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RESEARCH PAPER

A Nifedipine Coground Mixture with Sodium Deoxycholate. I. Colloidal Particle Formation and Solid-State Analysis

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ABSTRACT

Sodium deoxycholate (DCNa) is a bile salt that forms multimolecular inclusion compounds with a variety of organic substances. In this study, complex formulation of DCNa with nifedipine, a poorly water soluble drug, by grinding was investigated. The coground mixture was prepared with a vibration rod mill, and its solid state was characterized using powder X-ray diffraction, differential scanning calorimetry (DSC), and Fourier transform infrared (FTIR) spectroscopy. A laser diffraction particle size analyzer was also used to determine the particle size distribution curve in solution. When a nifedipine-DCNa (1:2 w/w) mixture coground for 30 min was dispersed into water and a pH 6.8 buffer solution, a semitransparent colloidal solution occurred immediately; 90% of the total particles formed in solution had a diameter less than 600 nm. Both powder X-ray diffraction peaks and DSC endothermic peak of nifedipine crystals were not found for the coground mixture, whereas a new exothermic peak was observed on DSC thermograms. The magnitude of this exothermic peak depended on the weight fraction of DCNa and the grinding time, indicating that nifedipine crystals changed into an amorphous state by complex formation with DCNa during the grinding process. In the FTIR spectrum of the coground mixture, the peaks of aromatic CH out-of-plane bend and dihydropyridine NH stretch of nifedipine were considerably weakened, suggesting that van der Waals interaction may be

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present between the drug and DCNa molecules. From these results, it is clear that the cogrinding method with DCNa is very useful for the formation of amorphous nifedipine in the solid state and the production of colloidal particles of the drug in solution.

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

INTRODUCTION

Poorly water soluble drugs tend to be eliminated from the gastrointestinal tract before they have had an opportunity to dissolve fully and be absorbed into the circulation. This characteristic results in low and erratic bioavailability and poor dose proportionality. It is important to enhance the dissolution rate of such drugs. Some dissolution-enhancing methods have therefore been applied for the production of pharmaceutical preparations (1). It is well known that both solid dispersion and cogrinding of poorly water soluble drugs with various kinds of polymers are useful for solubilization and enhancement of bioavailability because the crystalline drug is transformed into an amorphous form in a polymer network (2–4). The rationale behind such a strategy is that a highly disordered amorphous material has a lower energetic barrier to overcome to enter a solution than a regularly structured crystalline solid.

Compared with the solid dispersion method, the cogrinding method has no need for organic solvent processes involving environmental and health concerns or for fusion processes involving thermal instability and immiscibility. The cogrinding method is carried out under either dry or wet conditions with various milling devices, such as ball, jet, and hammer mills and the like. Since the dissolution rate of a drug is a function of its particle size, as well as its intrinsic solubility, previous studies with a number of poorly water soluble drugs have demonstrated that particle size reduction can lead to higher oral bioavailability, as reported for danazol (5), naproxen (6), nifedipine (7), and TA-7552 (8). These studies have involved mechanical size reduction of particles to smaller than 1 μm, which gives considerable potential for substantially enhancing bioavailability due to size reduction in the submicron range.

Many studies have shown that bile salts such as sodium deoxycholate (DCNa) markedly increase the dissolution rate and solubility of poorly water soluble drugs because the activity of bile salts is mainly due to micellar solubilization (9,10). DCNa has already been used for the cogrinding method with phenytoin, resulting in an amorphous formation and thereby an apparent increase in drug solubility (11–13). However, no detailed study has been reported on colloidal particle formation from coground products with DCNa in dissolution media. Recently, we found that when a coground mixture of DCNa with nifedipine, a poorly water soluble drug (10 μg/ml), was added to water, colloidal particles occurred readily. The purpose of this study was to investigate the characteristics of a nifedipine coground mixture with DCNa in the solid state using powder X-ray diffraction, differential scanning calorimetry (DSC), and Fourier transform infrared (FTIR) spectroscopy.

EXPERIMENTAL

Materials

Nifedipine and DCNa were purchased from Wako Pure Chemical Industries Company, Limited (Japan) and Kanto Chemical Industries Company, Limited (Japan), respectively. Chemical structures of these substances are shown in Fig. 1. 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 (0.5–2 g) were ground for 5–30 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. 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.

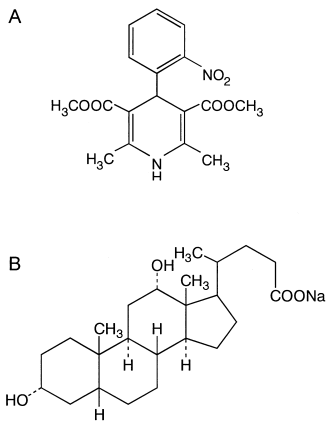

Figure 1. Chemical structure of (A) nifedipine and (B) DCNa.

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.

Thermal Analysis

Differential scanning calorimetry (DSC) was performed with a model DSC-220CU instrument (Seiko Denshi, Japan). The measurement conditions in an open-pan system were as follows: 5–10 mg sample weight; 10°C/min heating rate; N_2 50 ml/min gas flow rate.

Infrared Absorption Spectroscopic Analysis

The sample powder was dispersed in micronized KBr powder using a pestle and mortar without destruction of the sample particles. FTIR spectra were obtained by powder diffuse reflectance on an FTIR spectrophotometer (model 8020D, Shimadzu, Japan) and modified using the Kubelka-Munk equation.

Particle Size Analysis

The JP 13 second fluid (pH 6.8 buffer solution) 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 coground mixture containing nifedipine (about 50 mg) was dispersed in purified water and a pH 6.8 buffer solution (50 ml) and incubated at room temperature for 15–180 min with stirring. The particle diameter was then determined with a laser diffraction particle size analyzer (model SALD-1100, Shimadzu). The diameter of single-ground nifedipine was also measured in an aqueous solution of DCNa (0.2% w/v).

RESULTS AND DISCUSSION

Colloidal Particle Formation of the Coground Mixture in Water and pH 6.8 Buffer Solution

The particle size distribution curves of nifedipine-DCNa (1 : 1 and 1 : 2 w/w) coground mixtures in water and a pH 6.8 buffer solution are shown in Fig. 2. Since it is difficult to dissolve DCNa in acidic media, JP 13 second fluid (pH 6.8) was used in this study. The drug was coground with DCNa for 30 min. The particle size obtained in both media after 15 min was found to change from the micrometer order to the submicron order with an increase in the weight fraction of DCNa. Especially, concerning the 1 : 2 w/w coground mixture, a semitransparent colloidal solution was immediately observed after dispersal, and 90% of the total particles formed in solution had a diameter less than 600 nm.

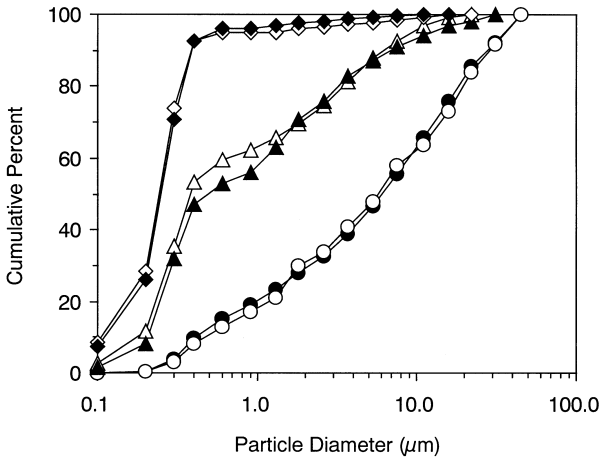

Figure 2. Particle size distribution curves of nifedipine-DCNa mixtures coground for 30 min. Weight ratio of nifedipine-DCNa (w/w): ○, ●, 1 : 0; △, ▲, 1 : 1; ◇, ◆, 1 : 2. Open symbols, in water; closed symbols, in pH 6.8 media. The stirring time was 15 min.

Figure 3 shows the particle size distribution curve of a nifedipine-DCNa (1 : 2 w/w) coground mixture dispersed for 180 min in water. Although a marked difference in the particle size distribution curves from 15 to 180 min was not observed, there was a very slight tendency for the particle size to increase with the stirring time; 86% of the total particles formed after 180 min had a diameter less than 600 nm. Considering that DCNa is easily soluble in water and the pH 6.8 buffer solution, these findings indicate that the nifedipine-DCNa (1 : 2 w/w) coground mixture rapidly forms colloidal particles of the drug in solution, and that they hardly aggregate over 3 h.

Many studies have reported enhancing the bioavailability of poorly water soluble drugs by size reduction to the submicron range (5–8). This is because particle size reduction can lead to an increase in specific area and consequently to an enhancement of dissolution rate. Therefore, it is possible to improve the bioavailability of nifedipine if the colloidal particles of the drug form rapidly from a nifedipine-DCNa coground mixture and then do not agglomerate in the gastrointestinal tract. Since DCNa has a low solubility in acidic media and a strong bitter taste, an enteric-coated dosage form is appropriate for the oral administration of this coground mixture. Further detailed investigations, such as the dissolution profile of the nifedipine-DCNa coground mixture, its changes under storage conditions, and DCNa function in the dissolution process are necessary.

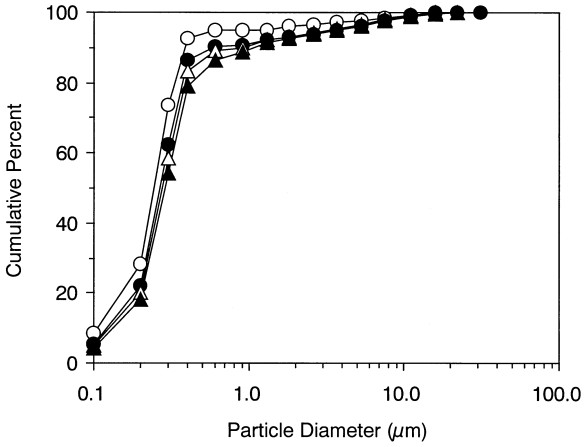

Figure 3. Effect of the stirring time on particle size distribution curves of nifedipine-DCNa (1 : 2 w/w) coground mixtures in water: ○, 15 min; ●, 60 min; △, 120 min; ▲, 180 min.

Amorphous Formation in the Coground Mixture

To clarify the physicochemical characteristics of nifedipine-DCNa (1 : 1 and 1 : 2 w/w) coground mixtures, powder X-ray diffraction and DSC measurements were done. Figure 4 shows powder X-ray diffraction patterns of the physical and coground mixtures. The DCNa used in this study revealed a halo pattern (curve b), indicating that DCNa was present in an amorphous state. The characteristic diffraction peaks of nifedipine crystals were clearly detected for the 1 : 2 w/w physical mixture (curve c), whereas they considerably decreased in the 1 : 1 w/w coground mixture (curve d) and disappeared from the 1 : 2 w/w coground mixture (curve e). These results suggest that nifedipine crystals in the coground mixture are transformed into an amorphous form by the grinding process with DCNa.

Figure 5 shows DSC thermograms of the physical and coground mixtures. An endothermic melting peak of nifedipine crystals and an exothermic recrystallization peak of DCNa powder were observed at 176°C and 214°C, respectively (curves a and b). The 1 : 2 w/w physical mixture showed a somewhat broadening of the drug endothermic peak and a disappearance of the DCNa exothermic peak (curve c). On the other hand, for the 1 : 1 w/w coground

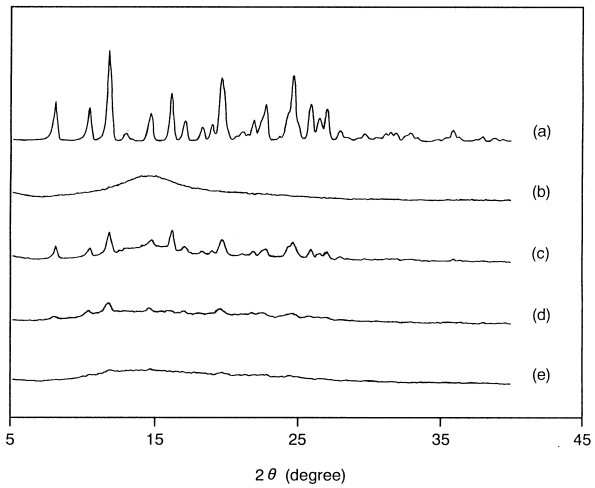

Figure 4. Powder X-ray diffraction patterns of nifedipine-DCNa coground mixtures: (a) nifedipine powder; (b) DCNa powder; (c) physical mixture (1:2 w/w); (d) coground mixture (1 : 1 w/w); (e) coground mixture (1 : 2 w/w). The grinding time of Fig. 4d and Fig. 4e was 30 min.

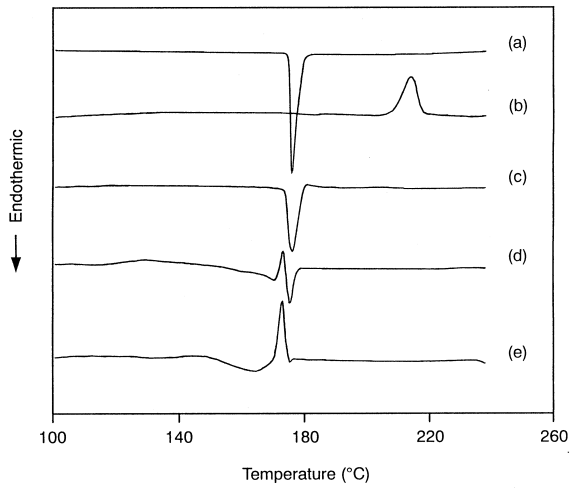

Figure 5. DSC thermograms of nifedipine-DCNa coground mixtures: (a) nifedipine powder; (b) DCNa powder; (c) physical mixture (1 : 2 w/w); (d) coground mixture (1 : 1 w/w); (e) coground mixture (1 : 2 w/w). The grinding time of Fig. 5d and Fig. 5e was 30 min.

mixture, the drug endothermic peak decreased, and a new exothermic peak occurred at 173°C (curve d). By increasing the weight fraction of DCNa in the coground mixture, the drug endothermic peak disappeared, and the magnitude of the exothermic peak increased (curve e). This exothermic peak may be attributable to an amorphous complexation between nifedipine and DCNa.

Using the single-ground nifedipine and the coground mixtures with 1 : 0.5–2 w/w of drug/DCNa, the heat ΔH of the endothermic melting peak of nifedipine crystals was plotted as a function of the weight percentage of DCNa in the coground mixtures (Fig. 6). The increase in the weight percentage of DCNa resulted in a linear reduction in the ΔH value of the drug. The ΔH value calculated by linear regression (correlation coefficient = 0.993) was zero at 66% w/w of DCNa, corresponding to the amount of DCNa in the 1 : 2 w/w coground mixture.

Figure 7 shows the DSC thermograms of 1 : 2 w/w coground mixtures for 5 to 30 min of grinding time. During the grinding process, even for only 5 min, the endothermic melting peak of nifedipine crystals disappeared, while a broadened endothermic peak occurred at 171°C (curve c). With an increase in the grinding time, the broadened endothermic peak at 171°C became unclear, and the exothermic peak due

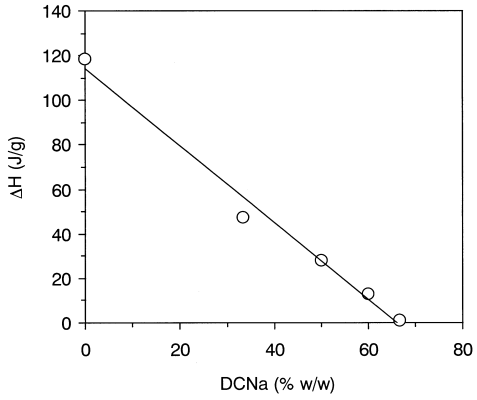

Figure 6. Plots of the heat ΔH of the endothermic melting peak of nifedipine crystals on DSC thermograms against the weight percentage of DCNa in coground mixtures. The grinding time was 30 min.

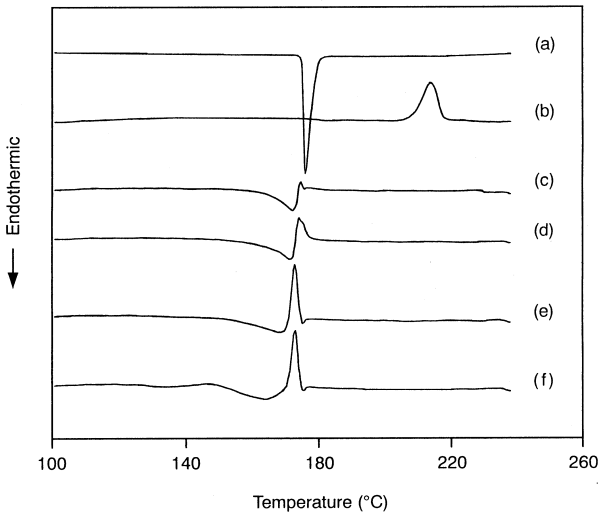

Figure 7. DSC thermograms of nifedipine-DCNa (1:2 w/w) coground mixtures: (a) nifedipine powder; (b) DCNa powder; (c), (d), (e), and (f) coground mixtures. The grinding times were as follows: (c) 5 min; (d) 10 min; (e) 20 min; (f) 30 min.

to complexation markedly increased (curves d and e). This exothermic peak did not change for grinding times of 20 min or above.

These results suggest that the weight fraction of DCNa and the grinding time are closely related to the complexation in the nifedipine-DCNa coground mixture. A grinding time of 30 min will be adequate for producing a complexation in proportion to the amount of DCNa.

It is said that aggregation due to van der Waals forces begins at 10 μm, becoming intense at 1 μm as crystals fracture across cleavage planes involving a valence type of force (14). However, the cogrinding technique for poorly water soluble drugs with hydrophilic low molecule additives, such as D-mannitol, can reduce the drug particle size to the submicron level, dispersing colloidal particles of the drug in biological fluids (8,15). D-Mannitol may help the micronization of drug crystals and prevent their aggregation. When grinding with polymers, such as microcrystalline cellulose, drug crystals can be transformed into an amorphous form, resulting in an increased dissolution rate and a drug supersaturation (3,16). Polymers, which have no effect on inhibiting drug crystallization, may decrease the drug concentration in dissolution media in a manner similar to that of solid dispersions. In the present study, it was particularly interesting that colloidal particles occurred immediately after the amorphous nifedipine-DCNa coground mixture was dispersed into solution. This phenomenon of producing colloidal particles from amorphous coground products has not been reported.

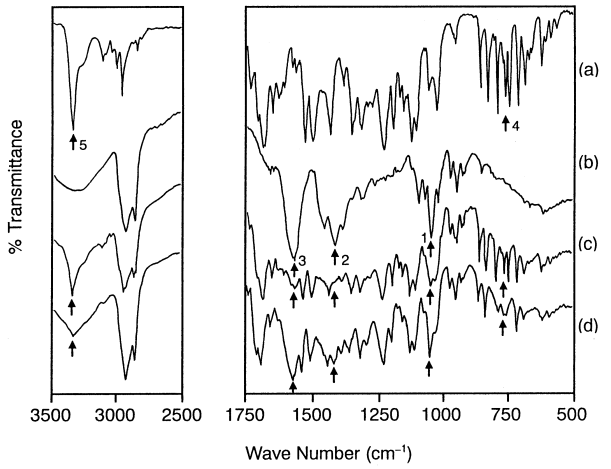

Figure 8. FTIR spectra of nifedipine-DCNa mixtures coground for 30 min: (a) nifedipine powder; (b) DCNa powder; (c) physical mixture (1 : 2 w/w); (d) coground mixture (1 : 2 w/w). 1, Attributable to the secondary alcoholic C–O stretch of DCNa; 2 and 3, attributable to the COO^- symmetric and asymmetric stretches of DCNa, respectively; 4, attributable to the aromatic CH out-of-plane bend of nifedipine; 5, attributable to the dihydropyridine NH stretch of nifedipine.

Intermolecular Interaction in Physical and Coground Mixtures

To characterize the nifedipine-DCNa interaction in the solid state, FTIR absorption spectra were obtained for physical and coground mixtures with 1 : 2 w/w of drug/DCNa (Fig. 8). Nifedipine crystals (curve a) showed a dihydropyridine NH stretching band at 3332 cm^{-1}, an esters C=O stretching band at 1685 cm^{-1}, an NO_2 stretching band at 1529 cm^{-1}, and an aromatic CH out-of-plane bending band at 745–795 cm^{-1}. DCNa powders (curve b) showed a CH stretching band at 2937 cm^{-1}, a COO^- asymmetric stretching band at 1559 cm^{-1}, a COO^- symmetric stretching band at 1406 cm^{-1}, and a secondary alcoholic C–O stretching band at 1043 cm^{-1}. For the physical mixture (curve c), the COO^- symmetric stretching peak of DCNa disappeared (arrow 2), and the peaks of the secondary alcoholic C–O stretch and COO^- asymmetric stretch of DCNa weakened (arrows 1 and 3, respectively). On the other hand, for the coground mixture (curve d), the peaks of the aromatic CH out-of-plane bend and dihydropyridine NH stretch of nifedipine considerably weakened (arrows 4 and 5, respectively). These findings suggest that some intermolecular interactions are present in physical and coground mixtures, and that complex formation in coground mixtures does not have the same interaction and structure as that in physical mixtures.

Yakou et al. (17) showed that phenytoin-DCNa coprecipitates (more than 1 : 5 w/w) were stable amorphous solids, and the complex formation of phenytoin-DCNa due to hydrogen bonding was confirmed by the IR spectral method. On the other hand, Otsuka and Matsuda (12) reported that DCNa enhanced the amorphous formation of phenytoin crystals during ball mill grinding, but the FTIR spectrum of a phenytoin-DCNa mixture coground for 3 h did not demonstrate significant differences from that of a physical mixture. These results imply that intermolecular interaction in coground products with DCNa is different from than in coprecipitates. Bile acids (e.g., colic acid and deoxycholic acid) have the ability to form multimolecular inclusion complexes with a variety of chemical compounds (18). Giglio (19) reported that, when a complex was formed between a guest and deoxycholic acid, the carbonyl groups of deoxycholic acid molecules formed hydrogen bonds with the OH groups of its adjacent molecules to build up the typical bilayer structure of the guest–deoxycholic acid complex. The outer surface

of the bilayer, covered with the methyl group of deoxycholic acid, is a hydrophobic channel in which guest molecules can be accommodated due to hydrophobic forces, rather than to electrostatic bonds. Candeloro de Sanctis et al. (20) and Jones et al. (21) also reported that aromatic guests interacted in a pile and formed contacts with deoxycholic acid molecules as a result of van der Waals forces. It is thus reasonable to assume that the changes in the COO^- symmetric and asymmetric stretching peaks of DCNa (Fig. 8, curve c) may be due to hydrogen bonding among DCNa molecules; the changes in the aromatic CH out-of-plane bending and dihydropyridine NH stretching peaks of nifedipine (Fig. 8, curve d) may be due to the van der Waals interaction between nifedipine and DCNa molecules.

However, Miyata et al. (18) reported no intercalation of guest molecules between the layers of deoxycholic acid molecules because the head-to-tail structure of deoxycholic acid resulted in rigid and stable sheets consisting of hydrogen-bonded networks. On the basis of our powder X-ray diffraction, DSC, and FTIR results before and after the grinding process with DCNa, we can assume the following. First, hydrogen-bonded networks occur rapidly in amorphous DCNa powder in the initial grinding stage, and thereby the hydrophobic side of DCNa assembles at nifedipine crystals. Second, the drug crystal size decreases with an increase in the grinding time; the van der Waals forces between nifedipine and DCNa molecules become larger, while the hydrogen bonding among DCNa molecules weakens. Third, the van der Waals forces promote the complex formation of nifedipine-DCNa, transforming the drug crystals into an amorphous state.

CONCLUSION

The cogrinding of nifedipine with DCNa was found to be effective with respect to the formation of colloidal particles when dispersed in water and a pH 6.8 buffer solution. The mechanochemical process promoted the amorphous formation of nifedipine in the solid state. The complexation between the drug and DCNa depended on the weight fraction of DCNa and the grinding time. The intermolecular interaction in the coground mixture may be attributable to the van der Waals attraction, which was different from that in a physical mixture. This cogrinding system was useful for the formation of amorphous nifedipine in the solid state and the production of colloidal particles of the drug in solution.

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