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*Research Article*

# Identification of Candesartan Cilexetil-L-Arginine Co-amorphous Formation and Its Solubility Test

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## Abstract

The formation of co-amorphous is one alternative that can be attempted to enhance the solubility of drugs. The study aimed to identify the co-amorphous formation between candesartan cilexetil (CAN) and l-arginine (ARG) and to know its effect on the solubility and dissolution rate of candesartan cilexetil. Initial prediction of co-crystal formation was undertaken by observing differences in crystal morphology between the candesartan cilexetil-l-arginine (CAN-ARG) mixture and each of its initial components due to crystallization in ethanol. The CAN-ARG co-amorphous was produced by the liquid-assisted grinding (LAG) method with the same molar ratio of the CAN and ARG mixture using ethanol as solvent. The co-amorphous formation of CAN-ARG was identified by powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) methods. The solubility and dissolution test was performed to know the impact of the co-amorphous CAN-ARG formation. The PXRD pattern of CAN-ARG of LAG result showed a very low peak intensity compared to pure CAN and ARG. The DSC thermogram of the CAN-ARG LAG result does not show any sharp endothermic peaks. The PXRD and DSC results reveal that CAN and ARG can form co-amorphous. The solubility and dissolution rate of candesartan cilexetil in co-amorphous CAN-ARG was better than that of pure CAN. It can be concluded that liquid-assisted grinding of CAN-ARG mixture is identified to form co-amorphous, which impacts increasing the solubility and dissolution rate of candesartan cilexetil.

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## INTRODUCTION

Solubility is one of the essential parameters besides permeability that affects the bioavailability of active pharmaceutical ingredients (APIs), which impact pharmacological responses. Increasing the solubility of an API can be attempted by converting it into a solid form with a higher solubility than pure API. One approach that can be taken to overcome the problem of poor solubility of an API is to change crystalline solids to amorphous ones. The alteration of a crystalline solid to an amorphous one can be accomplished by combining the API with an excipient, either a polymer or a small molecule[1,2]. Combining API with a polymer to form an amorphous solid is known as a solid dispersion. However, solid dispersion has a disadvantage due to the hygroscopicity of the polymer in large quantities[3,4]. Modifying a crystalline solid from API with a small molecule often results in an amorphous solid known as co-amorphous. The co-amorphous formation is effective in increasing the solubility of some APIs. Co-amorphous is formed when a crystalline API and a crystalline excipient undergo intermolecular interactions, thereby preventing the rearrangement of their respective molecular arrangements into

separated crystal lattices and producing an amorphous material[5,6]. Co-amorphous has better physical stability than the single amorphous form[7].

Candesartan cilexetil (CAN) is an ester prodrug of candesartan that is widely used as an antihypertensive with its mechanism of action by blocking the angiotensin II receptor. This API has poor solubility in water, but its permeability is high, so it is classified into class II in the biopharmaceutics classification system. This poor solubility can be a problem causing low dissolution and bioavailability[8]. Several amino acids have been identified as being able to form co-amorphous with some APIs with the effect of increasing their solubility[9]. One of the amino acids often used as a co-former in the co-amorphous formation is L-arginine (ARG). Several drugs have been successfully increased their solubility through the co-amorphous formation with L-arginine, including indomethacin[10-12], ibuprofen[13], and hydrochlorothiazide[14].

Co-amorphous can be prepared by grinding or solvent-based techniques. Co-amorphous formation often occurs due to inhibition of the crystallization process during co-crystals or salts preparation. Some co-crystal or salt formers (co-formers) can prevent the molecule rearrangement of an API from forming crystal due to the API-coformer intermolecular interaction[2,15]. Co-crystal and salt formed from an API and a crystalline co-former do not recrystallize immediately after the manufacturing process by the liquid-assisted grinding (LAG) process. The grinding of two or more compounds using liquid-assisted grinding (wet grinding) can cause crystal breakdown that can induce intermolecular interactions to form new solid phases, such as co-crystal[16,17,] salt[18], and co-amorphous[19,20]. This study aimed to identify the co-amorphous formation of candesartan cilexetil-L-arginine (CAN-ARG) and to know its effect on the solubility and dissolution rate of CAN.

## MATERIALS AND METHODS

### *Materials*

Candesartan cilexetil and L-arginine were purchased from Afine Chemical Limited, Hangzhou, China, and Merck, Indonesia, respectively. Solvents and reagents such as ethanol, hydrochloric acid, sodium hydroxide, and potassium dihydrogen phosphate were purchased from Merck, Indonesia. Instruments used in this study include a polarizing microscope (Olympus BX-53), automatic mortar grinder (Retsch RM 200), powder X-ray diffractometer (PRXD; Panalytical Empyrean), differential scanning calorimeter (DSC; Shimadzu DSC-6 plus), orbital shaker (IKA KS-260), dissolution tester (ZRS6G), and ultraviolet spectrophotometer.

### *Methods*

*Observation of crystal morphology by polarizing microscope*

Crystal morphology was observed using a polarizing microscope (Olympus BX-53) against CAN, ARG, and a mixture of CAN-ARG recrystallized in ethanol. The test was carried out by placing an amount of 1-3 mg of each CAN, ARG, and CAN-ARG on an object glass which was dropped with one drop of ethanol and allowed until the solvent evaporated. Observation of the crystal morphology of each sample was carried out using a polarizing microscope at a magnification of 200X.

*Preparation of CAN-ARG co-amorphous by LAG*

The co-amorphous preparation was carried out by the LAG method[21,22]. The co-amorphous preparation was carried out by grinding a mixture of 1.832 g (3 mmol) CAN and 0.522 g (3 mmol) ARG in an automatic mortar grinder (Retsch RM 200). The grinding was carried out for 10 minutes with the addition of five drops of ethanol until a soft and clear mass was formed. The soft mass was left in a desiccator to dry, powdered, and sieved through a 60 mesh.

*Detection of CAN-ARG co-amorphous formation by PXRD*

A total of 500 mg of LAG results from CAN-ARG that have been powdered are placed in a sample container and leveled. Scans were performed on a Panalytical Empyrean PXRD, using a Cu anode at a current of 30 mA and a voltage of 40 kV at a 2θ angle between 5 to 45°. The scanning under the same conditions was also performed on pure CAN and ARG as initial components.

*Detection of CAN-ARG co-amorphous formation by DSC*

About 3-5 mg of LAG powder from CAN-ARG was put in an aluminum crucible pan. The aluminum crucible pan containing the sample was positioned in the Shimadzu DSC-6 plus differential scanning calorimeter instrument and scanned at temperature intervals of 30-250°C at a scan rate of 10°/minute. The scanning under similar conditions was also executed on pure CAN and ARG as initial components.

*Solubility test*

The solubility tests were performed in the water at room temperature using the shaker method[23]. Each as much as 50 mg of CAN-ARG co-amorphous and pure CAN powder was placed into a vial. Five mL of water was put into the vial. The vial was placed in an orbital shaker at ambient temperature and shaken for two days at 250 rotations per minute (rpm). After shaking ends, the samples were filtered. The filtrate was measured using an ultraviolet spectrophotometer at 251 nm. Each test was repeated three times.

*Dissolution test*

The dissolution test was implemented as specified in the USP 40-NF 35 monograph of the CAN tablet[24]. The CAN-ARG co-amorphous powder was sieved through a 60-mesh sieve, and the equivalent of 32 mg of CAN was weighed for dissolution testing. Sampling was executed as much as 5 mL at 5, 10, 15, 20, 30, 45, and 60 minutes, and each sampling was replaced with the same medium and volume. Corrections to the calculations were made at each sampling point, and the amount of dissolved CAN was determined using an ultraviolet spectrophotometer. Dissolution tests with the same medium and conditions were also carried out on pure CAN. Each test was repeated six times.

## RESULTS AND DISCUSSION

*Crystal morphology*

The crystals morphology of the CAN-ARG mixture, pure CAN, and ARG after recrystallization in ethanol were shown in **Figure 1**. Identification was carried out by comparing the morphology of the CAN-ARG mixture with the respective crystal morphology of pure CAN and ARG. The observations with a polarizing microscope at a magnification of 200 times showed that the morphology of the recrystallized CAN-ARG does not show any colors due to the interference of light from the crystal lattice, but the result recrystallization only looks black. This situation indicates that CAN-ARG forms an amorphous solid phase. This is different from the crystal morphology of pure CAN and pure ARG, both of which show crystalline morphology, characterized by light polarization that causes the crystals to be colored. This initial microscopic indication can be used as the basis for CAN-ARG co-amorphous preparation by a LAG method using ethanol to accelerate the co-amorphous formation.

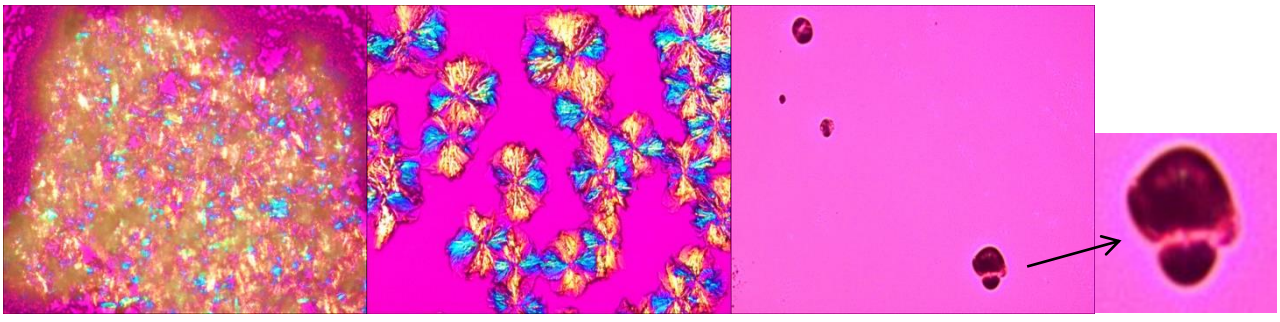


**Figure 1.** Morphology of (**a**) CAN, (**b**) ARG, (**c**) CAN-ARG, and (**d**) and further zoom of CAN-ARG mixture after recrystallized from ethanol compared its starting components observed by polarizing microscope at a magnification of 200x

*Preparation of CAN-ARG co-amorphous by LAG*

In the preparation of co-amorphous CAN-ARG, ethanol solvent was used because this solvent was able to dissolve both substances well. The addition of a solvent or solvent mixture in the wet grinding method helps accelerate the achievement of the amorphous state of each component, thereby increasing the movement of molecules that can accelerate the interaction[25]. In the preparation of CAN-ARG co-amorphous, ethanol was used as a solvent since this solvent could dissolve both substances well. Visually, the co-amorphous powder obtained after drying and grinding was white, the same as the powder of candesartan cilexetil starting material.

*Powder X-ray diffraction*

The X-ray diffractograms of the CAN-ARG LAG result and the two basic components were shown in **Figure 2**. The PXRD pattern of CAN showed the number of sharp peaks, which indicate that the CAN starting material was crystalline. The PXRD pattern of CAN powder corresponds to candesartan cilexetil form 1 reported by Matsunaga *et al*[26]. As with CAN, the PXRD pattern of ARG raw material was also crystalline. However, a different PXRD pattern was shown on the LAG result of CAN-ARG. The PXRD pattern of the LAG result of CAN-ARG showed a low peak intensity which indicates the formation of an amorphous phase. The high-intensity peaks previously present in the pure CAN and ARG disappeared. This situation indicates that CAN and ARG experienced intermolecular interactions during the LAG process, which prevented the CAN and ARG molecules from rearranging to form their respective crystal lattices and finally resulted in an amorphous material known as co-amorphous[27].

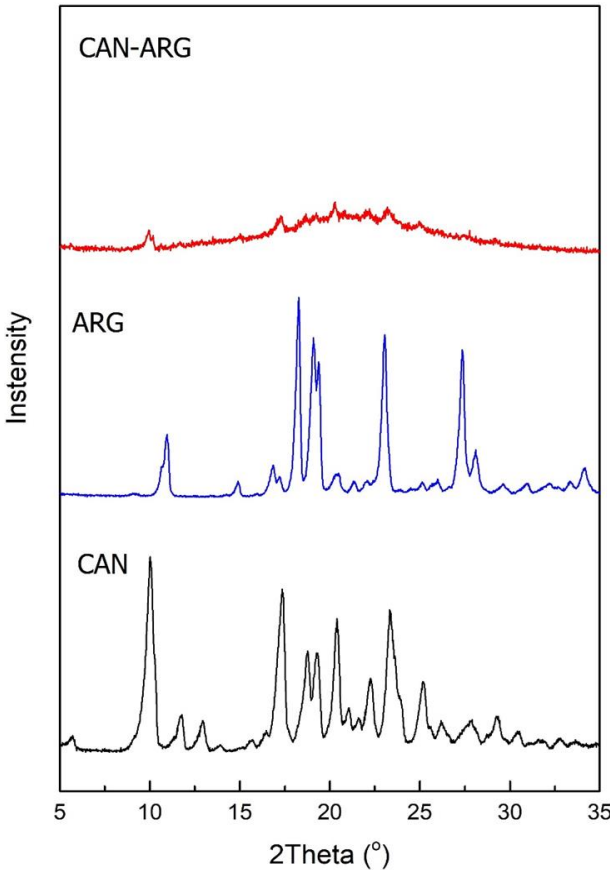


**Figure 2.** PXRD patterns of CAN-ARG LAG result, pure CAN, and pure ARG

*DSC thermograms*

DSC thermograms of CAN-ARG LAG result, pure CAN, and pure ARG were shown in **Figure 3**. The DSC thermogram showed that the thermal characteristics of the pure components of the starting material (CAN and ARG) were different from

those of the LAG of the CAN-ARG mixture. A sharp endothermic peak at 177.26°C in the DSC thermogram of CAN is due to the substance's melting, which corresponds to the melting point of the Form 1 polymorph[26,28]. The DSC thermogram of ARG showed two endothermic peaks, one sharp endothermic peak at 221.6°C corresponding to its melting point and another endothermic peak around 80-100°C due to the release of water molecules from the ARG raw material, which is slightly hygroscopic. DSC thermogram of the two starting components has sharp endothermic peaks that indicate both are crystalline. The DSC thermogram of the CAN-ARG grinding result did not show any sharp endothermic peaks indicating the co-amorphous formation. The formation of this co-amorphous was also confirmed by the presence of a glass transition (Tg) at 53.45°C, which is a characteristic of the amorphous form. The glass transition is the temperature at which an amorphous solid begins to change from a glassy to a liquid state when heated[29].

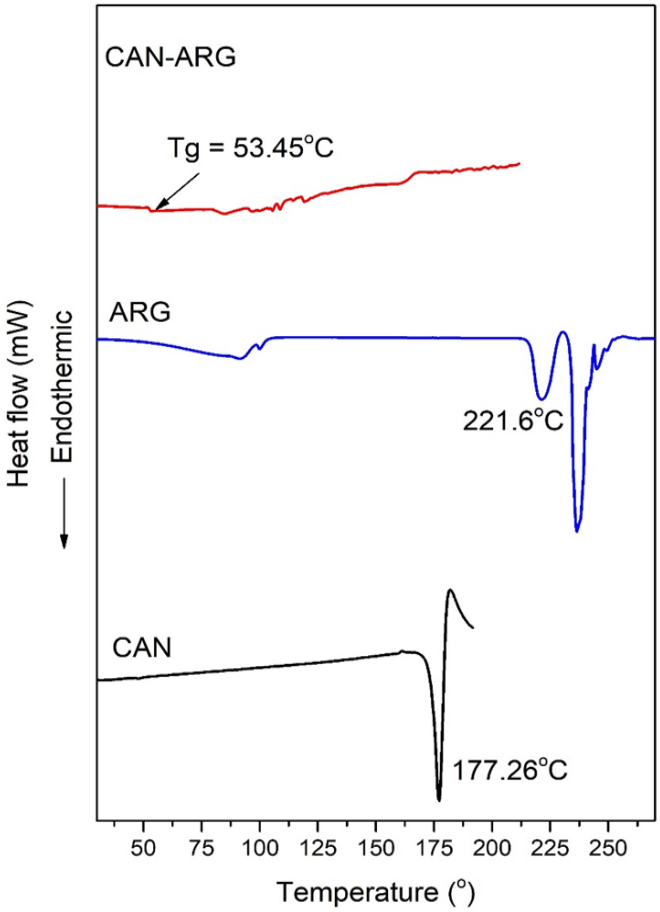


**Figure 3.** DSC thermograms of CAN-ARG LAG result, CAN, and ARG

*Solubility*

The solubility test aims to determine any changes in physicochemical properties that occurred due to the CAN-ARG co-amorphous formation. The solubility of CAN-ARG co-amorphous and pure CAN in water at room temperature were 2.837±0.080 and 0.009±0.001 mg/mL, respectively. The co-amorphous CAN-ARG showed 315-folds higher solubility than pure CAN. The increase in solubility through the formation of co-amorphous has been studied previously to increase the solubility of valsartan up to 1000-folds compared to the pure substance[30]. The possible reasons for this increase in solubility are that the co-amorphous form of CAN-ARG does not have a regular molecular arrangement, so the energy required to break the intermolecular bonds during the dissolution process is lower than that of CAN crystals. Candesartan is a weak acid, and the solubility of CAN in water depends on pH[31]. Therefore, the increase in solubility could also be due to the ionization of CAN in the presence of ARG (a weak base).

*Dissolution*

The dissolution rate profiles of CAN-ARG co-amorphous and pure CAN in 0.05 M phosphate buffer solution pH 6.5 containing 0.70% polysorbate 20 were shown in **Figure 4**. The dissolution profiles showed that the CAN released from the co-amorphous CAN-ARG had reached 100% in less than 20 minutes. In contrast, the CAN released from pure CAN was only 9.7% up to 45 minutes of testing. A significant increase in its solubility caused the increasing dissolution rate of CAN in the CAN-ARG co-amorphous after being co-amorphous.

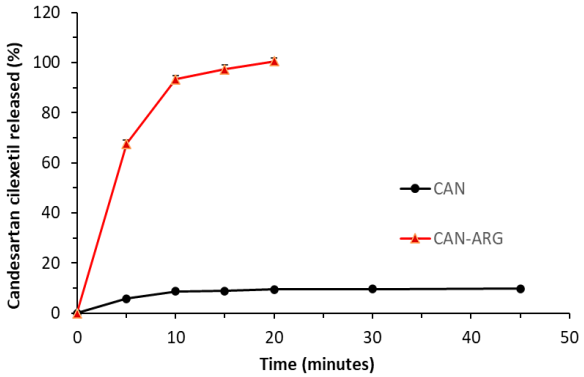


**Figure 4.** Dissolution profiles of CAN-ARG co-amorphous compared to pure CAN

## CONCLUSION

The co-amorphous formation between CAN and ARG has been identified by the polarizing microscope, PXRD, and DSC of the LAG, which shows a CAN-ARG co-amorphous formation between CAN and ARG. The CAN-ARG co-amorphous led to a significant improvement in the solubility and dissolution rate of CAN.

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## AUTHORS' CONTRIBUTION

All authors have an equal contribution in carrying out this study.

## DATA AVAILABILITY

None.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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