

Preparation of a Dispersible PEGylate Nanostructured Lipid Carriers (NLC) Loaded with 10-Hydroxycamptothecin by Spray-Drying

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Nanostructured lipid carriers (NLC) are based on mixture of solid lipids with spatially incompatible liquid lipids, which offer advantages of improving drug loading capacity and release properties. In the present study, hydroxycamptothecin (HCPT) loaded polyethylene glycol (PEG) modified NLC (PEG-NLC) was prepared by high pressure homogenize and spray drying method. PEG-NLC showed spherical particle with smooth surface in scanning electron microscopic (SEM) analysis. The crystallinity of lipid matrix within PEG-NLC was evaluated by powder X-ray diffraction and differential scanning calorimetry (DSC). The less ordered crystals or amorphous state of matrix were found in nanoparticles. A small, homogeneous particle size and high drug loading with fine entrapment efficiency of HCPT was obtained in PEG-NLC system. HCPT releasing from PEG-NLC showed a sustained release trend, and no significantly difference was found between two release curves of PEG-NLC before or after spray drying. After storage for 6 months, PEG-NLC powder after spray drying showed no significantly changes in particle size, drug loading and entrapment efficiency, crystal form and *in vitro* release. PEG modification statistically decreased the phagocytosis of NLC by RAW 264.7 cells, and spray drying process did not influence the cellular uptake of PEG-NLC. These results suggest that PEG-NLC prepared by spray drying is a stable and high-performance delivery system for HCPT.

Key words nanostructured lipid carrier; 10-hydroxycamptothecin; spray drying; polyethylene glycol; cellular uptake

10-Hydroxycamptothecin (HCPT) is one of the derivatives of camptothecin. It has strong antitumor activity against a wide range of tumors with a mechanism of targeting the nuclear enzyme topoisomerase I.¹⁾ However, due to the poor solubility in water and physiologically acceptable organic solvents, the development of HCPT dosage form is limited, and most available nanoparticle systems suffer from a low drug loading content. Moreover, the active lactone form of HCPT exists in a pH dependent equilibrium with less active open carboxylate form (Fig. 1).^{2,3)} In addition, the antitumor activity of CPTs is time-dependent. Their prolonged supply is very useful to obtain a higher efficacy.⁴⁾ The short half-life (about 30 min) of HCPT makes the administration frequently and intensifies the side effect of drug. Therefore, developing a suitable drug delivery system is meaningful to obtain a higher antitumor efficacy and decrease the side effect of HCPT.

The nanostructured lipid carriers (NLC) are presented as an improved generation of lipid nanoparticles,⁵⁾ which is developed from solid lipid nanoparticles (SLN) system. It consists of solid lipid matrices with spatially incompatible liquid lipids, results in a structure with more imperfections in crystal to accommodate the drug, and thus gets a higher drug loading capacity. NLC system combines many advantages of SLN, e.g. controlled drug release, biocompatibility and the possibility of production on large industrial scale. However,

it minimizes or avoids some potential problems associated with SLN, such as drug leakage during storage and limitation in drug loading capacity. Therefore, NLC is a promising carrier to increase the drug loading efficiency and prolong the half-life of HCPT. Additionally, nanoparticles with a polyethylene glycol (PEG) shell are reported to be able to prevent uptake by reticuloendothelial system (RES) and prolong the circulation time of drug.⁶⁾ It can prolong exposure of tumor cells to antitumor drug, enhance permeability and retention (EPR) effect,⁷⁾ and subsequently increase the therapeutic effect of antitumor drug.

The industrial development of nanoparticle systems is limited due to the problem of maintaining stability of suspensions for a prolonged time period.⁸⁾ The colloidal suspension, in general, does not tend to disperse, after several months of storage, aggregation can occur. Additionally, microbiological growth, drug leakage and/or other component degradation in aqueous environment is possible.^{9–11)} In order to improve the stability of SLN or NLC system, the freeze-drying process represents a good approach to increase chemical and physical stability of these systems over extended time periods.¹²⁾ However, nanoparticles are not easily lyophilized, they tend to collapse releasing the oil core. And freeze drying is usually a time consuming process.¹³⁾

The less cost-intensive spray drying technique is applied for SLN as an alternative method to lyophilization. It has been widely used in the chemical, food and pharmaceutical industries.¹⁴⁾ It converts a liquid into a dry system in one-step process and can produce fine, dust-free powders.

In our previous work,¹⁵⁾ PEG modified NLC (PEG-NLC) was developed as carrier for HCPT. The behaviors of PEG-NLC *in vitro* and *vivo* were studied. In the present study, a dry, fine-grained powder was obtained by spray drying of PEG-NLC. The physico-chemical properties, *in vitro* re-

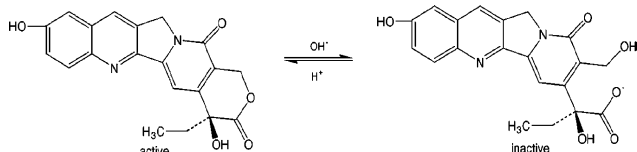


Fig. 1. The Structure of Hydroxycamptothecin and Equilibrium Reaction between the Active Form and Inactive Form

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lease and stability of the product was studied. The effect of PEG modification and spray drying process on the nanoparticle preventing phagocytosis by RES was evaluated on RAW264.7 cells.

Experimental

Materials Hydroxycamptothecin (HCPT, Shanghai Junjie Biotechnology Co., Ltd., China) was used as a model drug. Monostearin [Rikemal AS-105] (RIKEVITA, Japan) was chosen as solid lipid matrices of NLC. Purified Soybean Oil 788 (Lipoid, Germany) was selected as spatially incompatible liquid lipids for NLC. Lecithin [EPC] was obtained from Lipoid (Germany). Polyethylene glycol stearate with a polymerization degree of 40 (PEG molecule weight: 2000) was henceforth designed as PEG-40 stearate (Sigma, China). Plasdone K-29/30 (PVP K30) was a gift from ISP Technology, Inc. (U.S.A.). The pyrene was obtained from Aldrich (China). Other reagents were analytical grade or better.

Cell Culture Murine macrophage cell line RAW264.7, obtained from Academia Sinica Shanghai cell bank, was maintained in Dulbecco's modified eagle medium (DMEM) (Gibco, U.S.A.) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 U/ml streptomycin. Cultures were maintained at 37 °C, 5% CO₂, and 100% humidity.

Preparation of HCPT Loaded PEG-NLC by Spray-Drying PEG modified NLC (PEG-NLC) colloidal solution was prepared by melt-emulsification and homogenization technique. Briefly, 50 mg HCPT was dissolved in 1.5 g lecithin, then 4.0 g monostearin and 1.0 g soybean oil were added, and they were melted at 80 °C to form oil phase. The hot oil phase was dispersed in 94 ml aqueous solution containing 1.8 g PEG-40 stearate of the same temperature and a coarse emulsion was formed using Ultra Turrax® (IKA, Guangzhou, China) at 6500 rpm for 10 min. The hot coarse emulsion was then homogenized at pressure of 800 bar and temperature of about 60 °C with high pressure homogenizer (NS1001L, GEA, Italy) for 8 cycles. Subsequently, the dispersions were allowed to recrystallize at room temperature.

The PEG-NLC colloidal solution was blended with PVP-K30 (0.15 g/ml) as the matrix material. The resulting PEG-NLC was spray dried using Mobil Minor-H spray drying instrument (GEA, Italy) under the following conditions: the inlet temperature was 110 °C, the drying air flow rate was 80 kg/h, the atomizing air pressure was 100 kpa, and the outlet temperature was 40–50 °C.

Confirmation of PEG-NLC Morphology A scanning electron microscope (SEM, PHILIPS XL30, Netherland) was used to observe the morphology of PEG-NLC.

Thermal Analysis Differential scanning calorimetry (DSC) was carried out using 822c instrument (Mettler, U.S.A.). The operating conditions were: sample weight, 10 mg; heating rate, 10 °C/min.

Confirmation of the Crystal Form by X-Ray Diffraction Powder X-ray diffraction analysis was performed with a D/max 2550V diffractometer (Rigaku, Japan) using CuK α radiation, a voltage of 40 kV and a current of 150 mA. The scanning rate was 8°/min over a 2 θ range of 3–50°.

HPLC Analysis of HCPT The concentrations of HCPT lactone form were assayed based on the reversed-phase HPLC methods. HPLC analysis was performed using LC-20A (Shimadzu, Japan) system. The analytical column was ZORBAX SB-C18 5 μ m, 150 $\times$ 4.6 mm (Agilent, U.S.A.). The mobile phase consisted of 50:50 mixtures of aqueous triethylamine–acetate buffer (pH 6.0) and methanol. The flow rate was set at 1.0 ml/min and HCPT was monitored at 266 nm.

Particle Size of PEG-NLC Particle size and size distribution of PEG-NLC was determined by Nicomp 380 ZLS (Santa Barbara, California, U.S.A.). All samples before and after spray drying were dissolved by bi-distilled water to an adequate scattering intensity prior to the measurement.

Drug Encapsulation Efficiency and Drug Loading Content Determination Free HCPT (non-incorporated in the PEG-NLC) was separated by ultrafiltration centrifugation technique (Microcon YM-10, 10000MW, Millipore). 500 μ l HCPT loaded PEG-NLC was added to the Microcon YM-10 and centrifuged for 10 min at 4000 rpm. Free and total HCPT concentration of PEG-NLC was measured using HPLC. To determine the drug loading content, the PEG-NLC colloidal solution was withdrawn by ultracentrifugation and vacuum-dried at 40 °C. The residue was accurately weighted to obtain the weight of nanoparticles. The entrapment efficiency and drug loading content could be calculated by the Eqs. 1 and 2, respectively:

$$\text{entrapment efficiency (\%)} = \frac{W_T - W_F}{W_T} \times 100\% \quad (1)$$

$$\text{drug loading content (\%)} = \frac{W_T - W_F}{W_0} \times 100\% \quad (2)$$

where W_T and W_F are the weight of total drug in PEG-NLC and free drug in the ultrafiltrate after centrifugation. W_0 is the weight of nanoparticles.

In Vitro Release Study HCPT release from PEG-NLC was performed in phosphate buffer solution (PBS) (pH 7.4) using the dialysis bag method. 6 ml PEG-NLC (before spray drying and reconstituted) colloidal solution (0.5 mg/ml) was placed into the bag with a cut-off 12000–14000 Da. The bags were then immersed into 500 ml PBS (pH 7.4) at 37 °C, and the paddle rotation speed was set to 50 rpm. At selected time intervals 8 ml medium was taken out for analysis and the same amount of fresh medium was replenished. The sample was acidified with glacial acetic acid (50 μ l) and analyzed by HPLC. All the operations were carried out in triplicate.

Stability Study of PEG-NLC PEG-NLC samples (after spray drying) were kept in siliconized glass vial and placed in stability chamber (Binder, Germany) at 25 °C, 60% RH and 40 °C, 75% RH, respectively. PEG-NLC was sampling after 1, 2, 3 and 6 months. The particle size distribution and entrapment efficiency of reconstituted PEG-NLC were determined to evaluate stability of the formulation.

Uptake of PEG-NLC by RAW264.7 Cells RAW264.7 cells were seeded in 24-well plates at a density of 100000 cells per well and cultured in 1 ml DMEM. 50 μ l PEG-NLC labeled with 0.02% pyrene was added in the wells. At predefined time period of incubation at 37 °C, the supernatants were removed. Cells were washed thrice with PBS, viewed and photographed using fluorescence inverted microscope (Olympus IX51). The amount of label associated with the cells was assayed by dissolving cells in DMF and analyzed by fluorospectrophotometer (λ excitation: 375 nm, λ emission: 402 nm). The number of cells involved in the uptake was counted.

Results and Discussion

Morphology of PEG-NLC Scanning electron micrographs of PEG-NLC are shown in Fig. 2. The outer macroscopic morphology of PEG-NLC was spherical particle with

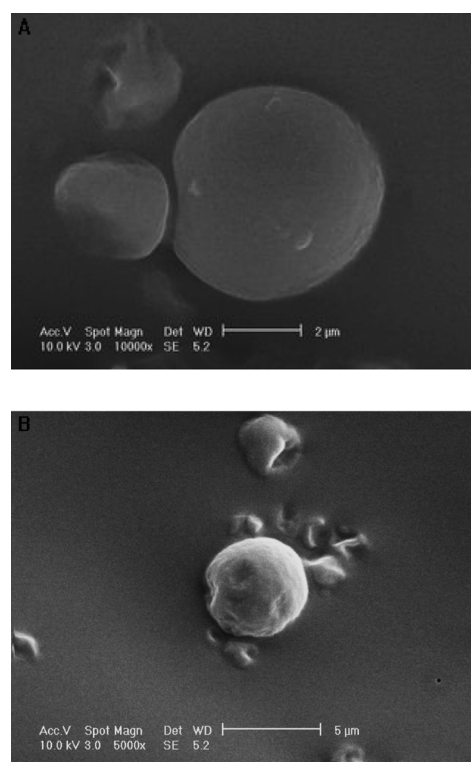


Fig. 2. The SEM Imaging of PEG-NLC after Spray Drying

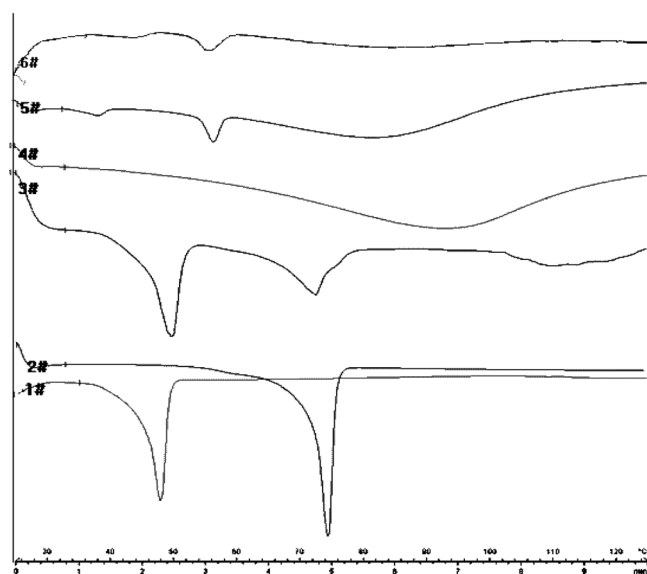


Fig. 3. DSC Scan of 1#-PEG-40 Stearate, 2#-GMS, 3#-Physical Mixture, 4#-PVP K30, and 5#-PEG-NLC Spray Drying Powder, 6#-PEG-NLC Powder Stored for 6 Months Heating from 25 to 125 °C at a Rate of 10 °C/min

smooth surface. The mean diameter of particles determined by laser particle size analyzer was $1.72 \pm 0.8 \mu\text{m}$. The adherence of small part of PEG-NLC was related to the water content of spray drying powder.

Crystal Form of PEG-NLC DSC was a tool to investigate the melting and recrystallization behavior of PEG-NLC. Figure 3 showed an overview of the melting process of PEG-40 stearate, GMS, physical mixture, PVP K30 and PEG-NLC spray drying powder. EPC showed a blunt peak in physical mixture at 102–120 °C, and PVP also had a wide peak at about 90 °C. For PEG-40 stearate and GMS, the melting process took place with maximum peak at 49.5 °C and 73.6 °C. These values were almost same in the physical mixture. However, there was a sharp decline of GMS and PEG-40 NLC melting point in PEG-NLC. The decreasing melting range could be correlated with impurities or less ordered crystals. For the less ordered crystal or amorphous state, the melt of the substance did not require or just required less energy than the perfect crystalline substance which needed to overcome lattice force. As a result, the higher melting enthalpy values should suggest high ordered lattice arrangement and *vice versa*. Therefore, one could conclude that the lipid within PEG-NLC should be in a less ordered arrangement or amorphous compared with GMS corresponding to the DSC analysis.

X-ray diffraction data listed in Fig. 4 were in good agreement with the results established by DSC measurements. The diffraction pattern of GMS showed remarkable difference from PEG-NLC with sharp peak. It revealed that the bulk matrix (GMS) was in good crystal. A typical amorphous state of PVP, which acts as solid carriers for spray drying, could be found from the diffraction pattern. It was clear that in PEG-NLC, the less order crystals or amorphous state were majority for the disappeared sharp peak of pure material. This would contribute to the higher drug load capacity that could be shown in the entrapment efficiency in the following experiment.

Characterizations of NLC The particle size, distribu-

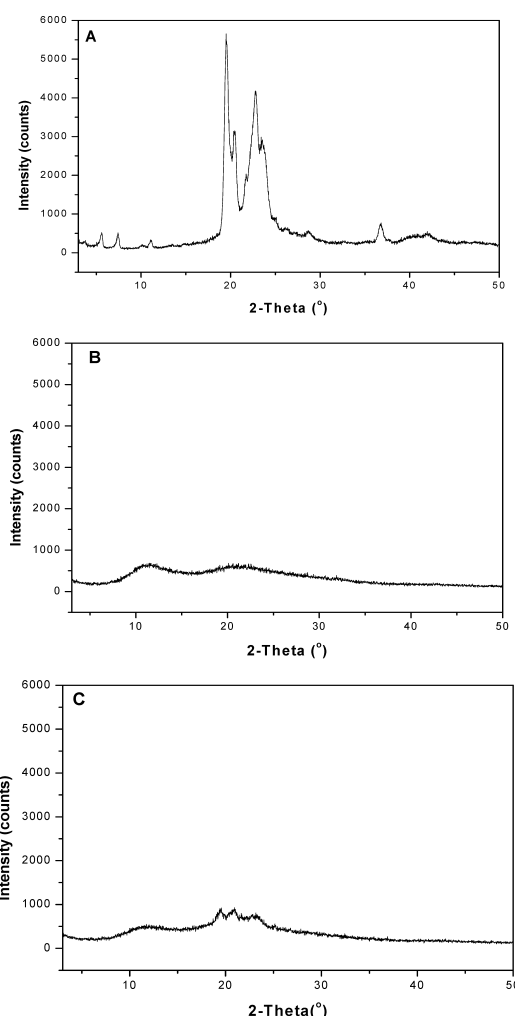


Fig. 4. X-Ray Diffraction Pattern of (A) GMS, (B) PVP K30 and (C) PEG-NLC after Spray Drying

Table 1. Particle Size (PS), Polydispersity Index (PI), Encapsulation Efficiency and Drug Loading Content of HCPT Loaded PEG-NLC before Spray Drying or Reconstitution^{a)}

	PEG-NLC before spray drying	Reconstituted PEG-NLC
PS (nm)	117.3 ± 2.8	216.6 ± 4.1
PI	0.147 ± 0.011	0.208 ± 0.008
Entrapment efficiency (%)	93.42 ± 1.71	92.70 ± 3.02
Drug loading content (%)	1.09 ± 0.11	1.02 ± 0.16

a) All data are expressed as mean $\pm$ S.D. ($n=3$).

tion, encapsulation efficiency and drug loading content of PEG-NLC before spray drying and reconstituted were compared (Table 1). The small and homogeneous particle size of PEG-NLC could still be maintained after spray-drying process, as revealed by slightly increased mean diameter ($216.6 \pm 4.1 \text{ nm}$ versus $117.3 \pm 2.8 \text{ nm}$) and PI (0.208 ± 0.008 versus 0.147 ± 0.011) of reconstituted PEG-NLC. As a consequence, PEG-NLC system got a high drug loading efficiency with fine entrapment efficiency ($>90\%$). The encapsulation efficiency and drug loading content of reconstituted PEG-NLC showed no significantly difference ($p>0.05$) compared with PEG-NLC before spray drying.

A general difficulty when spray drying NLC/SLN aqueous

solutions was the risk of solid lipid in NLC melting during the spray drying process. Kecht-Wyrsh postulates that spray drying of lipid particles is only possible when the boiling point of the spraying medium is below the melting point of the lipid.¹⁶⁾ In contradiction to this finding, spraying drying of monostearin PEG-NLC (mp 74 °C) could be performed successfully with PVP dissolved in water. During spraying, the evaporated moisture forms a skin around the PEG-NLC, which absorbs most of the heat. As temperature increases, the subsequent melting of heat-sensitive lipids is prevented. Generally, nanoparticles reach a maximum temperature during spraying, which is 30–35 °C or more below the outlet temperature of dryer.¹⁴⁾ Monostearin with a melting point of 74 °C will seldom melt during the spraying process. However, the inlet temperature of 110 °C almost reached the melting phase transition temperature of monostearin. At this temperature, solid lipids in PEG-NLC with small particle size are more capable to fuse compared with in larger ones due to their distinction in weight. Thus, a few part of small size nanoparticles fused in the system and led to mean diameter increasing of reconstituted PEG-NLC. Because diameters increased in small nanoparticles and have few changes in larger ones during spray drying, particle size trended to be uniform, and PI value would decrease.

In Vitro Release Study In order to evaluate the controlled release effect of PEG-NLC, HCPT released from PEG-NLC was investigated *in vitro* over 48 h. Release curves were showed in Fig. 5. It indicated that HCPT in PEG-NLC before spray drying and reconstituted PEG-NLC showed a sustained release trend with no burst release at initial stage. The half-life of HCPT in PEG-NLC could be prolonged

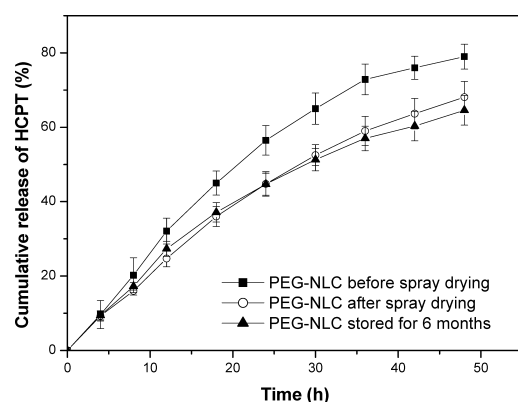


Fig. 5. Release Profile of HCPT in PEG-NLC before Spray Drying (■), PEG-NLC after Spray Drying (○) and PEG-NLC Stored for 6 Months (▲). The results are expressed as mean $\pm$ S.D. ($n=3$).

compared with free HCPT. There is no statistical difference found between these two drug release profiles ($p>0.05$). Reconstituted PEG-NLC trended to release HCPT more slowly compared with PEG-NLC before spray drying. The reason was that HCPT distributed homogeneously in the nanoparticle matrix. Its releasing from PEG-NLC depended on the co-effect of bulk erosion and diffusion. The particle size of reconstituted PEG-NLC (216.6 ± 4.1 nm) was slightly larger than PEG-NLC before spray drying (117.3 ± 2.8 nm) due to the small size nanoparticles fusion in spray process. Larger particle size will lead to slower rate of matrix erosion in PEG-NLC. Moreover, PVP was used as solid carriers in spray drying, this polymer will form a hydration layer outside the PEG-NLC. The hydration layer subsequently contributed to increasing of diffusion layer thickness. Therefore, the release rate of HCPT in PEG-NLC will decrease slightly after spray drying.

Stability Study of PEG-NLC To investigate the stability of PEG-NLC, the experiment was carried out over a period of 6 month. After 6 month of storage at 25 °C, 60% RH and 40 °C, 75% RH, no dramatic increase in the assay, related substance and size of PEG-NLC spray drying powder occurred (Table 2). The entrapment efficiencies of PEG-NLC had fallen by about 2% and 5% at 25 °C and 40 °C, respectively. This reduction could be explained from two aspects: Firstly, a few HCPT was hydrolyzed at 40 °C, 75% RH. Secondly, solid lipids were presented as imperfect crystal and amorphous type in NLC after the preparation. During storage, few more perfect crystalline β -modifications of solid lipids in NLC may be formed and leads to slightly drug expulsion. PEG-NLC spray drying powder after stored for 6 months in the condition of 25 °C, 60% RH, DSC analysis was carried out to determine the crystal form of it. The DSC files showed that there was no significantly change in crystal form of PEG-NLC during storage. The *in vitro* release of HCPT in PEG-NLC after stored for 6 months indicated that the release behavior also had no statistical change ($p>0.05$). The results revealed that PEG-NLC powder after spray drying had satisfactory stability after storage.

Uptake of PEG-NLC by RAW 264.7 Cells The rapid uptake of nanoparticles by the mononuclear phagocyte system (MPS) after intravenous administration is the major barrier preventing their use for delivering drugs to sites other than reticulo-endothelial system (RES), liver and spleen. Polyethylene glycol (PEG) has been used successfully to modify the surface of nanoparticles. The hydrophilic surface formed by PEG chains could prevent the attraction of opsonins, thereby prevent the uptake effect of RES.¹⁷⁾ It dra-

Table 2. Assay, Related Substance (Related sub.), Particle Size (PS), Polydispersity Index (PI), Encapsulation Efficiency (EE) and Drug Loading Content (LC) of HCPT Loaded PEG-NLC Placed for 6 Months^{a)}

Condition	25 °C, 60% RH					40 °C, 75% RH				
	Month	0	1	2	3	6	1	2	3	6
Assay (%)		99.8 $\pm$ 0.4	99.3 $\pm$ 0.7	99.4 $\pm$ 0.6	99.2 $\pm$ 0.3	99.0 $\pm$ 0.5	99.2 $\pm$ 0.3	99.5 $\pm$ 0.8	98.8 $\pm$ 0.7	98.3 $\pm$ 0.2
Related sub. (%)		0.3 $\pm$ 0.0	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.2
PS (nm)		217.2 $\pm$ 3.4	221.2 $\pm$ 1.6	226.7 $\pm$ 2.0	225.8 $\pm$ 4.1	228.0 $\pm$ 3.0	222.2 $\pm$ 4.9	225.1 $\pm$ 2.0	225.2 $\pm$ 4.2	231.1 $\pm$ 1.5
PI		0.204 $\pm$ 0.011	0.201 $\pm$ 0.009	0.205 $\pm$ 0.018	0.204 $\pm$ 0.010	0.209 $\pm$ 0.014	0.204 $\pm$ 0.018	0.214 $\pm$ 0.007	0.211 $\pm$ 0.020	0.217 $\pm$ 0.013
EE (%)		93.04 $\pm$ 2.21	92.72 $\pm$ 3.00	92.85 $\pm$ 1.90	92.03 $\pm$ 4.12	91.53 $\pm$ 2.71	92.30 $\pm$ 5.22	91.17 $\pm$ 3.72	90.42 $\pm$ 3.04	88.66 $\pm$ 4.31
LC (%)		1.12 $\pm$ 0.07	1.10 $\pm$ 0.12	1.03 $\pm$ 0.11	0.98 $\pm$ 0.10	0.92 $\pm$ 0.16	1.09 $\pm$ 0.06	1.00 $\pm$ 0.09	0.99 $\pm$ 0.13	0.90 $\pm$ 0.12

^{a)} All data are expressed as mean $\pm$ S.D. ($n=3$).

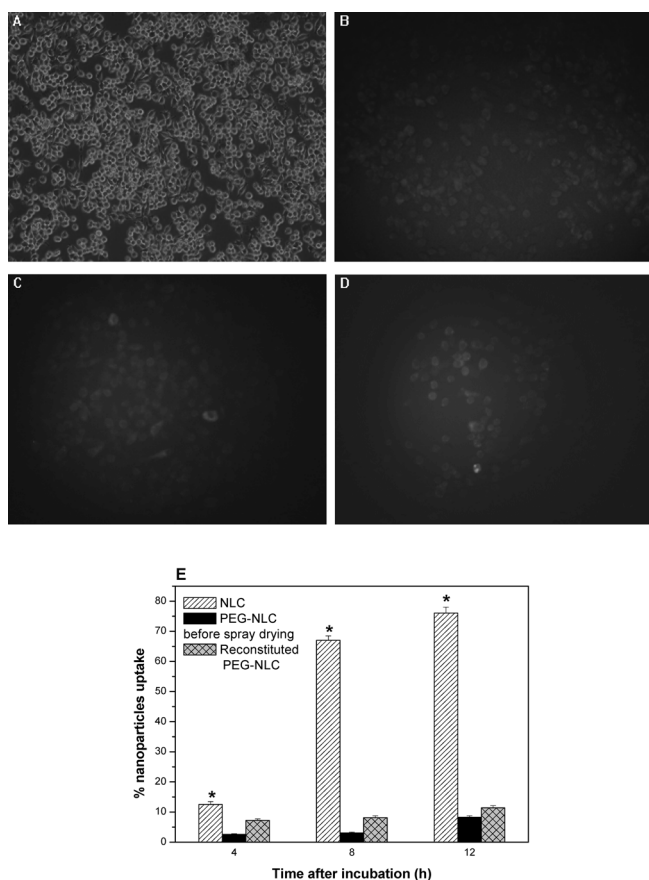


Fig. 6. Microscopy Images of the Uptake of Pyrene Labeled PEG-NLC and NLC by RAW264.7 Cells

(A) RAW 264.7 cell control culture. Fluorescence microscopy images of RAW 264.7 cells which were incubated for 12 h with (B) NLC without PEG modification, (C) PEG-NLC before spray drying, (D) reconstituted PEG-NLC, magnification=400 $\times$. The RAW 264.7 cell uptake efficiencies for different nanoparticles were presented in (E) after incubation (* $p < 0.01$ vs. PEG-NLC and PEG-NLC after spray drying). The results are expressed as mean $\pm$ S.D. ($n = 3$).

matically increases their circulating half-life and altered biodistribution, allowing them to reach tumor tissue.

RAW264.7 is a murine macrophage cell line. The experiment was performed to investigate whether PEG modification of NLC could avoid phagocytosis of macrophage, and whether spray drying process would influence the cellular uptake of PEG-NLC. Particles labeled with fluorescent dyes are frequently used to study cellular uptake. Other researchers had demonstrated that less than 0.6% of the incorporated dye could leach out from the nanoparticles over 48 h under *in vitro* sink conditions.¹⁸⁾ Pyrene is a suitable marker for nanoparticles for studying the cellular uptake behavior.¹⁹⁾

Pyrene was incorporated into PEG-NLC and NLC, the corresponding cellular uptake efficiency was monitored by fluorescence microscopy. Figures 6B, C and D showed the microscopic images of RAW 264.7 cells after 12 h incubation with pyrene labeled NLC, PEG-NLC before spray drying and reconstituted, respectively. It was evident that more NLC were engulfed by RAW 264.7 cells than PEG-NLCs after 12 h incubation. It can be observed from the figure that pyrene labeled NLC and PEG-NLC (blue) have been taken up by the cells, but they cannot enter the nuclei. Figure 6 E showed the cellular uptake efficiency for all the samples dur-

ing 12 h incubation. Obviously, more nanoparticles were taken up by RAW 264.7 cells as increase of hour because of the extended exposure time. The phagocytosis of RAW 264.7 showed no significant difference between PEG-NLC before spray drying and reconstituted ($p > 0.05$). NLC has highest cellular uptake efficiency (76.02% of NLC was uptaken in 12 h), and it was statistically higher than PEG-NLCs ($p < 0.01$).

According to literature, particle cellular uptake could be affected by many factors, such as particle size,²⁰⁾ different cell lines and cell densities, different compositions of the particles, surface properties (surface modification, surface charge).²¹⁾ In this experiment, PEG modification is the key character to determine the cellular uptake efficiency. PEG modification could prevent the attraction of opsonins, and subsequently avoid cellular uptake of nanoparticles. Spray drying process showed minimal effect on RAW 264.7 cellular uptake of PEG-NLC.

Conclusion

It was possible to obtain a dry, fine-grained powder from HCPT loading PEG-NLC applying the spray drying technique. PEG-NLC after spray drying had high drug loading content with fine entrapment efficiency, and maintained small, homogeneous particle size. It presented stable after 6 months storage. The DSC and X-ray diffraction analyses showed that the matrix cores of PEG-NLC are in less order crystals or amorphous state. *In vitro* release indicated PEG-NLC showed a sustained release of HCPT, and no significantly difference was found between PEG-NLC before spray drying and reconstituted. PEG modification statistically decreased the phagocytosis of NLC by RAW 264.7 cells, and spray drying process did not influence the cellular uptake of PEG-NLC.

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