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Application of ascorbic acid 2-glucoside as a solubilizing agent for clarithromycin: Solubilization and nanoparticle formation

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Abstract

Clarithromycin (CAM) was co-ground with L-ascorbic acid 2-glucoside (AA-2G), a newly developed food additive, to improve the solubility characteristics. The complete solubilizing effect of AA-2G was observed for the ground mixture with 1:1 molar ratio. When ground mixtures of CAM and AA-2G (2:1) were dispersed into water, not only the solubilization of CAM was observed but also nanoparticle formation with a mean particle diameter of 280 nm. The CAM particles obtained in this manner were stable in suspension for at least 7 days. Zeta potential analysis showed that positive charges on the particle surface may be contributing to the stability of the suspension. ^{1}H NMR spectrum of CAM dissolved in a phosphate buffer (pH 5.5) showed a signal derived from the *N*,*N*-dimethylamino group at 2.73 ppm, while that of an equimolar ground mixture of CAM with AA-2G in D_2O (pH 5.5) showed clearly two signals at 2.65 and 2.77 ppm derived from the splitting of the two methyl groups. The ^{13}C NMR spectrum of the equimolar ground mixture dissolved in D_2O exhibited two signals derived from *N*,*N*-dimethyl carbons of desosamine group at 37.2 and 42.3 ppm, whereas unprocessed CAM showed no resonance signal arising from those carbons. Moreover, the carbon resonance at 163 and 173 ppm arising from the ketone group in the CAM lactone ring shifted downfield to 177 and 180 ppm after the co-grinding with AA-2G. The formation of nanoparticles was only observed when CAM was co-ground with AA-2G in the molar ratio of 2:1, which might be attributable to a grinding-induced interaction in the solid-state via the ketone group in lactone ring of CAM.

Keywords: Clarithromycin; Ascorbic acid 2-glucoside; Solubilization; Nanoparticle; Co-grinding; Poorly water-soluble drug

1. Introduction

Clarithromycin (CAM), a 14-membered macrolide antibiotic, was developed with greatly improved acid stability compared to erythromycin (Nakagawa et al., 1992). CAM possesses potent antibacterial activity against clinically important respiratory pathogens such as penicillin-susceptible and -intermediate pneumococci. CAM has also been used to treat *Helicobacter pylori* infection (Gisbert and Pajares, 2003) and pediatric infections (Fujii et al., 1994). However, like many other macrolide antibiotics, CAM exhibits poor absorption and low bioavailability when administered orally. Because of its very low aqueous solubility (0.342 μg/mL H_2O at 25 °C), it is difficult to achieve an injectable CAM product in a clinically and commercially acceptable formulation.

The bioavailability of poorly water-soluble drugs can generally be improved by formulation techniques such as the preparation of binary systems with a hydrophilic carrier by mixing, melting or solvent methods (Aigner et al., 2002; Hassan et al., 2004). However, these methods can leave residual solvent, cause hydrolysis or bring about thermal decomposition of the pharmaceutically active component. Moreover, these processes are so complicated that good cost-performance cannot be easily accomplished. It has been reported that the solubility of a poorly water-soluble drug can be increased by altering the solvent's ability to solvate the drug, via intermolecular hydrogen bonding with pharmaceutically acceptable excipients, such as benzoate or benzoic acid (Simamora et al., 2001). However, it is necessary to add large amounts of excipients to cause solubilization of the drug. Therefore, a simpler and more practical pharmaceutical

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process has been sought for. The solubility improvement of poorly water-soluble drugs using safety-approved solubilizing agents is a very challenging target.

It has been widely known that ascorbic acid is a useful compound for solubilization in pharmaceutical formulations (Itoh et al., 2003). The molecule also acts an antioxidant, inhibiting oxidation at the cell membrane. The well-known susceptibility of ascorbic acid to thermal and oxidative degradation has led to considerable interest in its derivatives. L-Ascorbic acid 2-glucoside (AA-2G), structurally D-glucopyranosyl bonded to the second position of ascorbic acid, has been developed as one of the L-ascorbic acid derivatives. AA-2G has two beneficial properties; high stability against thermal and oxidative degradation and rapid conversion to ascorbic acid by α-glucosidase in the blood and liver cells (Yamamoto et al., 1990; Matsukawa et al., 2000). AA-2G has been approved as a newly developed food additive. Moreover, it is expected to be used in the development of lipid-soluble vitamins and as the principal component in cosmetic ingredients (Yamamoto et al., 2002). Some ascorbic acid derivatives have also been synthesized to improve drug solubility (Mandai et al., 1992). Variation in the alkyl chain of the fatty acid, which forms the ester linkage to the ascorbic acid can be used to improve surface activity, which would improve the solubilization of pharmaceutically active materials (Bilia et al., 2002).

In this study, we characterized a possible use of AA-2G for a newly developed formulation based on CAM. To clarify the solubilization mechanism, we have investigated the interactions of CAM with AA-2G in aqueous solution by ^{1}H NMR and ^{13}C NMR spectroscopy.

2. Materials and methods

2.1. Materials

Clarithromycin (Fig. 1) was received from Taisho Pharmaceutical Co., Ltd., Japan. L-Ascorbic acid 2-glucoside, L-tartaric acid, maleic acid, potassium dihydrogenphosphate and potassium hydroxide were of reagent grade and purchased from Wako Pure Chemical Industries, Ltd., Japan. All other chemicals used were of reagent grade. Clarithromycin (2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-5-(3,4,6-trideoxy-3-dimethylamino-β-D-xylo-hexopyranosyloxy)-3-(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyloxy)-11,12-dihydroxy-6-methoxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide) is an antibiotic approved for the treatment of common bacterial infections in non-immunocompromised individuals.

2.2. Methods

2.2.1. Preparation of physical mixture and ground mixture

CAM and AA-2G were physically mixed at a molar ratio of 2:1 or 1:1 in a glass vial using a vortex mixer. Ground mixtures were prepared by grinding in a vibration mill (CMT TI-200, Tochigi, Japan) for 30 min.

2.2.2. Powder X-ray diffraction (PXRD) measurement

Powder X-ray diffraction measurements were performed on a Rigaku Miniflex powder X-ray diffractometer (Rigaku, Japan). The measurement conditions were as follows: 30 kV voltage, 15 mA current, a scanning speed of 4° min^{-1} and a radiation source of CuKα.

2.2.3. Particle size analysis

The ground mixture was dispersed in distilled water and the suspension was sonicated for 2 min. The particle size was measured by the light-scattering method using a Microtrac FRA® (Nikkiso, Japan; measurement range, 0.1–700 μm) and by the dynamic light-scattering method using a Microtrac UPA® (Nikkiso, Japan; measurement range, 0.003–6 μm).

2.2.4. FT-IR spectroscopy

Infrared (IR) spectra were obtained with a Fourier transform JASCO 230 (FT)-IR spectrometer (JASCO Corporation, Japan). The samples were ground with potassium bromide and compressed to obtain disks, and the spectra were recorded at resolution of 4 cm^{-1}.

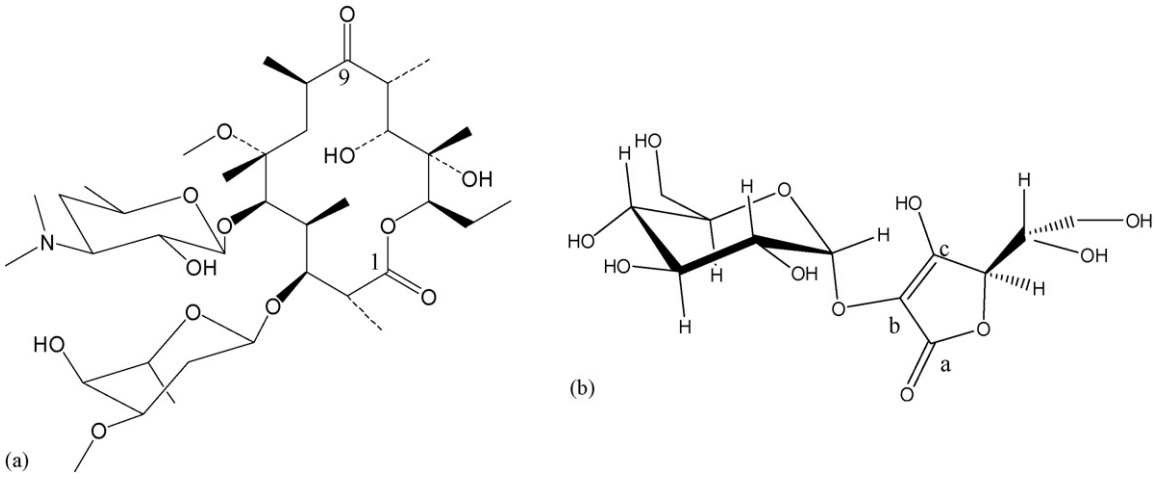

Fig. 1. Chemical structures of (a) clarithromycin (CAM) and (b) L-ascorbic acid 2-glucoside (AA-2G).

2.2.5. Quantitative determination of clarithromycin

The amounts of CAM in nanoparticle and solubilized forms were assessed by HPLC. The GM suspensions (100 mg CAM in 10 ml of distilled water) were filtered through an 11 μm membrane filter (Millipore, MA, USA). The filtrates containing particles smaller than 11 μm were filtered through a 0.8 μm membrane filters. Each filtered suspension was diluted with a mobile phase (*A*, 1/15 M monobasic potassium phosphate; *B*, acetonitirile; (*A*/*B*: 13/7, v/v)) to dissolve all the solid components. The filtrates containing nanoparticles (<0.8 μm) was further centrifuged at 230,000 *g* for 15 min (Himac CP 90α, Hitachi, Japan). The filtrate and the supernatant were appropriately diluted with the HPLC mobile-phase solution. The concentrations of CAM in these solutions were determined using an HPLC (LC-6A, Shimadzu Co., Kyoto, Japan) equipped with a UV detector (SPD-10AV), an integrator (C-R6A), and a reversed-phase column using Cosmosil 5C18- AR, 4.6 mm × 150 mm (Nacalai Tesque Co., Ltd., Kyoto, Japan) at 40 °C. The flow rate was adjusted so that the retention time for the CAM peak was about 8 min. The wavelength was set at 210 nm. The percentage recovery, which indicates the amount of CAM in the solution as nanoparticle and solubilized forms, was calculated according to Eq. (1).

$$\text{Recovery}\,(\%) = \frac{\text{Amount of CAM in filtrates}\,(<0.8\,\mu\text{m})}{\text{Total amount of CAM in the suspensions}} \times 100 \quad (1)$$

The percentage solubilization was calculated according to Eq. (2).

$$\text{Solubilization}\,(\%) = \frac{\text{Amount of CAM in supernatant}}{\text{Total amount of CAM in the suspensions}} \times 100 \quad (2)$$

Finally, the percentage of CAM in nanoparticles produced by grinding (nanoparticle (%)) was calculated as follows.

$$\text{Nanoparticle}\,(\%) = \text{Recovery}(\%) - \text{Solubilization}(\%) \quad (3)$$

2.2.6. Zeta potential

The zeta potential for each suspension containing CAM was determined using a NICOMP 380ZLS® (NICOMP Co., Ltd., USA). Each sample was analyzed in triplicate.

2.2.7. Stability study

The ground mixture of CAM and AA-2G was dispersed in distilled water, and the suspensions were then filtered through 0.8-μm membrane filters. The filtrate was stored at 25 °C and the particle size distribution was measured at set time intervals.

2.2.8. ^{1}H NMR and ^{13}C NMR measurement

The ^{1}H NMR and ^{13}C NMR spectrum for each specimen was measured at 24 °C on a JEOL JNM-LA500 spectrometer (JEOL, Japan) operating at 500 MHz for proton in D_2O solution. The measurement conditions of ^{1}H NMR were as follows: 90° pulse width, 6.40 μs; relaxation delay, 3.7232 s; scan, 256 times. The measurement conditions of ^{13}C NMR were as follows: 90° pulse width, 7.00 μs; relaxation delay, 2.0333 s; scan, 5120 times. Tetramethylsilane (TMS) was used as an internal standard. Fine adjustments to the pH of the buffer were made using potassium dihydrogenphosphate and potassium hydroxide solutions in D_2O.

3. Results and discussion

3.1. Solubilization of CAM by the equimolar co-grinding with AA-2G

To improve the dissolution properties of clarithromycin (CAM), we used ascorbic acid as a solubilizing agent. Ascorbic acid was found to be a useful compound to solubilize CAM. However, photodecomposition could be observed for ascorbic acid in aqueous media. To avoid photodecomposition, L-ascorbic acid 2-glucoside was used instead of ascorbic acid to improve the solubility characteristics of CAM. Fig. 2 shows the effect of AA-2G content on the solubility of CAM. Due to its hydrophobic properties, crystal of CAM hardly dissolved at all. When the physical mixture of CAM and AA-2G (molar ratio 1:1) was dispersed into aqueous media, the wettability and the aqueous dispersibility were substantially improved and 80% of CAM was solubilized. Furthermore, in the ground mixture almost all CAM dissolved into solution. Complete solubilization of CAM was also observed in mixtures containing high molar ratio of AA-2G (1:2 and 1:5, data not shown). It will be noted that the drastic change of CAM solubilization was not observed when other materials, such as L-tartaric acid and maleic

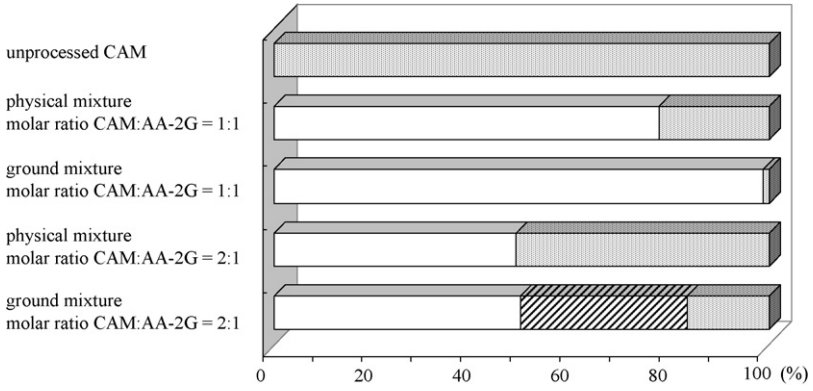

Fig. 2. Particle fractions of drug in CAM:AA-2G systems. □, Solubilization; ▨, 0.8 μm > particles; ▩, 0.8 μm < particles.

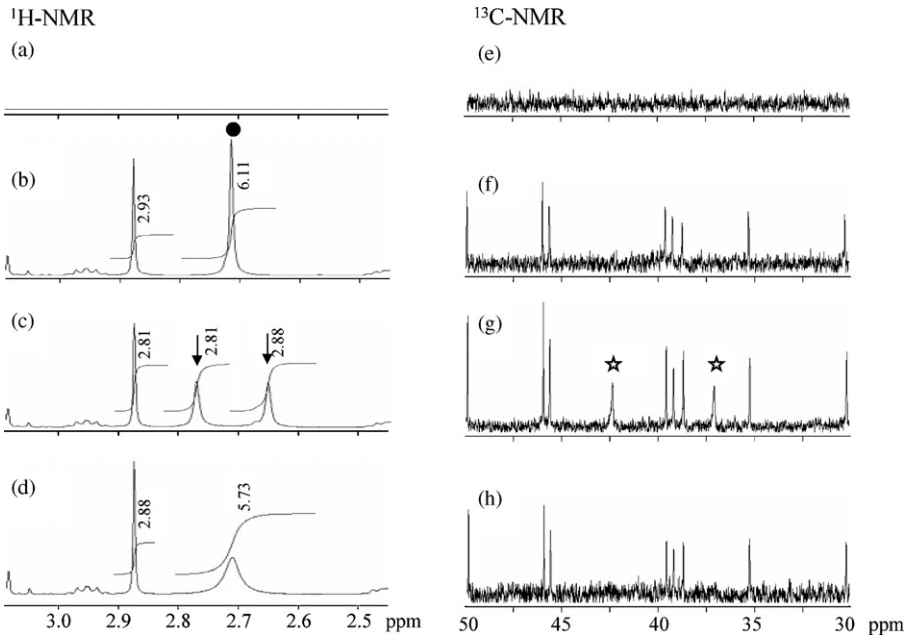

Fig. 3. ^{1}H NMR spectra and ^{13}C NMR spectra of CAM:AA-2G systems at pH 5.5. ^{1}H NMR spectra of (a) unprocessed AA-2G, (b) unprocessed CAM, (c) ground mixture (molar ratio CAM:AA-2G = 1:1), (d) ground mixture (molar ratio CAM:AA-2G = 2:1) and ^{13}C NMR spectra of (e) unprocessed AA-2G, (f) unprocessed CAM, (g) ground mixture (molar ratio CAM:AA-2G = 1:1), (h) ground mixture (molar ratio CAM:AA-2G = 2:1).

acid, were used as solubilizing agents. These findings suggest a specific interaction between AA-2G and CAM in aqueous media.

As reported by Nakagawa et al. (1992), the solubility of CAM was pH-dependent and the solubility increased when the pH in a phosphate buffer fell to 7 or less. The molecular state of CAM and AA-2G were assessed by ^{1}H and ^{13}C NMR spectroscopy to assess whether a specific interaction exists between CAM and AA-2G in aqueous solution. Fig. 3 shows the ^{1}H and ^{13}C NMR spectra of AA-2G, CAM, and the ground mixtures in a phosphate buffer (pH 5.5). In ^{1}H NMR spectrum, AA-2G shows no signal in the region from 2.5 to 3.0 ppm (Fig. 3a). On the other hand, unprocessed CAM shows a singlet signal at 2.73 ppm derived from the *N*,*N*-dimethylamino moiety of desosamine with the integral indicating six protons (Fig. 3b). This shows good agreement with results in literature (Steinmetz et al., 1992). Interestingly, the splitting of this signal into two broadened ones at 2.65 and 2.77 ppm was observed for the 1:1 ground mixture of CAM and AA-2G, the integral of each peak indicates three protons (Fig. 3c). The findings of the ^{13}C NMR spectra also support the interpretation of the ^{1}H NMR spectra. In the ^{13}C NMR of unprocessed CAM no obvious signal corresponds to the *N*,*N*-dimethylamino (Awan et al., 1992). However, the ^{13}C NMR spectrum of the 1:1 ground mixture clearly shows two sharp signals at 37.2 and 42.3 ppm (Fig. 3g) derived from the *N*,*N*-dimethylamino moiety of desosamine. These results indicate that in the 1:1 ground mixture a specific interaction takes place between the *N*,*N*-dimethylamino group of CAM and the hydroxyl group of AA-2G. The ^{13}C NMR spectra over the whole range (0–250 ppm) are also shown in Fig. 4. Unprocessed AA-2G exhibits a signal at 166 ppm corresponding to the C(c) in the lactone ring and at 173 ppm corresponding to the lactone carbonyl carbon C(a) (Fig. 4a). These signals were shifted downfield to 177 and 180 ppm, respectively, when the ground 1:1 mixture was dissolved in the phosphate buffer (Fig. 4c). This result implies the presence of an interaction such as a hydrogen-bond, involving the lactone carbonyl of AA-2G. A decrease in pH and also the interaction between CAM and AA-2G contribute to the solubilization of CAM.

3.2. Peculiar CAM nanoparticle formation by co-grinding with AA-2G at 2:1 molar ratio

When CAM crystals were ground with the AA-2G in the ratio 2:1, 50% of the CAM molecules dissolved, accompanied by the formation CAM nanoparticles (30%). As shown in Fig. 3d, a signal at 2.73 ppm was observed in ^{1}H NMR spectrum indicating that there is no significant interaction between *N*,*N*-dimethylamino group of CAM and AA-2G. However, resonances in the ^{13}C NMR spectra from C(c) and C(a) carbons of AA-2G shifted to downfield at 177 and 180 ppm, respectively. This indicates there might be a weak interaction between the hydroxyl group of CAM and the lactone carbonyl of AA-2G.

It is noteworthy that nanoparticle formation was only found for the 2:1 ground mixture. Fig. 5 shows the particle size distribution patterns of the suspensions obtained from CAM, physical mixtures of CAM and AA-2G, and its ground mixtures. The mean particle diameter of the unprocessed and ground CAM was approximately the same, 35 μm. The mechanochemical effect of grinding was not observed for CAM itself. After passing the filtrate of the ground mixture through a 0.8 μm filter, nanoparticles with a mean particle diameter of 0.28 μm were observed. As shown in Fig. 6, the volumetric mean particle size of the suspensions was almost constant after storage at 25 °C for 168 h, indicating that the particles are stable in water. From the zeta potential analysis, a positive charge of 25.2 ± 1.5 mV was

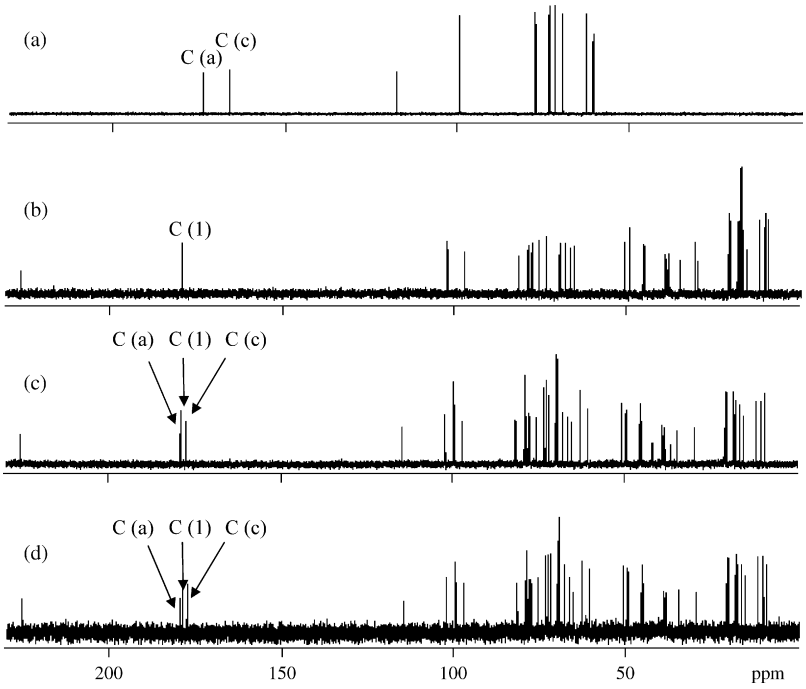

Fig. 4. ^{13}C NMR spectra of CAM:AA-2G systems at pH 5.5. (a) Unprocessed AA-2G, (b) unprocessed CAM, (c) ground mixture (molar ratio CAM:AA-2G = 1:1) and (d) ground mixture (molar ratio CAM:AA-2G = 2:1).

obtained for the suspended drug particles. Electrostatic repulsion due to positive charges on the nanoparticle surface may contribute to the stabilization of nanoparticles in suspension. The high positive charge on the particle surface was attributed to the positive charge on the nitrogen of desosamine, an amino sugar at aglycone position five of CAM.

To investigate mechanism of nanoparticle formation, molecular state of the ground mixture in solid-state was investigated by

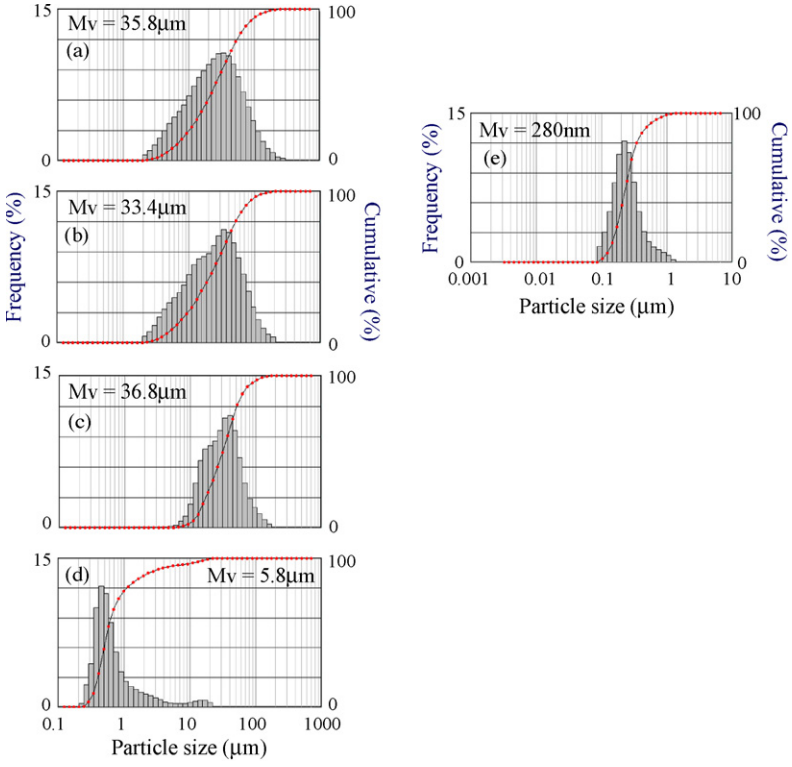

Fig. 5. Particle size distribution curves of CAM:AA-2G systems. (a) Unprocessed CAM, (b) ground CAM, (c) physical mixture (molar ratio CAM:AA-2G = 2:1), (d) ground mixture (molar ratio CAM:AA-2G = 2:1), (e) filtrate of (d) passing through 0.8 μm filter (measured by UPA®).

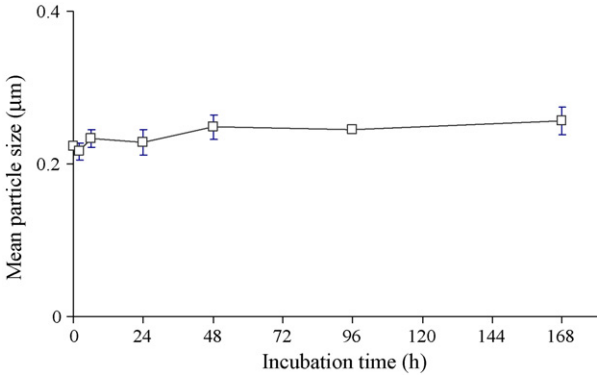

Fig. 6. Change in the mean particle size of CAM fine particles obtained from the 2:1 ground mixture with storage time (mean ± S.D., $n = 3$).

Table 1
Mean particle size of the CAM particles obtained from effect of pH and ground mixture systems

Samples	Mean particle size (μm)
Unprocessed CAM into distilled water	35.8
Ground CAM into pH 6.8 phosphate buffer	28.9
Ground CAM into AA-2G solution	23.2
Ground mixture (molar ratio CAM:AA-2G = 2:1) into pH 6.8 phosphate buffer	35.8
Ground mixture (molar ratio CAM:TA[a] = 2:1) into distilled water	12.1
Ground mixture (molar ratio CAM:MA[b] = 2:1) into distilled water	15.8
Ground mixture (molar ratio CAM:AsANa[c] = 2:1) into distilled water	8.7

[a] TA: L-tartaric acid.
[b] MA: maleic acid.
[c] AsANa: sodium ascorbate.

powder X-ray diffraction measurement and FT-IR spectroscopy. The PXRD patterns for the physical, 2:1 molar ratio mixtures of CAM and AA-2G, and for the mixtures obtained after grinding for 30 min are shown in Fig. 7. The co-grinding of CAM and AA-2G for 30 min changed the CAM crystals to an amorphous state. It is well-known that quite small crystalline particle cannot be detected as the X-ray diffraction shows a halo pattern (Srivastava et al., 2006). Since PXRD peaks of CAM crystals were not observed in both the 1:1 and the 2:1 ground mixtures of CAM and AA-2G, it can be concluded that, in the presence of AA-2G, CAM exists as an amorphous state or as fine crystallites.

When the ground, 2:1 mixture of CAM and AA-2G was dispersed into distilled water, the pH value decreased to 6.8. A pH of 8.6 was determined for CAM alone in solution. A phosphate buffer (pH 6.8) and an aqueous solution of AA-2G aqueous (pH 3.4) were prepared in order to examine the effects of pH dependency on nanoparticle formation (Table 1). It was found that the mean particle diameter was approximately 30 μm when ground CAM was dispersed into either the phosphate buffer (pH 6.8) or the AA-2G solution (pH 6.3). These results suggest that pH has no effect on particle formation. Moreover, no nanoparticle formation was observed when CAM was co-ground with sodium ascorbate, tartaric acid or maleic acid instead of AA-2G. In those system the mean particle diameter was estimated as several tens micrometer.

FT-IR spectra were measured in order to establish the molecular state in ground mixtures of CAM and AA-2G (Fig. 8). CAM crystals show characteristic peaks of $\nu_{C=O}$ stretching vibration from ketone group in a lactone ring (1692 cm^{-1}) and from $\nu_{O-C=O}$ stretching vibration in a lactone ring (1733 cm^{-1}). AA-2G crystals exhibit a peak from $\nu_{C=O}$ stretching at 1692 cm^{-1} shifted to lower frequency at 1685 cm^{-1} in the ground mixture. These findings indicate a grinding-induced interaction through

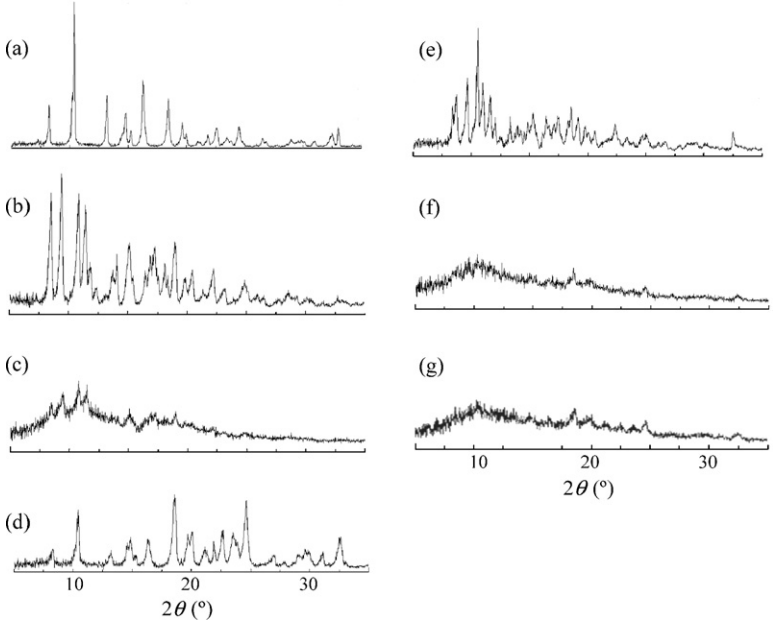

Fig. 7. PXRD patterns of CAM:AA-2G systems (ground for 30 min). (a) Unprocessed AA-2G, (b) unprocessed CAM, (c) ground CAM, (d) ground AA-2G, (e) physical mixture (molar ratio CAM:AA-2G = 2:1), (f) ground mixture (molar ratio CAM:AA-2G = 2:1) and (g) ground mixture (molar ratio CAM:AA-2G = 1:1).

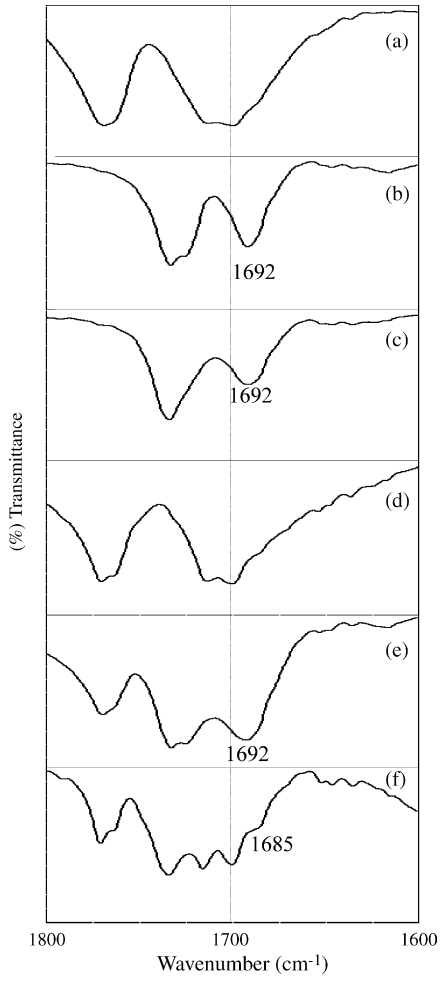

Fig. 8. FT-IR spectra of CAM:AA-2G systems. (a) Unprocessed AA-2G, (b) unprocessed CAM, (c) ground CAM, (d) ground AA-2G, (e) physical mixture (molar ratio CAM:AA-2G = 2:1) and (f) ground mixture (molar ratio CAM:AA-2G = 2:1).

the carbonyl group of CAM. Though it was difficult to show whether the interaction was still maintained in the solution, change of zeta potential of CAM from 4.2 to 25.2 mV by the co-grinding with AA-2G might reflect the presence of the interaction in aqueous solution. Intermolecular interactions induced by the co-grinding of drug and excipient usually occur on the surface of the drug particle produced, but this is not the case for all drug molecules. Additional characterization should be performed using methods such as FT-IR and solid-state NMR to establish the true nature of the interaction. In the present system solid-state NMR measurement would be a promising approach. However, we were unable to obtain clearly resolved data because of the high mobility of CAM. Further study is needed to clarify the mechanism of nanoparticle formation of CAM.

4. Conclusion

Co-grinding of CAM with AA-2G at a molar ratio of 1:1 or less was found to be an effective method to improve the solubility of CAM in aqueous solution. The ^{1}H and ^{13}C NMR spectra revealed that the solubilization effect was attributable to the interaction between *N*,*N*-dimethyl group of CAM and hydroxyl group of AA-2G. Nanoparticle formation of CAM was observed when the 2:1 ground mixture of CAM and AA-2G was dispersed into an aqueous media. Since both the solubilization and nanoparticle formation could be used to improve bioavailability, co-grinding with AA-2G is a promising method for modifying the dissolution properties of CAM.

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