Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 350 (2008) 338–343
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

# Preparation and characterization of intravenously injectable nimodipine nanosuspension

Ruolan Xiong, Weigen Lu *, Jun Li, Peiquan Wang, Rong Xu, Tingting Chen

*Division of Pharmaceutics, Shanghai Institute of Pharmaceutical Industry, ZhongShanBeiYi Road 1111, Shanghai 200437, China*

Received 13 April 2007; received in revised form 25 July 2007; accepted 26 August 2007
Available online 31 August 2007

**Abstract**

The purpose of this study was to develop an alternative, improved and better tolerated injectable nimodipine nanosuspension compared with commercially available ethanol solution. In this study, nimodipine nanosuspension was prepared by high-pressure homogenization (HPH). The effects of the production parameters such as pressure, cycle numbers and crushing principles on the mean particle size, 99% diameter and polydispersity of the nanosuspension were investigated. Characterization of the product was performed by scanning electron microscope (SEM) and differential scanning calorimeter (DSC). The safety of the nimodipine nanosuspension was discussed with special attention to contamination by microparticles and the increase in saturation solubility $C_s$. Irritability study in rabbits showed that this formulation provided less local irritation and phlebitis risks than the commercial ethanol product, which represented a promising new drug formulation for intravenous therapy of subarachnoid hemorrhage (SAH)-related vasospasm.


*Keywords:* Nimodipine; Nanosuspension; High-pressure homogenization; Crystal form; Vascular irritation

## 1. Introduction

Nimodipine is presently the only available therapy that could efficiently reduce the morbidity and mortality associated with delayed ischemic deficits in patients with subarachnoid hemorrhage (SAH)-related vasospasm. In clinical practice, the standard method of dosing calls for a 60 mg oral dose of nimodipine to be given every 4 h for 21 days (Toyota, 1999). The frequency of this dosing regimen reflects the low bioavailability of orally administered nimodipine due to the high first-pass metabolism in the liver. Pharmacokinetic studies showed that the bioavailability of orally administered nimodipine is between 2% and 28% in SAH patients (Vinge et al., 1986). Intravenous administration is an alternative to oral administration which could provide greater bioavailability than oral dosing. However, because of the low intrinsic solubility which is only 2.29 μg/ml (tested in the preformulation study, at 37 ± 0.1 °C), the clinical formulation for the intravenous administration of nimodipine contains about 40% solvent mixture: 23.7% (v/v) ethanol and 17% (v/v) PEG400, to achieve a sufficient nimodipine concentration. Furthermore, this formulation requires a time-consuming administration (infusion pump worked for about 10 h) accompanied with special equipments and nurse care (Lin and Zheng, 2000). With the existence of alcohol in the formulation, the patients would suffer from great pain and local irritation, and in some worse circumstance cause extreme phlebitis during infusion.

Recently following the swift development of high-pressure homogenization technology, preparation of nanosuspension is an attention attractive alternative for the i.v. administration of poorly soluble drugs. This cost-effective and technically simple approach is shown to yield a more physically stable and safer product than solvent mixtures products (Müller et al., 2001; Keck and Müller, 2006; Möschwitzer et al., 2004). There are several techniques for producing drug nanocrystals. Basically, one could differentiate the principals as top down and bottom up technologies. Typically, the drug nanocrystals are generated in an aqueous dispersion medium (e.g., by precipitation or a disintegration process), which should contain certain surfactant or polymer (e.g., poloxamer 188) to ensure the stability of drug colloidal system. Therefore, the nanosuspension could provide a better alternative to reformulate the existing drug formulations

* Corresponding author. Tel.: +86 21 55514600x108; fax: +86 21 65420806.
*E-mail address:* sipiluwg@163.com (W. Lu).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.08.036

without toxicologically less favorable excipients. The nanosuspension formulation is supposed to reduce the irritation and improve the compliance of patients.

This paper focused on the production of nimodipine nanosuspension by high-pressure homogenization, investigated the characteristics of the nimodipine nanosuspension, and compared the intravenous irritation of nimodipine nanosuspension with that of the ethanol solution (Nimotopss, Bayer).

## 2. Materials and methods

### 2.1. Materials

Nimodipine was purchased from Shandong Xinhua Pharmaceutical Factory, China. Poloxamer 188 was kindly given by BASF, Germany. Sodium deoxycholate and mannitol were purchased from Sinopharm Chemical Reagent Co. Ltd., China. Polysorbate 80 was obtained from Beijing Fengli Jingqin Commerce and Trade Co. Ltd., China. Nimotop was produced by Bayer, Germany. Lipid Emulsion 10% and 20% was given by Sichuan Shule Pharmaceutical Company, China. All other reagents were of the highest grade commercially available.

### 2.2. Preparation of nimodipine nanosuspension

The nimodipine coarse powder was firstly disintegrated into microparticles by fluid jet mill technology using MC One (Jetpharma SA, Switzerland). Then the nimodipine jet-milled powder (0.5%, w/v) was dispersed in an aqueous surfactant solution, containing 0.6% (w/v) poloxamer 188, 0.4% (w/v) sodium cholic acid and 4.0% (w/v) mannitol under magnetic stirring. The obtained pre-mixture was homogenized using three types of high-pressure homogenizers, including Microfluidizer processor M-110EH (MFIC, USA), Niro-Soavi NS1001L (ATS Co. Ltd., Italy), and EmulsiFlex C3 (Avestin Inc., Canada). At first, 200 bar with 2 cycles and then 500 bar with 5 cycles as a kind of pre-milling were applied, then 15–20 cycles at 1500 bar were run. All operations were carried out using a heat exchanger to maintain sample temperature at 25–30 °C.

For long-term stability, the nimodipine nanosuspension was lyophilized. Drying lasted for 15 h at −15 °C, with a secondary step of 3 h at −5 °C and the last step of 2 h at 20 °C The pressure during the dying was below 200 mTorr (Advantage, Virtis, USA). The final formulation was sterilized by γ-ray radiation. The absorbed dose of γ-ray was 12 kGy, and the radiation time lasted for 6 h. As nimodipine was a highly photolabile compound, all operations were performed under subdued light.

### 2.3. Particle size analysis

Particle size analysis was performed by photon correlation spectroscopy (PCS) using Nicomp 380/ZLS (PSS, Santa Barbara, CA, USA) and single-particle optical sensing (SPOS) using AccuSizer 780 Optical Particle Sizer (PSS, Santa Barbara, CA, USA). The diameters were calculated using volume distribution from Gaussian distribution. Diameters 99% mean that 99% of the particles were below the given size. The polydispersity index (PI) calculated from Gaussian distribution indicated the width of a particle distribution (e.g., 0.0 for a narrow, >0.3 for a very broad distribution). The additional analysis by SPOS provided the absolute number of particles per volume unit for the different size classes (>1 μm, >2 μm, and >5 μm) which was used to control particles that may lead to capillary blockade and embolism. The average values from three runs were used.

### 2.4. Morphology and crystal structure research

Morphological evaluations of nimodipine powder after jet milling and HPH were conducted through optical microscope (OM, Olympus-BX41, Japan) and SEM (JSM-6360LV, Japan). SEM samples were coated with gold under 20 mA for 80 s and the voltage was set at 20 kV. Thermal properties of the powder samples were investigated with DSC 7 (Perkin-Elmer, USA). Samples of about 5 mg were placed in standard aluminum pans and sealed with a lid. Heating scans by heat runs for each sample was set from 50 °C to 450 °C at 10 °C/min with a nitrogen purge of 20 ml/min.

### 2.5. Saturation solubility research

Retrodialysis method was used to quantify the increase in $C_s$ of nimodipine nanocrystals (particle size about 700 nm and 300 nm, respectively) compared with that of the nimodipine raw crystals (30–100 μm) and jet-milled microcrystals (1–10 μm). Nanocrystals with 700 nm particle size were centrifuged at 9000 rpm to get much smaller crystals with 300 nm. All the nimodipine crystals used for $C_s$ test were dispersed in the aqueous solution containing 0.6% (w/v) poloxamer 188, 0.4% (w/v) sodium cholic acid and 4.0% (w/v) mannitol. The bag filters containing 1 ml purified water were put into 10 ml suspension mentioned above. The containers were then placed in a water bath at a temperature of 37 ± 0.1 °C and shook at 150 $min^{-1}$. After 3 days, the solutions in the bag filters were drawn out and analyzed by HPLC (Agilent, USA). The mean of three values was used.

### 2.6. Vascular irritability study in rabbits

Experiments involving rabbits were approved by the Ethics Committee of Shanghai Institute of Pharmaceutical Industry (SIPI). The vascular irritability of nimodipine nanosuspensions was examined in New Zealand rabbit (weight 2.5–3.0 kg) in comparison with ethanol solution (Nimotop, Bayer) and physiological saline. Six rabbits were divided into two groups. One group of three rabbits was given nimodipine nanosuspension, and the other group was given Nimotop. Each animal was given 0.4 mg/kg test agents (nimodipine nanosuspensions or Nimotop) via the marginal ear vein at the right ear and physiological saline at the left one. The infusion was lasted for 5 consecutive days. Visual observations of the appearance of the veins were made at intervals until 24 h, and then the rabbits were sacrificed. After pathological section, the histopathological changes were observed and discussed.

## 3. Results and discussion

### 3.1. Process parameters—homogenization principles

In this research, three kinds of homogenizers with different crushing principles including microfluidisation and piston-gap were compared in preparing the nimodipine nanosuspension. The mean particle size and diameter 99% of nimodipine nanosuspensions prepared in the three homogenizers with same operation procedures (1500 bar × 20 cycles) were shown in Fig. 1. Obviously, the nimodipine nanosuspensions prepared by Niro-Soavi had the minimum particle size and smallest width of the size distribution; while the products from the Microfluidizer processor provided the maximum particle size and PI. Actually, Microfluidizer processor belonged to the microfluidisation principle. The suspension was accelerated and passed in an especially designed homogenization chamber with a high velocity. In the 'Z'-type chamber, the flow direction of suspension changed a few times leading to particle collision and shear forces. As the pressure and velocity in narrow chamber (about 70 μm in diameter) were both increased, the shear force increased dramatically. And at the turn of the chamber, the particles with high velocity collided frontally with the diamond chamber wall. However, the shear force as the mainly crushing force in Microfluidizer processor was especially suitable for the materials with flexibility such as emulsions but not for hard ones (e.g., drug crystals) according to our obtained experiences.

The other two kinds of homogenizer (Niro-Soavi and EmulsiFlex) both belonged to the piston-gap homogenizers. Prior to entering the gap, the suspension was contained in a cylinder with a relatively large diameter compared with the width of the following gap. According to the Bernoulli law, the liquid started boiling when entering the gap where the reduction of diameter led to a tremendous increase of the dynamic pressure and a simultaneous decrease of the static pressure. And the gas

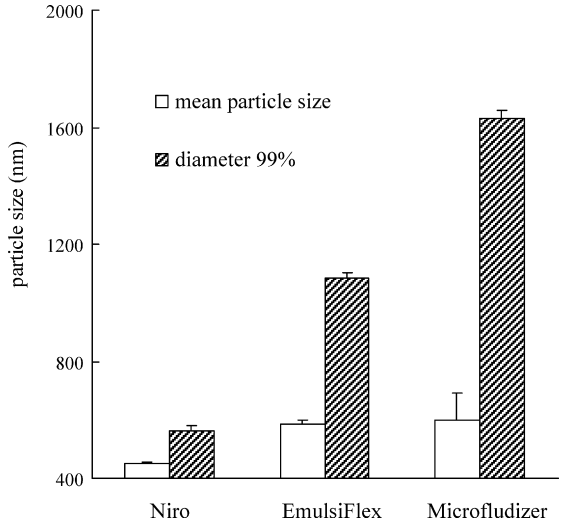


Fig. 1. The influence of homogenization equipments on the reduction of nimodipine nanosuspension. The mean particle size and diameter 99% as a function of different kinds of homogenizer including Niro-Soavi, EmulsiFlex and Microfluidizer ($n = 3$).

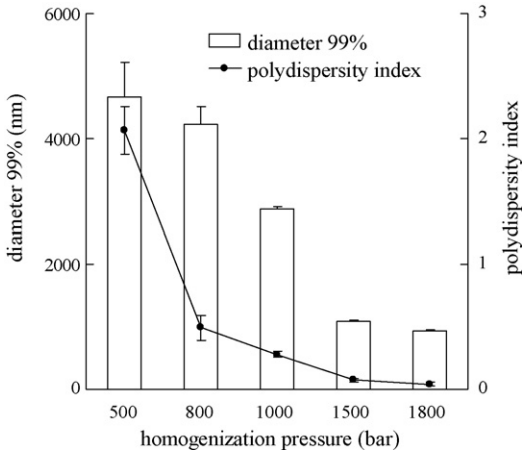


Fig. 2. The influence of homogenization pressure on the reduction of the nimodipine nanosuspension. Diameter 99% and polydispersity index as a function of the pressure using EmulsiFlex C3 ($n = 3$).

bubbles would implode (so-called cavitation) after leaving the narrow gap which caused particle disintegration. In addition, the collision and shear force also existed in the homogenization gap. For nimodipine, the cavitation was a more powerful and suitable crushing force for the preparation of nanocrystals compared with the shear forces in the Microfluidizer processor.

### 3.2. Process parameters—homogenization pressure and cycles

The influence of homogenization pressure on the reduction of the nimodipine microparticles was shown in Fig. 2. Applying a low homogenization pressure of 500 bar with 20 cycles lead to a decrease in the diameter 99% to 4.7 μm with PI ≫ 0.3. As the pressure increased from 800 bar to 1500 bar with the same cycle numbers, the particle size became much smaller. The homogenization pressure simultaneously narrowed the width of the size distribution, i.e. reduced the PI of the bulk population. Therefore, to obtain a higher monodispersity (smaller PI) the homogenization pressure needed to increase from 500 bar to 1500 bar. However, experiments with pressure increased up to 1800 bar failed to provide further decrease in particle size. This could be explained as that with decreased particle size, the remained crystals became more and more perfect. During a milling process, the crystals broke preferentially at weak points, which also meant imperfection. Thus, the force required to break the crystals increased rather exponentially with the decreasing of particle size. From this, for nimodipine particle with a given perfection of the crystal structure and hardness, only a certain size (about 450 nm) could be achieved when applying realistic production conditions (Keck and Müller, 2006).

To avoid blockage of homogenizer gap and chamber, the nimodipine coarse powder was homogenized respectively at 200 bar × 2 cycles and 500 bar × 5 cycles as a pre-milling. Then the homogenization pressure was increased to 1500 bar. The influence of cycle numbers at 1500 bar on the reduction of the microparticles was shown in Fig. 3. The mean particle size decreased clearly up to cycle number 5 and remained unchanged

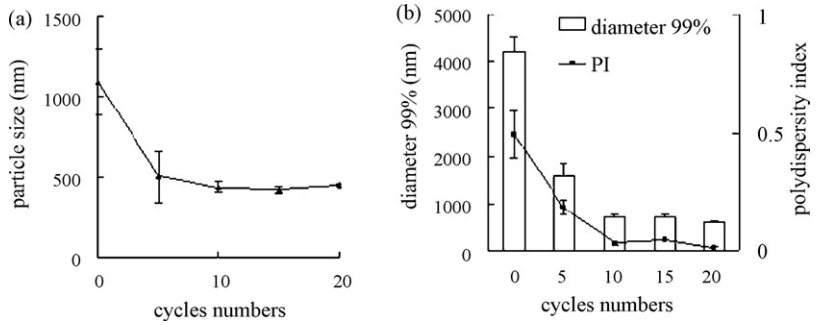


Fig. 3. The influence of cycle numbers at 1500 bar on the reduction of the nimodipine crystals using Niro-Soavi ($n = 3$). (a) The mean particle size (volume distribution from Gaussian distribution); (b) diameter 99% and PI.

when the cycle number was raised to 20. However, the diameter 99% and PI further decreased during 10–20 cycles, which elucidated that the nanocrystals could not become smaller, but could become more uniform during these cycles. This could be explained from the non-uniform power density distribution in the piston-gap of the homogenizer. There were zones of very high density, medium density and low density resulting from turbulent flow in the gap, the particles could be uniformly disintegrated only in the zone of medium power density. In the zone of low power density, some large particles could 'survive' in the homogenization cycles. With the increasing of cycles, those survived large particles obtained more chances to be disintegrated in the medium power density and caused the decreasing of the diameter 99% and PI values but without significant influence on the mean diameters (Keck and Müller, 2006).

### 3.3. Minimization of microparticulate content

According to 2005 Chinese Pharmacopeia for injectable emulsion, the number of particles larger than 1 μm should be less than 10%, and the maximum particle size should be below 5 μm. After all, the smallest size of blood capillaries was 5–6 μm which was so thin that blockade and embolism would probably happen for a number of large particles. However, it was still difficult to assess the maximum tolerable limit of nanocrystals in blood vessel (e.g., tolerable number as a function of microparticle size). It appeared more sensible to compare the microparticulate content of our prepared nimodipine nanosuspensions with that of a commercially available emulsion which was clinically intravenous administered chronically in large volumes, i.e. parenteral nutrition emulsion was intravenously administered for weeks even months in daily volume of 0.5 l or more (Peters et al., 2000). Fig. 4 depicts the number of particles (>1 μm, >2 μm, and >5 μm) per μl in the nimodipine nanosuspension (clinical content) compared with lipid emulsion 10% and 20%. The nimodipine nanosuspension had a considerably fewer number of big particles (>1 μm, >2 μm, and >5 μm) than the lipid emulsion (10% and 20%). Of course, the oil droplets were flexible and might therefore pass smaller capillaries. In addition, the emulsion would be metabolized by lipase within 3–4 h thus reducing the risk of embolism. Although the nanosuspension had fewer big particles, the nanocrystals were rigid and the risk for embolism should be paid special attention. While according to the Ostwald–Freundlich equation (Müller and Peters, 1998), the saturation solubility $C_s$ increased with particle size surface area increasing. Fig. 5 shows that $C_s$ of nimodipine doubled after entering the nanometer range. The increase in $C_s$ led to an increase in dissolution velocity ($dc/dt$) according to Noyes–Whitney theory (Müller and Peters, 1998). So once the nanoparticles could swiftly dissolve in the blood or into much smaller particles in several minutes, the risk of embolism would greatly decrease. Moreover, the safety of this formulation was also confirmed in animal studies: repeated infusions of nimodipine nanosuspension for 5 consecutive days at a dose of 0.4 mg/kg were well tolerated by rabbits without any discernible untoward side-effects.

### 3.4. Morphology and crystal form evaluation

SEM micrographs clearly showed the great differences between nimodipine microparticles and nanosuspensions (Fig. 6). The microparticles were found to be very large and especially irregular (Fig. 6a). While after HPH, big particles disappeared and the drug became small and uniform which was crucial for intravenous safety (Fig. 6b). However, the nanocrystals seem to be more rounded, perhaps because the particles were coated with a surfactant layer. In the suspension solution,

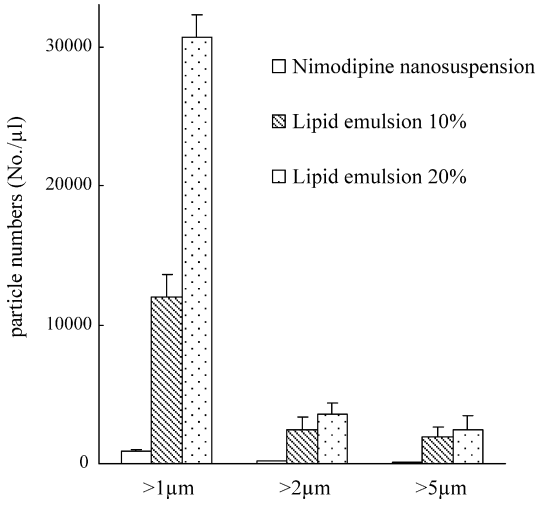


Fig. 4. Number of particles of various sizes in nimodipine nanosuspension, Lipid Emulsion 10% and 20% ($n = 3$).

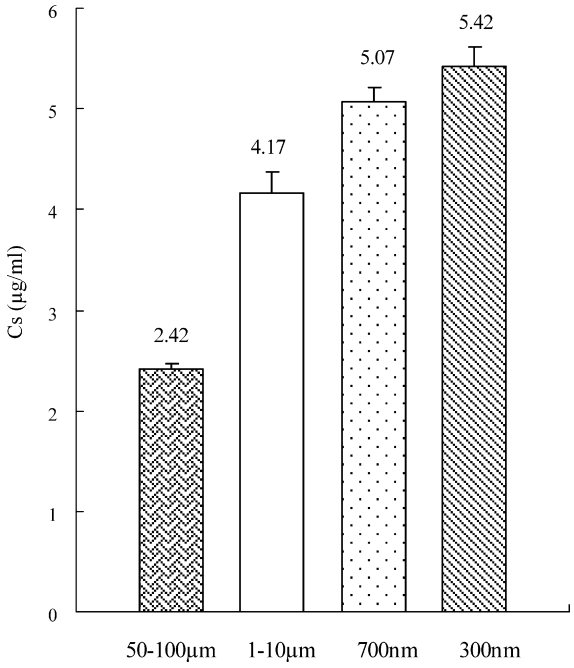


Fig. 5. The influence of particle size on nimodipine saturation solubility ($n = 3$).

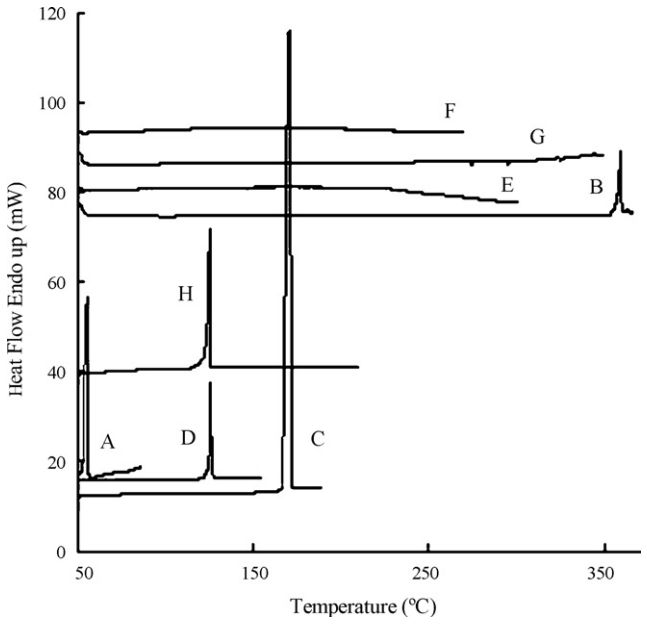


Fig. 7. DSC thermograms for (A) poloxamer 188, (B) sodium deoxycholate, (C) mannitol, (D) raw nimodipine, (E) freeze-dried excipients, (F) freeze-dried nanosuspension, (G) physical mixtute (mortar), and (H) pure nimodipine nanoparticles.

the surfactants used to stabilize the particles would adsorb to surface of the crystals by hydrophobic interaction. Therefore, after freeze-drying, the solidification of surfactants formed an amorphous layer on the surface of inner crystals (Müller and Jacobs, 2002).

On the other hand, the results of DSC (Fig. 7) also showed that the crystal form of the drug did not change during HPH preparation procedure. The poloxamer 188, sodium cholic acid and mannitol all had their visible melting points (A–C), but the melting point of the excipients disappeared after freeze-dried (E) which elucidated they formed amorphism. Moreover, the raw materials of nimodipine (D) had a typical melting point at 125.2 °C which represented that nimodipine raw materials belonged to polymorphic form I (Papageorgiou et al., 2006; Murali Mohan Babu et al., 2002). Both freeze-dried nimodipine nanocrystals and the physical mixture of drug and surfactants failed to give conspicuous melting point of nimodipine (F and G). After we removed the surfactants and gained the pure nanocrystals which was carefully washed, filtered by suction filtration and dried gently at 30 °C and 0.1 atm with anhydrous $CaCl_2$ for about 6 h, we found that the pure nimodipine nanocrystals did show a sharp absorption peak at 125.2 °C (H), which could be explained that nimodipine crystals dissolved gradually in the melted poloxamer 188 before reaching 125.2 °C (Lu, 2002). So obviously the HPH process did not change the crystal form of nimodipine with the reducing of particle size.

### 3.5. Vascular irritability study in rabbits

The rabbit ear vein irritation test was used to obtain a better appreciation of the level of irritation reduction due to drug delivery as nanosuspension compared with nimodipine ethanol solution. Deep respiration of animals occurred immediately following nimodipine ethanol solution infusion and lasted about 30 min post dosing, which were not observed in nimodipine nanosuspension group. And the rabbits struggled more frequently during infusion of Nimotop than infusion nanosuspension and saline. It must be attributed to high concentration of ethanol in the formulation. Macroscopical observation

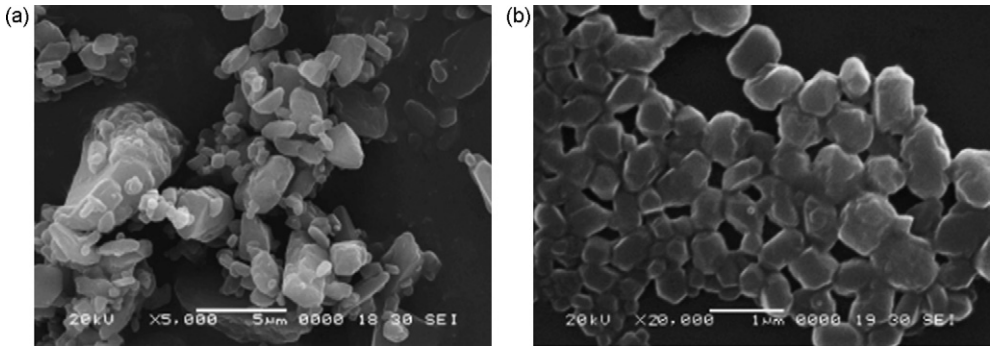


Fig. 6. SEM micrographs of nimodipine microparticles after jet mill (a) and freeze-dried nanosuspension (b).

revealed that the irritative activity of ethanol solution in blood vessels was great different from that of nanosuspension and saline, because the former induced the visible hemorrhage in the injection sites. Moreover, the histopathological damages with ethanol solution, including hemorrhage, congestion and edema in interstitium and slight necrosis in tissue, were observed microscopically.

Of course, it is reasonable to explain those irritative activities were caused by ethanol. But I think we cannot completely exclude the possibility of leaky damage. Especially when considering long-term of dosage regimen for about 10 h of treatment for SAH (Laslo et al., 2004), the high percentage of ethanol would inevitably cause great pain which would lead to the struggle of patients. Therefore, the ethanol in Nimotop could cause the vascular irritation and leaky damage, while the reformulation of nimodipine as nanosuspension would effectively remove the undesired side-effects caused by cosolvents.

## 4. Conclusions

It had been shown that high-pressure homogenization could be used to formulate nimodipine suspensions with crystals in the nanometer range. The particle size of nimodipine nanosuspension was decided by production pressure, number of cycles, crushing principles and the hardness of drug itself. Furthermore, the shape and crystal form as practical functions of the drug were researched.

The number of large particles in the nimodipine nanosuspension was much fewer than that of the fat emulsions for parenteral nutrition. And the saturation solubility was increased as the reduction of particle size into nanometer range, which led to the fast dissolution of drug nanocrystals. Therefore, the aqueous nanosuspension might be a good choice for intravenously administrating poor soluble nimodipine, which is proved to have the lower intravenous irritation and incidence of phlebitis than ethanol product.

## References

Keck, M.C., Müller, R.H., 2006. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization. Eur. J. Pharm. Biopharm. 62, 3–16.

Laslo, A.M., Eastwood, J.D., Urquhart, B., Lee, T.Y., Freeman, D., 2004. Subcutaneous administration of nimodipine improves bioavailability in rabbits. J. Neurosci. Methods 139, 195–201.

Lin, X.H., Zheng, X.P., 2000. Intravenous dosage regimen of Nimotop. J. Yichun Med. Coll. 12, 203–204.

Lu, B., 2002. New Techniques and New Dosage Forms of Drug, first ed. People's Medical publishing House, Beijing.

Möschwitzer, J., Achleitner, G., Pomper, H., Müller, R.H., 2004. Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. Eur. J. Pharm. Biopharm. 58, 615–619.

Müller, R.H., Jacobs, C., 2002. Buparvaquone mucoadhesive nanosuspension: preparation, optimisation and long-term stability. Int. J. Pharm. 237, 151–161.

Müller, R.H., Peters, K., 1998. Nanosuspension for the formulation of poorly soluble drugs I. Preparation by a size-reduction technique. Int. J. Pharm. 160, 229–237.

Müller, R.H., Jacobs, C., Kayser, O., 2001. Nanosuspensions as particulate drug formulation in therapy rationale for development and what we can expect for the future. Adv. Drug Deliv. Rev. 47, 3–19.

Murali Mohan Babu, G.V., Prasad, Ch.D., Ramana Murthy, K.V., 2002. Evaluation of modified gum karaya as carrier for the dissolution enhancement of poorly water-soluble drug nimodipine. Int. J. Pharm. 234, 1–17.

Papageorgiou, G.Z., Bikiaris, D., Karavas, E., Politis, S., Docoslis, A., Park, Y., Stergiou, A., Georgarakis, E., 2006. Effect of physical state and particle size distribution on dissolution enhancement of nimodipine/PEG solid dispersions prepared by melt mixing and solvent evaporation. AAPS J. 8, E623–E631.

Peters, K., Leitzke, S., Diederichs, J.E., Borner, K., Hahn, H., Müller, R.H., Ehlers, S., 2000. Preparation of a clofazimine nanosuspension for intravenous use and evaluation of its therapeutic efficacy in murine *Mycobacterium avium* infection. J. Antimicrob. Chemother. 45, 77–83.

Toyota, B.D., 1999. The efficacy of an abbreviated course of nimodipine in patients with good-grade aneurysmal subarachnoid hemorrhage. J. Neurosurg. 90, 203–206.

Vinge, E., Andersson, K.E., Brandt, L., Ljunggren, B., Nilsson, L.G., Rosendal-Helgesen, S., 1986. Pharmacokinetics of nimodipine in patients with aneurysmal subarachnoid haemorrhage. Eur. J. Clin. Pharmacol. 30, 421–425.