Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 343 (2007) 270–276
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Preparation and characteristic of vinorelbine bitartrate-loaded solid lipid nanoparticles

Jian You [a], Feng Wan [a,b], Fu de Cui [b], Yu Sun [b], Yong-Zhong Du [a], Fu qiang Hu [a,*]

[a] College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, PR China
[b] College of Pharmaceutical Sciences, Shenyang Pharmaceutical University, Shenyang 110016, PR China

Received 29 March 2007; received in revised form 21 May 2007; accepted 2 July 2007
Available online 13 August 2007

Abstract

A hydrophilic and temperature-induced degradation drug, vinorelbine bitartrate (VB)-loaded solid lipid nanoparticles (SLNs) were prepared by a cold homogenization technique. The physicochemical properties of the SLNs, with various lipid composition, drug content and altered homogenizing times, were investigated. The mean particle size of the SLNs ranged from 150 to 350 nm. The enhancement of lecithin content in lipid matrix resulted in smaller particle of SLNs. The atomic force microscopy (AFM) images displayed that the shape of the SLNs was irregular sphere with smooth surface. The drug entrapment efficiency (EE) could be improved with the increasing of lecithin or oleic acid content in lipid matrix, and reduced with the added amount of drug. The highest EE and drug loading capacity (DL) could reach up to 80 and 6.6%, respectively. The studies of drug release showed that the drug release could last for 48 h, and the rate was delayed by the addition of lecithin or oleic acid in the formulations. The physical stability experiment indicated that the SLNs were stable for 2 months under room temperature. Moreover, the cellular cytotoxicity of VB against MCF-7 cells could be improved by the entrapment of SLNs.

Keywords: Solid lipid nanoparticles; Vinorelbine bitartrate; Cold homogenization technique; Controlled release; Cellular cytotoxicity

1. Introduction

In recent years, it has become more and more evident that the development of new drugs alone was not sufficient to ensure progress in drug therapy. Exciting experimental data obtained *in vitro* were very often followed by disappointing results *in vivo* because of the insufficient drug concentration due to poor absorption, rapid metabolism and elimination, poor water solubility and high fluctuation of plasma levels due to unpredictable bioavailability after peroral administration. A promising strategy to overcome these problems involves the development of suitable drug carrier systems.

Solid lipid nanoparticles were developed at the beginning of the 1990s as an alternative carrier system to the existing traditional carriers, such as emulsions, liposomes and polymeric nanoparticles. The SLNs combined the advantages of other innovative carrier systems (e.g. physical stability, protection of incorporated labile drugs from degradation, controlled release and excellent tolerability) while at the same time minimizing the associated problems. Lipophilic drugs, with good compatibility with the lipids, have often been selected to incorporate into the SLNs for high drug loading and entrapment efficiency (Westesen et al., 1997; Cavalli et al., 1993; Miglietta et al., 2000; Ugazio et al., 2002). Many methods have been developed to prepare SLNs, such as high pressure homogenization (Lippacher et al., 2000; Lander et al., 2000; Siekmann and Westesen, 1994), solvent emulsification or evaporation (Sjostrom and Bergenstahl, 1992; Siekmann and Westesen, 1996), high speed stirring and ultrasonication (Speiser, 1990; Domb, 1993) and solvent diffusion method (Hu et al., 2005, 2006). The high pressure homogenization technique has emerged as a reliable and powerful technique for preparation of SLNs. In contrast to other techniques, scaling up represented no problem in most cases. Two general approaches of the homogenization step, the hot and the cold homogenization technique, can be used for the production of SLNs. Cold homogenization technique has been developed to overcome the following three problems of the hot homogenization technique (Mehnert and Mader, 2001):

* Corresponding author. Tel.: +86 751 88206742; fax: +86 751 88206742.
E-mail addresses: youjiandoc@zju.edu.cn (J. You), hufq@zju.edu.cn (F.q. Hu).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.07.003

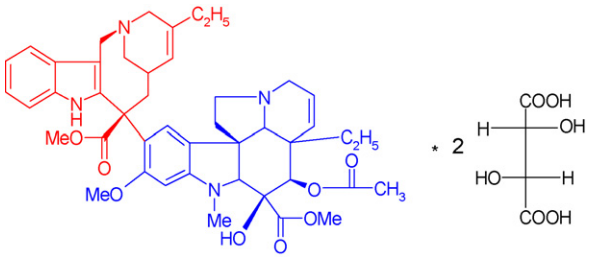

Fig. 1. The chemical structure of vinorelbine bitartrate. The catharanthine moiety of compound was colored red and the vindoline moiety is colored blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

(1) temperature-induced drug degradation;
(2) drug distribution into the aqueous phase during homogenization;
(3) complexity of the crystallization step of the nanoemulsion leading to several modifications and/or supercooled melts.

Vinorelbine bitartrate (VB) is a semi-synthetic vinca alkaloid (the molecular formulation is shown in Fig. 1) that indicates a significant antitumor activity through disrupting microtubule. VB has been shown activity in many tumor types and is currently registered for the treatment of advanced breast cancer (ABC) and non-small cell lung cancer (NSCLC) in most countries (Mano, 2006). Recently, the development and impressive success of the monoclonal antibody trastuzumab for patients with Her-2 overexpressing/amplified ABC (Slamon et al., 2001; Marty et al., 2005) has in a way revived the interest in vinorelbine in this setting. Otherwise, the taxanes associated with significant toxicity also urge researchers to study vinorelbine bitartrate again. However, vinorelbine bitartrate is easy to be degraded on condition of high temperature, and possesses excellent hydrophilicity, which takes so many difficulties in designing its SLNs formulation.

The aim of this study was to assess the feasibility of preparing SLNs loading VB by cold homogenization technique, and the physicochemical properties of obtained VB-loaded SLNs, such as particle size, zeta potential, surface morphology, drug loading capacity, drug entrapment efficiency, *in vitro* drug release behavior and anticancer activity. The effects of composition of lipid materials and charged amount of drug on EE and *in vitro* drug release behavior were investigated in detail.

2. Materials and methods

2.1. Materials

Glyceryl monostearate (GMS) was provided by Shanghai Chemical Reagent Co. Ltd. (China); lecithin E80 and oleic acid were purchased from Lipoid (Germany); Vinorelbine bitartrate was kindly donated from Hangzhou Huadong Pharmaceutical Co. Ltd. (China); Poloxamer 188 (F_{68}) was purchased from BASF (Germany). Methylthiazolyldiphenyltetrazolium bromide (MTT), RPMI-1640 medium, fetal bovine serum, penicillin–streptomycin solution, phosphate-buffered saline (PBS, pH 7.4), trypsin–EDTA solution were purchased from Sigma (USA). Dimethyl sulfoxide (DMSO) and ethanol were analytical reagent grade. De-ionized (DI) water (Millipore) was used throughout the experiments.

2.2. Preparation of VB-loaded SLNs

VB-loaded SLNs were prepared by cold homogenization technique. The desired amounts of GMS were melted with water bath (60 °C). VB, lecithin and oleic acid were dissolved in 1 ml ethanol, and the organic solution was then added into melted GMS by drop-by-drop under 60 °C water bath. The mixture was cooled by pouring the mixture in liquid nitrogen to remove ethanol. The obtained solid dispersion was ground to form microparticles. The microparticles were suspended in 40 ml aqueous phase containing 1% F_{68} and 20% sugar, and homogenized at 15,000 r/min for 0.5 h by a homogenizer (Polytron PT4000, Switzerland), followed by homogenization at 20,000 psi using a high pressure homogenizer (Emulsiflex C-5, AVESTIN, Canada) under an ice-bath condition.

2.3. Characterization of VB-loaded SLNs

2.3.1. Measurement of particle size and zeta potential for VB-loaded SLNs

Particle size and zeta potential of VB-loaded SLNs were measured by photon correlation spectroscopy using Zetasizer (PCS3000, Malvern, English). Samples were diluted appropriately with aqueous solution containing 1% F_{68} and 20% sugar.

2.3.2. Surface morphology observation by AFM

The surface morphology of prepared SLNs was visualized 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. The samples were diluted 10 times with distilled water and then dropped onto freshly cleaved mica plates, followed by vacuum drying during 24 h at 25 °C.

2.3.3. Determination of drug entrapment efficiency

The VB contents were measured by high performance liquid chromatograph (HPLC). The chromatographic system consisted of a Waters 515 HPLC pump equipped with a 20 μl loop. Waters Symmetry C_{18} (5 μm, 3.9 mm × 150 mm) analytical column was used. Detector used was Waters 2487 UV-Dural λ Absorbance Detector operated at 267 nm. Mobile phase was methanol/0.05 M KH_2PO_4 solution (pH 4.5) (60:40), and flow rate was kept at 0.8 ml/min.

The drug entrapment efficiency was calculated from the ratio of the drug amount incorporated into the SLNs to the total charged drug amount. Ultracentrifugation was carried out using Centrisart, which consist of filter membrane (molecular weight cutoff 10,000 Da) at the base of the sample recovery chamber. About 0.5 ml of SLNs dispersion was placed in the outer chamber and the sample recovery chamber placed on top of the sample. The unit was centrifuged at 3500 rpm for 15 min. The SLNs along with encapsulated drug remained in the outer

Table 1A
Composition and characteristics of SLNs for different formulations

Formula	Composition (mg)				Characteristics			
	GMS	L	OA	Drug	Size (nm)	Particle size distribution (nm)	Zeta (mV)	EE (%)
A	200	90	14	14	225.3	One peak: 28.1–445.9	19.6	77.2
B	200	90	14	42	272.6	One peak: 47.0–746.4	21.2	54.7
C	200	90	–	14	158.2	One peak: 35.4–561.4	18.3	54.6
D	200	30	12	12	345.6	Two peaks: 57.9–190.8; 317.3–626.8	18.6	68.3
E	200	30	12	36	319.1	Two peaks: 28.4–35.8; 89.9–714.7	21.9	42.7
F	200	30	–	12	341.7	Two peaks: 116.0–146.0; 581.7–732.4	19.2	53.3

chamber and dispersion medium moved to the sample recovery chamber through filter membrane. The amount of the drugs in the dispersion medium was estimated by HPLC analysis.

2.3.4. Stability studies

VB-loaded SLNs dispersion was stored at room temperature for 2 months under a sealed condition. The mean particle size, zeta potential and drug entrapment efficiency were determined.

2.4. In vitro drug release studies

The drug release from SLNs was performed by using the dialysis bag method. Phosphate buffer (PBS, pH 7.4) was used as dissolution medium. The dialysis bag (molecular weight cutoff 7000 Da) could retain nanoparticles and allow the diffusion of free drug into dissolution media. The bags were soaked in DI water for 12 h before use. A 1 ml of VB-loaded SLNs dispersion was poured into the bag with the two ends fixed by clamps. The bags were placed in a conical flask and 10 ml dissolution media was added. The conical flasks were placed into a thermostatic shaker (HZQ-C; Haerbin Dongming Medical Instrument Factory, Haerbin, China) at 37 °C at a rate of 60 times/min. At 0.5, 1, 2, 4, 8, 12, 24, 36, 48 h after test, the medium in the conical flask was completely removed for analysis and fresh dialysis medium was then added to maintain sink conditions. The drug contents in samples were analyzed by the HPLC method. All the operations were carried out in triplicate.

2.5. In vitro anticancer activity assay

Anticancer activity of VB-loaded SLNs was assayed against MCF-7 breast cancer cell by the MTT method. Control experiments were carried out using the complete growth culture medium only (serving as non-toxic control). MCF-7 cells (1 ml) at a density of 5×10^5 cells/ml were seeded in a 24-well plate in the complete growth culture medium. After culturing for 24 h, the media were exchanged with 1 ml culture medium containing a specified concentration of VB-loaded SLNs, or free drug or blank SLNs dispersion. After specified intervals (4, 8, 12, 24, 36, 48 h), the culture medium from each well was removed and the cells were washed twice with ice-cold PBS. Then, 1 ml of the complete growth culture medium and 60 μl MTT solution (5 mg/ml in PBS) were then added to each well. After 4 h of further incubation, the media were removed and the formazan crystals were solubilized with 1 ml DMSO for 15 min. The amount of formazan was then determined from the optical density at 570 nm by a microplate reader (Tecan GENios). The results were expressed as percentages relative to the result obtained with the non-toxic control.

3. Result and discussion

3.1. Preparation of VB-loaded SLNs

In cold homogenization technique, melted lipid materials containing VB was rapidly cooled by pouring the mixture in liquid nitrogen, which promoted to form a solid dispersion (molecular dispersion) of the drug in lipid matrix (zur Mühlen et al., 1998). The preparation recipes and some properties of the VB-loaded SLNs are listed in Table 1A. For comparison, SLNs of formula A were also prepared by solvent diffusion method, according to literature (Hu et al., 2006). A higher drug entrapment efficiency (close to 80%, Table 1A) of VB-loaded SLNs was obtained by cold homogenization technique, which was higher than that of SLNs produced by solvent diffusion method (only about 50%). The drug molecules dispersed into lipid matrix had less chance to contact with aqueous medium in preparing process by cold homogenization technique, while the drug diffusion into aqueous medium occurred easily in the solvent diffusion process. Fig. 2A and Table 1B showed the effect of cycle times of high pressure homogenization on the particle size of blank SLNs and particle size distribution prepared by cold homogenization technique, respectively. It was clear that the particle size decreased significantly in the initial four cycles, and almost kept constant in the further cycles.

Table 1B
The effect of cycle time on particle size distribution of blank SLNs

Cycle times	Particle size distribution (nm)
1	Three peaks: 59.6–70.7; 196.0–387.1; 1074.0–1789.1
2	Two peaks: 155.7–184.6; 607.1–719.6
3	Two peaks: 146.9–174.2; 572.8–579.0
4	One peak: 37.5–676.7
5	One peak: 52.5–396.9
6	One peak: 59.6–344.8
7	One peak: 80.1–312.4
8	One peak: 51.9–328.9
9	Two peaks: 96.8–114.8; 318.5–377.6
10	Two peaks: 114.7–191.1; 447.2–628.4
11	Two peaks: 90.9–247.3; 369.1–673.0

GMS, L and OA mean glyceryl monostearate, lecithin E80 and oleic acid, respectively. All plots were average values ($n = 3$).

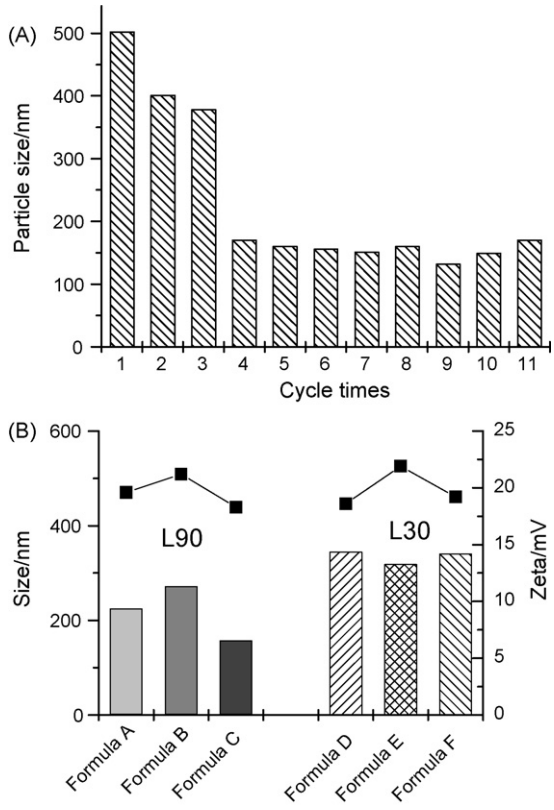

Fig. 2. (A) The effect of cycle times of high pressure homogenization on particle size of SLNs prepared by cold homogenization technique. (B) VB-loaded SLNs: mean particle sizes and zeta potentials. SLNs contain 90 mg lecithin (L90) and 30 mg lecithin (L30). Columns and squares represent particle size and zeta potential, respectively. All plots were average values ($n = 3$).

Particle sizes and zeta potentials of VB-loaded SLNs with different formulas are shown in Table 1A and Fig. 2B. In all formulations, the particle sizes ranged from 150 to 350 nm, and zeta potentials were about +20 mV. The particle sizes of SLNs containing 90 mg lecithin were obviously smaller than those of SLNs containing 30 mg. No obvious difference in zeta potentials of SLNs was found among the formulation with different lipid compositions and drug content. The increasing of lecithin content in SLNs formulations could reduce the melt point of lipid matrix and interface tension between lipid matrix and dispersion medium (aqueous phase), consequently favor the formation of SLNs with smaller particle size (De Labouret et al., 1995). These results were in agreement to general theory of high pressure homogenization (Jenning et al., 2000). However, it was found that the oleic acid (OA) had no significant effect on particle size of SLNs. It may be due to the lower OA content used in formulations.

Zeta potential is a key factor to evaluate the stability of colloidal dispersion. It was currently admitted that zeta potentials above 30 mV were required for full electrostatic stabilization (Ney, 1973). However, many experiments demonstrated that not only electrostatic repulsion dominated the stability of nanoparticles, the use of steric stabilizer also favored the formation of stable nanoparticle dispersion (Cavalli et al., 2000; Heurtault et al., 2003). In these studies, it seemed that the value of zeta potential of VB-loaded SLNs was not sufficient to keep the particles dispersing stably. However, the particle size did not change significantly within 2 months (Table 2), which should contribute to the following two points. One was the zeta potential was still high enough to maintain the particles dispersing uniformly in the system. In general, particles could be dispersed stably when absolute value of zeta potential was above 30 mV due to the electric repulsion between particles. However, the SLNs coating with Poloxamer 188 led to the decrease of zeta potential. As a steric stabilizer, Poloxamer 188 can easily compensate for missing electrostatic repulsion to stabilize the dispersion for long time (Schwarz et al., 1994). The other was the sugar could help to stabilize the dispersion by increasing the viscosity of the dispersion medium like a dispersion agent.

3.2. AFM observation

Atomic force microscopy (AFM) was adapted to investigate the non-hydrated state of SLNs because the sample preparation did not need pre-treatment and the result was closer to that obtained by photon correlation spectroscopy (PCS) than by scanning electron microscopy (SEM) (Dubesa et al., 2003). Fig. 3 indicated the AFM images of VB-loaded SLNs for formula A. The shape of particle were irregular sphere (Fig. 3A) with smooth surface (Fig. 3B) unlike that of lipid nanoparticles containing liquid lipid prepared by solvent diffusion method (Hu et al., 2005), which showed a regular sphere with smooth surface. The difference in shape between the lipid nanoparticles prepared by cold homogenization and solvent diffusion method may be due to the different formation mechanisms of nanoparticles. The cold homogenization technique prepares the nanoparticles owing to the powerful mechanical force and shearing force, which can result in the irregular shape of nanoparticles. While with the solvent diffusion to dispersion medium in solvent diffusion procedure, the lipid matrix has to form the nanoparticles with sphere shape to minimize the surface energy. Fig. 3C indicated that the VB-loaded SLNs had about 120 nm length and width with about 7–10 nm height. The lower height of SLNs could be resulted from the collapse of nanoparticles during the process of AFM samples preparation.

Table 2
Physical stability of formula A ($n = 3$)

	Size (nm)		Distribution (nm)		Zeta (mV)		EE (%)	
	0 day	2 months	0 day	2 months	0 day	2 months	0 day	2 months
Formula A	225.3	212.5	28.1–445.9	38.5–540.2	19.6	16.7	77.2	72.3

Data were average values ($n = 3$).

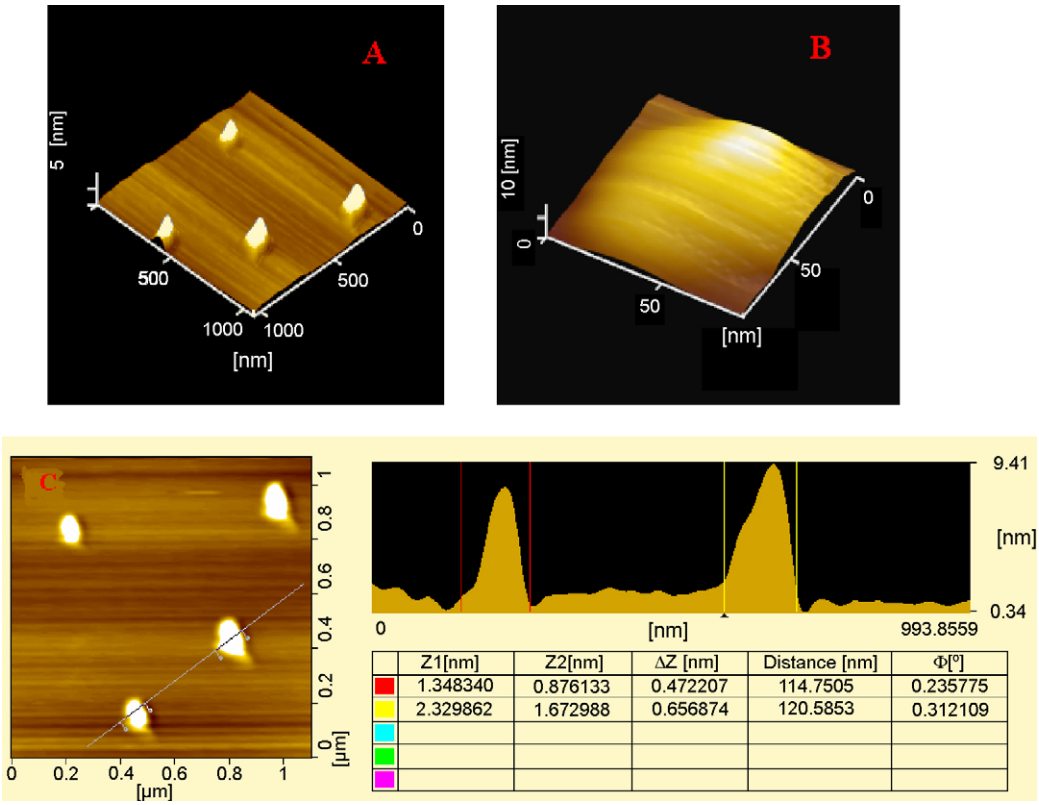

Fig. 3. AFM images of SLNs for formula A: (A) multi-particles; (B) zoom-in of single particle surface; (C) the analysis of particle size.

3.3. Drug entrapment efficiency

The effects of lecithin and OA content in lipid matrix on the drug entrapment efficiency of VB-loaded SLNs were shown in Table 1A. The lecithin and OA played the important roles to improve the drug incorporation. The formulation without OA (formula C and F), the drug entrapment efficiencies were lower than that of formulation with the same lecithin and OA contents (formula A and D), respectively. The incorporation of OA into lipid matrix could increase the solubility of VB in the lipid matrix. Koukoulitsa et al. (2006) had reported that the vinorelbine was amphoteric molecular, which can associate with phospholipid by hydrophobic and hydrophilic interactions between them. The drug entrapment efficiency of the SLNs with OA content enhanced with the increasing of lecithin content, and highest drug entrapment efficiency (77.2%) was achieved in formula A containing 90 mg lecithin and 14 mg OA. This result suggested the complex formation between lecithin and drug. However, for the formula without OA (formula C and F), no clear difference in drug entrapment efficiency was found in the formula with different lecithin content. These results indicated the incorporation of OA into lipid matrix could reduce the viscosity of lipid matrix, and consequently improve the combination between drug and lecithin (Levy et al., 1994). Comparing formula A with B or formula D with E, it was clear that the drug entrapment efficiency decreased with the increasing of the charged amount of drug. The stability test of SLNs under room temperature indicated that the drug entrapment efficiency of formula A had no significant change during 2 months (Table 2).

3.4. In vitro drug release behaviors of VB-loaded SLNs

The *in vitro* drug release profiles of VB-loaded SLNs with different formulations are shown in Fig. 4. All of the drug release could last to 48 h. The VB-loaded SLNs with higher lecithin content and OA (fomula A and B) displayed the slowest drug release rate. The slower drug release rate of the formula with lecithin could also contribute to the complex formation between lecithin and drug. Furthermore, the interaction between drug and lecithin could improve the compatibility of VB with GMS, and so enhanced the residence of the drug in lipid matrix. The VB-loaded SLNs without OA indicated fastest drug release rate (fomula C and F), and no obvious difference was observed in the formulation with different lecithin content. This suggested that the combination between drug and lecithin in this case was very weak. The drug release behaviors of the formulations with higher drug loading were also investigated. It was found that their release behaviors were close to these with lower drug loading, which might contribute to near uniform distribution of the drug in lipid nanoparticles. The drug release behavior of the SLNs prepared by cold homogenization technique was also compared with that of SLNs produced by solvent diffusion method (Fig. 4B). The SLNs produced by solvent diffusion method indicated faster drug release with a burst release. This may be caused by the formation SLNs with drug-enriched shell due to the solvent diffusion during the preparing process. Table 3 displayed the difference of particle sizes before and after release experiments. The particle sizes of all formulations decreased obviously after release experiments, which demonstrated that

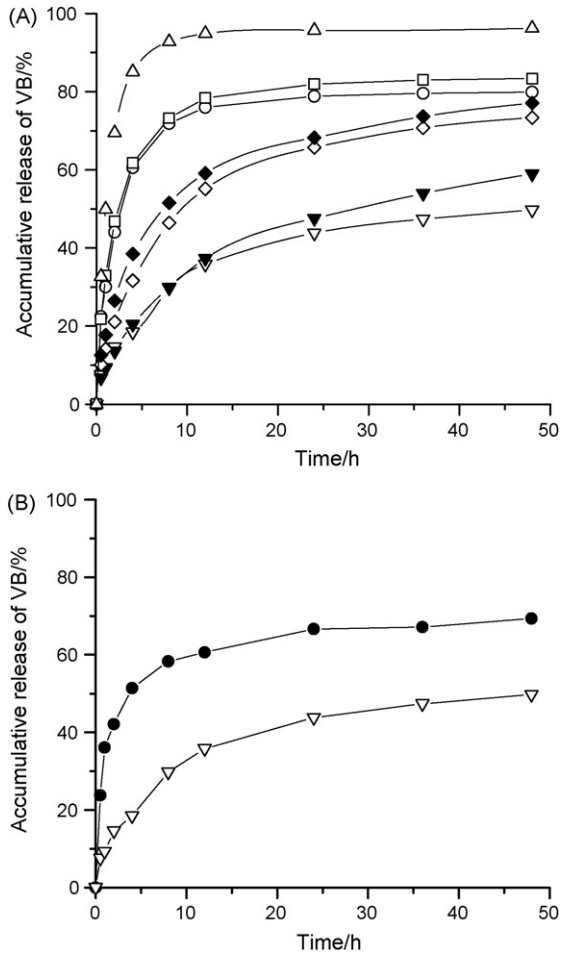

Fig. 4. (A) *In vitro* drug release profiles for different formulations: (△) free drug; (▽) formula A; (▼) formula B; (◯) formula C; (◇) formula D; (◆) formula E; (□) formula F. (B) *In vitro* drug release profiles for formula A, prepared by cold homogenization technique (▽) and by solvent diffusion method (●). All plots were average values ($n = 3$).

the corrosion of lipid materials was one of the drug release mechanism.

3.5. In vitro anticancer activity assay

The cellular cytotoxicity assays of the blank SLNs and VB-loaded SLNs were carried out using MCF-7 breast cancer cell as a model cell. The inhibition and viability of cells were evaluated by MTT assay. Fig. 5 shows the cellular inhibitions of free VB

Table 3
Particle sizes before and after release experiment

Formula	Before release experiment (nm)	After release experiment (nm)
A	225.3	173.4
B	272.6	185.9
C	158.2	147.2
D	345.6	285.6
E	319.1	240.8
F	341.7	296.0

Data were average values ($n = 3$).

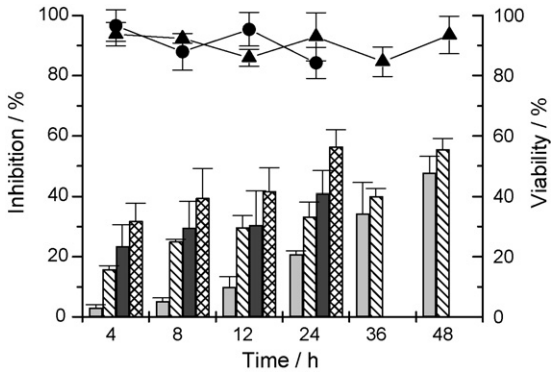

Fig. 5. The cellular inhibition of 10 μg/ml free drug, SLNs containing 10 μg/ml VB, 20 μg/ml free drug and SLNs containing 20 μg/ml VB and the cellular viability of 250 μg/ml blank SLNs and 500 μg/ml blank SLNs: (▒) free drug 10 μg/ml; (▨) drug-loaded SLNs 10 μg/ml; (■) free drug 20 μg/ml; (▩) drug-loaded SLNs 20 μg/ml; (▲) blank SLNs 250 μg/ml; (●) blank SLNs 500 μg/ml. All plots were average values ($n = 3$).

and VB-loaded SLNs and the cellular viability of blank SLNs with different concentration and incubation time. Even the cells were incubated with 500 μg blank SLNs for 24 h, the cellular viability was above 80%. This result suggested that the blank SLNs had lower cellular cytotoxicity. Comparing with free VB, the cellular cytotoxicity of VB-loaded SLNs increased significantly at the same incubation condition, which should contribute to the increased intracellular drug concentration via the transport of SLNs, as the reported (Chawla and Amiji, 2003). As we can see from Fig. 5, the cell inhibition increased with increasing concentration of VB-loaded SLNs or free VB.

4. Conclusion

The SLNs loading hydrophilic drug (VB) was successfully prepared by cold homogenization technique. The *in vitro* drug release behavior and drug entrapment efficiency could be adjusted through altering the amount of lecithin and OA. OA played important role in drug release behavior and drug entrapment efficiency of the SLNs. In the presence of OA, the incorporated lecithin could combine with drug by hydrophobic and hydrophilic interactions, consequently improved the drug entrapment efficiency, and delayed the drug release rate. The highest 6.6% VB loading was achieved in present method. The cellular cytotoxicity tests demonstrated that the blank SLNs had low cellular cytotoxicity, and the cellular cytotoxicity of VB can be enhanced significantly by the entrapment of lipid matrix.

References

Cavalli, R., Caputo, O., Gasco, M.R., 1993. Solid lipospheres of doxorubicin and idarubicin. Int. J. Pharm. 89, 9–12.

Cavalli, R., Caputo, O., Gasco, M.R., 2000. Preparation and characterization of solid lipid nanospheres containing paclitaxel. Eur. J. Pharm. Biopharm. 10, 305–309.

Chawla, J.S., Amiji, M.M., 2003. Cellular uptake and concentrations of tamoxifen upon administration in poly(epsilon-caprolactone) nanoparticles. AAPS Pharmsci. 5, 1–7.

De Labouret, A., Thioune, O., Fessi, H., et al., 1995. Application of an original process for obtaining colloidal dispersions of some coating polymers.

Preparation, characterization, industrial scaling up. Drug Dev. Ind. Pharm. 21, 229–241.

Domb, A.J., 1993. Lipospheres for controlled delivery of substances. United States Patent USS 188,837.

Dubesa, A., Parrot-Lopeza, H., Abdelwahed, W., et al., 2003. Scanning electron microscopy and atomic force microscopy imaging of solid lipid nanoparticles derived from amphiphilic cyclodextrins. Eur. J. Pharm. Biopharm. 55, 279–282.

Heurtault, B., Saulnier, P., Pech, B., et al., 2003. Physico-chemical stability of colloidal lipid particles. Biomaterials 24, 4283–4300.

Hu, F.-Q., Jiang, S.-P., Du, Y.-Z., Yuan, H., et al., 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., Mäder, K., Gohla, S.H., 2000. Solid lipid nanoparticles (SLN) based on binary mixtures of liquid and solid lipids: a ^{1}H NMR study. Int. J. Pharm. 205, 15–21.

Koukoulitsa, C., Kyrikou, I., Demetzos, C., Mavromoustakos, T., 2006. The role of the anticancer drug vinorelbine in lipid bilayers using differential scanning calorimetry and molecular modeling. Chem. Phys. Lipids 144, 85–95.

Lander, R., Manger, W., Scouloudis, M., Ku, A., Davis, C., Lee, A., 2000. Gaulin homogenization: a mechanistic study. Biotechnol. Progr. 16, 80–85.

Levy, M.Y., Schutze, W., Fuhrer, C., et al., 1994. Characterization of diazepam submicron emulsion interface: role of oleic acid. J. Microencapsulation 11, 79.

Lippacher, A., Muller, R.H., Mader, K., 2000. Investigation on the viscoelastic properties of lipid-based colloidal drug carriers. Int. J. Pharm. 196, 227–230.

Mano, M., 2006. Vinorelbine in the management of breast cancer: new perspectives, revived role in the era of targeted therapy. Cancer Treat. Rev. 32, 106–118.

Marty, M., Cognetty, F., Maraninchi, D., et al., 2005. Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. J. Clin. Oncol. 23, 4265–4274 (Epub. May 23, 2005).

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

Miglietta, A., Cavalli, R., Bocca, C., Gabriel, L., Gasco, M.R., 2000. Cellular uptake and cytotoxicity of solid lipid nanospheres (SLN) incorporating doxorubicin or paclitaxel. Int. J. Pharm. 210, 61–67.

Ney, P., 1973. Zetapotentiale and flotierbarkeit von mineralien. Springer, New York.

Schwarz, C., Mehnert, W., Lucks, J.S., Muller, R.H., 1994. Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. J. Control. Release 30, 83–96.

Siekmann, B., Westesen, K., 1994. Melt-homogenized solid lipid nanoparticles stabilized by the nonionic surfactant tyloxapol. I. Preparation and particle size determination. Pharm. Pharmacol. Lett. 3, 194–197.

Siekmann, B., Westesen, K., 1996. Investigations on solid lipid nanoparticles prepared by precipitation in o/w emulsions. Eur. J. Pharm. Biopharm. 43, 104–109.

Sjostrom, B., Bergenstahl, B., 1992. Preparation of submicron drug particles in lecithin-stabilized o/w emulsions. I. Model studies of the precipitation of cholesteryl acetate. Int. J. Pharm. 88, 53–62.

Slamon, D.J., Leyland-Jones, B., Shak, S., et al., 2001. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N. Engl. J. Med. 344, 783–792.

Speiser, P., 1990. Lipidnanopellets als Tragersystem fur Arzneimittel zur peroralen Anwendung. European Patent EP 0167825.

Ugazio, E., Cavalli, R., Gasco, M.R., 2002. Incorporation of cyclosporine A in solid lipid nanoparticles. Int. J. Pharm. 241, 341–344.

Westesen, K., Bunjes, H., Koch, M.H.J., 1997. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J. Control. Release 48, 223–236.

zur Mühlen, A., Schwarz, C., Mehnert, W., 1998. Solid lipid nanoparticles (SLN) for controlled drug delivery–drug release and release mechanism. Eur. J. Pharm. Biopharm. 45, 149–155.