Effect of surfactants or a water soluble polymer on the crystal transition of clarithromycin during a wet granulation process

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ABSTRACT

To generate products containing a stable form of clarithromycin (CAM) (form II) regardless of the initial crystal form of CAM or type of granulation solvent, the effects of five surfactants, or a water-soluble polymer (macrogol 400) were determined on the crystal transition of CAM. The metastable form (form I) was kneaded with water, after adding surfactants, or a water-soluble polymer. Form II was also kneaded with ethanol, after adding the same additives. The resulting samples were analyzed by powder X-ray diffraction. Form I was completely converted to form II by a wet granulation using water with additives bearing polyoxyethylene chains such as polysorbate 80 (PS80), polyoxyl 40 stearate or macrogol 400. The granulation of the form II using ethanol with these additives did not result in a crystal transition to form I. Furthermore, CAM tablets were manufactured using granules with PS80, and these crystal forms and dissolution behaviors were investigated. As a result, the wet granulation of CAM with PS80 gave CAM tablets containing only form II and PS80 did not have any adverse effects on tablet characteristics. Therefore, these data suggests that the crystal form of CAM can be controlled to be form II using a wet granulation process with additives bearing polyoxyethylene chains regardless of the initial crystal form of CAM or type of granulation solvent.

ABBREVIATIONS: CAM, clarithromycin; SDS, sodium lauryl sulfate; LCT, soybean lecithin; SFE, sucrose fatty acid ester; PS80, polysorbate 80; POS40, polyoxyl 40 stearate; PEG400, macrogol 400; PXRD, powder X-ray diffraction; L-HPC, low-substituted hydroxypropylcellulose.

INTRODUCTION

Clarithromycin (CAM), which is a 14-membered semi-synthetic macrolide antibiotic, is more stable to acidic conditions than erythromycin (Nakagawa et al, 1992) and exhibits a broad range of antimicrobial activities. CAM is widely used for the treatment of a variety of different infections, including *Helicobacter pylori* infection. Several tablet-based and pediatric formulations (i.e., granules for oral suspension) containing CAM have been developed and marketed throughout the world (Yajima et al, 1999; Yajima et al, 2002). The total annual sales of generic CAM products in Japan equates to more than 340 million dollar (35 billion yen). It is noteworthy that it has been more than 20 years since branded CAM products were available to buy in Japan.

Nine crystal forms of CAM have been reported in the literature, including form 0 (ethanol solvate) (Spanton et al, 1999), form I (metastable form) (Liu et al, 1999; Noguchi et al, 2012; Tozuka et al, 2002), form II (stable form) (Tozuka et al, 2002; Liu et al, 1998; Suh et al, 2002; Sohn et al, 2000; Tian J et al, 2011), form III (acetonitrile solvate) (Liu et al, 2003), form IV (hydrate) (Avrutov et al, 2003; Jacco, 2012), form V (Gruss et al, 2008), a hydrochloride salt (Parvez et al, 2000; Noguchi et al, 2014) and a methanol solvate (Iwasaki et al, 1993). Polymorphic crystals generally exhibit significant differences in their individual physicochemical properties, including their solubility, stability and bioavailability properties. These differences can have a significant impact on the therapeutic properties of medicinal agents, and the selection of the optimal crystal

form of a medicinal agent is therefore one of the most important factors governing the development of pharmaceutical formulations. The CAM products currently marketed in Japan are formulated using the most thermodynamically stable from of the CAM crystals, which is form II (Liu et al, 1998; Suh et al, 2002; Tian J et al, 2011). The purification of form II is typically achieved by the conversion of crystal form 0 or I to form II using temperatures greater than 80 °C under vacuum conditions (Liu et al, 1999; Liu et al, 1998; Suh et al, 2002; Sohn et al, 2000; Tian J et al, 2011). The development of a novel process for the preparation of form II that avoids the use of high temperature conditions could therefore reduce the costs associated with the manufacture of these products, as well as production cost of the active pharmaceutical ingredient.

Concerns remain that form II of CAM could undergo a crystal transition during the manufacturing process to give the metastable form of CAM. Although various pharmaceutical techniques have been used to produce solid dosage forms such as wet granulation, dry granulation and direct tableting, wet granulation may be the most appropriate technique for the CAM formulation process, because this technique can improve the surface condition of CAM with a highly adhesive property. During the wet granulation process, an organic solvent can also be used in addition to water for the formulation of CAM to induce the uniform granulation of CAM powders with a water-insoluble property. However, when the wet granulation of form II CAM powders is

performed in the presence of an organic solvent, such as ethanol, the CAM crystals can be converted from form II to form I via form 0 (Spanton et al, 1999). It is therefore critically important to suppress the crystal transition of CAM from form II to any of its other forms and to promote the crystal transition to form II during the wet granulation of CAM in the presence of an organic solvent.

To overcome the problems listed above, we focused on the use of surfactants, because several surfactants have been reported to induce the solution-mediated crystal transition of drug compounds (Roderiguez-Hornedo and Murphy, 2004). In this study, we have established a simple technique to enhance the crystal transition of CAM from form I to form II, whilst preventing the crystal transition of the form II crystals during the pharmaceutical manufacturing process by means of additives bearing polyoxyethylene chains.

MATERIALS AND METHODS

Materials

Forms I and II of CAM were obtained from Kyonbo pharmaceutical Co., Ltd (Chungchongnam, Korea) and Ercros Industrial S.A. (Barcelona, Spain), respectively. Sodium lauryl sulfate (SDS), which is an anionic surfactant, was obtained from Sigma Aldrich (Tokyo, Japan). Soybean lecithin (LCT), which is an amphoteric surfactant, was obtained from Nacalai Tesque (Tokyo, Japan). Sucrose fatty acid ester (SFE), polysorbate

80 (PS 80) and polyoxyl 40 (POS40) stearate, which are non-ionic surfactants, were obtained from Mitsubishi-Kagaku Foods Co. (Tokyo, Japan), Kanto Chemical (Tokyo, Japan) and NOF Co. (Tokyo, Japan), respectively. Macrogol 400 (PEG400), which is a water-soluble polymer, was obtained from NOF Co. Corn starch, which is used as a filler, was obtained from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Low-substituted hydroxypropyl cellulose (L-HPC; used as a disintegrant) was obtained from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Light anhydrous silicic acid (used as a plasticizer) was supplied by Freund Co., Ltd (Tokyo, Japan). Magnesium stearate (used as a lubricant) was purchased from Taihei Chemical Industrial Co., Ltd (Tokyo, Japan). Ethanol (>95%) was obtained from the Japan Synthetic Alcohol Co., Ltd (Kanagawa, Japan). All of the other reagents used in the current study conformed to the standards defined in the 16th Edition of the Japanese Pharmacopoeia (JP16).

Methods

Preparation of wet granules using form I and purified water

Five gram samples of form I were mixed with 0, 0.05, 0.25, 0.5 or 1.0 g of each surfactant or PEG400. The resulting mixtures were then treated with 0.75, 1.5 or 3.0 mL of purified water, before being kneaded for 2 min using a mortar and a pestle at room temperature. The wet granulated powders were subsequently sieved through a 2360-μm screen, and the resulting granules were dried in an oven at 50 °C for 40 min. The dried

granules were then sieved through a 1000-μm screen, and the resulting sieved powders were subjected to powder X-ray diffraction (PXRD) analysis.

Preparation of wet granules using form II and ethanol

Five gram samples of form II were mixed with 0 or 0.25 g of each surfactant or PEG400. The resulting mixtures were then treated with 0.75, 1.5 or 3.0 g of ethanol before being kneaded for 2 min at room temperature using a mortar and a pestle to give the corresponding granulated powders, which were sieved and dried according to the procedure described above for the "preparation of wet granulation powders of form I with purified water".

Preparation of wet granules using form II and purified water or using form I and ethanol

Five gram samples of form II or form I were mixed with 0 or 0.25 g of each surfactant or PEG400. The resulting mixtures containing form II were then treated with 1.5 g of water, and these containing form I were then treated with 1.5 g of ethanol before being kneaded for 2 min at room temperature using a mortar and a pestle to give the corresponding granulated powders, which were sieved and dried according to the procedure described above for the "preparation of wet granulation powders of form I with purified water".

Preparation of the CAM tablets

PXRD

The crystal forms of the CAM found in the granulation powders and tablets were analyzed using a Bruker PXRD system (Bruker AXS Co., Ltd., Kanagawa, Japan). The granulation powders and tablets were gently ground into fine powders using a mortar and pestle before being packed into sample cups. The packed sample cups were then subjected to PXRD analysis using CuK_α radiation (λ = 1.5418 Å), with the tube voltage and amperage set to 40 kV and 40 mAV, respectively. Scanning was conducted at room temperature between 2θ angles of 2° and 40° with a scanning step width of 0.015° and a scanning speed of 0.1 s/step.

Difference scanning calorimetry (DSC)

The crystal forms of the CAM found in the granulation powders were analyzed using DSC equipment (DSC-50, Shimadzu Corp., Kyoto, Japan). The all measurements were performed using an empty aluminum pan as a reference under a nitrogen gas atmosphere. The solid samples (10.0 ± 1.0mg) were weighed into aluminum pan and heated with a rate of 10 °C/min. The analysis of forms I and II of CAM were achieved at the range of 40 to 250 °C. Whilst the case of granulation powders were carried out at the range of 40 to 180 °C in order to observe the exothermic peak coming from the crystal transition of CAM

Fourier-transform infrared spectroscopy (FT-IR)

Infrared absorption spectrum of the granulation powders including CAM were measured by Attenuated Total Reflection (ATR) method using FT-IR equipment (FT/IR-6600, JASCO Corp., Tokyo, Japan). In measurement case of PS80 alone, since PS80 is a liquid at normal temperature, the mixture with PS80 and KBr was measured. All samples were scanned in the range of 400 to 4000 cm^{-1}.

Preparation of the CAM tablets

The compositions of the tablets are summarized in **Table 1**. Form I or II of CAM, corn starch, L-HPC and light anhydrous silicic acid were weighed at 250 tablets per batch, according to **Table 1**. A ground sample of form I CAM was obtained by grinding the material in a hammer mill (PULVERISETTE 14, Fritsch Japan Co., Ltd., Yokohama, Japan). The particle sizes of the intact and the hammer milled CAM samples are shown in **Table 2**. The weighed powders were mixed for 1 min using a mortar and a pestle. The resulting mixture was then treated with a granulation solvent (water or ethanol) either with or without PS80 before being kneaded with a mortar and pestle. After the wet granulation process, the powders were sieved through a 2360-μm screen. The sieved powders were then kneaded with water and ethanol, which were used as granulation solvents, before being dried for 1–2 h in an oven at 70 and 50 °C, respectively. The dried granules were sieved through a 1000-μm screen, and the resulting sieved powders were

placed into a plastic bag together with light anhydrous silicic acid and magnesium stearate, where they were mixed by shaking (60 times). The granules were compressed into tablets using a manual tableting hydraulic press (Kikusui Seisakusho Ltd., Kyoto, Japan) with a two-stage R-plane punch of 10 mm in diameter. The granules were compressed with a compression force of 6.0 kN. The weight of the resulting tablets was 320 mg, with each tablet containing 200 mg of CAM.

Measurement of the thickness and hardness properties of the CAM tablets

The thickness and hardness properties of the CAM tablets manufactured in the current study were measured using a dial gauge (Ozaki MFG. Co., Ltd., Tokyo, Japan) and hardness measuring apparatus (Freund Co., Ltd., Tokyo, Japan), respectively.

Disintegration Test

Disintegration tests were performed according to the procedure described in JP16 using a disintegration apparatus (Miyamoto Riken Ind. Co., Ltd., Osaka, Japan). Purified water was used as the test medium at 37.0 ± 0.5 °C.

Dissolution Test

The dissolution test was carried out according to the paddle method described in JP 16 using a dissolution apparatus (NTR-6100A, Toyama Sangyo Co., Ltd., Tokyo, Japan). Hydrochloric acid (pH 1.2) and phosphate buffer saline (pH 6.8) solutions were used as

the dissolution media in accordance with the procedure described in JP16. The paddle rotation speed and temperature were set at 50 rpm and 37.0 ± 0.5 °C, respectively. Twenty milliliter aliquots of the sample solution were withdrawn at predetermined time intervals and replaced with an equal volume of the dissolution medium. Each sample solution was filtered through a 0.45-μm membrane filter and diluted by 3-fold using a 0.2 mol/L potassium dihydrogen phosphate solution.

The amount of CAM in the dissolution media was determined by high-performance liquid chromatography (HPLC) (Shimadzu Corp., Kyoto, Japan) using an Inertsil ODS-2 column (4.6 × 150 mm; GL Science Inc., Tokyo, Japan), which was kept at 50 °C in a column oven. The wavelength of the UV detector was set to 210 nm. The column was eluted with a mobile phase consisting of a mixture of 1/15 M potassium dihydrogen phosphate and acetonitrile (13:7, *v*/*v*) at a flow rate of 1.3 mL/min. The injection volume was set at 100 μL. For the quantification of CAM at pH 1.2, the sum of the peaks corresponding to CAM and its degradation product (Morimoto et al, 1990) were regarded as the total amount of CAM. The dissolution amount of CAM in solution at pH 6.8 was calculated from the area of the peak corresponding to CAM.

Statistic

The differences in the hardness and disintegration time properties between the tablets made from F1 and those that were made from the other formulations evaluated in

229 the current study were statistically analyzed using an F-test and a Student t-test or the

230 Welch t-test. Differences with $p < 0.01$ were considered to be statistically significant.

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RESULTS AND DISCUSSION

Effect of surfactants or PEG400 on crystal transition of form I by wet granulation with purified water

The PXRD patterns of forms I and II, as well as the granulation powders derived from form I using only water, are shown in **Fig. 1**. The specific diffraction peaks of forms I and II were observed at 2θ = 5.0°, 6.5°, 7.9° and 10.2°, and 2θ = 8.5°, 9.4°, 10.8° and 11.4°, respectively. When form I was granulated with water containing no surfactants, PXRD analysis revealed the appearance of the specific diffraction peaks of form II, as well as those belonging to form I (**Fig. 1**), which indicated that the wet granulation of form I with water did not lead to its complete transformation to form II.

Several additives were also investigated in terms of their ability to induce the crystal transition of CAM. The PXRD patterns of samples obtained by the wet granulation of form I with water in the presence of surfactants or PEG400 are shown in **Figs. 2 and 3**. During the wet granulation with SDS, the wettability of the form I CAM powders improved considerably compared with the corresponding granulation process without the surfactant. In the kneaded CAM samples containing SDS concentrations in the range of 5 to 10 wt%, the material existed in the capillary state. However, the capillary state changed into a slurry state as the amount of SDS was increased to 20 wt%. Although the properties of the kneaded samples changed depending on the amount of SDS that had been added to the mixture, form I was not completely transformed to form II under any

of these conditions (**Fig. 2a**). Incomplete transitions from form I to form II were also observed when the wet granulation process was performed with water in the presence of LCT or SFE (**Fig. 2b and 2c**). Incidentally, as for the diffraction peaks which were observed at $2\theta = 2.5°$ on powder granulated with SDS or SFE, Since the diffraction peak of around 2.5° was observed on only powders of SDS or SFE (The detail results are not shown), we think this peak come from the each surfactants. As for the peak at 4.5°, we think this peak basically come from form 0. However, in the case of powder with SDS, since the diffraction peak of around 4.5° was observed on only powders of SDS (The detail result is not shown), we think this peak come from not only form 0 but also SDS.

The wet granulation of form I with water in the presence of PS80 led to an improvement in the dispersion state of the form I particles, which improved further as the amount of PS80 added to the mixture was increased. Furthermore, the PXRD patterns of the wet granulation powders prepared with PS80 showed that the specific diffraction peaks belonging to form I of CAM completely disappeared following the addition of more than 5wt% PS80 (**Fig. 3a**). This result therefore indicated that form I was completely transformed to form II under these conditions. A complete transitions from form I to form II was also observed when the wet granulation process was performed with more than 5wt% POS40 or PEG400 (**Fig. 3b and 3c**). Taken together, these results indicate that the degree of crystal transition from form I to form II is dependent on the types of surfactant

and water soluble polymer added to the wet granulation process.

The amount of water added to the granulation of CAM is one of the most important factors governing the extent of the crystal transition from form I to form II, and several previously published reports have demonstrated that form II can be obtained by heating an aqueous slurry of either form 0 or I at 70–80 °C (Liu et al, 1998; Suh et al, 2002; Tian J et al, 2011). The results of the current study demonstrated that a portion of form I could be transformed to form II following a wet granulation process using 30 wt% water (**Fig. 1**), which suggested that the crystal transition of CAM from form I to form II could potentially be controlled by varying the amount of water added to the wet granulation process. With this in mind, we investigated the influence of the amount of water added to the wet granulation process on the crystal transition of form I. Form I crystals of CAM were treated with different amounts of water (i.e., 15, 30 and 60 wt%) to give pendular, funicular or capillary and slurry states of form I, respectively. The ratios of surfactants and PEG400 to form I were fixed to 5 wt%, which was determined to be the minimal amount needed for complete crystal transition from form I to form II, as mentioned above (**Fig. 3**). The PXRD patterns of the various samples obtained in the experiments are shown in **Figs. 4 and 5**. Wet granulation processes with water and water in the presence of SDS resulted in a decrease in the intensities of the specific diffraction peaks belonging to form I, while the intensities of the specific diffraction peaks belonging to form II

290 increased in proportion to the amount of water added to the process (**Fig. 4a and 4b**).
291 Furthermore, when form I was kneaded with 60 wt% water, the kneaded state of form I
292 changed from a pendular state to a slurry state, and form I was completely converted to
293 form II. Although there was an increase in the intensities of the specific diffraction peaks
294 belonging to form II when form I was kneaded with 60 wt% water in the presence of LCT,
295 a complete transition from form I to form II was not observed, and peaks belonging to an
296 unknown crystal form were also observed around 2θ=6° (**Fig. 4c**). Specific diffraction
297 peaks belonging of form II were barely observed by PXRD when SFE was added to the
298 granulation process, even when the amount of water was increased from 5 to 60 wt% (**Fig.**
299 **4d**). These results suggested that the addition of SFE did not induce the transition of the
300 CAM crystals from form I to form II. However, the kneading state of the form I crystals
301 mixed with 15 wt% water containing PS80, POS40 or PEG400 was found to be pendular,
302 which was the same as that found following the addition of LCT or SFE. In all of these
303 cases, the intensities of the specific diffraction peaks belonging to form I decreased,
304 whereas those belonging to form II increased (**Fig. 5**). When 30 or 60 wt% water was
305 added during the kneading of form I with PS80, POS40 or PEG400, the PXRD patterns
306 of the resulting powders were identical to that of form II (**Fig. 5**), which indicated that
307 form I was completely converted to form II when the amount of water in the granulation
308 process was over 30 wt%.

Incidentally, the diffraction peaks were observed at 2θ = 6°, 8° and 10.5° on the granulated powders of form I using water as shown in Fig.1, Figs. 2 and 4. The diffraction peaks of 6° and 8° did not be correspondent with that of form I and II as well as other known crystal forms. Therefore, we think these peaks come from a new crystal form. In the near future, we'd like to demonstrate in accordance with other analytical techniques. As for the peak at 10.5 degree, we think this peak basically come from form IV. Since form IV is hydrate, we think that form IV was slightly formed during wet granulation process using water.

The results obtained thus far can be summarized as follows. When the wet granulation of form I was carried out using only water or water containing SDS, the transformation of the form I crystals to form II crystals was incomplete when less than 30 wt% water was added to the granulation process. With regard to LCT or SFE, the extent of the transformation of the crystals from form I to form II was lower than that observed for the wet granulation in the absence of these agents, even when the kneaded material reached to slurry state. In contrast, the addition of PS80, POS40 or PEG400 to the wet granulation process led to the complete transformation of the crystals from form I to form II when 30 wt% of water was added as a solvent. These results suggested that a robust granulation process for the complete transformation of the CAM crystal from form I to form II was developed, and this new process was less dependent on the amount of water

328 added to the granulation by using PS80, POS40 or PEG400

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330 ***Effect of a surfactant or PEG400 on the crystal transition of form II by wet granulation***

331 ***with ethanol***

332 It was envisaged that the wet granulation of form II in ethanol would induce to the

333 transformation of the CAM crystals from form II to form I. Given that PS80, POS40 and

334 PEG400 promoted the conversion of the CAM crystals from form I to form II, it was

335 expected that the addition of these additives to a wet granulation process involving form

336 II would suppress the conversion of the crystals to form I, even when ethanol was used

337 as a solvent. The PXRD patterns of samples obtained following a wet granulation process

338 using ethanol with form II in the presence or absence of surfactants and PEG400 are

339 shown in **Fig. 6**. When the granulation process was without any additives, PXRD analysis

340 of the resulting powders revealed specific diffraction peaks belonging to form 0 (i.e., 2θ

341 = 4.7° and 6.6°) and form I (i.e., 2θ = 7.9° and 10.2°), as well as those belonging to form

342 II, which indicated that form II was being converted to forms 0 and I when it was

343 subjected to a wet granulation process using ethanol. The conversion of from II crystals

344 to crystals of forms 0 and I was also observed when the wet granulation process was

345 conducted with SDS, LCT or SFE. In contrast, in the addition of PS80, POS40 or PEG400

346 to the wet granulation of form II CAM crystals appeared to stabilize the crystals and the

PXRD patterns of these samples were almost identical to that of the form II material. Taken together, these results show that the addition of PS80, POS40 or PEG400 to the wet granulation of CAM with ethanol prevented the conversion of the form II crystals to forms 0 and I crystals.

The influence of the amount of ethanol added to the wet granulation process was also investigated in terms of its impact on the crystal transition of form II. The ratio of ethanol to the form II crystals was varied (i.e., 15, 30 and 60 wt%), whilst the ratio of surfactant or PEG400 to the form II crystals was fixed at 5 wt%. The PXRD patterns of these samples are shown in **Figs. 7 and 8**. When the wet granulation of form II was carried out using only ethanol, a portion of form II was transformed to form 0 and I following the addition of more than 30 wt% ethanol (**Fig. 7a**). The result of the PXRD analysis revealed that the intensities of the specific diffraction peaks belonging to forms 0 and I increased as the amount of ethanol increased during the wet granulation of the from II crystals in the presence of SDS, LCT or SFE (**Figs. 7b–d**). In contrast, the PXRD patterns of the form II powders granulated with PS80 were identical to that of form II, even when the amount of ethanol was increased from 5 to 60 wt% (**Fig. 8a**). The PXRD patterns of the powders granulated with POS40 were identical to that of form II for ethanol charges of up to 30 wt% (**Fig. 8b**). However, trace amounts of the specific diffraction peaks belonging to form 0 were detected when 60 wt% ethanol was added to the granulation

process. As for PEG400, specific diffraction peaks belonging to form 0 were detected at very low intensities following the addition of 30 and 60 wt% ethanol. Interestingly, none of the diffraction peaks belonging to form 0 were observed when the granulation was conducted with 15 wt% ethanol in the presence of PEG400. It is noteworthy that the degree of crystal transition from form II to another crystal form in these powders was lower than that observed for the powders kneaded with SDS, LCT or SFE (**Fig. 8c**). Taken together, these result indicated that the kneading of form II crystals with 30 wt% ethanol in the presence of PS80 or POS40 were the optimal kneading conditions in terms of suppressing the conversion of the form II crystals to another crystalline form. Furthermore, the addition of PS80 allowed for the addition of a larger amount of ethanol when the kneading condition reached the slurry state, with the CAM crystals remaining unchanged as form II crystals. These results therefore suggest that the addition of PS80 provides a robust granulation process capable of stabilizing the form II crystals.

The results listed above revealed that the addition of PS80, POS40 or PEG400 accelerated the crystal transition from form I to form II when the wet granulation of form I was conducted with water, and that the same additives also inhibited the crystal transition from form II to form I when the wet granulation of form II crystals was conducted with ethanol. From a structural perspective, it is noteworthy that all three of these additives contained a polyoxyethylene chain as part of their chemical structure.

Taken together, these results suggested that the crystal transition of CAM was related to the interaction of the polyoxyethylene chain of the additives and the CAM molecule.

Effect of a surfactant or PEG400 on the crystal transition of form II by wet granulation with purified water or form I by wet granulation with etnanol

Form II is stable form, when the wet granulation of form II with water and additives mentioned above was carried out, the crystal transition did not occur as shown in **Figs. 9**. On the other hand, the crystal transition from form I to Form II will be hard to induce when the wet granulation of Form I with ethanol which leads CAM crystal form to form 0 was carried out. However, it was expected that the addition of additives contained a polyoxyethylene chain to a wet granulation process involving form I would progress the conversion of the crystals to form II, even when ethanol was used as a solvent. The PXRD patterns of samples obtained following a wet granulation process using ethanol with form I in the presence or absence of surfactants and PEG400 are shown in **Figs. 10**. As a result, PS80 or POS40 were transformed from form I to form II even if ethanol was used as granulation solvent. On the other hand, PEG400 and other additives were transformed to form 0 and form II could not be obtained. These results show that not only the polyoxyethylene chain but also a hydrophobic group will be necessary for the crystal transition from Form I to Form II occurring by ethanol.

Taken together, form I transforms to form II spontaneously. Furthermore, since this reaction rate is enhanced by water, it is thought that a hydrogen bond might be involved in this transition. As for the transition from form I to form II on wet granulation using water with the additives bearing polyoxyethylene chain, we guess that these additives works catalytically and is reduced the activation energy of this transition reaction which a hydrogen bond relate. Moreover, since form 0 is formed by ethanol binding to the CAM molecule, ethanol molecule has a high affinity in CAM molecule. The molecule of polyoxyethylene chain resembles that of ethanol. Accordingly, the polyoxyehylene chain will have a high affinity to CAM molecule. As for transition from form II to form I on granulation using ethanol with these additives, since molecule of the polyoxethylene chain bind to the CAM molecule faster than ethanol, form II might be maintained. As for transition from form I to form II on granulation using ethanol, since the solubility to ethanol of form I will be higher than that of form II, form 0 might be easy to be formed on this granulation condition. However, when the additives having not only the polyoxethylene chain but also the hydrophobic group are added, since the hydrophobic group bind to the hydrophobic part of the CAM like a micelle, form 0 might is hard to be formed, and then form I might transform to form II by the catalytic reaction of the polyoxethylene chain.

The evaluation of interaction between the polyoxyethylene chain and the CAM molecule by DSC and FT-IR

As mentioned above, we think the crystal transition of CAM is related to the interaction of the polyoxyethylene chain of the additives and the CAM molecule. Accordingly, we had investigated the nature of interactions between excipients and drug substance by using DSC and FT-IR. Especially we focused on the crystal transition from I to form II by using water. Form I crystals of CAM were kneaded with 30wt% water and 5wt% additives as the analysis samples.

As a result of DSC applied for the powder of intact form I and form II crystals, an exothermic peak derived from form I was observed at around 125°C and that from form II was not observed, and the powders granulated with form I and PS80, PEG400 or PS40 in presence of water did not show the exothermic peak as showing in **Figs. 11**. These results indicated that form I was transformed to form II by granulating with PS80, PEG400 or PS40, and these results were similar to the results of PXRD as mentioned in **Figs. 3**.

In addition, as for a result of FT-IR, form I and II were distinguished by comparing the IR spectrum at range of 3300 to 3470cm^{-1} which are guess to come from the stretching vibration of O-H bond, 2770 to 2980 cm^{-1} which are guess to come from the stretching vibration of C-H bond, and 1250 to 1450 cm^{-1} which are guess to come from the

deformation vibration of C-H bond as showing **Figs. 12**. As for spectrum at range of 3300 to 3470cm^{-1}, although only one broad peak was observed at 3459cm^{-1} in Form I, two different peaks were observed at 3467cm^{-1} and 3395cm^{-1} in Form II on **Figs. 12**. These mean that the forming hydrogen bonds are different between form I and II. Furthermore, the hydrogen bonds forming in form II will be stronger than that of Form I because form II has the absorption band having lower frequency than form I in these ranges. As for spectrum at range of 2770 to 2980cm^{-1} and 1250 to 1450 cm^{-1}, since these absorption bands were guessed to come from C-H bond of methyl group, it was thought that the position of methyl group in crystal form are different between form I and form II due to the difference of strength on the hydrogen bond.

IR spectrum of the powders granulated with form I and PS80, PEG400 or PS40 in presence of water corresponded to that of Form II, while in the powders granulated with other surfactants, IR spectrum did not completely correspond to that of Form II (the detail results were not shown). In order to discuss deeply regarding the interaction of the polyoxyethylene chain and the CAM molecule, the IR spectrum of the powders granulated with form I, water and PS80 which is transformed from I to form II by wet granulation with water or ethanol was compared with that of PS80 alone and the physical mixture of these. The results are showed in **Figs. 13.** The portion of form I in the physical mixture was observed to transform to form II. As for the absorption band at 1298 cm^{-1},

461 although this was observed in PS80 alone, the physical mixture and the granulated
462 powders were not observed this at here. Since this absorption band was guessed to come
463 from the deformation vibration of O-H bonds binding to the polyoxyethylene chain in
464 PS80, these O-H bonds will be relate to the interaction of the polyoxyethylene chain and
465 the CAM molecule. Furthermore, as for the absorption band of around 1250cm^{-1},
466 although this was observed in PS80 alone at 1249cm^{-1}, the broad peak was observed in
467 physical mixture and the granulated powders at 1243cm^{-1}. Since this absorption band was
468 guessed to come from the stretching vibration of C-O including in the polyoxyethylene
469 chain, the difference of absorption band coming from C-O between PS80 alone and the
470 physical mixture or the granulated powders will be demonstrated the interaction of the
471 polyoxyethylene chain and the CAM molecule.

472 In conclusion, we think that the crystal transition of CAM was caused by hydrogen
473 bond being formed between the polyoxyethylene chain and the CAM molecule.

474 Further studies using nuclear magnetic resonance analysis should therefore be
475 conducted to develop a deeper understanding of the nature of the interaction between
476 CAM and these three additives (i.e., PS80, POS400 or PEG400).

477

478

Evaluation of quality of CAM tablets obtained from the composition including PS80

It was found the crystal transition of CAM could be controlled by adding PS80 to the wet granulation process. Next, tablets were made from these granules using a compression process with forms I and II of CAM, which were generated using a wet granulation process with ethanol or water in the presence of PS80. In this time, we confirmed that form I or II also did not transformed to another crystal form by mixing with placebo formulation constructed from corn starch, L-HPC, light anhydrous silicic acid and magnesium stearate as showing in **Figs. 14**. The PXRD patterns of the tables were then investigated to confirm the influence of compression process on the crystal state of the CAM (**Fig. 15**). The PXRD pattern of F1, which contained form II granules that had been kneaded with water containing PS80, was identical to that of form II. Tablets F2, F3 and F4 were made from formulations containing form I. F2 was produced by the wet granulation of form I with water in the absence of PS80. PXRD analysis of this material revealed that the specific diffraction peaks belonging to form I were still present, although the intensities of the specific diffraction peaks belonging to form II had increased, which demonstrated that the complete transition to form II crystals had not been induced under these conditions. However, the addition of PS80 led to the complete transition to form II crystals, regardless of the particle size of the form I crystals, because the PXRD patterns of F3 and F4 were identical to that of form II (**Fig. 15**; F3 and F4).

F5 and F6 were derived from a formulation involving the wet granulation of form II crystals with ethanol. Specific diffraction peaks belonging to forms 0 and I were observed in F5, which was prepared in the absence of PS80. In contrast, the PXRD pattern of F6, which was formulated in the presence PS80, was identical to that of form II. These results demonstrated that the addition of PS80 effectively suppressed the conversion of form II crystals to forms 0 and I during the formulation process. Taken together, these results demonstrate that the formulation of CAM tablets using a wet granulation process with water and form I or ethanol with form II in the presence of at least 5 wt% PS80 to CAM gave CAM tablets in their most stable form (i.e., form II) with none of the other forms being detected, even after the compression process.

Table 3 shows the pharmaceutical properties of each tablet. For the F3, F4 and F6 tablets, the hardness and disintegration time were significantly higher and shorter, respectively, than those of F1 ($p<0.01$). As mentioned above, the crystal form of CAM in all four of these formulations including F1 was form II, so the significant differences in the properties of these tablets can therefore be explained in terms of the differences in the localization of PS80 either on or inside the wet granules. When PS80 is localized on the surfaces of the granules, the plasticity of the granulated particles and the wicking time of the tablets constructed from these granules would decrease. The uniform localization of PS80 inside the granules would lead to improvements in the plasticity and the wicking

time of the tablets. The hardness and disintegration time properties of the tablets would therefore be dependent on the uniformity of PS80 distribution in each formulation.

Finally, the influence of the PS80 on the dissolution behavior of the CAM tablets was investigated because of the differences in the localization of PS80 on/inside the wet granules. Various tablets were subjected to the dissolution test in different media (pH values of 1.2 and 6.8) (**Fig. 16**). The dissolution behavior of the CAM tablets dissolved at pH 1.2 was consistent with a zero-order release pattern that was independent of the crystal form of CAM in final tablet and the addition of PS80 (**Fig. 16a**). It has been reported that CAM tablets form a gel-like structure on their surface under low pH conditions, and that this gel structure can prevent gastric fluid from penetrating the tablet (Fujiki et al, 2011). This process could be involved in the zero-order release pattern observed during the *in vitro* dissolution test at pH 1.2. In contrast, as for the dissolution behavior of the F1, F3, F4 and F6 tablets, which were prepared as form II, at pH 6.8, F6 showed the fastest dissolution rate, whereas F1 had the slowest initial dissolution rate. These results correlated well with the particle size of CAM and the disintegration time of each tablet, which suggested that there were differences in the localization tendency of the PS80 on/inside the granules for the F1, F3, F4 and F6 tablets. Furthermore, the rate of dissolution of CAM from the F4 tablets was higher than that of the F3 tablets. Because the F4 tablets were manufactured by the grinding of form I CAM particles with a smaller

particle size than the form I particles used in F3, this result demonstrates that the rate of dissolution of CAM can be controlled based on the particle size of the form I crystals. The dissolution rates of the F2 and F5 tablets were slower than those of the other tablets. The extremely slow dissolution rate of F5 could be attributed to the delayed disintegration of the tablet, because the F5 tablet only started to disintegrate after ~30 min. This delayed disintegration could have been caused by the high form I content of the F5 tablet, as shown in **Fig. 15**, because fine needle-shaped crystals were reported to be formed on the surface of tablets containing form I during disintegration test, which may have led to a delay in the disintegration time by inhibiting the penetration of the solution into the tablet (Fujiki et al, 2015). Although the disintegration time of the F2 tablets was short, the dissolution rate was slow. This result suggested that the solubility of another crystal form transformed from form I was lower than that of form II (**Fig. 16b**).

Based on the results described above, it is clear that the dissolution behavior of CAM tablets manufactured with PS80 showed a zero-order release pattern at pH 1.2, which was attributed to the formation of a gel on the surface of these tablets. The dissolution of CAM occurred much more rapidly at pH 6.8. These dissolution behaviors would therefore be ideal for the adsorption of CAM in the intestine *in vivo*.

CONCLUSIONS

In this study, we have shown that form I crystals of CAM can be completely

converted to the corresponding form II crystals using a wet granulation process with water in the presence of PS80, POS40 or PEG400, which all possess a polyoxyethylene chains as part of their molecular structure. Furthermore, the crystal transition of form II to any other form of CAM could not be induced by the wet granulation of form II crystals with ethanol in the presence of these additives. An evaluation of the crystal transition and physicochemical properties of CAM tablets following the addition of PS80 to the formulation process revealed that the CAM tablets contained form II crystals regardless of the crystal form of CAM or the type of solvent used for the granulation. These tablets could be used to control the dissolution behavior of CAM at pH 6.8, whilst maintaining a zero-order release pattern at pH 1.2.

This pharmaceutical technology is simpler and lower in cost than conventional techniques which could induce the conversion of form II CAM crystals to form I crystals. This technology could also make it possible to develop products containing only form II CAM crystals, even if special functional polymers for improved pharmaceutical properties were used with ethanol. It is envisaged that this technology will expand the scope of formulation development.

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Table captions

Table 1. Compositions of the CAM tablets.

Table 2. Particle sizes of the untreated CAM and the hammer milled CAM samples.

Table 3. Physical properties of the CAM tablets.

641 **Table 1.**

Ingredient		Formulation					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
Granulation	Form II of CAM - intact	200.0	—	—	—	200.0	200.0
	Form I of CAM - intact	—	200.0	200.0	—	—	—
	Form I of CAM - hammer milling	—	—	—	200.0	—	—
	Corn starch	52.4	62.4	52.4	52.4	62.4	52.4
	L-HPC	40.0	40.0	40.0	40.0	40.0	40.0
	Light anhydrous silicic acid	5.0	5.0	5.0	5.0	5.0	5.0
	Polysorbate 80	10.0	—	10.0	10.0	—	10.0
	Granulation solvent	Water	Water	Water	Water	Ethanol	Ethanol
Before compression	Light anhydrous silicic acid	3.0	3.0	3.0	3.0	3.0	3.0
	Magnesium stearate	9.6	9.6	9.6	9.6	9.6	9.6
Total		320.0	320.0	320.0	320.0	320.0	320.0

642 The each value represents the amount (mg/tablet) of each additives added in CAM tablet.

643

644 **Table 2.**

Form of CAM	**Particle size** (μm)			
	Intact		Ground	
	D_{50}	D_{90}	D_{50}	D_{90}
II	3.1 ± 0.4	12.9 ± 0.7	—	—
I	25.1 ± 2.6	100.3 ± 8.2	16.5 ± 0.9	47.2 ± 3.2

645 Each data represents the mean value ± S.D. (n=3).

646

647

648 **Table 3.**

Formulation	Hardness (N)	Thickness (mm)	Disintegration time (min)
F1	60.0 ± 2.6	5.03 ± 0.02	9.3 ± 0.5
F2	117.7 ± 8.3*	5.25 ± 0.02	1.0 ± 0.2*
F3	93.7 ± 1.5*	5.10 ± 0.01	3.6 ± 0.2*
F4	107.0 ± 7.2*	5.14 ± 0.01	2.6 ± 0.1*
F5	132.0 ± 5.0*	5.06 ± 0.03	28.0 ± 3.0*
F6	83.3 ± 5.1*	5.07 ± 0.01	4.4 ± 0.8*

649 Each data represents the mean value ± S.D. (n=3). * p<0.01, compared with F1.

Figure legends

Fig. 1. PXRD patterns of forms I and II, and the wet granulation powders of form I prepared with 30 wt% water in the absence of the surfactants

Open and closed circles represent the diffraction peaks characteristic to forms I and II, respectively.

Fig. 2. PXRD patterns of the wet granulation powders of form I prepared with 30 wt% water in the presence of (a) SDS, (b) LCT and (c) SFE.

Open and closed circles represent the diffraction peaks characteristic to forms I and II, respectively.

Fig. 3. PXRD patterns of the wet granulation powders of form I prepared with 30 wt% water in the presence of (a) PS80, (b) POS40 and (c) PEG400.

Open and closed circles represent the diffraction peaks characteristic to forms I and II, respectively.

Fig. 4. Effect of the amount of water added during the granulation of form I (a) in the absence of surfactants, and in the presence of (b) 5 wt% SDS, (c) 5 wt% LCT and (d) 5 wt% SFE, on the PXRD patterns of the resulting wet granulation powders.

Open and closed circles represent the diffraction peaks characterized by forms I and II, respectively.

Fig. 5. Effect of the amount of water added during the granulation of form I CAM crystals in the presence of (a) 5 wt% PS80, (b) 5 wt% POS40 and (c) 5 wt% PEG400, on the PXRD patterns of the resulting wet granulation powders.

676 Open and closed circles represent the diffraction peaks characterized by forms I and II,
677 respectively.
678
679 **Fig. 6. PXRD patterns of the wet granulation powders of form II prepared with 30**
680 **wt% ethanol in the absence or presence of a surfactant or polymer.**
681 Open circles, closed circles and open triangles represent the diffraction peaks
682 characterized by forms I, II and 0, respectively.
683
684 **Fig. 7. Effect of the amount of ethanol added during the granulation of form II in**
685 **(a) the absence of surfactants, and in the presence of (b) 5 wt% SDS, (c) 5 wt%**
686 **LCT and (d) 5 wt% SFE, on the PXRD patterns of the resulting wet granulation**
687 **powders.**
688 Open circles, closed circles and open triangles represent the diffraction peaks
689 characterized by forms I, II and 0, respectively.
690
691 **Fig. 8. Effect of the amount of ethanol added during the granulation of form II in**
692 **the presence of (a) 5 wt% PS80, (b) 5 wt% POS40 and (c) 5 wt% PEG400 on the**
693 **PXRD patterns of the resulting wet granulation powders.**
694 Open circles, closed circles and open triangles represent the diffraction peaks
695 characterized by forms I, II and 0, respectively.
696
697 **Fig. 9. PXRD patterns of the wet granulation powders of form II prepared with 30**
698 **wt% water in the absence or presence of a surfactant or polymer.**
699 Open circles, closed circles and open triangles represent the diffraction peaks
700 characterized by forms I, II and 0, respectively.
701

Fig. 10. PXRD patterns of the wet granulation powders of form I prepared with 30 wt% ethanol in the absence or presence of a surfactant or polymer.

Open circles, closed circles and open triangles represent the diffraction peaks characterized by forms I, II and 0, respectively.

Fig. 11. DSC thermograms of initial form Ⅰ and form Ⅱ , the wet granulation powders of form I prepared with 30 wt% water in the presence of additives bearing polyoxyethylene chains.

Fig. 12. IR spectrums of initial form Ⅰ and form Ⅱ.

Fig. 13. Comparison of IR spectrums of initial form Ⅰ, PS80, the physical mixture of form Ⅰ and PS80, and the wet granulation powders of form I prepared with 30 wt% water in the presence of PS80.

Fig. 14. PXRD patterns of the physical mixture of form I or form II and a placebo.

Open circles and closed circles represent the diffraction peaks characterized by forms I and II, respectively.

Fig. 15. PXRD patterns of the various CAM tablets and a placebo, which did not contain any CAM.

Open circles, closed circles and open triangles represent the diffraction peaks characterized by forms I, II and 0, respectively.

Fig. 16. Dissolution behaviors of various CAM tablets at (a) pH 1.2 and (b) pH 6.8.

Each point represents the mean value ± S.D. (n=3).