Drug Development and Industrial Pharmacy, 33:569–575, 2007

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040600975147

informa
healthcare

Preparation of Azithromycin Nanosuspensions by High Pressure Homogenization and its Physicochemical Characteristics Studies

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ABSTRACT The azalide azithromycin was proved to be clinically effective against Gram-positive and Gram-negative bacteria. But the low bioavailability caused by its poor solubility and gastrointestinal response limited its application in clinic. With the purpose of increasing its saturation solubility and dissolution velocity, azithromycin was produced as nanosuspensions by high pressure homogenization. Nanosuspensions could increase the drug-loading and reduce the administration dosage, thus the gastrointestinal response could be minimized. In order to enhance the stability of the nanosuspensions, we got the freeze-dried powder by lyophilization. After dispersed in distilled water, the nanoparticles had a bulk population of about 400 nm and a spherical figure (watched by transmission electron microscopy). The analysis of differential scanning calorimetry and powder X-ray diffraction demonstrated that the crystal state of azithromycin had changed. In vitro release studies showed that the dissolution rate of nanosuspension, compared with micronized powder, had been obviously increased.

KEYWORDS Azithromycin, High pressure homogenization, Nanosuspensions, Lyophilisation

INTRODUCTION

The azalide azithromycin (Fig. 1), which is derived from erythromycin, contains a methyl-substituted nitrogen in the lactone ring. It possesses a broad spectrum of activities against Gram-positive and Gram-negative bacteria and is mainly used in clinic for the treatment of common respiratory and skin/skin structure infections (Hoepelman et al., 1995). It is also proved to be clinically effective for the treatment of urethritis and cervicitis caused by Chlamydia trachomatis (Amsden et al., 2001).

However, the success use of azithromycin in clinic is limited by a low bioavailability due to its insolubility in water and some gastrointestinal responses, such as diarrhea (Glenda et al., 2002). One promising way to improve its

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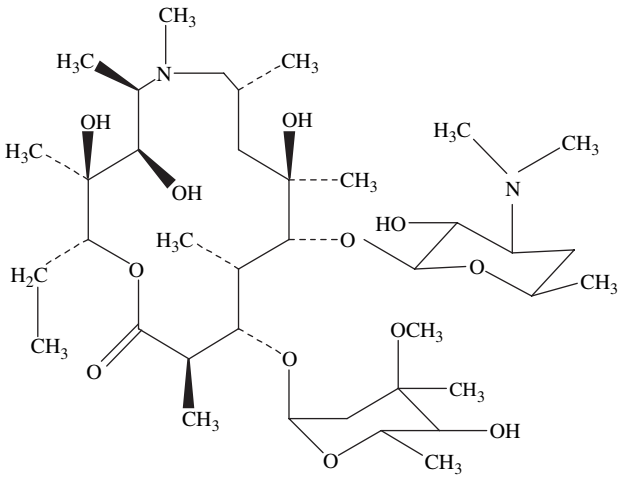

FIGURE 1 **Structure of Azithromycin.**

solubility and dissolution behavior is to reduce the particle size down to the nanometer range (Müller et al., 2002), which thus leads to an increased surface area and an augmented dissolution velocity (Keck et al., 2006). Besides, when the particle size reach to the nanometer range, it will be able to adhere to the intestinal wall, which leads to a prolonged residence and contact time in the GIT, and overcomes the loss of drug due to diarrhea (Jacobs et al., 2001), so a high bioavailability could be obtained. Moreover, because of the increased drug-loading, the administration dosage is reduced, thus the gastrointestinal irritation, compared with the present formulation, has been minimized.

A very easy method to produce nanoparticles is high pressure homogenization, which is a particle size reduction process generally applied in food, cosmetic, pharmaceutical industry (Müller et al., 1998). With the high pressure homogenization, the reduction of particle size from μm to nm brings about an improved in solubility and dissolution behavior of drugs. Various surfactants can be used to avoid aggregating, by covering the surface of the particles and supporting the electrostatic repulsion or steric stabilization between the particles, (Grau et al., 2000). In order to enhance the stability, the nanosuspensions can be lyophilized into powder forms (Kocbek et al., 2006).

At present, some poorly-soluble drugs have been made into nanosuspensions to overcome the poor bioavailability, but there were no relative reports about macrolides. The aim of this study was to create an azithromycin nanosuspensions by high pressure homogenization and hope to solve the puzzles about the application of azithromycin in clinic. The systems were mainly investigated on their physicochemical properties and in vitro release.

MATERIALS AND METHODS

Materials

The drug azithromycin was purchased from Beijing TaiYang Pharmaceutical Co., Ltd. Pluronic F68 was obtained from Sigma (St. Louis, MO). Lecithin was provided by Beijing Shuangxuan Pharmceutical Co., Ltd. Tween 80 was purchased from ShangHai Gen-Tech Co., Ltd.

Preparation of Nanosuspensions

To produce nanosuspensions of a poorly soluble drug, it was preferential to start with a very fine drug powder. To avoid blocking the homogenization gap, it was recommended performing so-called premilling (Bernhard et al., 1999). Firstly, the surfactants were dissolved or dispersed in distilled water by using an electric stirrer (JJ-1, Jiangsu, China). The drug powder was dispersed in the aqueous surfactant solution using again the electric stirrer (10 min, 1000 rpm). Then the pre-mix was passed through a Lab 40 homogenization (NS 1001 L, Niro Soavi S.P.A., Italy), 2 cycles were performed at 100 bar, 5 cycles at 500 bar, and finally 15 cycles at 1500 bar. Samples were drawn at each pressure for particles size analysis. At last, in order to avoid particle growth during storage time, the nanosuspensions was transferred into freeze-dried powder forms by a lyophilizer.

Particle Size Analysis

The morphological examination of the systems was performed by transmission electron microscopy (TEM) (JEM-1200EX, Japan). Firstly, the lyophilized powder was dispersed in distilled water using supersonic wave (SK8200 HP, Shanghai, China). Then the samples were stained with 2% (w/v) phosphotungstic acid and placed on copper grids with films for viewing by TEM.

The DSC Analysis

Thermal behavior of the systems was investigated with a differential scanning calorimeter (CDR-4P, Shanghai, China). Approximately 20 mg of sample

was placed in open aluminium pans, and heated at scanning rate of 10°C/min between 25°C and 500°C, using nitrogen as blanket gas. The apparatus is magnesia calibrated. To evaluate the internal structure modifications after forming of formulations, analysis was performed on azithromycin, pluronic F68, their physical mixtures and the freeze-dried nanosuspensions.

The X-Ray Diffraction Analysis

The crystalline state of the samples was estimated by an X-ray diffractometer (D/max r-B, Rigaku, Japan). The experiments were performed in symmetrical reflection mode with a Cu line as the source of radiation. Standard runs using a 40 kV voltage, a 40 mA current and a scanning rate of 4°/min over a 2θ range of 2.5–40° were used. The samples analyzed were the same as the DSC experiments.

Stability Studies

Freeze-dried powder was placed into three ampouls respectively and sealed for storage at 4°C for one year. The particle size and morphous were determined.

In Vitro Drug Release

The release of azithromycin from nanosuspensions was evaluated over 24 h by a dialysis system using Intellective Dissolution Meter (Tianjin, China) . Each dialysis bag (pore size:2.4 nm, SERVA, Heidelberg, Germany) was loaded with 5 ml of sample and soaked in 200 mL phosphate buffer solution (pH 7.4) containing 1% sodium lauryl sulfate (SLS) at 37°C and under 100 rpm stirring. SLS was used to increase the solubility of azithromycin in the buffer solutions and prevent adsorption of the azithromycin on the dialysis bag (Chawla et al., 2002), and the dialysis bag allows the free drug into the dissolution media. At predetermined intervals took out of 3 mL dissolving medium, and immediately restored with 3 mL of fresh buffer to maintain the sink condition. The amount of released drug was assessed by a UV detector (UNICOTM UV-2102 PCS) at 284.7 nm. The sulfuric acid was used as color developing reagent. To evaluate the different release rate between nanosuspensions and azithromycin crystals, the release behavior of nanosuspensions and pure micronized azithromycin solution was compared. All measurements were performed in triplicate.

RESULTS AND DISCUSSION

Screening of Formulations with Different Types and Concentration of Surfactants

Three different surfactants which were suitable for oral administration were chosen and compared. The composition of screening formulation A-D was shown in Table 1.

All formulations showed a similar appearance right after the production. But one month later the formulation A and C were hardly redispersible by hand shaking. To avoid side effects, such as anaphylactic shock or other allergic reactions due to the excess of surfactants, it was important to reduce the amount of surfactants without loosing the stability of suspensions (Müller et al., 2002). In conclusion, we chose the formulation B as the optimized formulation in spite of the similar apparent stability of formulation B and D.

Process Parameters—Cycle Numbers and Pressure

The suspension passes the very small homogenization gap with a very high velocity. Due to the narrowness of the gap, the streaming velocity of the suspension increases tremendously, which means the dynamic fluid pressure increases. Simultaneously the static pressure on the fluid decreases below the boiling point of water at room temperature. In consequence, water starts boiling at room temperature due to the high pressure, gas bubbles are formed which implode when the fluid leaves the homogenization gap. These cavitation forces are strong enough to break the drug microparticles to drug nanoparticles (Müller et al., 2001).The power density (w/m^3) is a factor which determined achievable dispersions, i.e., the fineness of

TABLE 1 Composition of Azithromycin Nanosuspensions (w/w, %)

Formulation	Azithromycin	Pluronic F68	Tween 80	Lecithin
A	1.0	0.2	–	0.1
B	1.0	0.3	–	–
C	1.0	–	0.2	0.1
D	1.0	1.0	–	–

the drug nanocrystals. The power density Pv is defined as the energy W dissipated in the homogenization volume V related to the time t: Pv =W/tV (Moschwitzer et al., 2004). Based on this equation, the factors determining Pv are the homogenization pressure and the width of the homogenization gap. So larger pressure and longer time (more cycles) can lead to smaller size. But we should increase the applied pressure step by step from lower pressure to the maximal pressure to avoid block of the gap, i.e., 2 cycles were performed at 100 bar, 5 cycles at 500 bar, and finally 15 cycles at 1500 bar.

In order to obtain a narrow size distribution it is necessary to run several processes through the homogenizer (homogenization cycles). A typical number of homogenization cycles reported for nanosuspension are between 10 and 20, depending on the hardness of the drug to be processed. A narrow size distribution is essential to prevent particle growth caused by different saturation solubility in the vicinity of differently sized particles, which was explained in Ostwald ripening (Lindfors et al., 2006).

Transmission Electron Micrographs

The morphological characterization of nanosuspension under different pressure are shown in Fig. 2–4. It's obvious to find that after homogenization the drug power are transformed into small spherical nanoparticles step by step. Through homogenization the diameter of drug nanoparticles become smaller, and the surface appearance becomes larger than the pure substance.

At first two homogenization cycles under 100 bar (shown in Fig. 2), we can see that the drug powder is dispersed into microparticles. Fig. 3 shows the particles which are passed through five cycles under 500 bar, the progress of forming of nanoparticles can be observed. After 15 cycles under 1500 bar, the spherical particles, which posses a mean diameter 400 nm, are produced (shown in Fig. 5). Comparing all the particles in total progress, we can easily see the effect of high pressure and cycle numbers. The size distribution was shown in Fig. 6.

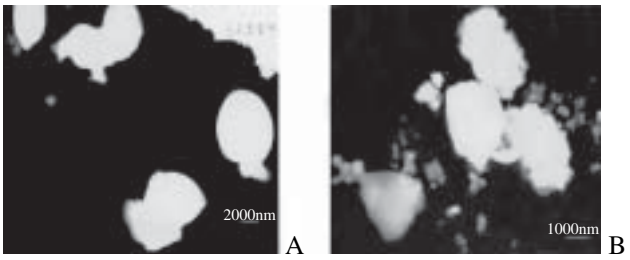

FIGURE 2 TEM Micrographs of the Nanosuspensions After 2 Cycles at 100 bar (A 5000×, B 10,000×).

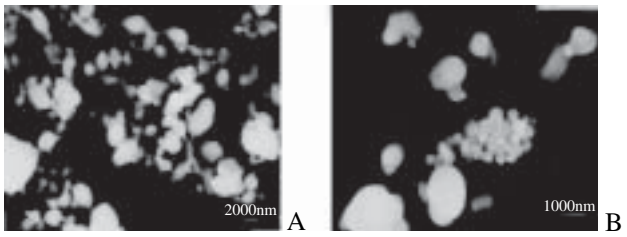

FIGURE 3 TEM Micrographs of the Nanosuspensions After 5 Cycles at 500 bar (A 5000×, B 10,000×)

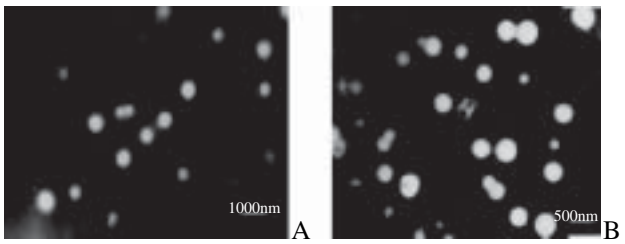

FIGURE 4 TEM Micrographs of the Nanosuspensions After 15 Cycles at 1500 bar (A 10,000×, B 20,000×).

FIGURE 5 TEM Micrographs of Final Nanosuspensions (100,000).

Differential Scanning Calorimetry

When nanosuspensions are being produced, the material phases of the drug and the excipients might change. The drug might present as a solid solution or dispersed among the adjuvant as a molecular or a crystalline (Dubernet, 1995). The studies on the thermal behavior and crystallinity could help to ascertain the

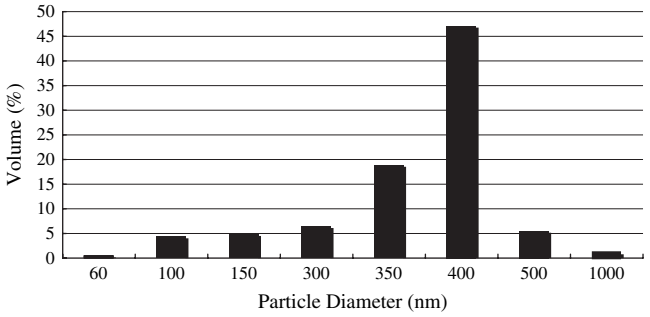

FIGURE 6 **Size Distribution of Azithromycin Nanosuspensions.**

physicochemical status of the entrapped drug inside the excipient and assess the interaction amongst different components during the fabrication process.

The results of DSC (Fig. 7) demonstrated that the nanosuspension was quite different from the simple mixture of its ingredients, i.e., azithromycin and pluronic F68. For instance, we could find an endothermic peak at 91.5 ~ 98.5°C as the characteristic peak of azithromycin, and an endothermic peak at 75.0 ~ 87.5°C as the characteristic peak of pluronic F68. Whereas in the DSC results of nanosuspensions the peaks mentioned above had disappeared or changed greatly, and presented some new characteristic peaks, such as the two new endothermic peaks at 54.7 ~ 63.3°C and 241.4 ~ 270.8°C. According to the DSC analysis, we could conclude that the material phases of the nanosuspensions were quite different from the simple mixture of azithromycin and pluronic F68. The formation of the new material phases testified the formation of nanosuspensions. This result indicated that azithromycin was not in a crystalline state but in an amorphous state, in which the drug was likely to have higher energy and therefore showed increased solubility, dissolution rates and higher bioavailability (Corrigan et al., 2003; Hancock et al., 2002).

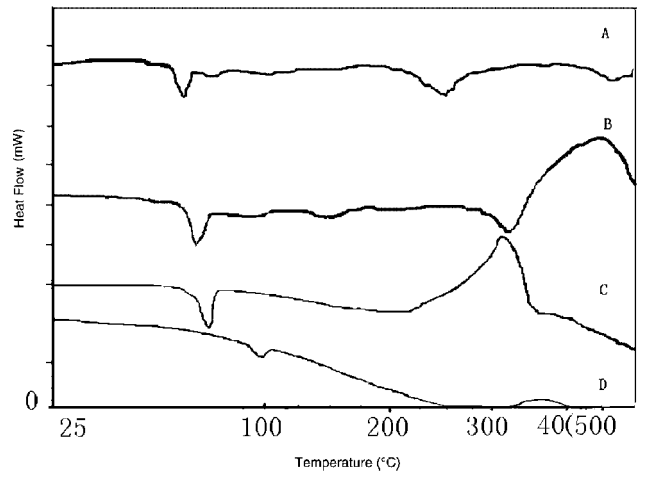

FIGURE 7 **DSC Thermograms of A-Lyophilized Powder; B-Physical Mixture of Azithromycin and Pluronic F68; C-Pluronic F68; D-Azithromycin.**

X-Ray Diffractometry

The results of the X-Ray scattering (Fig. 8) demonstrated that the azithromycin-nanosuspensions were quite different from the simple mixture of its ingredients, i.e., pluronic F68, azithromycin. From the analysis of the simple mixture by X-Ray scattering, we could find the characteristic peaks of pluronic F68, azithromycin obviously. For example, we could find two diffraction peaks at 18.980° ($d = 4.6720$ Å), 23.200° ($d = 3.8380$ Å), as the characteristic peak of pluronic F68, and five diffraction peaks at 7.800° ($d = 11.3253$Å),

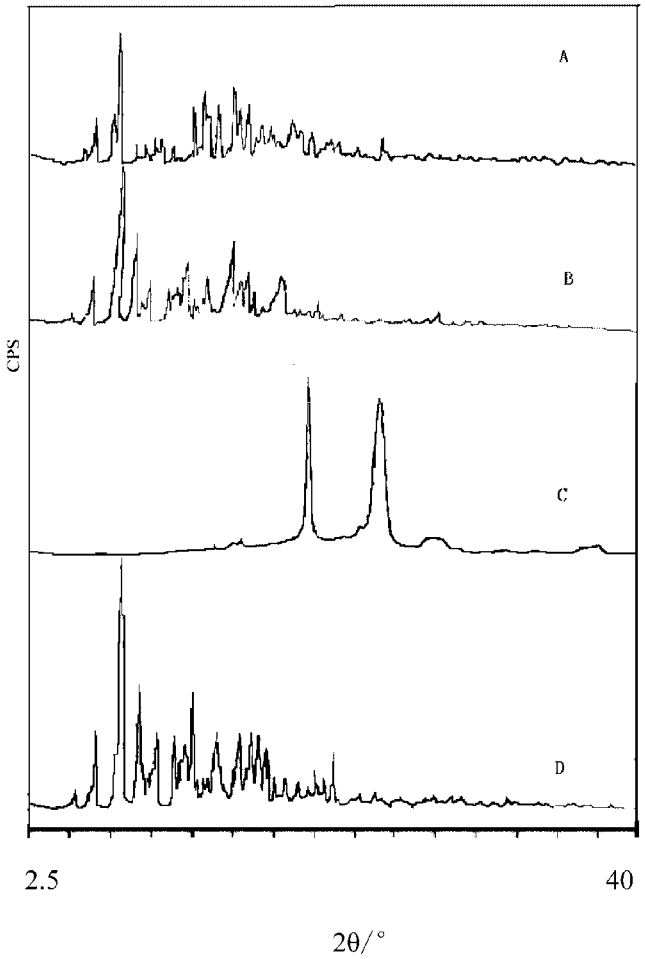

FIGURE 8 **X-ray Diffraction Patterns of A-Lyophilized Powder; B-Physical Mixture of Azithromycin and Pluronic F68; C-Pluronic F68; D-Azithromycin.**

9.780° (9.0365 Å), 11.140° (7.9361 Å), 12.400° (7.1324 Å), 15.280° (5.7939 Å), as the characteristic peak of azithromycin. Whereas in the result of the azithromycin-nanosuspensions, some characteristic peaks of azithromycin and pluronic F68 disappeared, and the parameters of other characteristic peaks, such as the peak intensity, peak position and interplanar spacing, had changed obviously, there presented some new characteristic peaks, such as the diffraction peaks at 11.920° (7.4185 Å), 16.300° (d = 5.4336 Å), 19.700° (d = 4.5028 Å), 30.300° (d = 2.9474 Å), Therefore, through the X-ray diffraction, we could confirm the formation of the new material phases. The result of X-ray was agreed with that of DSC.

Stability Studies

After 1 year of storage at 4°C, no dramatic increase in the particle size occurred (Table 2). The transmission electron micrographs showed that the particles still had a spherical figure. It certified that the freeze-dried powder possessed long-term stability which attributed to no exist of Ostwald ripening occurring in the aqueous suspensions and repulsions offered by the pluronic F68 covering the surface of the particles.

In Vitro Drug Release

The dissolution profiles of freeze-dried azithromycin nanosuspensions in comparison with a reference mixture of micronized azithromycin with pluronic F68, were shown in Fig. 9. The dissolution rate was markedly enhanced in the nanometre-sized system, as more than 65% of the drug dissolved in 5 hr, as opposed to only 20% of micronized drug. This could be attributed to the increased surface area of the drug and possible better contact between nanosuspensions and dissolution medium.

TABLE 2 **Effects of Storage Time (at 4°C) on Particle Size of Three Nanosuspensions Freeze-Dried Powder**

Samples	Storage time	Average particle size (nm)
A	0 day	384.2
	6 months	390.7
	12 months	408.5
B	0 day	402.8
	6 months	413.6
	12 months	431.8
C	0 day	394.2
	6 months	409.5
	12 months	417.6

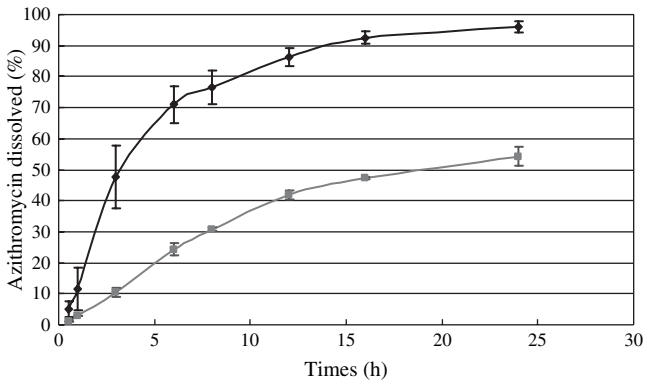

FIGURE 9 **Release Profile of Azithromycin from Nanosuspension (—♦—) and Micronized Powder (—■—) at pH 7.4.**

According to Noyes–Whitney equation, an increase in saturation solubility and decrease in particle size lead to an increased dissolution rate (Bernhard et al., 1999). The bioavailability of azithromycin is truly dissolution rate limited, so particle size reduction can significantly improve the performance of the drug. So formulation of poorly water-soluble drugs as nanometresized drug particles has a dramatic effect on dissolution rate, drug solubility and consequently bioavailability.

CONCLUSION

It was shown that it was possible to obtain an azithromycin nanosuspensions with fine solubility and dissolution properties, and the nanosuspensions possessed a high drug-loading (1%), which could reduce the administration dosage and gastrointestinal response. By the transformation of the nanosuspensions into lyophilized powders, the physical stability of this system could be further enhanced. All of these can increase the clinic activity of azithromycin and allow more efficient therapy compared with the present formulations.

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