Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 348 (2008) 146–152
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Nimodipine loaded lipid nanospheres prepared by solvent diffusion method in a drug saturated aqueous system

Fu-Qiang Hu, Yu Zhang, Yong-Zhong Du, Hong Yuan *

College of Pharmaceutical Science, Zhejiang University, 388 Yuhangtang Road, Hangzhou 310058, China

Received 19 March 2007; received in revised form 6 June 2007; accepted 10 July 2007
Available online 25 July 2007

Abstract

To overcome the disadvantages such as lower drug entrapment efficiency (EE) of lipid nanospheres prepared by conventional solvent diffusion method, a solvent diffusion method in drug saturated aqueous system was developed. Nimodipine was used as a model drug to incorporate into lipid nanospheres. The monostearin (MS) solid lipid nanoparticles (SLN) produced by conventional method under different production temperature only showed 24.40–30.21 wt% EE, and relatively higher EE was achieved when the production temperature was 0 °C. The EE could be enhanced by the incorporation of liquid lipid (caprylic/capric triglycerides, CT) into SLN and the employing of drug saturated dispersion medium. The nanostructured lipid carrier (NLC) with higher CT content indicated the highest EE as the drug saturated aqueous solution was used as dispersion medium. The differential scanning calorimetry (DSC) results demonstrated the present method could improve the drug encapsulation into lipid nanospheres. In vitro drug release experiments indicated the present preparation method could delay the drug release rate from lipid nanospheres, and the drug release rate could adjust by the CT content in lipid nanospheres. The highest drug loading (DL) was reached up to 4.22 wt% when 8 wt% drug was charged in the preparation of lipid nanospheres.

Keywords: Nimodipine; Lipid nanospheres; Solvent diffusion method; Monostearin; Caprylic/capric triglycerides; Drug entrapment efficiency

1. Introduction

In recent years, lipid nanospheres have been attracted great interest because of their potential application in medicine as drug delivery carriers. These carriers have obvious advantages, such as improving the therapeutic effect (Lu et al., 2006), good tolerability (Müller et al., 1996b), biodegradable (Müller et al., 1996c), a high bioavailability for ocular administration (Cavalli et al., 2002), and a targeting effect to brain (Yang et al., 1999).

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are two main types of lipid nanospheres. SLN is a colloidal carrier system for controlled drug delivery, follow by the development of emulsion, liposomes, microparticles and nanoparticles based on synthetic or natural polymers (Müller et al., 2000). They combine the advantages of emulsions, liposomes and polymeric nanoparticles. The solid matrix can protect incorporated active ingredients against chemical degradation and provide the highest flexibilities in the modulation of the drug release profiles. Moreover, the SLNs are composed of well physiologically tolerated excipients and can be produced on large industrial scale by high pressure homogenization (Olbrich et al., 2001; Müller and Lucks, 1996a). However, there are also some potential limitations, i.e. limited drug loading capacity, drug expulsion during storage due to the crystallization of lipid matrix (Müller et al., 2000).

NLC composed of solid lipid matrix with certain content of liquid lipid are a new generation of lipid nanoparticles. The incorporation of liquid lipids into solid lipid matrix leads to great imperfections in the crystal lattice of nanoparticles, thus leading to improved drug loading capacity and reduced drug expulsion during storage (Jenning et al., 2000; Jenning and Gohla, 2001; Souto et al., 2004).

The high pressure homogenization is the main method for the preparation of lipid nanospheres due to the large scale production ability. However, the high temperatures used in the process could affect the stability of the drug and the carriers (Mehnert and Mader, 2001), and the higher emulsifier concentration was also believed to cause the burst drug release (Mühlen and Mehnert,

* Corresponding author. Tel.: +86 571 88208439; fax: +86 571 88206742.
E-mail address: yuanhong@zju.edu.cn (H. Yuan).

0378-5173/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2007.07.025

1998). Due to these reasons, the solvent diffusion method in an aqueous system was developed to prepare the SLN in our group (Hu et al., 2002, 2004, 2005, 2006). This method had two advantages: the one is no needs for emulsifiers; and the other is simple preparation procedure. In our continuous research, however, it was found that the drug entrapment efficiency and drug loading were very lower when some lipophilic drugs with lower molecular weight were used as model drugs. The lower drug entrapment efficiency and drug loading were contributed to the incompatibility between drug and lipid matrix, and the drug solubility in aqueous dispersion medium (although it is very lower).

Nimodipine, a second-generation dihydropyridine calcium antagonist with apparent selectivity for cerebral blood vessels is poorly water-soluble, and has lower bioavailability for oral administration. In clinic, the injection of nimodipine ethanol solution was usually used. It exist presents the safe problem. It was reported that lipid nanospheres are a promising sustained release system lipophilic drugs after oral administration to increase the bioavailability (Mehnert and Mader, 2001).

In present research, a solvent diffusion method in drug saturated aqueous system was developed to prepare lipid nanospheres. The nimodipine was used as model drug. Monostearin (MS) and caprylic/capric triglycerides (CT) were chosen as the solid and liquid lipid material of lipid nanosphere, respectively. The properties such as particle size, drug entrapment efficiency, drug loading and in vitro drug release behavior of prepared nimodipine loaded lipid nanospheres were compared with that of nimodipine loaded lipid nanospheres produced by conventional solvent diffusion method. Moreover, the effect of production temperature, liquid lipid content and the charged amount of drug on the properties of nimodipine loaded lipid nanospheres were investigated in details.

2. Materials and methods

2.1. Materials

Monostearin was purchased from Shanghai Chemical Reagent Co., Ltd. (China). Caprylic/capric triglycerides was purchased from Lanxi Wumei Chemical Co. Ltd. (Zhejiang, China). Nimodipine was purchased from Xinhua Pharmaceutical Factory (Shandong, China). Sodium dodecyl sulfate (SDS) was purchased from Shantou Xilong Chemical Factory (Guangdong, China). Acetone was purchased from Hangzhou Chemical Reagent Co. Ltd. (Zhejiang, China). Ethanol was purchased from Hangzhou Changzheng Chemical Factory (Zhejiang, China). Methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). All of reagents were analytical reagent grade.

2.2. Preparation of nimodipine loaded lipid nanospheres

For the preparation of nimodipine loaded lipid nanosphere, weighted nimodipine and lipid materials (MS with or without CT) were dissolved into 5 ml mixed organic solvent of ethanol and acetone (1:1, v/v) at 50 °C. This resultant organic solution was then quickly dispersed into 100 ml distilled water (0, 25 and 60 °C) or nimodipine saturated aqueous solution (0 °C). The nimodipine loaded lipid nanospheres dispersions were obtained under mechanical agitate (DC-40, Hangzhou Electrical Engineering Instruments, China) with 400 rpm for 5 min. After the pH of dispersion was adjusted to 1.2 by hydrochloric acid, the dispersion was then centrifuged (3K30, Sigma, Germany) at 20,000 rpm for 15 min. The precipitate of lipid nanospheres was re-dispersed into 100 ml 0.2 wt% SDS aqueous solution to remove the drug adsorbed on the surface of lipid nanospheres. Then, the nimodipine loaded lipid nanospheres were collected by centrifugation at 20,000 rpm for 15 min, and subjected to further use.

2.3. Particle size and zeta potential determination

The particle size and zeta potential of resulted nimodipine loaded lipid nanospheres were measured by Zetasizer (3000HS, Malvern Instruments, UK). For the preparation of sample, the nimodipine loaded lipid nanospheres dispersions were diluted 10 times by distilled water.

2.4. Surface morphology observation

The surface morphology of obtained nimodipine loaded lipid nanospheres was observed by an atomic force microscopy (SPA 3800N, SEIKO, Japan). Explorer atomic force microscope was in tapping mode, using high resonant frequency (F_0 = 129 kHz) pyramidal cantilevers with silicon probes having force constants of 20 N/m. Scan speeds were set at 2 Hz. Prior to AFM observation, the nimodipine loaded lipid nanospheres dispersions were diluted 20 times by distilled water, and dropped on freshly mica piece, followed by vacuum drying for 24 h at 25 °C.

2.5. DSC analysis

Differential scanning calorimetry (DSC) analysis was performed using Q100 DSC (TA, USA). For DSC measurement, 10 mg of powdered nimodipine loaded lipid nanospheres were put in the aluminum pans. A scan rate of 10 °C/min was employed in the 30–290 °C temperature range.

2.6. Determination of nimodipine content

The nimodipine contents were measure by high performance liquid chromatograph (HPLC, Agilent 1100 series, USA). A Diamohsil C18 column (200 mm × 4.6 mm) with 5 μm particles was used. The mobile phase was consisted of methanol, acetonitrile and ultrapure water (35:38:27, v/v/v), and the flow rate was 1.0 ml/min. The column effluent was detected at 237 nm with a variable wavelength detector (G1314A, JP11615541, UV detector, USA). The column temperature was maintained at 37 °C. The calibration curve for the quantification of nimodipine was linear over the range of standard concentration of nimodipine at 2–80 μg/ml with a correlation coefficient of R^2 = 0.9998. The limit of detection was 1 ng/ml.

2.7. Determination of drug entrapment efficiency (EE) and drug loading (DL)

As mentioned in the preparation of nimodipine loaded lipid nanospheres, the nimodipine content in the two supernatants were measured by HPLC method. One supernatant was separated after the nanosphere dispersion was precipitated by the pH adjust of dispersion. Other supernatant was separated after the separated lipid nanospheres were washed by 0.2 wt% SDS solution. The drug entrapment efficiency (EE) and drug loading (DL) of nimodipine loaded lipid nanospheres were then calculated from formula (1) and (2):

$$\mathrm{EE} = \frac{(W_\mathrm{T} - W\mathrm{s}_1 - W\mathrm{s}_2)}{W_\mathrm{T}} \times 100\% \quad (1)$$

$$\mathrm{DL} = \frac{(W_\mathrm{T} - W\mathrm{s}_1 - W\mathrm{s}_2)}{(W_\mathrm{T} - W\mathrm{s}_1 - W\mathrm{s}_2 + W_\mathrm{L})} \times 100\% \quad (2)$$

where W_T was the total amount of charged drug. In the solvent diffusion method using drug saturated dispersion medium, the drug content in drug saturated aqueous solution was taken account into the calculation of EE and DL of lipid nanospheres. $W\mathrm{s}_1$ was the amount of drug in supernatant after the first centrifugation, $W\mathrm{s}_2$ was the amount of drug in supernatant after the second centrifugation, W_L was the total amount of charged lipid.

2.8. In vitro drug release studies

After 20 mg nimodipine loaded lipid nanospheres were washed by 0.2 wt% SDS solution, the collected nimodipine loaded lipid nanospheres were redispersed into 100 ml 0.2 wt% SDS solution. The dispersions were shaken horizontally (Incubator Shaker HZ-88125, Hualida Laboratory Equipment Company, China) at 37 °C and 60 strokes per min. One milliliter of the dispersion sample was withdrawn at different time intervals. The samples were centrifuged at 20,000 rpm for 10 min. The nimodipine contents in the supernatants were measured by HPLC method.

2.9. Statistics

Data were expressed as means of three separate experiments, and were compared by analysis of variance (ANOVA). A p-value < 0.05 was considered statistically significant in all cases.

3. Results and discussion

3.1. Preparation of nimodipine loaded solid lipid nanoparticles by conventional solvent diffusion method

At first, the nimodipine loaded SLN were prepared by conventional solvent diffusion method. The preparation recipes and the properties of resultant nimodipine loaded SLN are shown in Tables 1 and 2 (Run 1, 2 and 3). The effect of preparation temperature on the physicochemical properties of resultant nimodipine loaded SLN was investigated. From Table 2, it was found that the volume average diameter and its polydispersity index of the SLN increased from 240.1 to 443.7 nm, and from 0.162 to 0.243, as the preparation temperature was reduced from 60 to 0 °C. The lower temperature of dispersion medium led the lower diffusion rate of organic dispersion phase, and consequently formed the relatively large particles having wide size distribution. All of obtained SLNs were very stable. Notice the absolute values of zeta potentials for the SLN prepared under different production temperature were higher than 50.

Table 2 also shows the results of EE and DL for the SLNs prepared under different production temperature (Run 1, 2 and 3). It could be seen that all of the EEs of SLNs were below about 30 wt%. Because the solubility of nimodipine in distilled water is very low (only 1.6 μg/ml under 0 °C), the EEs of SLNs were higher than 80 wt% (data not shown) if only the drug content in supernatant which separated after the nanosphere dispersion was precipitated by the pH adjust of dispersion, was taken account into the calculation of EE for SLN. The little solubility of drug in dispersion medium could lead the drug diffusion toward dispersion medium, and consequently caused the drug distribution on the surface of lipid nanospheres. Due to this reason, the collected SLNs were further washed by 0.2 wt% SDS solution to remove the drug located on the surface of SLNs. By the washing with SDS solution, the real EEs of SLNs were significantly reduced (below 30 wt%). The lower EE of SLN prepared under 60 °C may be caused from two reasons: The one is the higher drug solubility in the dispersion medium under 60 °C; the other is more drug content located on the SLN surface due to the large

Table 1
Recipes for the preparation of nimodipine loaded lipid nanospheres

Run no.	Nimodipine (mg)	MS (mg)	CT (mg)	Continuous phase	T (°C)
1	5	95	0	Distilled water	60
2	5	95	0	Distilled water	25
3	5	95	0	Distilled water	0
4	5	95	0	Drug saturated aqueous solution	0
5	5	75	20	Distilled water	0
6	5	75	20	Drug saturated aqueous solution	0
7	5	85	10	Drug saturated aqueous solution	0
8	1	79	20	Drug saturated aqueous solution	0
9	3	77	20	Drug saturated aqueous solution	0
10	8	72	20	Drug saturated aqueous solution	0
11	10	70	20	Drug saturated aqueous solution	0

Table 2
Properties of resultant nimodipine loaded lipid nanospheres

Run no.	Volume average diameter (nm)	PI (−)	ζ (mV)	EE (wt%)	DL (wt%)
1	240.1 ± 7.3a	0.162 ± 0.048	−77.3 ± 4.6	27.26 ± 0.63	1.41 ± 0.03
2	305.7 ± 10.3a	0.226 ± 0.079	−52.7 ± 4.1	24.40 ± 0.67	1.27 ± 0.03
3	443.7 ± 12.7a,b	0.243 ± 0.064	−60.7 ± 2.1	30.21 ± 0.76c,e	1.57 ± 0.04d,f
4	476.4 ± 9.4g	0.389 ± 0.089	−50.4 ± 3.8	42.47 ± 0.25e,h	2.19 ± 0.01,f,i
5	177.5 ± 4.8b	0.304 ± 0.071	−69.5 ± 3.2	50.35 ± 0.49c	2.58 ± 0.03d
6	171.2 ± 6.3g	0.316 ± 0.093	−66.4 ± 2.3	62.32 ± 0.29h,j	3.18 ± 0.01i
7	270.6 ± 14.1g	0.259 ± 0.057	−57.1 ± 3.2	56.21 ± 0.30h	2.87 ± 0.02i
8	191.7 ± 7.9	0.342 ± 0.061	−69.2 ± 2.9	82.24 ± 0.85j	0.82 ± 0.01
9	185.7 ± 6.4	0.276 ± 0.042	−54.1 ± 1.9	70.64 ± 0.56j	2.14 ± 0.02
10	194.6 ± 8.2	0.119 ± 0.046	−68.7 ± 2.6	50.71 ± 0.40j	4.22 ± 0.03
11	253.0 ± 7.5	0.053 ± 0.028	−71.4 ± 3.1	38.65 ± 0.46j	4.12 ± 0.05

PI, ζ, EE and DL indicate the polydispersity index of particle size, zeta potential, drug entrapment efficiency and drug loading, respectively. Data are represented with mean ± S.D. ($n = 3$). (a–j) show significant difference ($p < 0.05$).

specific surface area (Notice the particle size of SLN prepared under 60 °C was smallest.). To obtain the relatively higher EE of lipid nanospheres, the lipid nanospheres were prepared under 0 °C in the later stage.

Fig. 1 shows the in vitro nimodipine release behaviors of SLNs prepared by conventional solvent diffusion method under different preparation temperature. It was clear that the drug release rate in the initial stage was slowed by reducing the preparation temperature. The nimodipine loaded SLN prepared under 0 °C had the slowest in vitro drug release rate. The slowest drug release rate may be due to the large particle size ($p < 0.05$) of SLN prepared under 0 °C.

3.2. Preparation of nimodipine loaded nanostructured lipid carriers by conventional solvent diffusion method

The incorporation of liquid lipids into solid lipid matrix could lead to great imperfections in the crystal lattice of nanoparticles, thus leading to improved EE, DL and reduced drug expulsion during storage. When the content of liquid lipid was higher than the solubility of liquid lipid in solid lipid, the phase separation occurred and the nanocompartments of liquid lipid could be formed. For a number of drugs, the solubility in liquid lipid was higher than that in solid lipid. Consequently, the higher EE and DL can be achieved in NLC (Müller et al., 2002).

In present research, the CT was used as a liquid lipid to incorporate into solid lipid matrix to improve the EE and DL of lipid nanospheres. The preparation recipe and properties of resulted NLC is listed in Tables 1 and 2 (Run 5), respectively. Comparing with the results of SLN (Run 3), the EE and DL of NLC with 20 wt% CT content was increased from 30.21 to 50.35 wt%, and from 1.57 to 2.58 wt%, respectively. Moreover, the particle size of resulted NLC was decreased by the incorporation of 20 wt% CT into solid lipid matrix. As reporting in previous research (Hu et al., 2005), the smaller particle size of NLC was contributed to the lower surface tension of mixed lipid matrix than solid lipid matrix. Fig. 2 shows the in vitro drug release behaviors of SLN and NLC prepared by conventional solvent diffusion method. The in vitro drug release rate of NLC was faster than that of SLN, which contributed two factors: the one is smaller particle

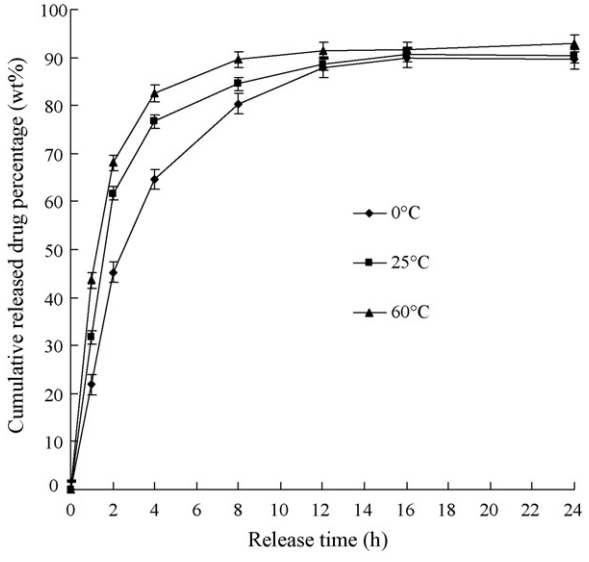

Fig. 1. In vitro drug release profiles of nimodipine loaded SLN prepared by conventional solvent diffusion method under different production temperature.

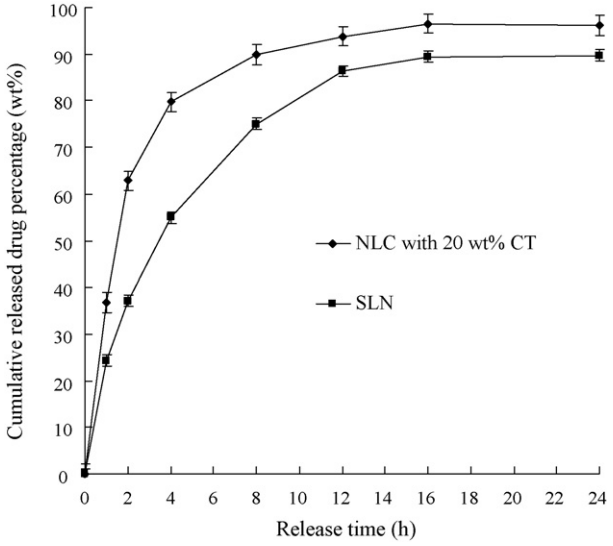

Fig. 2. In vitro drug release profiles of SLN and NLC prepared by conventional solvent diffusion method.

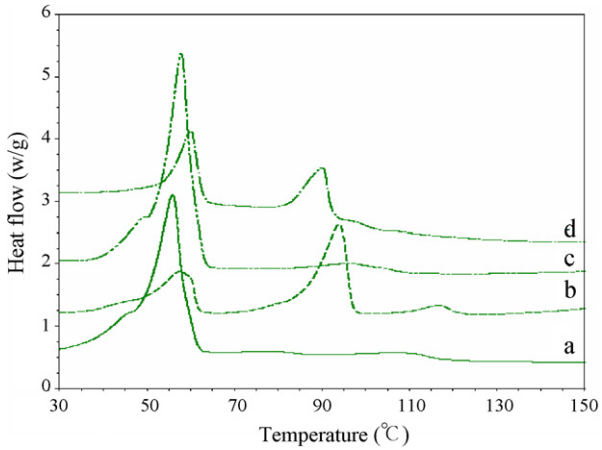

Fig. 3. DSC curves of NLCs. (a) NLC prepared by conventional solvent diffusion method after washing with 0.2 wt% SDS solution; (b) NLC prepared by conventional solvent diffusion method before washing with 0.2 wt% SDS solution; (c) NLC prepared by solvent diffusion method in a drug saturated aqueous system after washing with 0.2 wt% SDS solution; (d) NLC prepared by solvent diffusion method in a drug saturated aqueous system before washing with 0.2 wt% SDS solution;.

size of NLC; the other is the faster drug diffusion rate via mixed matrix of solid lipid and liquid lipid than that of through solid lipid matrix.

3.3. Preparation of nimodipine loaded lipid nanospheres by solvent diffusion method in a drug saturated aqueous system

Although the solubility of nimodipine in the dispersion medium, distilled water is very low, it could lead the drug diffusion toward dispersion medium, and consequently caused the drug distribution on the surface of lipid nanospheres based on the properties difference between drug and lipid matrix. If the dispersion medium was saturated by drug previously, the drug diffusion toward dispersion medium in the lipid nanospheres production process could be limited. Herein, A solvent diffusion method in drug saturated aqueous system was developed to prepare nimodipine loaded SLN and NLC. The preparation recipes and the properties of resulted SLN and NLC were also listed in Tables 1 and 2 (Run 4 and 6). It was obvious that the EE of SLN and NLC were highly improved by the employing drug saturated aqueous solution in steady of distilled water. Fig. 3 shows the DSC curves of NLC prepared by two methods (Run 5 and 6) before and after the washing with 0.2 wt% SDS solutions. The melting point of MS and nimodipine was 56–58 °C and 124–128 °C, respectively. So the peaks between 50 and 60 °C in DSC curves were the peaks for mixed lipid matrix of MS and CT, and the peaks near 90 °C in DSC curves were the peaks for nimodipine. It could be seen that the nimodipine peaks appeared before the NLCs (curves b and d) were washed by 0.2 wt% SDS solutions. After the NLCs were washed by 0.2 wt% SDS solutions (curves a and c) no obvious nimodipine peaks were found. It means the drug located on the surface of NLC could be removed by the washing with 0.2 wt% SDS solution. Moreover, comparing with the curves b and d, the peak area ratio of nimodipine to lipid matrix for curve b was higher than that for curve d. This result demonstrated the nimodipine content on the surface of NLC was reduced significantly when the drug saturated dispersion medium was used. Due to this reason, the EE of NLC was highly improved.

It was also found the volume average diameter of NLC was smaller than that of SLN as the solvent diffusion method in a drug saturated aqueous system was used, and no obvious differences of particles size for SLN and NLC were found between two preparation methods. Usually, atomic force microscopy (AFM) was employed to gain the size, shape (Gualbert et al., 2003) and surface morphological information (Shahgaldian et al., 2003) of nanoparticles. The AFM images of nimodipine loaded SLN and NLC prepared by solvent diffusion method in drug saturated dispersion medium are shown in Fig. 4 (a) and (b). For the SLN (Fig. 4 (a)), the length and width were between 300 and 400 nm and the height was between 50 and 60 nm. For the NLC (Fig. 4(b)), the length and width were between 50 and 100 nm and the height was between 5 and 6 nm. The observed flattened particles in AFM image may result from the collapse of nanoparticles during vacuum drying process (Montasser et al., 2002). The lower height of NLC was due

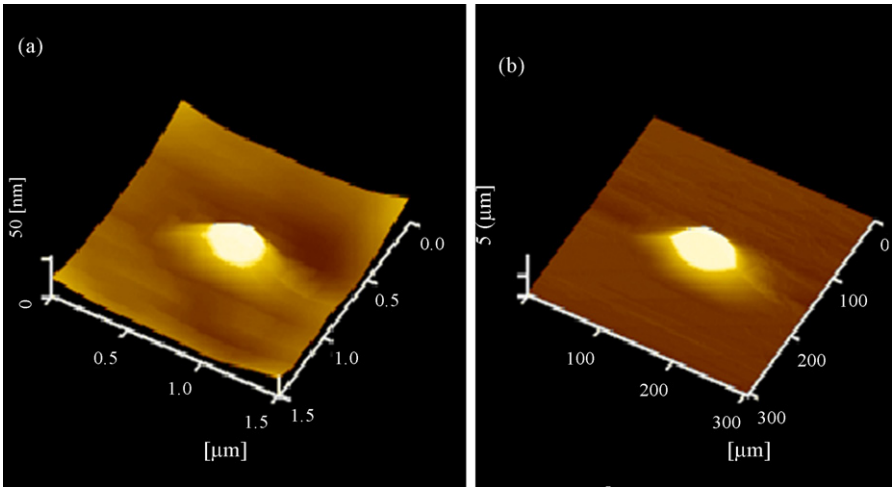

Fig. 4. Atomic force microscopy (AFM) images of SLN (a) and NLC (b) prepared by solvent diffusion method in a drug saturated aqueous system.

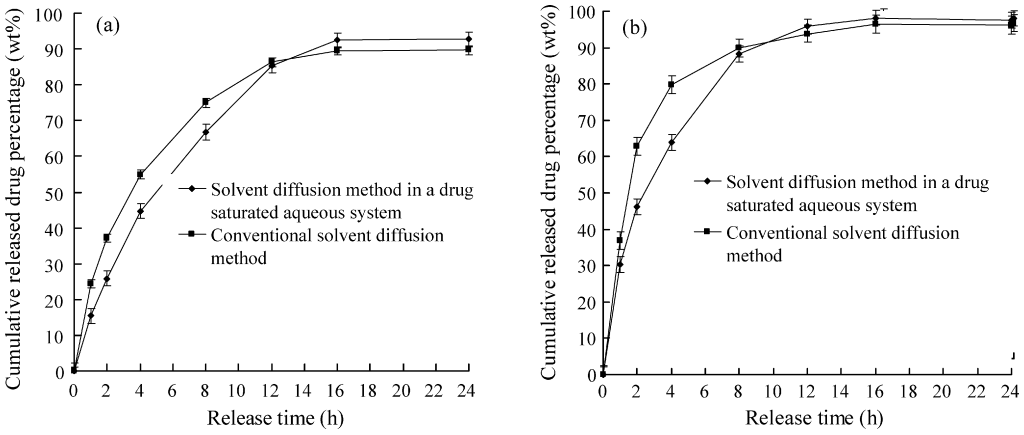

Fig. 5. In vitro drug release profiles of SLN (a) and NLC (b) prepared by conventional solvent diffusion method and a solvent diffusion method in drug saturated aqueous system.

to the incorporation of CT with lower melting point into solid matrix.

Fig. 5 (a) and (b) show the in vitro drug release behaviors of SLN and NLC prepared by two different methods. The in vitro drug release rates of SLN and NLC prepared by solvent diffusion method in drug saturated aqueous system in the initial stage were slower than that of SLN and NLC prepared by conventional solvent diffusion method, respectively. Because the particle sizes of SLN or NLC prepared by two preparation methods were almost same, so the slower drug release rate of SLN and NLC prepared by solvent diffusion method in drug saturated aqueous system could be contributed the good entrapment efficiency of drug into lipid matrix.

To investigate the effect of CT incorporated in to lipid nanospheres on the properties of lipid nanospheres prepared by solvent diffusion method in a drug saturated aqueous system, the nimodipine loaded NLC with 10 wt% CT content was prepared (Run 7). Comparing the NLC (Run 7) with the SLN (Run 4) and NLC with 20 wt% CT content (Run 6), it was clear that the volume average diameter of lipid nanospheres decreased, and the EE of lipid nanospheres increased as the CT content in lipid nanospheres was enhanced. Fig. 6 shows the in vitro drug release behaviors of lipid nanospheres with different CT content prepared by solvent diffusion method in drug saturated aqueous system. The in vitro drug release rate was enhanced with the CT content in lipid nanospheres. The faster in vitro drug release rate of NLC with higher CT content could contribute to the smaller particle size and the faster drug diffusion through the mixed lipid matrix with higher CT content.

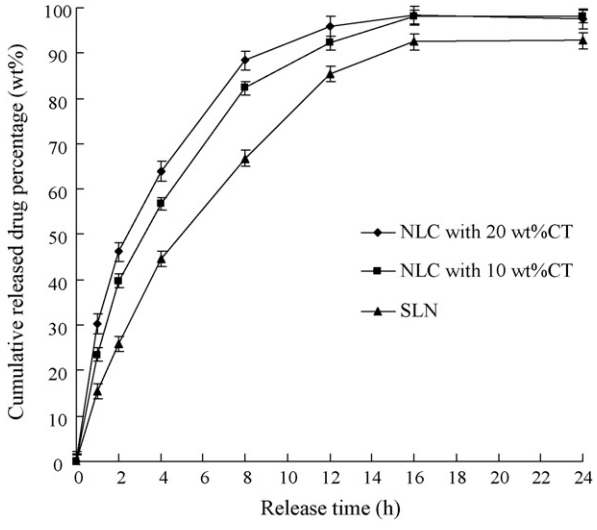

Fig. 6. In vitro drug release profiles of lipid nanospheres with different CT content prepared by solvent diffusion method in a drug saturated aqueous system.

In order to further investigate the drug loading capacity of the NLC prepared by solvent diffusion method in a drug saturated aqueous system, the NLCs were prepared by adding different amount of drug. The recipes and the properties of NLCs are shown in Tables 1 and 2 (Run 8–11). From Table 2, it can be seen the EE of NLC decreased with increasing the charged amount of drug, and the highest 4.22 wt% drug loading was achieved when the charged amount of drug was 8 wt%.

4. Conclusions

A solvent diffusion method in drug saturated aqueous system was developed to prepared nimodipine loaded lipid nanospheres, to improve the EE and DL of the lipid nanospheres. The lower preparation temperature and the incorporation of liquid lipid into lipid matrix could increase the EE of lipid nanospheres. Comparing with the lipid nanospheres prepared conventional solvent diffusion method, the EEs of SLN and NLC prepared by present method were highly improved. The increase of CT content in the NLC could enhance the EE. The EE of NLC was reduced by increasing the charged amount of drug, and highest 4.22 wt% drug loading of the NLC was achieved when 8 wt% drug was added. We believe this method could improve the entrapment of other lipophilic drugs with lower molecular weight into lipid matrix.

Acknowledgements

We gratefully acknowledge the Analysis Center of Zhejiang Univercity. We would like to thank Yi-qing Ye for the AFM analysis and Ling-sheng Cheng for the DSC analysis.

References

Cavalli, R., Gasco, M.R., Chetoni, P., Burgalassi, S., Saettone, M.F., 2002. Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. Int. J. Pharm. 238, 241–245.

Gualbert, J., Shahgaldian, P., Coleman, A.W., 2003. Interactions of amphiphilic calix[4]arene-based solid lipid nanoparticles with bovine serum albumin. Adv. Drug Deliv. 257, 69–73.

Hu, F.Q., Yuan, H., Zhang, H.H., Fang, M., 2002. Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. Int. J. Pharm. 239, 121–128.

Hu, F.Q., Hong, Y., Yuan, H., 2004. Preparation and characterization of solid lipid nanoparticles containing peptide. Int. J. Pharm. 273, 29–35.

Hu, F.Q., Jiang, S.P., Du, Y.Z., Yuan, H., Ye, Y.-Q., Zeng, S., 2005. Preparation and characterization of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system. Colloids Surf. B Biointerfaces 45, 167–173.

Hu, F.Q., Jiang, S.P., Du, Y.Z., Yuan, H., Ye, Y.-Q., Zeng, S., 2006. Preparation and characteristics of monostearin nanostructured lipid carriers. Int. J. Pharm. 314, 83–89.

Jenning, V., Andreas, F.T., Gohla, S.H., 2000. Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. Int. J. Pharm. 199, 167–177.

Jenning, V., Gohla, S.H., 2001. Encapsulation of retinoids in solid lipid nanoparticles (SLN). J. Microencapsul. 18, 149–158.

Lu, B., Xiong, S.B., Yang, H., Yin, X.B., Chao, R.B., 2006. Solid lipid nanoparticles of mitoxantrone for local injection against breast cancer and its lymph node metastases. Eur. J. Pharm. Sci. 28, 86–95.

Mehnert, W., Mader, K., 2001. Solid lipid nanoparticles production, characterization and applications. Adv. Drug Deliv. Rev. 47, 165–196.

Montasser, I., Fessi, H., Coleman, A.W., 2002. AFM imaging of novel type of polymeric colloidal nanostructures. Eur. J. Pharm. Biopharm. 54, 281–284.

Mühlen, A.Z., Mehnert, W., 1998. Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles. Pharmazie 53, 552–559.

Müller, R.H., Lucks, J.S., 1996a. Arzneistoffträger aus festen Lipidteilchen, Feste Lipidnanosphären (SLN). European Patent 0 605 497.

Müller, R.H., Maaßen, S., Weyhers, H., Specht, F., Lucks, J.S., 1996b. Cytotoxicity of magnetite loaded polylactide, polylactide/glycolide particles and solid lipid nanoparticles (SLN). Int. J. Pharm. 138, 85–94.

Müller, R.H., Rühl, D., Runge, S.A., 1996c. Biodegradation of solid lipid nanoparticles as a function of lipase incubation time. Int. J. Pharm. 144, 115–121.

Müller, R.H., Mäader, K., Gohla, S., 2000. Solid lipid nanoparticle (SLN) for controlled drug delivery-review of the state of the art. Eur. J. Pharm Biopharm. 50, 161–177.

Müller, R.H., Radtke, M., Wissing, S.A., 2002. Nanostructured lipid matrices for improved microencapsulation of drugs. Int. J. Pharm. 242, 121–128.

Olbrich, C., Bakowsky, U., Lehr, C.M., Muller, R.H., Kneuer, C., 2001. Cationic solid-lipid nanoparticles can efficiently bind and transfect plasmid DNA. J. Control. Release 77, 345–355.

Shahgaldian, P., Silva, E.D., Anthony, W., 2003. Para-acyl-calix-arene based solid lipid nanoparticles (SLNs): a detailed study of preparation and stability parameters. Int. J. Pharm. 253, 23–38.

Souto, E.B., Wissing, S.A., Barbosa, C.M., et al., 2004. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. Int. J. Pharm. 278, 71–77.

Yang, S.C., Lu, L.F., Cai, Y., Zhu, J.B., Liang, B.W., Yang, C.Z., 1999. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J. Control. Release 59, 299–307.