Preparation, Optimization and Characteristic of Huperzine A Loaded Nanostructured Lipid Carriers

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The objective of the present work was to study the preparation, optimization and characteristics of Huperzine A (Hup A), an effective Traditional Chinese Medicine treatment of Alzheimer's disease (AD), loaded nanostructured lipid carriers (NLC). NLC were successfully prepared by a modified method of melt ultrasonication followed by high pressure homogenization using Cetyl Palmitate (CP) as the solid lipid, Miglyol®812 as the liquid lipid, Soybean phosphatidylcholine (Spc) and Solutol HS15® as the emulsifiers. The best formulation was optimized with a three-factor, three-level Box–Behnken design. Independent variables studied were the amount of the mixed lipid, the amount of the emulsifier mixture and lipid/drug ratio in the formulation. The dependent variables were the particle size, entrapment efficiency (EE) and drug loading (DL). Properties of NLC such as the morphology, particle size, zeta potential, EE, DL and drug release behavior were investigated, respectively. As a result, the designed nanoparticles showed nearly spherical particles with a mean particle size of 120 nm and −22.93±0.91 mV. The EE (%) and DL (%) could reach up to 89.18±0.28% and 1.46±0.05%, respectively. Differential scanning calorimetry (DSC) of Hup A loaded NLC indicated no tendency of recrystallisation. *In vitro* release studies showed a burst release at the initial stage and followed by a prolonged release of Hup A from NLC up to 96 h. The results suggest that the presented Hup A loaded NLC system is a potential delivery system for improving drug loading capacity and controlled drug release.

Key words Huperzine A; nanostructured lipid carrier; Alzheimer's disease; melt ultrasonication-high pressure homogenization; Box–Behnken design

Solid lipid nanoparticles (SLN) introduced in 1991 represent an alternative carrier system to traditional colloidal carriers, such as emulsions, liposomes and polymeric micro- and nanoparticles, especially for the delivery of lipophilic compounds.[1] Main advantages of SLN are biocompatibility, drug targeting, modified release, lack of organic solvent during the production process and possibility of production on large industrial scale.[2—4] However, it has some limitations, such as limited drug loading capacity and drug expulsion during storage.

To overcome the disadvantages of SLN, nanostructured lipid carriers (NLC) have been developed in recent years.[5] NLC composed of solid lipid matrix with a certain percentage of liquid lipid are a new and improved generation of lipid nanoparticles.[6] Compared with SLN, the NLC are produced by controlled mixing of solid lipids with spatially incompatible liquid lipids leading to special nanostructures with improved drug incorporation and release properties.[7,8] Using spatially liquid lipids result in massive crystal order disturbance and imperfections to more room for the accommodation of guest molecules. Hu *et al.* prepared NLC based on a mixture of stearic acid (SA) and 30 wt% oleic acid, they found that the drug loading capacity increased and release prolonged.[9]

Huperzine A (Hup A), a lycopodium alkaloid isolated from the Chinese medicinal herb *Huperzia serrata* (THUNB.) TREV., is a reversible, potent, and selective inhibitor of acetylcholinesterase.[10,11] Clinical trials in China demonstrated that Hup A was efficient and reliable in treatment of patients with mild to moderate Alzheimer's disease (AD).[12] For most of patients, the daily repeated oral administration is convenient, but it was difficult for Alzheimer's patient suffered from memory disorder not to miss scheduled self-medication. In addition, there was the side-effect on gastrointestinal tract reported, such as, nausea and anorexia.[13] Therefore, long term and parenteral formulations of Hup A against AD are a promising strategy of improving therapeutic efficacy. Hup A used as a lipophilic model drug is very suitable using nanoparticles as drug carriers. The aim of this study was to design and evaluate the feasibility of preparing Hup A loaded NLC by a method of melt ultrasonication-high pressure homogenization. To prepare NLC, cetyl palmitate (CP) and Miglyol®812 were chosen as the solid and liquid lipid material, respectively. Solutol HS15® as a nonionic surfactant for injection solution and soybean phosphatidylcholine (Spc) were chosen as emulsifiers. Hup A loaded NLC were optimized by employing Box–Behnken design. The physicochemical properties such as surface morphology, particle size, zeta potential, entrapment efficiency, drug loading, differential scanning calorimetry (DSC) and drug release behavior of Hup A loaded NLC were investigated in detail.

Experimental

Materials Huperzine A (Hup A) was provided by Ningbo Traditional Chinese Pharmaceutical Co., Ltd. (China). Cetyl palmitate (CP, mp 54—55 °C) was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Soybean Phosphatidylcholine (Spc) was obtained from Shanghai Taiwei Pharmaceutical Co., Ltd. (China). Miglyol®812 (caprylic/capric triglycerides) and Solutol HS15® (polyoxyethylene-660-12-hydroxystearat) were kindly donated by SASOL Chemie GmbH and BASF (Germany), respectively. The water used for all experiments was distilled water.

Preparation of Hup A loaded NLC The NLC was prepared by a modified method of melt ultrasonication and high pressure homogenization combined technique. Hup A was dispersed in the 2—5% (w/w) mixed lipid phase (consisted of CP and 20% Miglyol®812 content) maintained at around 10 °C above the melting temperature of mixed lipid (52 °C) for avoiding the lipid memory effect and making new crystallization possible.[14] 3—5%

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(w/w) hot aqueous phase (Spc/Solutol HS15® of 1 : 1 as emulsifier mixture) was heated to the same temperature then added drop by drop into the molten lipid phase under magnetic stirrer with 1000 rpm for 5 min. A hot pre-emulsion thus obtained was ultrasonicated using a ultrasonic probe (JY92-II ultrasonic processor, Ningbo Scientz Biotechnology Co., Ltd., China) at 400 W for 6 min then homogenized using a Nanomizer SYSTEM YSNM-1500 (CONTROL UNIT, YOSHIDA) applying 70 MPa and three homogenization cycles. The obtained dispersion cooled at room temperature was filtered through a millipore filter (0.45 μm).

Experimental Design A three-factor, three-level Box–Behnken experimental design was used to optimize the procedure. It was suitable for investigating the quadratic response surfaces and for constructing second order polynomial model using Design Expert® (Version 7.1.5, Stat-Ease Inc., Minneapolis, U.S.A.), thus enabling optimization of a process with a small number of experimental runs (15 runs). The design consisted of replicated center points and the set of points lying at the midpoints of each edge of the multidimensional cube that defines the region of interest. The non-linear computer-generated quadratic model is given as

$$Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_{11}X_1^2+b_{22}X_2^2+b_{33}X_3^2 \quad (1)$$

where Y is the measured response associated with each factor level combination; b_0 is an intercept; b_1 to b_{33} are regression coefficients computed from the observed experimental values of Y; and X_1, X_2 and X_3 are the coded levels of independent variables. The terms X_1X_2 and X_i^2 (i=1, 2 or 3) represent the interaction and quadratic terms, respectively.[15,16] The dependent and independent variables were shown in Table 1 along with their low, medium and high levels, which were selected based on the results from preliminary experimentation. The amount of the mixed lipid (CP+20% Miglyol®812; X_1), the concentration of the emulsifier mixture (Spc+Solutol HS15®; X_2) and mixed lipid/drug ratio (X_3) used to prepare the 15 formulations and the observed responses were given in Table 2.

Transmission Electron Microscopy (TEM) The morphology of the Hup A loaded NLC was observed using a TEM (JEM-1200, JEOL Co., Ltd., Tokyo, Japan). A drop of the nanoparticle dispersion was finely spread on a copper grid coated carbon with films and negatively stained with 2% (w/v) phosphotungstic acid for viewing.

Particle Size and Zeta Potential Analysis Particle size was measured by laser diffractometry (LD) using a Coulter®LS 230 (Bekman Coulter Co., Ltd., U.S.A.) at room temperature. The average particle size was expressed as the median volume diameter. The zeta potential was carried out using zeta potential analyzer (Delsa 440SX; Bekman Coulter Co., Ltd., U.S.A.). Before measurement, NLC dispersions were diluted 20-fold with the distilled water for size determination and zeta potential measurement. The measurements were performed in triplicate.

Determination of Entrapment Efficiency and Drug Loading The drug entrapment efficiency (EE) was determined by measuring the concentration of free drug in the dispersion medium with ultrafiltration technique[17] (Microncon YM-10, U.S.A.). One half milliliter of undiluted sample of NLC was placed in the outer chamber and the sample recovery chamber placed on top of the sample and centrifuged at 5000 rpm for 10 min. The free drug from the sample recovery chamber was estimated by HPLC using a Diamonsil C_{18} column (4.6×250 mm, 5 μm), at 312 nm. The mobile phase was methanol/watert/riethylamine (45 : 55 : 0.02, v/v/v, acetic acid adjusted pH to 6.0) and the flow rate was 1 ml/min. Thus EE was calculated from the amount of free drug and the amount of initial drug used for the assay.

Entrapment efficiency (EE) and drug loading (DL) could be achieved by the following Eqs. 2 and 3.

$$\text{EE (\%)}=\frac{W_{\text{initial drug}}-W_{\text{free drug}}}{W_{\text{initial drug}}}\times 100\% \quad (2)$$

$$\text{DL (\%)}=\frac{W_{\text{initial drug}}-W_{\text{free drug}}}{W_{\text{mixed lipid}}}\times 100\% \quad (3)$$

DSC Analysis Differential scanning calorimetry (DSC) analysis was performed on a DSC60 detector (Shimadzu Co., Japan). Approximately 5 mg of active was weighted into an aluminium pan and sealed hermetically. DSC scan was recorded from 30 to 300 °C at a heating rate of 10 °C/min under a nitrogen purge, using an empty pan as reference.

The DSC measurements were carried out on the following samples: (a) Hup A; (b) CP bulk powder; (c) mixed lipid (CP+20% Miglyol®812); (d) physical mixtures of Hup A and mixed lipid; and (e) lyophilized NLC with loaded Hup A.

***In Vitro* Release Study** *In vitro* release studies were performed using the dialysis bag method,[18,19] which modified to maintain a sink condition and achieve satisfactory reproducibility. A 2 ml of Hup A loaded NLC dispersion was first poured into the dialysis bag (molecular weight cut off 12000—14000) with the two ends fixed by thread and placed into the preheated dissolution media, containing 100 ml of a pH 7.4 phosphate buffer solution (PBS). The suspension was stirred at 37±0.5 °C by using a RC Drug Dissolution Tester (Tianjin Medical Instrumental Factory, China) with paddle rotating at 50 rpm. Five hundred microliters of the sample was withdrawn at fixed time intervals and the same volume of fresh medium was added accordingly. Samples were analyzed by HPLC as described above. Free Hup A dissolvd in pH 7.4 PBS was used as a control. All the operations were carried out in triplicate.

Table 1. Variables in Box–Behnken Design

Factor	Levels		
	Low (−1)	Medium (0)	High (+1)
X_1=mixed lipid (%)	2	3.5	5
X_2=emulsifier mixture (%)	3	4	5
X_3=lipid/drug ratio	40	60	80
Dependant variables		Constraints	
Y_1=particle size (nm)		Minimize	
Y_2=entrapment efficiency (%)		Maximize	
Y_3=drug loading (%)		$1.2 \leq Y_3 \leq 1.6$	

Table 2. The Composition and Observed Responses in Box–Behnken Design for Hup A Loaded NLC

Batch	Independant variables			Dependant variables		
	X_1 (%)	X_2 (%)	X_3 (%)	Y_1 (nm)	Y_2 (%)	Y_3 (%)
1	3.50	5.00	80.00	124	90.16	1.13
2	3.50	4.00	60.00	133	87.34	1.45
3	5.00	3.00	60.00	360	60.56	1.01
4	2.00	4.00	80.00	123	81.23	1.02
5	5.00	5.00	60.00	227	84.71	1.41
6	3.50	3.00	80.00	171	69.82	0.88
7	2.00	5.00	60.00	118	81.71	1.35
8	3.50	3.00	40.00	176	68.72	1.72
9	5.00	4.00	40.00	287	77.94	1.95
10	2.00	4.00	40.00	122	81.62	2.04
11	3.50	5.00	40.00	120	85.16	2.13
12	3.50	4.00	60.00	135	88.18	1.46
13	5.00	4.00	80.00	258	80.87	1.01
14	3.50	4.00	60.00	132	87.42	1.45
15	2.00	3.00	60.00	129	78.28	1.29

Results and Discussion

Optimization Data Analysis and Validation of Optimization Model A three-factor three-level Box–Behnken design as the response surface methodology (RSM) requires 15 experiments. The independent variables of the 15 experimental runs and their responses were given in Table 2. Based on the experimental design, the factor combinations resulted in different formulation of NLC. The ranges of the responses, Y_1—Y_3 (particle size, entrapment efficiency and drug loading, respectively) were 118—360 nm, 60.56—90.16% and 0.88—2.13%, respectively. The fitted models could be viewed as regression equations as in Table 3, generated by the software. Only statistically significant ($p<0.05$) coefficients were included in the equations.[20]

Positive sign before a factor in polynomial equations rep-

resents that the response increases with the factor, while a negative sign means the response and factors have reciprocal relation. As shown in Table 3, from these equations it was quite clear that the particle size (Y_1) had positive effects on the amount of mixed lipid (X_1), while inverse relationship with the amount of emulsifier mixture (X_2) and lipid/drug ratio (X_3). The results showed that when increasing mixed lipid concentration from 2 to 5% (w/w), the mean particle size increased from 118 to 360 nm. As at higher concentrations, the mixed lipid phase led to the formation of aggregates upon addition of particle size. This is caused by a decrease of ultrasonication efficiency resulting in an increase in particle agglomeration. With the amounts of the emulsifier (X_2) increasing, the mean particle size rapidly decreased then slightly changed. And also the emulsifiers concentration (X_2) and lipid/drug ratio (X_3) had a positive effect on the response the entrapment efficiency (Y_2). The encapsulation efficiency was found to vary from 60.56 to 90.16% depending mainly upon the emulsifier (X_2) concentration. The drug loading (Y_3) was observed to be positive effects on the mixed lipid (X_1) and emulsifier concentration (X_2) whereas it could be retarded by increasing lipid/drug ratio (X_3).

In addition to the close agreement between the predicted and adjusted R^2 values for each response, the value of R^2 was also observed to be >0.99 (Table 3) for all the regression equations generated, suggesting the statistical validity and significance of these equations for optimisation of NLC.

Three-dimensional (3D) plots depicting the effects of mixed lipid (X_1), emulsifier mixture (X_2) and lipid/drug ratio (X_3) on the response the entrapment efficiency (Y_2) were shown in Figs. 1—3, based on the model polynomial functions to assess the change of the response surface. These types of plots were useful to study the effects of two factors on the response at a time, when the third factor was kept at a constant level. All the relationships among the three variables were non-linear, and it was observed that mixed lipid (X_1) and emulsifier mixture (X_2) played the important roles to improve the drug incorporation. Hup A was poorly soluble in water and in lipid, however, the solubility could be enhanced by the addition of emulsifier. In Fig. 2, the effect of varying the amount of mixed lipid on the entrapment efficiency was studied when emulsifier mixture was kept constant. The result showed that the entrapment efficacy rapidly increased then slightly changed as the amount of mixed lipid increased due to the insolubility of the Hup A in CP. In Fig. 3, the entrapment efficacy was significantly increased with increasing the amount of emulsifier mixture at a constant amount of mixed lipid. This might be due to increased solubility of Hup A in the aqueous phase as the percentage of emulsifier mixture increased. Thus, a part of Hup A was incorporated in the surfactant layer at the surface of the NLC leading to a high entrapment efficacy.

The optimized formulation was achieved with 3.42% (w/w) mixed lipid, 4.57% (w/w) emulsifier mixture and mixed lipid/drug ratio of 59.86 : 1 (w/w), respectively. There-

Table 3. Summary of Results of Regression Analysis for Responses Y_1, Y_2 and Y_3 for Fitting to Quadratic Model

Quadratic model	R^2	Adjusted R^2	Predicted R^2
Response (Y_1)	0.9935	0.9819	0.8972
Response (Y_2)	0.9917	0.9768	0.8731
Response (Y_3)	0.9984	0.9955	0.9746

Regression equations of the fitted quadratic model[a)]

$$Y_1 = 133.33 + 80.00X_1 - 30.88X_2 - 3.62X_3 - 30.50X_1X_2 - 7.50X_1X_3 + 2.25X_2X_3 + 62.46X_1^2 + 12.71X_2^2 + 1.71X_3^2$$

$$Y_2 = 87.65 - 1.72X_1 + 8.67X_2 + 1.08X_3 + 3.93X_1X_2 + 0.83X_1X_3 + 0.98X_2X_3 - 5.32X_1^2 - 7.27X_2^2 - 1.92X_3^2$$

$$Y_3 = 1.45 + 0.026X_1 + 0.15X_2 - 0.47X_3 - 0.13X_2^2 + 0.14X_3^2$$

a) Only the terms with statistical significance are included.

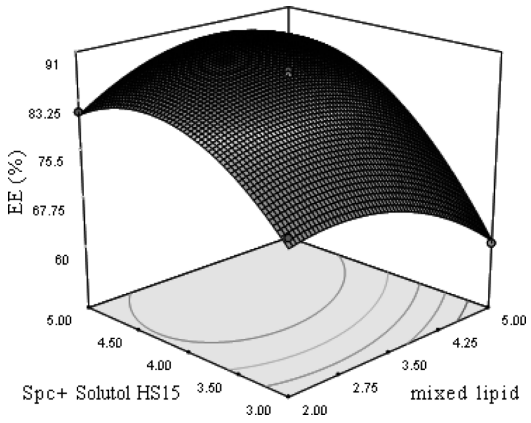

Fig. 1. Response Surface Plot of Mixed Lipid (X_1) and Spc+Solutol HS15 Concentration (X_2) on Response Y_2

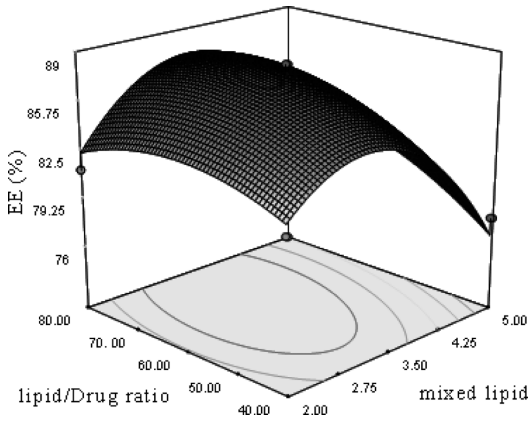

Fig. 2. Response Surface Plot of Mixed Lipid (X_1) and Lipid/Drug Ratio (X_3) on Response Y_2

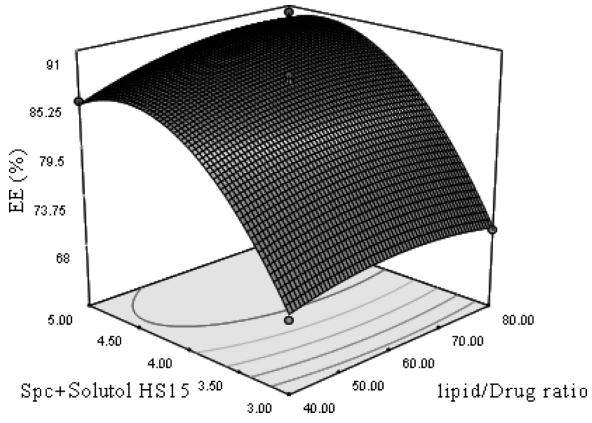

Fig. 3. Response Surface Plot of Spc+Solutol HS15 Concentration (X_2) and Lipid /Drug Ratio (X_3) on Response Y_2

fore, a new batch of NLC with the predicted levels of formulation factors was prepared to confirm the validity of the optimization procedure. Table 4 demonstrated that the observed values were mostly similar with predicted values within 5% of predicted error.

Preparation, Particle Size, Zeta Potential and Entrapment Efficiency Hup A loaded NLC were successfully prepared by a modified method of melt ultrasonication followed by high pressure homogenization. The results showed that sonicating the coarse emulsion for 6 min could effectively decrease particle size with narrow size distribution, then three homogenization cycles at 70 MPa was sufficient to achieve quite physically stable distributions. Melt ultrasonication followed by homogenization selected for all the formulations was a reliable, simple and reproducible method for preparing NLC. The TEM micrographs revealed that Hup A loaded NLC were spherical in shape with smooth surfaces and uniformly distributed around 100 nm in diameter (Fig. 4).

The optimized formulation was achieved with 3.42% (w/w) mixed lipid, 4.57% (w/w) emulsifier mixture and 0.57 mg/ml Hup A, respectively. The results indicated that the encapsulation efficiency of 89.18±0.28% with particle size, drug loading and zeta potential of 121.67±3.21 nm, 1.46±0.05% and −22.93±0.91 mV, respectively. A unimodal with a relatively narrow size distribution was observed and LD50% and LD90% were lower than 121 and 145 nm, respectively. Furthermore, the proportion of the emulsifier mixture was of great impact on the particle size and the efficacy of drug incorporation (data was not shown). With Spc as emulsifier and Solutol HS15® as co-emulsifier, the total concentration of the emulsifier mixture was kept constant. The results discovered that an increase of Solutol HS15® ratio could decrease particle size but lower stability, and an increase of Spc ratio could improve stability but bigger diameter of nanoparticles. Hence, Hup A loaded NLC were homogeneous and stable, with high entrapment efficiency at the ratio 1 : 1 (Spc/Solutol HS15®). The zeta potential was measured in the original dispersion medium (emulsifier mixture) which was diluted with distilled water for the physical stability of the dispersion. Hup A loaded NLC presented a negative surface charge that would enhance the stability of the systems. A combination of Spc and a non-ionic surfactant (Solutol HS15®) leads to the formation of close-packed mixed film, which confers improved stability, which is attributed to the steric stabilization of the non-ionic surfactant.[21,22]

In the study CP was mixed with a 20% content of Miglyol®812. In general the solubility of drugs is higher in liquid lipids (oils) compared with solid lipids. These results indicated the incorporation of 20% Miglyol®812 into lipid matrix could reduce the viscosity of lipid matrix, thus increasing the total drug loading capacity.

DSC Analysis DSC uses the fact that different lipid modifications possess different melting points, which is widely used to investigate the status of the lipid.[23—25] Figure 5 showed the DSC curves for Hup A, CP bulk, mixed lipid (CP+20% Miglyol®812), physical mixtures of Hup A and mixed lipid and lyophilized NLC loaded with Hup A. The melting point of Hup A was 232.69 °C (curve a). CP bulk and mixed lipid showed a single melting peak at 56.43 °C and 54.00 °C, respectively (curves b and c). It could be seen the melting point of CP would be depressed when Miglyol®812 was added. The thermograms of the physical mixtures of Hup A and mixed lipid showed characteristic endothermic peaks at 52.95 °C and 224.94 °C (curve d). Hup A was found to be reduced in intensity and shifted to probably because of encapsulation in mixed lipid. There was no melting peak for Hup A in lyophilized Hup A loaded NLC (curve

Table 4. Comparative Levels of Predicted and Observed Responses for Optimized Formulation

Response	Observed	Predicted	Predicted error[a] (%)
Y_1 (nm)	121.67±3.21	117	+4.27
Y_2 (%)	89.18±0.28	90.17	−1.10
Y_3 (%)	1.46±0.05	1.5	−3.33

a) Predicted error (%)=(observed value−predicted value)/predicted value×100%.

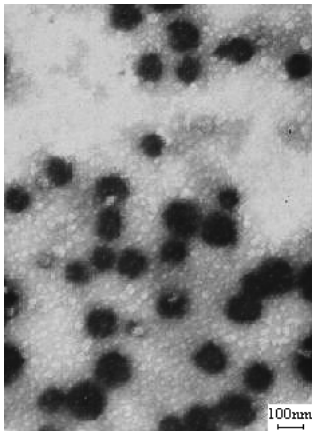

Fig. 4. Transmission Electron Microphotographs of Hup A Loaded NLC Consisting of Cetyl Palmitate 3.42% (w/w), Spc/Solutol HS®15 4.57%, Lipid/Drug Ratio of 59.86 : 1 (×1500)

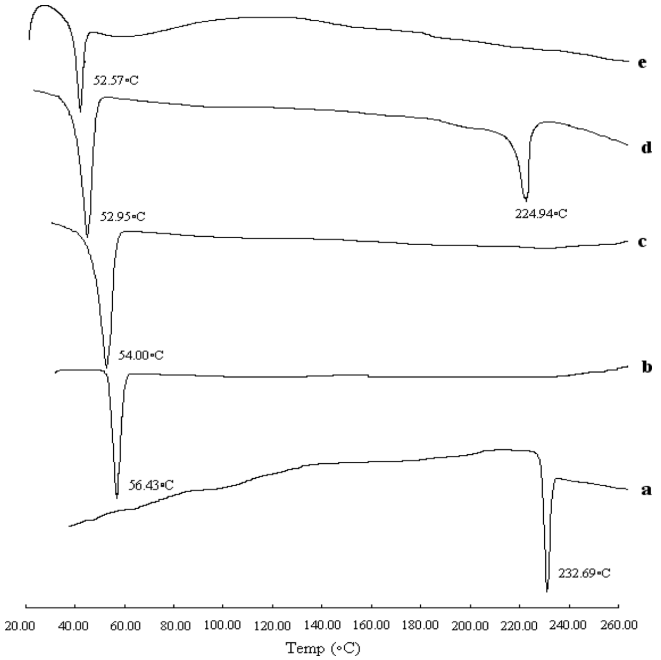

Fig. 5. Differential Scanning Calorimetric Heating Curves of (a) Hup A; (b) CP Bulk; (c) Mixed Lipid (CP+20% M812); (d) Physical Mixtures (Hup A and Mixed Lipid); and (e) Hup A Loaded NLC

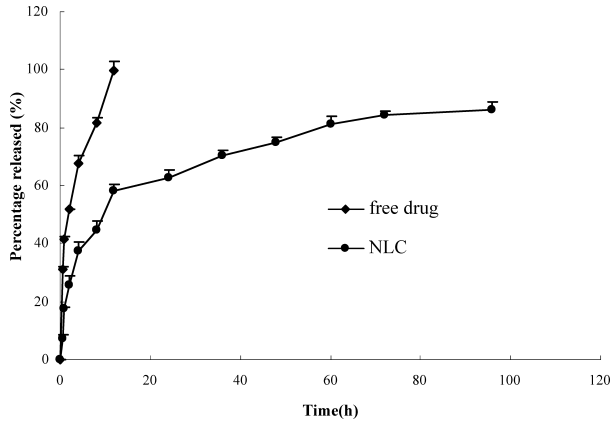

Fig. 6. *In Vitro* Drug Release Profiles of Hup A from NLC and Free Drug ($n=3$)

pH 7.4 PBS was used as dialysis medium.

e). This suggested that Hup A dispersed homogeneously in NLC and no crystallization of HupA occurred during the NLC preparation process. Therefore, one could conclude that the lipid within NLC should be in a less ordered arrangement compared to the bulk CP corresponding to the DSC analysis. While the incorporation of Miglyol®812 to CP could lead to massive crystal order disturbance, it indicated that matrix of lipid particles had great imperfections in the crystal lattice and leaves enough space to accommodate drug molecules. Thus a highly disordered configuration of lipids would improve the entrapment efficiency and drug loading.

Drug Release Behavior The drug release behavior of Hup A loded NLC was investigated using a dialysis membrane in pH 7.4 PBS (37±0.5 °C). In Fig. 6 free Hup A exhibited a rapid release of 99% of drug within 12 h, whereas Hup A from NLC founded the relative burst drug release at the initial stage and followed by sustained release over 96 h. The release behavior of Hup A from the mixed lipid matrices mainly depended on the co-effect of diffusion or bulk erosion. Furthermore, NLC had been prepared not to remove existing free drug, the initial burst related to free drug could not be ignored.

It was possible to modify the release profiles as a function of lipid matrix, surfactant concentration and production parameters (*e.g.* temperature).[26] During the production of NLC, Hup A partitioned from the oil phase to the water phase. With increasing temperature of the water phase and increasing emulsifier concentration, the saturation solubility of Hup A in the water phase would increase. During the solidifying process, the solubility of Hup A in the water phase decreased continuously that meant a re-partitioning of Hup A into the lipid phase occurred. With reaching the recrystallization temperature of the lipid, Hup A enriched core started to form this lipid phase. Continuing to reducing the temperature, the already crystallized core was not accessible anymore for Hup A, thus a fraction of Hup A in the outer shell and on the particle surface was released in the form of a burst, Hup A incorporated into the particle core was released in a prolonged way. And the release data obtained was fitted with the equations of first-order, Higuchi and Weibull,[11] respectively, in which Weibull equation fitting results was the best ($y=0.5603x-1.7544$, $r=0.9842$). Furthermore, the incorporation of 20% Miglyol®812 into solid lipid matrix caused the NLC became more imperfect and allowed loaded drugs easier to release, so increased the drug release rate when liquid lipid was included in NLC matrices.[27,28]

Conclusion

In the study, Hup A loaded NLC were successfully prepared by a modified method of melt ultrasonication followed by high pressure homogenization. The results showed that it was possible to formulate lipid nanoparticles with good properties with mixed lipid consisting of CP and Miglyol 812. The optimized formulation was obtained by using a three-level three-factorial Box–Behnken experimental design. It was found that the optimized formulation was achieved with 3.42% mixed lipid (X_1), 4.57% emulsifier mixture (X_2) and lipid/drug ratio of 59.86 : 1 (X_3) and the observed responses were close to the predicted values for the optimized formulation. The DSC analyses showed that the matrix cores of Hup A loaded NLC were less ordered arrangement of crystals. So many imperfections could offer space to accommodate the drug molecules, and increase the drug loading capacity. *In vitro* release tests exhibited an initial burst release followed by a prolonged release and the release pattern of drug was found to follow Weibull equations. The main reasons leading to initial burst were correlated to a small part of drug-enriched shell model. Most of Hup A was encapsulated in the lipid core to obtain a prolonged release. Future experiments are aimed at evaluating the *in vitro* (PC12 cell line) and *in vivo* efficacy of these newly developed formulations.

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