In vitro dissolution enhancement of micronized l-nimodipine by antisolvent re-crystallization from its crystal form H

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In order to enhance solubility and dissolution rate in water, micronized l-nimodipine (NMD) has been successfully prepared by antisolvent re-crystallization process using acetone as solvent and deionized water as antisolvent. The effects of five experimental parameters on the mean particle size (MPS) of NMD nanosuspension were investigated. It was found that the MPS of NMD nanosuspension decreased significantly when the concentration of NMD–acetone solution increased from 50 to 150 mg/mL along with the increase of volume ratio of antisolvent to solvent from 1 to 3, and then increased slightly with the following increase of them. By contrast, the MPS decreased with the increased feed rate of NMD–acetone solution and the amount of surfactant, from 1 to 3 mL/min and 0.025% to 0.2%, respectively. Thereafter, the MPS did not show any obvious change. The precipitation temperature had no significant effects on MPS. The optimum micronization conditions were determined as follows: NMD–acetone solution concentration of 150 mg/mL, the volume ratio of antisolvent to solvent of 3, the flow rate of NMD–acetone solution of 9 mL/min, the preparation temperature of 15 °C and the amount of the surfactant of 0.2%. Under optimum conditions, micronized NMD with a MPS of 708.3 nm was obtained. The micronized product was characterized using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), high performance liquid chromatography–mass spectrometry (LC–MS), X-ray diffraction (XRD), differential scanning calorimetry (DSC), and thermo gravimetric (TG), to verify the influences of micronization process on the final product. The results showed that the chemical structure of micronized NMD was not changed, but the crystalline structure had undergone transition during precipitation, which changed from form H into L. The dissolution test showed that micronized l-NMD exhibited enhanced dissolution rate and solubility of 5.22 folds compared to raw H-NMD. These results suggested that micronized l-NMD may have potential value to become a new oral NMD formulation with high bioavailability.

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1. Introduction

Nimodipine (Fig. 1), isopropyl (2-methoxyethyl)-1,4-dihydro-2,6-dimethyl 1-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylate, is a drug substance of the 14-dihydropyridine type that was developed by Bayer AG. It was widely used in the treatment of ischemic cerebrovascular diseases, hypertension, etc. (Haws and Heistad, 1984) owing to the ability of selectively regulating calcium channels to increase cerebral blood flow. Nimodipine has also been used in other cerebrovascular disorders, such as ischemic stroke and multi-infarct dementia. However, NMD is a poorly water-soluble drug. Pharmacokinetic studies showed that its oral bioavailability is between 2% and 28% in SAH patients (Langley and Sorkin, 1989). So the poor water solubility of NMD restricts its use in clinic. The relatively low bioavailability also has formed a major challenge during drug formulation. Nimotop, NMD tablet marketed by Bayer, one of its best oral formulation, has to be taken 2 tablets (30 mg each) for every 4 h for patients according to clinical practice (Fu et al., 2013). The frequency of this dosing regimen reflects low bioavailability of NMD due to poor water solubility and high first-pass metabolism in the liver (Xiong et al., 2008). Meanwhile, NMD is a kind of polycrystalline drug existing two polymorphic forms including H and L. According to the study performed by Grunenberg et al., the dissolution rate of H-NMD was faster than l-NMD, but the result was opposite in the aspect of thermodynamically stability (Grunenberg et al., 1995).

In order to improve its oral bioavailability, some new delivery systems of NMD have been reported such as solid dispersion...
2. Materials and methods

2.1. Materials

The raw nimodipine (purity 99.7%, l-nimodipine) was purchased from Shanxi Ruicheng Pharm. Middle Product Co. Ltd. (Ruicheng, China). Acetone (purity >99.5%) was purchased from Shanghai branch of Sigma–Aldrich (Shanghai, China). Deionized water was prepared with Hitech-K Flow Water Purification System (Hitech Instruments Co. Ltd., Shanghai, China). Tween 80 was obtained from Bodi Chemical Co. Ltd. (Tianjin, China). Methanol, ethanol and acetonitrile were of high performance liquid chromatography grade purchased from J&K Scientific Ltd (Beijing, China).

2.2. Preparation of NMD nanosuspension

NMD nanosuspension was prepared by antisolvent re-crystallization technique. The experimental processes for preparation were illustrated in Fig. 2. In this process, acetone and deionized water were chosen as solvent and antisolvent of NMD, respectively. A certain amount of raw NMD was dissolved in acetone and the solution was then conducted ultrasonic treatment using TI-H-5 (Elma, Singen, Germany) for 10 min to make sure that the drug can be completely dissolved. Different volume ratios of Tween 80 were added into deionized water, which was used as antisolvent. At different temperatures, a certain concentration of NMD–acetone solution was injected into the antisolvent using a peristaltic pump at a various flow rate. The solutions were mixed with a high-speed FSH-II Adjustable High-Speed Homogenizer Stirrer (Jiangsu Zhengji Instruments, Jintan, People's Republic of China) at 10,000 rpm for 10 min. Then NMD nanosuspension was obtained.

2.3. Optimization of NMD nanosuspension

Single factor analysis was chosen for the optimization of operating conditions for NMD micronization by the antisolvent re-crystallization process. Through preliminary experiments, we found that the following factors had important effects on the MPS of NMD nanosuspension: the concentration of NMD–acetone solution, the volume ratio of antisolvent to solvent, feed speed of NMD–acetone solution, precipitation temperature and amount of surfactant. In order to investigate the impacts of these factors on the MPS, a series of single factor experiments were designed, namely, different concentration of NMD–acetone solution, volume ratio of antisolvent to solvent, feed rate, precipitation temperature and amount of surfactant. In order to evaluate the chemical and physical stability of the ultimate product, NMD nanosuspension was prepared into freeze-dried powder using a Gamma 2-20 apparatus (Christ, Germany). Three milliliters of NMD nanosuspension was successively added into each glass vial, pre-frozen at −40 °C for

![Fig. 1. The chemical structure of NMD.](image)

![Fig. 2. Diagram of the experimental processes to prepare the micronized NMD.](image)
Table 1

<table>
<thead>
<tr>
<th>Levels</th>
<th>Factors</th>
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<tr>
<td></td>
<td>(A) Concentration of NMD–acetone solution [mg/ml]</td>
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<td>1</td>
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<tr>
<td>2</td>
<td>100</td>
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<tr>
<td>3</td>
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<td>4</td>
<td>200</td>
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<td>5</td>
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2 h, and then subsequently lyophilized at −60 °C for 48 h to obtain freeze-dried micronized NMD powder.

2.5. Characterization of product

2.5.1. Morphology and MPS

The MPS of NMD nanosuspension was detected by DLS (Zetasizer, Brookhaven Instruments, USA). 3 mL NMD nanosuspension sample was added into a sample cell to detect MPS. The sample was diluted with mixture having the same composition with antisolvent process if necessary. Every measurement was repeated at least three times.

The MPS of micronized NMD was measured by SEM (Quanta 200, FEI Co., Eindhoven, The Netherlands). The samples were coated with gold for 4 min using a sputter coater before analysis. The MPS of micronized NMD was measured with imagePro Plus 6.0 software (Media Cybernetics, Inc., USA). From the SEM images of every experiment, 100 particles were randomly selected and long diameter was measured. The mean value was calculated as MPS.

2.5.2. Fourier transform infrared spectroscopy (FT-IR)

The FTIR spectrum of samples was obtained by IRRafinity-1 (SHIMADZU, Japan) and recorded in the wavenumber range of 4000–500 cm⁻¹ at a resolution of 4 cm⁻¹. The micronized and raw NMD were diluted with KBr mixing powder at 1% and pressed to self-supporting disks separately.

2.5.3. High performance liquid chromatography–mass spectrometry (LC–MS)

The raw and micronized NMD were dissolved separately in methanol and the concentration of them was 1 mg/mL. The sample was diluted if necessary. LC–MS spectra were obtained by analyst 1.4 of API3000 (AB, USA). The mass spectrometer was operated in negative ion mode.

2.5.4. X-ray diffraction (XRD)

X-ray diffraction analysis was employed to detect the crystallinity of NMD, which was conducted using an X-ray powder diffractometer (Philips, Xpert-Pro; The Netherlands) with Cu Ka1 radiation generated at 100 mA and 50 kV during the range from 5° to 60° of 2θ.

2.5.5. Differential scanning calorimetry (DSC)

Thermal analysis was carried out using DSC (TA instruments, model DSC 204) for raw and micronized NMD. Analysis was performed for 2.0 mg samples at a temperature heating rate of 10 °C/min and a temperature range of 30–250 °C.

2.5.6. Thermal gravimetric (TG)

The thermogravimetric properties of the raw and micronized NMD were analyzed by a thermogravimetric analyzer (NETZSCH TG 209 F3; PerkinElmer, USA). The test conditions were as follows: approximately 3.0 mg of sample was weighed into an open aluminum pan. The experiment was performed at a heating rate of 10 °C/min and a nitrogen flow rate of 50 mL/min. The percentage weight loss of the samples was monitored from 30 to 600 °C.

2.5.7. Gas chromatograph (GC)

The residual amount of acetone in the micronized NMD was analyzed using an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5 (5% phenyl methyl siloxane) capillary column (30 m × 320 mm × 0.25 μm, nominal) equipped with a G1540N-210 flame ionization detector. 1.0 g micronized NMD was exactly weighed and placed in a 10 mL volumetric flask, and 10 mL of ethanol was added. The flask was shaken for 60 min by ultrasonic agitation at 20 °C. After being shaken, sample was centrifuged at 10,000 rpm for 10 min, and 1 μL supernatant was injected into GC injection port. The conditions of GC analysis of acetone were as follows: the oven temperature was initially maintained at 60 °C for 2 min, and then raised at a rate of 4 °C/min to 80 °C, holding for 2 min. The temperature of injector was set at 200 °C and the detector temperature was set at 250 °C. Nitrogen gas was used as carrier gas at a flow rate of 25 mL/min, and 5 μL samples were injected manually in split mode, with a split ratio of 13:1. The hydrogen gas and air flow rates were 30 and 125 mL/min, respectively.

2.5.8. Dissolution evaluations

The dissolution study of raw and micronized NMD was performed by paddle method. The solution temperature was set at 37.0 ± 0.5 °C and paddle speed was 100 rpm/min. Deionized water was used as dissolution medium. 20 mg of raw and micronized NMD was weighed accurately, and then joined into 200 mL dissolution medium accordingly. Samples (3 mL) were withdrawn at 1, 2, 3, 4, 6, 8, 10, 12, and 24 h, filtered using 0.22-μm filters, and then the same volume of dissolution medium was complemented. The filtrate was injected into HPLC system with a Jasco UV-975 ultraviolet (UV) detector (Easton, MD, USA) using a Diamond C18 column (4.6 mm × 250 mm, 5 μm; Dikma Technologies, Beijing, People’s Republic of China) with a mobile phase consisting of methanol, acetonitrile and deionized water (35:38:27, v/v/v), flow rate of 1 mL/min, and UV detection wavelength of 235 nm. The concentration of NMD was calculated relative to a NMD reference of y = 36,734x + 145,508 and R² = 0.998 (x is the concentration of NMD in μg/mL and y is the peak area).

3. Results and discussion

3.1. Effects of experiment parameters on MPS of NMD nanosuspension

3.1.1. Concentration of NMD–acetone solution

Fig. 3(a) showed the effects from drug concentration in the range of 50–250 mg/mL on MPS of NMD nanosuspension. The MPS of NMD nanosuspension rapidly decreased with increased concentration. The smallest MPS of 179.1 nm was obtained at 150 mg/mL. Thereafter, MPS increased with increased concentration. The possible reasons for this phenomenon are as follows: the
Fig. 3. The effect of each parameter on the MPS of micronized NMD. (a) Concentration of the NMD–acetone solution; (b) the volume ratio of antisolvent to solvent; (c) feed rate; (d) precipitation temperature; (e) the amount of surfactant.

precipitation process consists of two major events, nucleation and crystal growth. Compared to crystal growth rate, nucleation rate is more dependent on supersaturation degree, and the nucleation rate greatly affects final particle size (Boistelle and Astier, 1988; De Yoreo and Vekilov, 2003). A higher drug concentration would create a higher supersaturation level and nucleation rate, resulting in small particle size (Chen et al., 2004). However, the MPS of NMD nanosuspension had slightly increased when the concentration of NMD–acetone solution was increased to 250 mg/mL. This phenomenon may be caused by agglomeration under high concentration of NMD condition. So, the optimal concentration of NMD–acetone solution was determined at 150 mg/mL.

3.1.2. The volume ratio of antisolvent to solvent

Fig. 3(b) showed the effects of volume ratio of antisolvent to solvent on MPS of NMD nanosuspension. From this figure, it can be seen clearly that with the increasing volume ratio of antisolvent to solvent the MPS of NMD nanosuspension decreased rapidly at first and then increased slowly. The minimum MPS of 84.0 nm with a yield of 96.0% was obtained at 3 of volume ratio. During the process of antisolvent re-crystallization, the supersaturation degree of the system has great impacts on the MPS of precipitates. In general, the higher the supersaturation degree, the smaller the particle size of crystals is (Lee et al., 2005; Shekunov and York, 2000). Under the same concentration of NMD, the solubility of NMD in the mixed solution becomes smaller and smaller with the increasing proportion of antisolvent in the system. This caused the increase of supersaturation degree, which resulted in decreased MPS during re-crystallization. However, the particle agglomeration could occur due to the high nucleus concentration of drug when the volume ratio exceeded 3. And as a result the NMD nanosuspension has larger MPS. So, the
optimal volume ratio of antisolvent to solvent was selected at 3.

3.1.3. Feed rate

Fig. 3(c) showed the effects of feed rate on MPS of NMD nanosuspension. With increasing feed rate, the MPS of NMD nanosuspension rapidly decreased and then changed slightly. Explanations were given as follows: the degree of supersaturation and the speed of reaching it both have great influences on the MPS during the antisolvent re-crystallization. When the supersaturation degree of the system is a certain value, which the supersaturation degree generated in a short time, can lead to rapid nucleation and formation of smaller MPS (Barrett et al., 2011; Strey et al., 1994). Under the same feeding volume, the faster the feed rate, the shorter the time of generating supersaturation is, thereby the smaller the MPS of NMD particles is. Fig. 3(c) showed the MPS of nanosuspension basically kept a constant when the speed of feeding rate was more than 3 mL/min. The most likely reason is that the precipitation system could reach supersaturation in a short time with higher feed rate. High feed speed can improve the efficiency of industrial production. Consequently, the optimal feed rate was chosen at 9 mL/min.

3.1.4. Precipitation temperature

Fig. 3(d) showed the effects of precipitation temperature on MPS. As shown in the figure, the MPS decreased when the precipitation temperature dropped from 30 to 15 °C. It can be explained that the decrease in solubility of NMD and narrow width of metastable zone at low temperatures favors higher nucleation rate (Münster and Rotsch, 2000). Decrease in solubility and increase of supersaturation of NMD in acetone occurred as a consequence of reducing precipitation temperature, resulting in smaller particles being produced. On the one hand, the increase of supersaturation degree due to decrease in NMD solubility could be helpful to obtain small power particles. On the other hand, increased viscosity of the solvent hindered the diffusion between solvent and antisolvent, resulting in nonuniform supersaturation. As a result, the MPS of nanosuspension increased at 10 °C. As a result, the optimal precipitation temperature was chosen at 15 °C.

3.1.5. The amount of surfactant

Fig. 3(e) showed the effects of the amount of surfactant on MPS. Tween 80, a nonionic surfactant, was selected as the surfactant during antisolvent re-crystallization. When the amount of Tween 80 increased from 0.025 to 0.2%, the MPS of NMD suspension decreased significantly. With the volume percent of Tween 80 increased to 0.4%, the MPS changed slightly, which indicated that the NMD particle surface was already sufficiently enveloped by Tween 80. So the optimal volume percent of surfactant added into antisolvent is 0.2%.

3.1.6. The optimum conditions of antisolvent re-crystallization

From the results of single factor experiments, except precipitation temperature, other four factors have significant effects on the MPS of NMD nanosuspension. The optimum conditions of antisolvent re-crystallization were chosen as follows: the concentration of NMD–acetone solution of 150 mg/mL, the volume ratio of antisolvent to solvent of 3, the feed rate of NMD solution of 9 mL/min, the precipitated temperature of 15 °C and the amount of Tween 80 of 0.2% (v/v). Under these conditions, NMD nanosuspension with a MPS of 26.7 nm and a yield of 92.5% were obtained. The micronized NMD was obtained after NMD nanosuspension was freeze-dried like Section 2.4. The morphology and MPS of micronized NMD were investigated by SEM. The SEM results showed that micronized NMD had a MPS of 708.3 nm.

3.2. Morphology and MPS

SEM of raw and micronized NMD particles was shown in Fig. 4. From Fig. 4a, it can be seen that raw NMD particles exhibit coarse plate shape crystals with a large size of 134.4 μm. However, micronized NMD particles in Fig. 4b present hexagon or broad rectangle shape with a MPS of 708.3 nm. The description of its polymorphs including crystal form H and L was in accord

![Fig. 4](image1.png)

**Fig. 4.** SEM images and PSD of micronized NMD precipitated from acetone under optimum condition. (a) Raw NMD; (b) micronized NMD; (c) PSD of NMD nanosuspension and redissolved micronized NMD.

![Fig. 5](image2.png)

**Fig. 5.** FTIR spectra of (a) micronized NMD; (b) raw NMD.
with the report of Grunenberg et al. (1995). Fig. 4c showed the particle size distribution of NMD nanosuspension and redissolved micronized NMD. Compared with NMD nanosuspension, the MPS of micronized NMD was significantly increased. The major reason for this phenomenon is due to the agglomeration of NMD particles during drying process.

3.3. Chemical structure

The FTIR spectra of raw and micronized NMD were taken to obtain information on the change of chemical structure after antisolvent re-crystallization processing. As presented in Fig. 5, the spectrum of two samples has some slight differences, which are in detailed shown in Table 2. These small differences could be caused by differences of intramolecular hydrogen bonds and intermolecular Van der Waals force, which also can suggest the change of crystalline structure of NMD after antisolvent re-crystallization processing.

The chemical structures of two NMD samples were further evaluated by using LC–MS to determine molecular weights. As shown in Fig. 6, it can be seen that no modification occurred in molecular weight. Calculated from the mass spectrum, the molecular weight of raw and micronized NMD is 418 and 418.3, respectively. This is the same as the molecular weight of 418.4 reported in literature (Kane and Robinson, 1998). Combining the results of FTIR and LC–MS, we can determine the chemical structure of NMD did not change during the antisolvent re-crystallization process.

3.4. The crystal structure

The raw and micronized NMD were taken to perform XRD analysis to obtain the information on crystalline change after antisolvent re-crystallization processing. Fig. 7a showed the XRD results for raw and micronized NMD particles. As can be seen, the two samples had very different diffractograms, especially at low 2θ angles. The raw NMD showed several characteristic peaks at 2θ = 6.48°, 12.52°, 13.00°, 17.31°, 20.31°, 26.21°, while these of micronized NMD at 2θ = 10.19°, 15.19°, 19.37°, 20.72°, 25.31°, 26.86°. Compared with the data in the previous literature (Urbanetz and Lippold, 2005), it can be concluded that raw NMD and micronized NMD are severally polymorphic form H and L.
3.5. Thermal stability

The basic requirement of pharmaceutical preparation is safety, utility and stability, and stability is the most important factor to ensure that the preparation is safe. Polymorphism generally exists in solid drugs, which is a very important factor for their stability. In the case of polymorphism, different polymorphs will have different stability in the solvent with the stable polymorph (Kitamura, 2002). According to previous reports, NMD exists in two polymorphic forms, namely crystal form H (modification I) and L (modification II). And l-NMD is the thermodynamically stable crystal form at ambient conditions compared with H-NMD (Grunenberg et al., 1995).

In order to further confirm the physical state of micronized NMD, DSC was also performed to analyze samples. As can be seen from Fig. 7b, it showed that the melting points of raw NMD and micronized NMD were 124.5 °C and 117.0 °C separately. This is agreed with the corresponding results of polymorphic form H and L of NMD (Grunenberg et al., 1995; Urbanetz and Lippold, 2005). Combining the results of XRD and DSC analysis, we can conclude that micronized l-NMD was obtained successfully by antisolvent re-crystallization from its original crystal form H.

The TG curves, used to examine the thermal weight losses of raw and micronized NMD, are shown in Fig. 8. With the temperature increased from 30 to 204 °C, raw and micronized NMD had no loss weight. This shows that the two forms of crystal were relatively stable within 204 °C. With the temperature increased from 204 °C to 330 °C, the weight of them decreased quickly and they had the same weight loss rate. This phenomenon results from NMD thermal decomposition and then weight loss when the temperature increased to 204 °C. When the temperature increased to 597 °C, the weight of them was no further loss. In conclusion, the weight loss of raw H-NMD and micronized l-NMD showed no significant difference within the temperature range from 30 to 597 °C. These results shows that the two kinds of crystal are all have good thermal stability. According to previously literature reported, the thermal stability of l-NMD is better than H-NMD (Grunenberg et al., 1995). However, the results of this study showed that the thermal stability of micronized l-NMD, which obtained by antisolvent re-crystallization, and raw H-NMD are the same. The reason may be that with small MPS has higher specific surface area and hence has higher specific surface energy, which subsequently leads to an easier vaporization and earlier decomposition energy. Therefore, two opposite effect including changing of crystal type and micronization led to the above results.

3.6. Solvent residue

It is necessary to analysis residual solvent in pharmaceutical products because residual organic solvents represent a potential risk for human health due to their toxicity. In this study, micronized NMD was prepared by the antisolvent precipitation process using the International Conference on Harmonization (ICH) class III antisolvent acetone with low toxicity as the solvent. Fig. 9 showed the results of acetone residues using GC. From the chromatograms of the acetone standard solution (Fig. 9a), in which acetone eluted at 2.462 min, a regression equation between peak (y) and acetone concentration (x) can be fitted as y = 2940.698x – 7.168 (R² = 0.999). According to the regression equation, the residual acetone content in micronized NMD is 2150 ppm. The ICH limit for acetone in class III solvents is 5000 ppm or 0.5%, therefore, the micronized NMD meets the ICH requirements and is suitable for pharmaceutical use.

Table 2

Differences of FTIR spectral data of raw and micronized NMD.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wavenumber (cm⁻¹)</th>
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<tr>
<td>Raw NMD</td>
<td>3299 3225 3096 1522 1401 1344 1268 1046 1022 1004</td>
</tr>
<tr>
<td>Micronized NMD</td>
<td>3271 3215 3088 1530 1409 1352 1275 1053 1035 1011</td>
</tr>
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</table>

Fig. 7. Results of XRD and DSC of micronized and raw NMD. (a) XRD; (b) DSC.

Fig. 8. TG curves of the micronized and raw NMD.
3.7. Dissolution rate and solubility

Dissolution rate of raw NMD (H-nimodipine/modification I) and micronized NMD (l-nimodipine/modification II) were shown in Fig. 10 and Table 3. The micronized NMD showed a more rapid dissolution than raw NMD. As can be seen, the dissolution rate of raw NMD and micronized NMD were 1.16% and 27.18% at 60 min, respectively and then they were increased to 15.7% (15.7 μg/mL) and 81.9% (81.9 μg/mL) after 24 h. The solubility of micronized NMD is 5.22 times of raw drugs. According to several research teams’ reports, the solubility of H-NMD and l-NMD were 0.86 μg/mL and 0.44 μg/mL (Grunenberg et al., 1995; Riekes et al., 2012). In other words, the solubility of H-NMD is 1.95 times of l-NMD. However, the result in this work demonstrates that the dissolution rate and solubility of micronized l-NMD is 5.22 times of H-NMD, which could be ascribed to the enlarged surface of the micronized drug particles according to Noyes–Whitney equation (Kesisoglou et al., 2007; Van Eerdenbrugh et al., 2008). The solubility of a drug substance in aqueous media may have a crucial bearing on its bioavailability. Many previous studies have already reported that the increased dissolution rate of poorly soluble drugs in vitro could enhance its bioavailability in vivo (Kesisoglou et al., 2007; Wong et al., 2006).

4. Conclusions

Micronized l-nimodipine (NMD) has been successfully prepared by antisolvent re-crystallization process using acetone as solvent and deionized water as antisolvent. The MPS of NMD nanosuspension decreased significantly with the increased concentration of NMD–acetone solution from 50 to 150 mg/mL, the volume ratio of antisolvent to solvent from 1 to 3, and then increased slightly with the increasing of them. By contrast, the MPS decreased with the increased feed rate of NMD–acetone solution and the amount of surfactant, from 1 to 3 mL/min and 0.025% to 0.2%, respectively. Thereafter, the MPS did not show any obvious change. Under the optimum conditions, micronized NMD with a MPS of 708.3 nm was obtained. The chemical structure of micronized NMD did not change, but the crystalline structure had undergone transition during precipitation, which changed from form H into l. The dissolution test showed that the micronized l-NMD exhibited enhanced dissolution rate and solubility of 5.22 times compared to the raw H-NMD. These results suggest that micronized l-NMD may have great potential value to become a new oral NMD formulation with high bioavailability.

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Table 3

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Polymorphs</th>
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<tr>
<td></td>
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<tr>
<td>1</td>
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