to be bound with alterations in the pharmacokinetic characteristics after i.v. administration [9]. So a suitable carrier is required to reduce these adverse reactions.

Lipid microspheres (LMs) mainly consist of oil, phospholipid and water. Usually, drugs are incorporated in oil which is also formed in the internal phase. This kind of structure could protect drugs from hydrolysis, reduce irritation and other side effects, and sustain drug release [10,11]. LMs are biocompatible, biodegradable, and simply applied for large-scale manufacturing [12]. In addition, tumor is different from normal tissue that could increase drug retention due to enhanced permeation and retention effect [13]. Besides, tumors need higher energy which could be supplied by LMs [14]. Thus, the encapsulation of TM-2 into LMs could increase drug accumulation in tumor and improve therapeutic efficacy. What’s more, our previous study had shown that TM-2 was insoluble in water but lipophilic, so LMs seem to be an attractive carrier for it [6].

The stable pH for TM-2 is 5.5–6.0, and TM-2 is liable hydrolysis in excessive acid media and alkaline media [6]. So pH is a key factor during the preparation of TM-2 LMs. While in our previous study, when TM-2 LMs were prepared, the final pH varied within a wide range, which made the quality of the final product uncontrollable. Moreover, the oil phase of previous TM-2 LMs was media chain triglyceride (MCT) only; there was some disadvantage compared with mixed oil. In this paper, a new formulation containing MCT and long chain triglyceride (LCT) for Timataxel lipid microspheres (TM-2 LMs) was prepared. The pH was well controlled by NaH_{2}PO_{4} and phospholipid, and the effect of different phospholipid on the stability of TM-2 LMs was studied. The stability of TM-2 LMs was investigated in detail, including autoclaving stability, freezing and thawing stability, dilution stability and acceleration stability. Finally, the pharmacokinetic characteristics of TM-2 LMs were investigated.

2. Materials and methods

2.1. Materials, reagents, and animals

Timataxel (TM-2, purity >99%) was kindly supplied by School of Pharmacy, Fudan University (Shanghai, China). Cabazitaxel (CBZ, purity >98%) was synthesized in the Medicinal Chemistry Lab of Yantai University (Yantai, China). Long chain triglyceride (LCT) and media chain triglyceride (MCT) were purchased from TieLing Beiya Pharmaceutical Co. (Tieling, China). Egg lecithin PL-100M was provided by Shanghai Advanced Vehicle Technology Pharmaceutical Co., Ltd. (China). Lipid E80 and Lipid S100 were purchased from Lipid KG (Ludwigshafen, Germany). Glycerol was purchased from Zhe-Jiang Suichang Glycerol Plant (Zhejiang, China). All other chemicals and reagents were of HPLC or analytical grade.

Male Sprague-Dawley (SD) rats, weighing 200 ± 10 g, were kindly provided by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The animal study was conformed to the Guideline for Animal Experimentation of Shenyang Pharmaceutical University.

2.2. Preparation of TM-2 LMs

TM-2 LMs were prepared by high-pressure homogenization method. Considering the drug loading (1 mg/ml), 10% (w/v) oil phase was selected for further study according to our previous
study [6]. The specific steps were as follows: firstly, the phospholipid was dispersed in the mixture of MCT and LCT at 70 °C in a constant temperature water bath, then when the phospholipid dissolved, TM-2 was added. At the same time, the aqueous phase was prepared by combining a mixture of glycerol and NaH₂PO₄, heated to 75 °C. After that, to gain a coarse emulsion, the water phase was slowly added into the oil phase with high speed shearing mixing at 10,000 r/min for 3 min. Then the volume was adjusted to 100 ml and the pH was adjusted to 5.0 with 0.1 mol/l phosphate solution. Subsequently, the coarse emulsion was passed through a high pressure homogenizer (AH 100D; ATS Engineering, Inc., China) to obtain the final emulsion. At last, TM-2 LMs were sealed in vials under nitrogen gas and sterilized at 121 °C for 8 min.

2.3. Characterization of TM-2 LMs

The particle size distribution (PSD) was measured by Nicomp™ 380 Particle Sizing system (Zeta Potential/Particle Sizer NICOMPTM 380ZLS, Santa Barbara, California, USA), which was also used to determine the zeta-potential of the LMs. A digital pH-meter type PB-10 (Sartorius, Germany) was used to measure the pH of TM-2 LMs.

The drug content was determined by high-performance liquid chromatography (HPLC) method. The HPLC system consisted of a Chromaster-5110 pump, a Chromaster-5210 autosampler, a Chromaster-5310 column oven and a Chromaster-5410 UV detector (Hitachi Company, Japan). The mobile phase consisted of acetonitrile-water (60:40, v/v) with a flow rate of 1.2 ml/min, and the wavelength of the UV and the column temperature were 227 nm and 35 °C, respectively. The samples were prepared as follows: 1 ml TM-2 LMs were transferred into a 25 ml volumetric flask and diluted with water. The EE was calculated as follows:

\[ \text{EE} (\%) = \frac{(C_{\text{total}} - C_{\text{water}}) \times 0.9}{C_{\text{total}}} \times 100\% \]

In the equation, \( C_{\text{total}} \) refers to the content of drug in TM-2 LMs, \( C_{\text{water}} \) refers to the content of TM-2 in aqueous phase.

2.4. Stability assessment

2.4.1. Autoclaving

TM-2 LMs were prepared according to Section 2.2. After being sealed in vials, TM-2 LMs samples were autoclaved at 121 °C for 8, 10, and 15 min, respectively. Finally, the drug content, pH and particle size were determined to evaluate the stability of TM-2 LMs according to Section 2.3.

2.4.2. Freezing and thawing test

TM-2 LMs were prepared according to Section 2.2. Then the samples were stored at −20 °C for 12 h following thawing at room temperature for 12 h. After that, the physical appearance and PSD were determined according to Section 2.3. The freezing and thawing test would last for three cycles.

2.4.3. Stability against dilution

Four groups of TM-2 LMs samples were prepared according to Section 2.2 to study the dilution stability. The first two groups samples were diluted fivefold with 5% glucose, and then stored at room temperature and 4 °C, respectively. The other two groups samples were diluted with 0.9% saline following the steps above. The particle size was determined at intervals of 0, 1, 2, 4, 6, 8, 10, 12, and 24 h according to Section 2.3.

2.4.4. Acceleration stability test

Acceleration stability test consisted of two parts: stress test and storage test. The stress test was carried out on the condition of 60 °C or 4500 lx. TM-2 LMs prepared as in Section 2.2 were divided into two parts and stored at 60 °C or 4500 lx for 10 d, respectively. The samples were selected every 5 d to evaluate PSD, ζ-potential, pH value, EE and drug content. One new batch of TM-2 LMs was prepared to investigate the storage stability at 25 °C for 3 months. Parameters similar to the stress test were monitored at the time intervals of 0, 1, 2 and 3 months.

2.5. Evaluation of TM-2 LMs in vivo

A pharmacokinetic study was carried out to evaluate the characteristic of TM-2 LMs (1 mg/ml) in vivo as compared with TM-2 aqueous injection (1 mg/ml). TM-2 LMs were prepared as in Section 2.2. As for TM-2 solution, 60 mg TM-2 was formulated in polyisorbate 80 (1.56 g) then diluted with 13% ethanol (w/w), and 5% dextrose solution was added to get the final solution.

The animals were randomly divided into two groups with six animals in each group. Then, TM-2 LMs were administrated to the first group via the caudal vein at a dose of 8 mg/kg, and TM-2 solution was given to the latter group via the caudal vein at the same dose level. After dosing, blood samples were collected into heparinized tubes at 0.083, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, and 24 h, then centrifuged immediately at 4000 r/min for 10 min. The harvested plasma samples were stored at −20 °C until analysis.

The method of sample preparation was reported previously [16]. In short, 100 µl plasma samples were spiked with 10 µl IS (CBZ, 1000 ng/ml methanol solution) working solution. And then the mixture was extracted with 2 ml methyl tert-butyl ether by vortexing for 10 min. 1.8 ml supernatant was collected after centrifugation at 13,000 r/min for 10 min and evaporated to dryness at 35 °C under a stream of nitrogen. The residue was redissolved in 100 µl of the mixture of acetonitrile/water (60/40, v/v) followed by centrifugation for 10 min at 13,000 r/min. Finally, 5 µl acquired supernatant was injected. The content of TM-2 in plasma was determined on an ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA) and a Waters Xevo TQ triple quadrupole mass spectrometer (Waters Corp., MA, USA) equipped with an electrospray ionization source. The analysis time was 3 min. The mobile phase was
composed of acetonitrile (A) and water (B, containing 5 mM ammonium acetate). During the first 1 min, solution A increased from 60 to 95% and held for 1.3 min. Then solution A decreased to the initial rate and kept there until 3.0 min. The capillary voltage was 3.0 kV; the cone voltage was 35 V for TM-2 and 33 V for CBZ. The collision energy was 25 eV and 22 eV for TM-2 and CBZ, respectively.

The pharmacokinetic parameters of TM-2 were analyzed by DAS 2.1 software supplied by the Pharmacological Society of China (Beijing, China). SPSS 19.0 (Statistical Package for the Social Science) was used to analyze and compare the pharmacokinetic parameters between TM-2 LMs and TM-2 solution by an independent samples t-test.

3. Results and discussion

3.1. Preparation of TM-2 LMs

Lipid microspheres have been particularly attractive as a part of parenteral nutrition therapy for decades. However, there are some adverse effects associated with LCT limiting its application, including the increasing of pulmonary artery pressure, suppressing gastrointestinal function, and so on [17,18]. MCT is rapidly cleared, carnitine-independent transport and eventually transfer to ketone bodies [17,19]. Neuro-toxicity and Candida albicans connected with MCT could not be ignored [17,20]. When an emulsion containing a mixture of LCT and MCT was infused, there was no effect on reticuloendothelial syndrome and acute pancreatitis [21]. Thus the emulsion of mixed oil could reduce the risk associated with pure MCT or LCT based lipid emulsions. As time goes by, drug has the tendency to separate out of emulsion. So an adequate solubility for drug in oil is necessary. Our previous study showed that TM-2 had a much higher solubility in MCT (>100 mg/ml) than in LCT (25.36 mg/ml) [6]. Thus, a large amount of MCT may prevent drug from precipitating. Taken the safety and stability into consideration, a mixture of MCT and LCT (7.5:2.5, w/w) was chosen as the oil phase.

Considering that TM-2 was preferred to an acid environment [6], 2 mM/l NaHCO3 was chosen. Four representative formulations with different phospholipids were prepared to develop optimum TM-2 LMs. The results that were listed in Table 1 indicated that the particle size of formulation with different phospholipid changed markedly during the manufacture of TM-2 LMs except PL-100M. To investigate this phenomenon, the compound of phospholipid was changed. The major composition of different phospholipid was shown in Table 2. Compared with E80 and S100, the content of PE and cholesterol in PL-100M was much higher. Considering that cholesterol could regulate the phase state of the membrane [22], it was suspected that cholesterol led to the stable state. So the particle size of TM-2 LMs containing E80 was compared with that of TM-2 LMs containing E80 and cholesterol. The results were listed in Fig. 2. It could be concluded that regardless of the presence of cholesterol, the particle size changed markedly after sterilization. Thus, cholesterol was not the decisive factor. The head groups of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were different, the latter was smaller [23]. Phospholipids were arranged on the surface of lipid microsphere droplets, the larger head group of PC made it need more space than chains, while the space for the head group of PE was the same with chains. So the existence of PE would make the phospholipid layer close and in order, and heating would bring out less change to it. This could be proved by comparing the formulation of S100 with E80. There was almost no PE in S100, and the coarse emulsion was creaming after 2 min, while E80 was stable. So PE may be the key factor leading to the stability of TM-2 LMs. After 2 months, there were visible oil droplets when the amount of PL-100M was 1.2%. Hence, 1.4% PL-100M was chosen to prepare TM-2 LMs.

The stable pH for TM-2 is 5.5–6.0, while the pH of LMs would decrease during storage due to the hydrolysis of phospholipid and the release of free fatty acid. So the final pH of TM-2 LMs, which is about 6.0, is better. The effect of phospholipid on pH was different during the preparation of LMs. The pH of LMs containing PL-100M increased. The difference of pH may be connected with the characteristic of PL-100M. PL-100M is made from a suspension whose pH is 6.5–9.5 containing two different kinds of phospholipid, which is different from E80 or S100 [24]. So the pH of final emulsion would rise up to 6.0 from 5.0.

3.2. Stability assessment

3.2.1. Autoclaving

It is known to us that shorter sterilization with higher temperature is better for the stability of drug than longer sterilization with lower temperature. Thus, 121 °C steam sterilization was employed. The results were presented in Table 3. LMs are thermodynamically and dynamically unstable, so harsh autoclaving condition would bring out adverse effects on the stability of TM-2 LMs. The content and pH of TM-2 LMs

| Table 1 – The effect of phospholipid on particle size of TM-2 LMs before/after sterilization (mean ± SD; n = 3). |
|-----------------|-----------------|-----------------|-----------------|
| Phospholipid    | Particle size (nm) | Before sterilization | After sterilization |
| E80 1.2%        | 248.4 ± 75.3    | 498.0 ± 231.4    |
| S100 1.2%       | ND              | ND              |
| PL-100M 1.2%    | 244.6 ± 50.9    | 246.2 ± 76.3    |
| PL-100M 1.4%    | 235.1 ± 66.8    | 239.8 ± 26.1    |

<table>
<thead>
<tr>
<th>Table 2 – The content of PC, PE and Cholesterol in different commercial natural-extracted phospholipid.</th>
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<td>Phospholipid</td>
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<tr>
<td>PL-100M</td>
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<tr>
<td>E80</td>
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<tr>
<td>S100</td>
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</table>

The data of PC and PE contents are quoted from inspection report of bought commercial lecithin.
decreased as time went by. The decrease of pH may connect with the hydrolysis of phospholipid and the oil phase. The degradation of phospholipid followed Arrhenius kinetics [25], high temperature sterilization led to the release of free fatty acid which finally resulted in the decrease of the pH of TM-2 LMs. The hydrolysis of phospholipid may cause the leakage of drug which was susceptible to hydrolysis in water, so the drug content decreased.

3.2.2. Freezing and thawing test

The freeze-thawing test is a method to evaluate the stability of LMs. As shown in Fig. 3, the particle size was slightly larger after 3 cycles. This was different from previous study that freezing resulted in a much larger droplet size due to the condensation of lipid droplets [26]. This may be partially due to the existence of LCT. Oil could penetrate into the surface membrane, which would change the mean spontaneous curvature of membrane that plays an important role in the stability of emulsion [26]. With the existence of LCT, less MCT would penetrate into the surface of emulsion, then the spontaneous curvature of membrane would have a less decrease. In addition, it was reported that during the freeze-thawing cycles, salt combined with glycerin may suppress ice crystal formation and maintain the density of droplets similar to that in solution [27]; as a result, the droplet coalescence would be suppressed.

3.2.3. Stability against dilution

Lipid microspheres are occasionally demanded to be diluted, frequently with saline (0.9%) or glucose (5%) solution. The result of particle size that was shown in Fig. 4A and B revealed that 0.9% NaCl resulted in a larger particle size at room temperature, while the other three groups almost remained unchanged. The similar result has been shown in a previous study [28], and it revealed that this phenomenon was related to the reduced electrostatic repulsion. The result illustrated that once diluted, the lipid microspheres should be injected as soon as possible, although the change was within permitted limit.

3.2.4. Acceleration stability test

The influence of heat and light on the degradation of TM-2 LMs was investigated under the circumstance of 60 °C and 4500 lx, respectively, and the result was given in Table 4. The pH dramatically decreased, especially at 60 °C, while other parameters almost kept unchanged. This may be because the elevated temperature accelerated the degradation of phospholipid, and thus resulted in the formation of free fatty acids. The result indicated that TM-2 LMs were relatively stable. However, to

<table>
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<tr>
<th>Time (h)</th>
<th>Before sterilization</th>
<th>After sterilization</th>
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<th>After sterilization</th>
<th>Before sterilization</th>
<th>After sterilization</th>
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<tr>
<td>8</td>
<td>6.07 ± 0.04</td>
<td>6.07 ± 0.05</td>
<td>218.0 ± 61.7</td>
<td>220.0 ± 61.0</td>
<td>98.75 ± 0.17</td>
<td>97.1 ± 0.18</td>
</tr>
<tr>
<td>10</td>
<td>6.07 ± 0.06</td>
<td>225.9 ± 65.7</td>
<td>96.26 ± 0.16</td>
<td>92.37 ± 0.11</td>
<td></td>
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</tr>
<tr>
<td>15</td>
<td>6.03 ± 0.58</td>
<td>217.4 ± 68.0</td>
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minimize the degradation of drug, it was suggested that TM-2 LMs should be stored avoiding high temperature and light. The results of TM-2 LMs stored at 25 ± 2 °C were shown in Table 5. During 3 months, the change of TM-2 LMs was in qualified range over the study period, indicating that TM-2 LMs were a stable intravenous administration system.

3.3. Pharmacokinetic study

A pharmacokinetic study was designed to compare the difference between TM-2 LMs and TM-2 solution. The curves of the mean plasma concentration-time were shown in Fig. 5, and the corresponding pharmacokinetic parameters were listed in Table 6. From Fig. 5, the concentration of TM-2 in plasma decreased rapidly regardless of TM-2 LMs or TM-2 solution. The AUC of TM-2 LMs was 3663.71 μg/l h with 1712.52 μg/l h for TM-2 solution. The clearance for TM-2 LMs and TM-2 solution was 2.26 l/h/kg and 4.77 l/h/kg, respectively. However, the half-lives (t½) of the two carriers were similar. All in all, the TM-2 LMs group had higher AUC and AUC, smaller clearance (CL) and lower apparent volume of distribution (P < 0.05). The analytical data suggested that TM-2 in both drug carriers followed a triphasic model which was consistent with previous findings [16].

Compared with the TM-2 solution group, the TM-2 LMs group had a much higher AUC, which stated in previous study that
when lipophilic drugs were given in lipid microspheres, it demonstrated higher plasma concentrations following i.v. administration than in solution [29]. What made the difference between them was probably the drug carrier. TM-2 solution was prepared by dissolving drug in polysorbate 80 which was quickly eliminated after i.v. administration [3], thus it may result in a precipitation of TM-2 with different particle size. Particle size of droplets had an effect on drug distribution. The smaller one (0.2–0.7 μm) may be trapped into liver, spleen, and lung, while the larger one (>7 μm) would be taken in the capillary bed of the lung [30]. So TM-2 solution would distribute wider in vivo than TM-2 LMs and then contribute to a decrease in AUC.

Table 6 – The main pharmacokinetic parameters in rats of TM-2 after intravenous administration of TM-2 LMs and TM-2 solution at a dose of 8 mg/kg (mean ± SD; n = 6).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TM-2 LMs</th>
<th>TM-2 solution</th>
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<tbody>
<tr>
<td>AUC_{0-t} (μg/l h)</td>
<td>3405.89 ± 611.13</td>
<td>1433.32 ± 174.69</td>
</tr>
<tr>
<td>AUC_{0-∞} (μg/l h)</td>
<td>3663.71 ± 762.62</td>
<td>1712.52 ± 217.20</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>13.76 ± 7.58</td>
<td>14.22 ± 7.28</td>
</tr>
<tr>
<td>CL (l/h/kg)</td>
<td>2.26 ± 0.44</td>
<td>4.77 ± 0.76</td>
</tr>
<tr>
<td>V (l/kg)</td>
<td>42.58 ± 18.86</td>
<td>93.67 ± 39.28</td>
</tr>
<tr>
<td>C_{max} (μg/l)</td>
<td>6450.00 ± 1948.51</td>
<td>1563.62 ± 207.02</td>
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**Fig. 4** – (A) The influence of saline (0.9%) on the particle size of TM-2 LMs at the temperature of 4 °C and room temperature after diluted fivefold (mean ± SD; n = 3). (B) The influence of glucose (5%) on the particle size of TM-2 LMs at the temperature of 4 °C and room temperature after diluted fivefold (mean ± SD; n = 3).
In addition, the release of TM-2 seemed to be a solubility-limited process. TM-2 was lipophilic and located in the internal phase of LMs which was stable in a certain period of time. So drug was released slowly from LMs to the blood. And when the release of drug reached a saturated state, the release was stopped, following a dynamic balance. As a result, TM-2 loaded in LMs penetrated into the tissue slowly than in solution and contributed to a higher AUC.

The larger clearance of TM-2 solution may be associated with polysorbate 80. The quick elimination of polysorbate 80 may lead to the increase of unbound drug (not bound to polysorbate 80 and to plasma protein) which caused a faster clearance in patient [31]. Similar to Taxotere®, TM-2 solution was made by adding polysorbate 80. So it was suspected that polysorbate 80 would increase the clearance of TM-2, and the similar result was got in the comparison of Larotaxel solution and lipid microsphere [30].

4. Conclusions

A TM-2 LM was successfully prepared, then the final formula was composed of TM-2 0.10%, oil phase 10.00% (MCT:LCT = 7.5:2.5), PL-100M 1.40%, NaH₂PO₄ 0.024%, glycerol 2.25%, and water to a total volume of 100 ml. PE may contribute to the stability of TM-2 LMs. The stability of TM-2 LMs was investigated thoroughly. The optimum sterilization condition for TM-2 LMs was 121 °C for 8 min. Freezing and thawing test indicated that TM-2 LMs could undergo dramatic temperature fluctuations. If dilution is necessary, glucose (5%) seemed to be a better choice than saline. Stress test for 10 d suggested that high temperature and light should be avoided during the storage of TM-2 LMs. And storage test indicated that TM-2 LMs were stable for at least 3 months. The pharmacokinetic study revealed that TM-2 LMs could increase the AUC and decrease the clearance and apparent volume of distribution. So LMs could be a suitable drug delivery for TM-2.

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