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RESEARCH PAPER

A Nifedipine Coground Mixture with Sodium Deoxycholate. II. Dissolution Characteristics and Stability

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ABSTRACT

Nifedipine is a poorly water soluble drug that demonstrates low bioavailability. In a previous study, a coground mixture of nifedipine with sodium deoxycholate (DCNa), a bile salt, immediately produced colloidal particles when dispersed in water. In this study, the effect of the weight fraction of DCNa, grinding time, dissolution media, and storage conditions on colloidal particle formation in solution was investigated. The coground mixture was prepared with a vibration rod mill, and its solid state was characterized using powder X-ray diffraction. A laser diffraction particle size analyzer was used to determine the particle size distribution curve in water. The size of particles formed in solution decreased with an increase in the weight fraction of DCNa and grinding time. A nifedipine-DCNa (1 : 2 w/w) mixture coground for 30 min was used in the experiments. Colloidal particle formation from the coground mixture was also observed in dissolution media of water and a pH 6.8 buffer solution at 37°C. Most precipitates passed through a filter with a pore size of 0.8 μm, but the particle size distribution in water was different from that in the pH 6.8 buffer solution. DCNa exhibited not only micellar solubilization for drug crystals, but also a retarding effect on drug crystal growth in a supersaturated solution. The latter effect could serve to form colloidal particles in solution. When stored under 75% relative humidity at 40°C for 1 month, the amorphous coground

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mixture crystallized, and the particle size in water markedly increased. Therefore, the weight fraction of DCNa, grinding time, dissolution media, and humidity during storage influence the dissolution characteristics of nifedipine from a coground mixture.

Key Words: *Coground mixture; Colloidal particle; Humidity; Nifedipine; Sodium deoxycholate*

INTRODUCTION

A much greater improvement in the bioavailability of poorly water soluble drugs would be expected to enhance their dissolution in gastrointestinal fluids. Nifedipine, a calcium channel agent, is a poorly water soluble drug that has low bioavailability when orally administered in crystalline form (1). Many studies have therefore been done in an attempt to enhance the dissolution rate of nifedipine by solid dispersion and cogrinding techniques (1–5). These techniques are effective for transforming the crystalline drug into an amorphous solid or for reducing the drug particle size to the submicron range in an inert carrier such as a water-soluble polymer.

Amorphous formation of poorly water soluble drugs in solid dispersions and coground mixtures will serve to enhance an intrinsic solubility of drugs, resulting in a faster dissolution rate and then a supersaturated solution in dissolution media (1,6). In general, the deposition process of a drug from a supersaturated solution depends on two factors: nucleation and subsequent crystallization (7,8). If the carriers used in the above techniques have a low inhibitory effect on drug crystallization, microparticulate crystals of the drug will precipitate from the supersaturated solution (2,9). However, this drug deposition process varies with the combination of drug and carrier. Yano et al. (10,11) reported that YM022 solid dispersion with hydroxypropylmethylcellulose and polyoxyethylene hydrogenated caster oil immediately formed colloidal particles in the dispersed solution, contributing to a large increase in bioavailability.

Sodium deoxycholate (DCNa) is a bile salt; it markedly increases the dissolution rate and solubility of poorly water soluble drugs due to micellar solubilization (12). Sakurai et al. (13) showed that amorphous mefenamic acid formed in solid dispersions with DCNa brought about a supersaturated solution, and then the apparent increased solubility of the drug decreased rapidly. Otsuka and Matsuda (14,15) also reported that most of crystalline phenytoin was transformed into an amorphous form during a grinding process with DCNa, resulting in an enhancement of the apparent solubility of the drug. Interestingly, its supersaturated state did not decrease.

In a previous study (16), we reported that nifedipine crystals were transformed into an amorphous form by grinding with DCNa, and colloidal particles occurred rapidly when the coground mixture was added to a solution. The present study was designed to evaluate the effect of the weight fraction of DCNa, grinding time, dissolution media, and humidity during storage on colloidal particle formation. The ability of DCNa to dissolve drug crystals and to retard drug crystal growth in dissolution media was also examined.

EXPERIMENTAL

Materials

Nifedipine and DCNa were purchased from Wako Pure Chemical Industries Company, Limited (Japan) and Kanto Chemical Industries Company, Limited (Japan), respectively. All other chemicals used were reagent grade. All experiments were carried out under subdued light to prevent light degradation of nifedipine.

Preparation of Coground Mixtures

Nifedipine (1 g) and DCNa (0.5–3 g) were ground for 5–40 min using a vibration rod mill (sample mill TI-100, CMT, Japan). The sample chamber was made of aluminum oxide, and the sample capacity was 10 ml. The environmental conditions were $23^{\circ}C \pm 2^{\circ}C$ and $50\% \pm 10\%$ relative humidity (RH). Nifedipine (1 g) was ground for 30 min as a single-ground sample, and the drug was also mixed with DCNa (2 g) uniformly in a plastic bag by hand for 5 min to make a physical mixture.

Powder X-Ray Diffraction Analysis

Powder X-ray diffraction patterns were measured at room temperature with an X-ray diffractometer (model JDX-8030, JEOL, Japan). The measurement conditions were as follows: copper target; nickel filter; 40-kV voltage; 30-mA current; 1° receiving slit; 0.6-s time constant; 4°/min scanning speed.

Particle Size Analysis

The coground mixture containing nifedipine (about 50 mg) was dispersed in purified water (50 ml) and incubated at room temperature for 15 min with stirring. The particle diameter was then determined with a laser diffraction particle size analyzer (model SALD-1100, Shimadzu, Japan). The diameter of single-ground nifedipine was also measured in an aqueous solution of DCNa (0.2% w/v).

High-Performance Liquid Chromatographic Analysis of Nifedipine

Assay of nifedipine was determined using high-performance liquid chromatography (HPLC) (model LC-10A, Shimadzu) under the following conditions: C18 reversed-phase column (YMC-Pack ODS-H80); 230-nm detection wavelength; 0.05 M phosphate buffer (pH 3.0)/methanol/tetrahydrofuran (60 : 32 : 8 v/v) mobile phase; 1.3 ml/min flow rate.

Dissolution Studies

The JP 13 second fluid (pH 6.8) was obtained as follows: 118 ml of 0.2 M NaOH solution was added to 250 ml of 0.2 M KH_2PO_4 solution and diluted with water to 1000 ml. The dissolution profiles of a nifedipine-DCNa (1 : 2 w/w) coground mixture containing 50–500 mg of the drug were measured using the JP 13 paddle method in purified water and JP 13 second fluid (500 ml) at 37°C with constant stirring at 50 rpm. Aliquots (5 ml) of the solution were withdrawn, filtered with 0.2, 0.45, and 0.8 μm cellulose acetate–type membrane filters (Advantec, Japan) and diluted with methanol. The apparent concentration of nifedipine was determined using the HPLC system under the above conditions.

Drug Solubility Studies

Excess amounts of nifedipine were added to purified water and a pH 6.8 buffer solution containing various concentrations of DCNa. After shaking for 48 h at 37°C, samples were withdrawn, filtered with 0.2 μm cellulose acetate–type membrane filters, diluted with methanol, and assayed with HPLC at 237 nm.

Crystallization Studies

Crystallization studies were performed using an apparatus for dissolution tests according to JP 13 (paddle method, 150 rpm) at 37°C. A methanol solution (2 ml) containing 50 mg of nifedipine was added to purified water and a pH 6.8 buffer solution of 500 ml containing 100–300 mg of DCNa. Samples were filtered with 0.8 μm cellulose acetate–type membrane filters, diluted with methanol, and assayed with HPLC at 237 nm.

Stability Studies

The nifedipine-DCNa (1 : 2 w/w) coground mixture was stored for 1 month in airtight glass containers at 40°C or in a chamber with controlled temperature and humidity (model PR-1F, Tabai Espec, Japan) at 40 °C/75% RH. Powder X-ray diffraction and particle size analyses were carried out.

RESULTS AND DISCUSSION

Effect of Weight Fraction of Sodium Deoxycholate and Grinding Time on Colloidal Particle Formation in Water

In the previous study (16), we reported that amorphous formation of nifedipine in coground mixtures was dependent on the complexation between the drug and DCNa during grinding. This complexation in coground mixtures was confirmed as a new exothermic peak on differential scanning calorimetry thermograms, assuming that the van der Waals forces promote complex formation on Fourier transform infrared spectra. Its magnitude was closely related to the weight fraction of DCNa and grinding time.

In this study, the effect of the weight fraction of DCNa and grinding time on colloidal particle

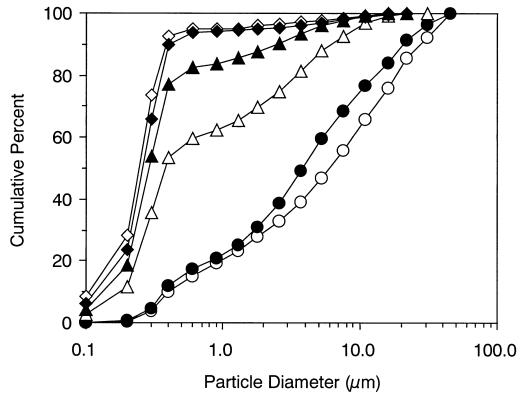

Figure 1. Effect of the weight ratio on particle size distribution curves of nifedipine-DCNa mixtures coground for 30 min. Nifedipine/DCNa (w/w): ○, 1 : 0; ●, 1 : 0.5; △, 1 : 1; ▲, 1 : 1.5; ◇, 1 : 2; ◆, 1 : 3.

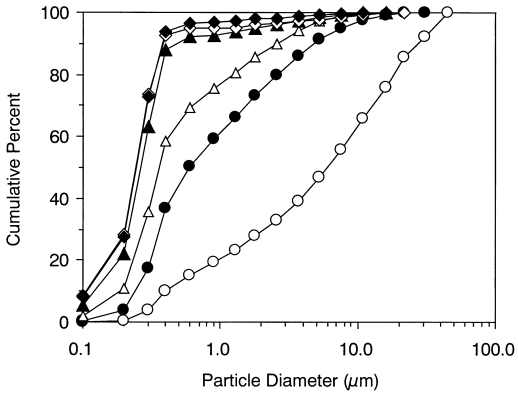

Figure 2. Effect of the grinding time on particle size distribution curves of nifedipine-DCNa (1 : 2 w/w) coground mixtures: ○, 30 min (1 : 0 w/w); ●, 5 min; △, 10 min; ▲, 20 min; ◇, 30 min; ◆, 40 min.

formation in water was examined. The particle size distribution curves of nifedipine coground mixtures containing different weight fractions of DCNa are shown in Fig. 1. These mixtures were coground for 30 min. The particle size was found to change from the micron order to the submicron order with an increase in the weight fraction of DCNa. There was no difference in the particle size distribution curves for 1 : 2 w/w or above the weight ratio of drug/DCNa, of which samples immediately formed semitransparent colloidal solutions after dispersal in water.

Next, with regard to the nifedipine-DCNa (1 : 2 w/w) coground mixture, the relationship between particle size and grinding time was investigated (Fig. 2). The particle size distribution curves of the drug were also apparently affected by the grinding time, and 90% of the total particles had a diameter less than 600 nm for mixtures coground for 20 min or more. In the previous study, after the mixture was coground for 30 min and dispersed in water, a marked difference in its particle size distribution curve was not observed for 180 min.

These results indicate that the weight fraction of DCNa and the grinding time are important for the formation of colloidal particles in solution. Consequently, in the experiments discussed below, a nifedipine-DCNa (1 : 2 w/w) mixture coground for 30 min was used.

Dissolution Studies

Figure 3 shows the dissolution profiles of the nifedipine-DCNa coground mixture in water and a pH 6.8 buffer solution at 37°C. Since it is difficult to dissolve DCNa in acidic media, JP 13 second fluid (pH 6.8) was used in this study. Membrane filters with three different pore sizes (0.2, 0.45, and 0.8 μm) were also used to confirm the formation of colloidal particles in dissolution media and to determine the apparent concentration of the drug through each filter. Although the solubility of nifedipine was about 10 μg/ml in both dissolution media at 37°C, the 1 : 2 w/w coground mixtures containing 50 and 500 mg of nifedipine were each added to 500 ml of medium (Figs. 3A and 3B, respectively), corresponding to from 10 to 100 times the intrinsic drug solubility.

The dissolution of the coground mixtures was rapid in both dissolution media, and each apparent concentration of the drug through different filters was nearly constant up to 120 min. The drug concentrations through the pore sizes of 0.2, 0.45, and 0.8 μm after 120 min were as follows: For the case shown in Fig. 3A, concentrations were 36, 59, and 87 μg/ml in water and 25, 33, and 77 μg/ml in pH 6.8 media, respectively; in the case shown in Fig. 3B, concentrations were 23, 107, and 836 μg/ml in water and 37, 45, and 928 μg/ml in pH 6.8 media, respectively. These findings suggest that more than 80% of the drug added to the solution formed colloidal particles, and that the composition of the dissolution media influenced the particle size distribution.

It is also thought that the bioavailability of nifedipine may be improved if colloidal particles of

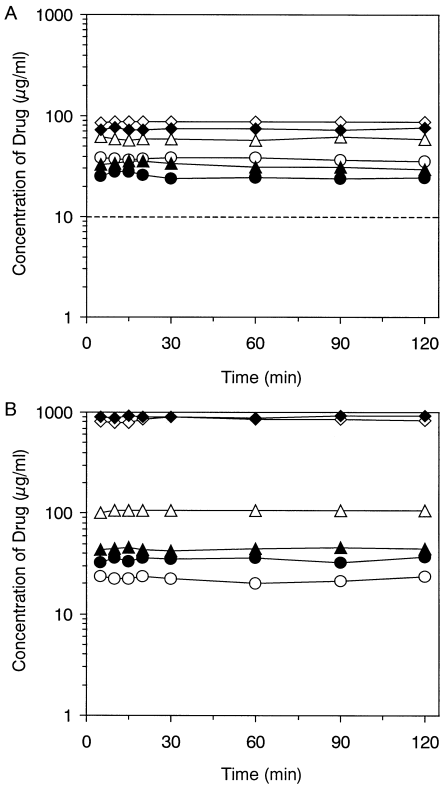

Figure 3. Dissolution profiles of nifedipine-DCNa (1 : 2 w/w) mixtures coground for 30 min. Amount of drug added to dissolution media: (A) 50 mg; (B) 500 mg. Filter pore size: ○, ●, 0.2 μm; △, ▲, 0.45 μm; ◇, ◆, 0.8 μm. Open symbols, in water; closed symbols, in pH 6.8 media. The drug solubility without DCNa is 10 μg/ml.

the drug form rapidly from the coground mixture with DCNa and subsequently do not aggregate in the gastrointestinal tract, as shown in Fig. 3. Since DCNa has a low solubility in acidic media and a strong bitter taste, an enteric-coated dosage form is appropriate for oral administration. However, it should be kept in mind that, for nifedipine, increasing the bioavailability is often associated with a high and early plasma peak that is deleterious on long-term administration (17).

Solubility and Crystallization Studies

Many investigations have demonstrated the solubilizing property of DCNa for poorly water soluble materials (12,13); solubility increases linearly with the concentration of DCNa, based on micellar solubilization. Figure 4 shows a plot of the concentration of nifedipine against the different concentrations of DCNa solution at 37°C. A filter with a pore size of 0.2 μm was used to determine the apparent solubility of the drug. An approximately linear relationship was shown in both water and the pH 6.8 buffer solution; theoretically, 1.6 g DCNa was necessary to double the intrinsic drug solubility. However, as shown in Fig. 3A, the drug concentration through a pore size of 0.2 μm in dissolution media with 100 mg of DCNa was about three times the intrinsic drug solubility. This implies that the micellar solubilizing effect of DCNa is not appreciably associated with the enhancement of drug solubility. In addition, the coground mixture has amorphous nifedipine (16), which induces a supersaturation phenomenon in dissolution media, as reported in many studies on nifedipine solid dispersions (1,2). Thus, the apparent increased solubility of nifedipine as shown in Fig. 3 may be attributable to amorphous formation of the drug in the coground mixture.

Further evidence of the DCNa effect on colloidal particle formation in dissolution media was observed in the crystallization behavior of nifedipine from its supersaturated solution with DCNa (Fig. 5). After a methanol solution containing 50 mg of nifedipine was added to water and a pH 6.8 buffer solution with or without DCNa at 37°C, the drug concentration was measured through a pore size of 0.8 μm to confirm colloid formation. The paddle rotation was three times that of the dissolution studies to accelerate drug crystallization (18). The drug concentration

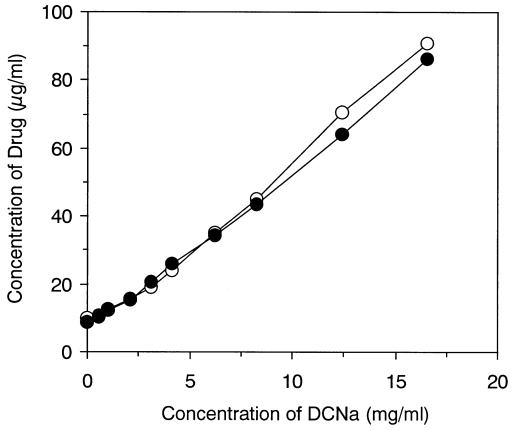

Figure 4. Effect of DCNa on aqueous solubility of nifedipine at 37°C. Filter pore size 0.2 μm. Open symbols, in water; closed symbols, in pH 6.8 media.

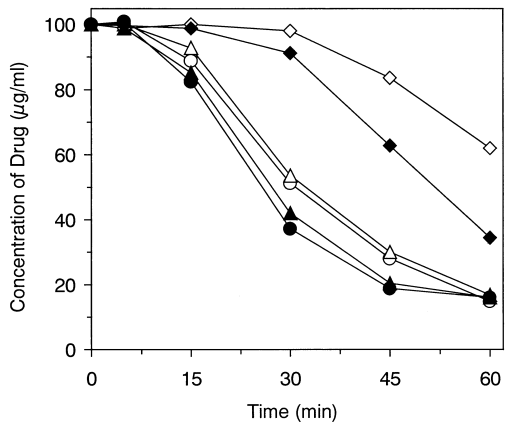

Figure 5. Crystallization behavior of nifedipine in aqueous DCNa solution at 37°C. Amount of DCNa contained in solution: ○, ●, 0 mg; △, ▲, 100 mg; ◇, ◆, 300 mg. Filter pore size 0.8 μm. Open symbols, in water; closed symbols, in pH 6.8 media.

in the solution with 100 mg of DCNa decreased with time similar to that without DCNa. On the other hand, in the solution with 300 mg of DCNa, the decrease in drug concentration was much slower than that without DCNa, indicating the retarding effect of DCNa on drug crystal growth to the micron order in the supersaturated solution. Since the drug concentration through each pore size did not decrease with time, as shown in Fig. 3, it can be assumed that the retarding effect of DCNa on nifedipine crystal growth acts at a sufficiently early stage of dissolution.

If aqueous dispersions of hydrophobic solids are to resist aggregation (coagulation and flocculation), they must be stabilized (7); stabilizing factors include the presence of adsorbed macromolecules or nonionic surfactants and electric charges on the particle surface due to adsorbed ions such as ionic surfactants. Hasegawa et al. (2) reported that the inhibitory effect of cellulose polymers on nifedipine crystallization from supersaturated solutions was due to polymer adsorption on the solid-water interface through hydrophobic interaction at the stage when a hydrophobic drug-crystal surface was formed. In fact, bile acids contain both a hydrophobic and a hydrophilic side, and this property is mainly responsible for their remarkable chemical behavior (19). Thus, it may be assumed that the hydrophobic site of DCNa is rapidly adsorbed on the drug crystal surface, retarding the drug crystal growth. At the same time, the electric charge on the surface can be induced by the anionic group of the hydrophilic side of DCNa to stabilize colloidal particles in solution.

From characteristics of the general behavior of solid dispersion in water, it is noted that solid dispersion may cause a supersaturated condition, and that microparticulate crystals of a drug may form after dispersal of solid dispersion into water. Yano et al. (10) reported that a spray-dried solid dispersion composed of YM002 (a poorly water soluble drug), hydroxypropylmethylcellulose 2910, and polyoxyethylene hydrogenated castor oil 60 produced colloidal particles after reconstitution in purified water. They also reported that the colloid formation of this solid dispersion in water was due to a different behavior from the general pattern described above (20). Similarly, our results differ from the general pattern of amorphous systems; no work has been done on colloid formation from an amorphous solid prepared by a cogrinding method. The intermolecular interaction between the drug and DCNa formed during the grinding process can be expected to facilitate the adsorption of DCNa on the drug crystal surface in solution.

Stability Studies

Since molecules in an amorphous state are thermodynamically metastable compared with those in a crystalline state (21), the potential for crystallization of amorphous parts in coground mixtures during storage is always present. It is well known that amorphous solids are liable to be hygroscopic, and small amounts of adsorbed moisture can plasticize amorphous solids, thereby leading to a decrease in their glass transition temperature and an increase in molecular mobility (22,23). The effect of humidity during storage was therefore investigated on the formation of colloidal particles in water for a nifedipine-DCNa (1 : 2 w/w) coground mixture. Figure 6 shows the particle size distribution curves of coground mixtures stored at 40°C and 40°C/75% RH for 1 month. The particle size distribution curve of the sample stored at 40°C did not change against that of the initial sample, whereas the particle size of the sample stored at 40°C/75% RH increased markedly.

The physical stability of the nifedipine-DCNa (1 : 2 w/w) coground mixture was evaluated by powder X-ray diffraction analysis (Fig. 7). There were no differences in the powder X-ray diffraction

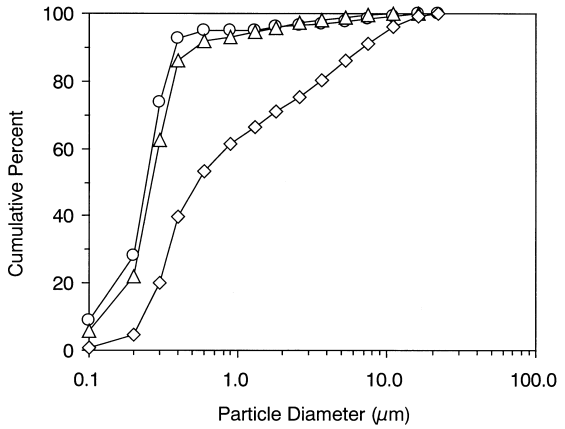

Figure 6. Particle size distribution curves of nifedipine-DCNa (1 : 2 w/w) coground mixtures stored at 40°C or 40°C/75% RH for 1 month: ○, initial; △, 40°C; ◇, 40°C/75% RH.

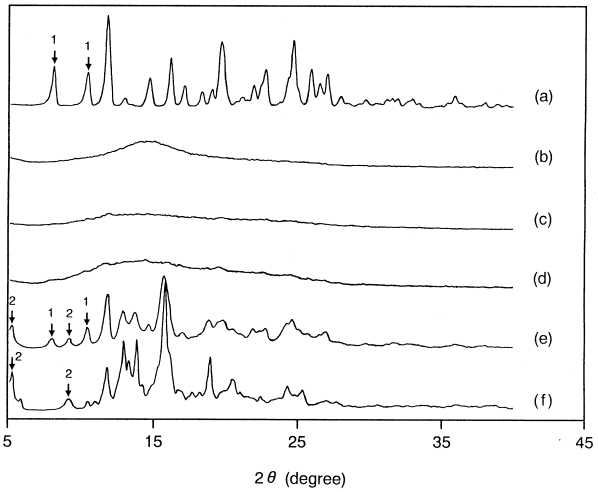

Figure 7. Powder X-ray diffraction patterns of nifedipine-DCNa (1 : 2 w/w) coground mixtures stored at 40°C or 40°C/75% RH for 1 month: (a) nifedipine powder; (b) DCNa powder; (c) initial; (d) 40°C; (e) 40°C/75% RH; (f) DCNa powder stored at 40°C/75% RH. 1, Crystalline nifedipine peaks; 2, crystalline DCNa peaks.

patterns of the coground mixtures before and after storage at 40°C for 1 month (curves c and d, respectively). On the other hand, both the coground mixture and the DCNa powder stored at 40°C/75% RH for 1 month showed distinct peaks (curves e and f, respectively). Peaks of 8.1° and 10.5° 2θ in crystalline nifedipine (arrow 1) and 5.4° and 9.1° 2θ in crystalline DCNa (arrow 2) were observed in the coground mixture. These findings suggest that the presence of humidity during storage decreases the complex stability of nifedipine-DCNa in the coground mixture, resulting in the crystallization of amorphous nifedipine and DCNa, subsequently increasing the particle size in solution.

Forming colloidal particles from coground mixtures with DCNa in solution is anticipated to have general applicability to many poorly water soluble drugs to enhance their oral bioavailability. However, it should be noted that humidity during storage carries the risk of decreasing the colloidal particle formation of coground mixtures when dispersed in the gastrointestinal tract.

CONCLUSION

The cogrinding of nifedipine with DCNa immediately produced colloidal particles when dispersed in water and a pH 6.8 buffer solution. The particle size distribution depended on the weight fraction of DCNa and the grinding time, but the initial particle size was not changed with time in the dissolution studies. DCNa possessed not only micellar solubilization for nifedipine crystals, but also a retarding effect on drug crystal growth in a supersaturated solution. The latter effect in solution could serve to form colloidal particles. Humidity during storage promoted the crystallization of amorphous nifedipine and DCNa in a coground mixture and thereby increased the particle size in solution.

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