Development and Validation of a Method for Determination of Encapsulation Efficiency of CPT-11/DSPE-mPEG2000 Nanoparticles

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Abstract

Herein we report development and validation of the method for evaluating the encapsulation efficiency of a micelle-based nanosystem composed of irinotecan hydrochloride (CPT-11) and an amphiphilic molecule DSPE-mPEG2000. The results showed that the centrifugation method can be used for separation of free drug, a critical step in measuring encapsulation efficiency, and the EE of three batches of CPT-11/DSPE-mPEG2000 micelles was 90.0% ± 1.0%. The results also indicated that the conditions used in the process have to be optimized to acquire reliable data.

Keywords: Irinotecan hydrochloride; Micelle; Encapsulation efficiency; DSPE-mPEG2000

Introduction

Nano-sized systems especially nanoparticles (NPs) have been extensively explored for encapsulating anti-cancer drugs of low solubility and facilitating better delivery of drug molecules to tumors through the enhanced permeability and retention (EPR) effect. The introduction of NPs can thus substantially reduce undesired side effects of drug formulations through removal of excipients associated with the side effects and enhance therapeutic efficacy by altering the biodistribution profiles and pharmacokinetics of the free drug [1]. The encapsulation of drug molecules within the “Trojan horse” nanosystems is the key in harnessing the power of nanotechnology for development of better anti-cancer therapeutics [2]. Because of this, the determination of the encapsulation efficiency (EE) which is defined as the percentage of drug molecules successfully entrapped within the NPs is regarded as a key step in characterizing the quality of the nanoformulations [3,4].

Among the various types of NPs currently being investigated, micelle is a relatively simple system formed by the spontaneous self-assembly of block copolymers in aqueous solution [5,6]. The micelle is usually comprised of a hydrophobic core and surrounding hydrophilic corona. The drug molecules can be entrapped within the core of the micelle system through various interactions, mainly hydrophobic-hydrophobic, and be protected from premature release. For example, Genexol-PM, a micelle form of the paclitaxel composed of polyethyleneglycol (PEG)-polylactide, has shown both increased efficacy and less toxicity and has now been approved for marketing in several countries and currently in late stage clinical trials in the USA. In addition, there are ongoing clinical studies for other micelle-based anti-cancer drug formulations such as NK911, NK012, NK105 (all three are developed by Nippon Kayaku Co. Ltd.), SP1049C (Supratech Pharma Inc.), and BIND-014 (Bind Therapeutics) [7].

Micelles are formed when the concentration of block copolymers goes above the critical micelle concentration (CMC) [8]. This process is usually driven by the hydrophobic-hydrophobic interaction. Below the CMC, the block copolymers will exist in a freely dispersed state. Above the CMC, the assembled structures and the free molecules are in a state of dynamic equilibrium and the system is thermodynamically stable. Therefore, a critical step in the analysis of EE is to separate unentrapped, free drug molecules from the drug incorporated into the micelle.

This paper reports the determination of EE for a micelle system composed of irinotecan hydrochloride (CPT-11) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-mPEG2000). The solution form of CPT-11 is approved to be used alone or with other drugs to treat metastasized colorectal cancer and a nanoposposomal form of irinotecan (developed by Merrimack Pharmaceuticals) has just got approved by US FDA for treatment of metastatic pancreatic cancer [7]. The DSPE-mPEG2000 is an amphiphilic molecule that is regarded as a GRAS (generally regarded as safe) material by FDA and has been widely used in pharmaceutical research [9]. In this paper, we examined three strategies including dialysis method, centrifugation method and a gel chromatography method for separation of unentrapped drug molecule from the micellar system [3,10,11]. The amount of the drug was characterized using high performance liquid chromatography (HPLC) coupled with UV-Vis detector or UV spectrometry. Herein we want to highlight the importance of method validation during the analysis of EE. The results indicated that among the three methods investigated, the centrifugation method is the most appropriate one for separation of free drug for this micelle system and the EE of three batches of CPT-11/DSPE-PeG2000 micelles was determined to be 90.0% ± 1.0%.

Methods

Synthesis of CPT-11/DSPE-mPEG2000 micelle

Irinotecan hydrochloride-encapsulated DSPE-mPEG2000 micelles were prepared by a green method. Briefly, 166.7 mg of DSPE-mPEG2000 was dissolved in 1 mL of lactose aqueous solution (5 wt.%) under stirring to form a clear and colorless system. Then 40 mg of irinotecan hydrochloride was added to a 10 mL glass vial. The solution was dialyzed at 4°C against deionized water for 24 hours.

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Lyophilized CPT-11/DSPE-mPEG2000 powder in medium and has been widely used to separate molecules based on their molecular weight. In the elution process, smaller molecules can enter the pores and have to travel longer distances when move through the column. On the other hand, bigger structures cannot diffuse freely and therefore elute earlier. Gel filtration chromatography has been used to remove unencapsulated molecules from nanoparticles especially liposomes [12].

Free CPT-11 solution was loaded on the column and the recovery rate was determined. HPLC was used to measure the amount of CPT-11 in the fractions collected. The results indicated that by using 5% glucose as eluting solution only 10% of drug was recovered from the gel column and a sample recovery of 98.4% was achieved in the case of PBS. We further evaluated PBS as the eluting buffer. We built a simple gel column and a sample recovery of 98.4% was achieved in the case of glucose as eluting solution only 10% of drug was recovered from the gel column and a sample recovery of 98.4% was achieved in the case of PBS.

Results and Discussion

Preparation and characterization of CPT-11/DSPE-mPEG2000 nanoparticle

The CPT-11/DSPE-mPEG2000 micelles were prepared using a direct solution processing strategy. This “green” synthesis method does not involve any organic solvent and enables the scale-up of nanoparticle production to levels practical for clinical applications. The particles showed a monodispersed size of 15.1 ± 0.8 nm based on DLS. Due to the anionic nature of polyethylene glycol, the particles are negatively charged (ζ potential=-4.6 ± 1.3 mV). The TEM images were shown in Figure 1.

The gel filtration chromatography method

The gel filtration chromatography, also referred to as size exclusion chromatography, employs porous gel particles suspended in aqueous medium and has been widely used to separate molecules based on their molecular weight. In the elution process, smaller molecules can enter the pores and have to travel longer distances when move through the column. On the other hand, bigger structures cannot diffuse freely and therefore elute earlier. Gel filtration chromatography has been used to remove unencapsulated molecules from nanoparticles especially liposomes [12].

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The results (Table 2) showed that with more concentrated drug solution, the micelles based on their different ability to cross through the dialysis bag and failed to escape. Therefore, the dialysis method does not fit this nanoparticle formulation.

**The centrifugation method**

Centrifugation method was used to separate free molecules from the micelles based on their different ability to cross through the membrane with different sizes of pores upon centrifugation [13]. We first evaluated the recovery of free CPT-11 solution in the procedure. The results (Table 1) showed that with more concentrated drug solution, higher recovery rates can be achieved. This might be attributed to the fact that the membrane on the centrifugation filter can only hold a certain amount of drug and when the original concentration is high enough, this absorbed part became negligible. The data also indicated that the MWCO of the membrane does not significantly affect the recovery rate. For further study, we chose the 30000 MWCO and 0.3 to 1 mg/mL of CPT-11 concentration. We then evaluated the influence of centrifugation time on recovery. The results (Table 2) showed that centrifugation time has minimal effect on the recovery and 20 min was chosen so that relatively larger amount of sample can be obtained for analysis. To further confirm that free drug in the presence of micelles can be separated using this strategy, we briefly mixed empty micelles with free drug solution and then performed the above procedure to recover the free drug and the results showed that up to 99% of free drug could be recovered.

We then used the aforementioned method for analysis of EE of the CPT-11/DSPE-mPEG2000 micelles. The micelle dry powder was suspended in three different media including 0.9% NaCl solution, deionized water (DI water) and 5% glucose at a concentration of 8 mg/mL of CPT-11. The results (Table 3) showed that the EE in 0.9% of NaCl solution is 76.57% while the EE in DI water and 5% glucose are 95.22% and 93.32%, respectively. We hypothesized that the encapsulation of CPT-11 within the DSPE-mPEG2000 core might be partially attributed to the electrostatic interaction between the two components and the stability of the micellar structure might be disrupted to some extent due to the ionic strength in 0.9% NaCl solution. Based on this result, 5% glucose was chosen as the dispersion medium for further study.

We then tested the EE of the CPT-11/DSPE-mPEG2000 micelles at different concentrations of CPT-11. The results (Table 4) showed that the EE remained unchanged until the CPT-11 concentration fell below 0.18 mg/mL. However, previous results showed that at low CPT-11 concentration, below 0.3 mg/mL, the absorption of the drug on the membrane of centrifugation filter became substantial compared to the free drug solution and therefore cannot be neglected. Because of this, we chose the 2.8 mg/mL of CPT-11 as the testing condition due to the fact that the free drug concentration in this solution is around 0.3 mg/mL and the result is therefore most accurate.

We then used this method to test three batches of CPT-11/DSPE-mPEG2000 micelles and results showed an EE of 90.0% ± 0.1%. The solution was incubated at 4°C in the darkness and the EE did not show EE of the CPT-11/DSPE-mPEG2000 micelles in different medium.

| Conc. of CPT-11 (mg/mL) | Medium   | EE      |
|-------------------------|----------|---------|
| 0.9% NaCl solution      | 76.6%    |
| DI water                | 95.2%    |
| 5% glucose              | 93.3%    |

**Table 3:** EE of the CPT-11/DSPE-mPEG2000 micelles in different medium.

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**Figure 2:** The real-time absorption measured using the gel filtration chromatography as separation method for free CPT-11.
any change, indicating that the micelle system is relatively stable when suspended in the medium (Table 5). We also incubated the dry powder of CPT-11/DSPE-mPEG2000 micelles at 60°C and the results also showed that EE remained the same over 6 months.

**Conclusion**

We examined three methods for evaluating the EE of a CPT-11/DSPE-mPEG2000 micellar system. Through validation of methods and conditions, an ultracentrifugation method was established to measure the EE of the CPT-11/DSPE-mPEG2000 micelles. Our results showed that an appropriate condition has to be used to achieve more accurate and solid data when evaluating encapsulation efficiency of nanoparticle-based drug formulations.

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