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Research Article

IDENTIFICATION OF PHYSICAL INTERACTION BETWEEN ANTI MALARIAL DRUGS COMBINATION ARTESUNATE-AMODIAQUINE HYDROCHLORIDE

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ABSTRACT

Identification of solid state to investigate the possibility of physical interaction between Antimalarial Artemisinin base Combination Treatment (ACT) AS and AQ by hot contact method Kofler, cold contac method (crystallization reaction) and biner phase diagram confirmation had been carried out. The results of hot contact method Kofler shown formation a new crystalline habit as long and thin needle shaped on contact zone (mixing zone) between AS and AQ. It had a different melting point in compared to its single component. Crystallization reaction (cold contact methods) between two of supersaturated solution of component AS and AQ in methanol solvent also indicated the growth of crystal habit as similar as hot contact method Kofler. Confirmation by biner phase diagram shown the specific diagram for cocrystalline phase. Solid state interaction between AS and AQ was analysed by powder X-ray diffraction, FTIR spectrophotometric, microscopic SEM and thermal DTA, TG-DSC. Microscopic analysis by SEM shown significantly the change of habit and morphology of crystal as long and thin needle shaped. The difference of powder X-ray diffraction (PXRD) interferences peaks were observed in addition to PXRD interference peaks of each component and its physical mixtures that proved formation of cocrystalline phase. DSC Thermogram indicated a new endothermic peak corresponding to melting point of a new cocrystalline phase at temperature 160,4 °C.

Keywords: Artesunate, Amodiaquine HCl, Physical interaction, Cocrystall, Kofler contact methods, Cold contact methods, Biner phase diagram, Thermal analysis, Crystallography.

INTRODUCTION

One of Artemisinin Combination Treatment base (ACT) Artesunate (AS) and Amodiaquine (AQ) is the current mainstay of treatment and are recommended by the WHO as an antimalarial drug (27,28). AS and AQ generally used independently as a combination of drugs (co blistered) that cause non optimalization of the treatment or non-compliance in the use of drugs (3). The use of fixed-dose combination form is very rare (16,17,21). Between AS and AQ are incompatible. AS degraded due to several things: moisture content, processing temperature and the possible influence of 4-aminokuinolin (7).

Intermolecular interaction in binary system most likely to occur in the two materials which similar. Similarities are generally based on the molecular formula and the internal structure or level of crystallinity lattice symmetry.

Interactions are often found in pharmaceutical technology include: an eutectic mixture, solid solutions (mixed crystals) and molecular compounds (cocrystal) (6,22).

Combination between active ingredient and excipients can lead to make transformation and solid-solid interactions in physics and chemistry (1,4,5). Interaction between ingredients in the drug dosage forms can cause the formation of new impurities, problems in the preparation and manufacturing processes, changes in the nature of the physicochemical properties of drug substance (5). Intermolecular interaction as a consequence of the physics of binary systems that occur in the combination of AS - AQ is not known clearly.

This study aims to reveal the kinds of AS - AQ intermolecular interactions with microscopic identification approach using thermal contact and crystallization reaction. Characterization of the result of interaction was studied by thermal analysis DSC / DTA, FTIR spectroscopic, PXRD crystalography and SEM.

This research started with researching and characterizing each single compound artesunate and amodiaquine HCl finding the possibility of polymorphisms that will provide physical consequences of the AS - AQ binary system. Some methods for producing crystalline forms like evaporation of solvent (methanol, ethanol, chloroform, n - hexane), cold crystallization method (Freeze Drying in the water solvent) and grinding had been done. The changing nature of the crystals was observed by means of powder X - ray diffraction (PXRD), thermal analysis DTA/DSC, HSM (Hot Stage Microscope), SEM and FTIR. The next stage was to construct the phase diagram of physical mixture of AS - AQ in various composition. Further characterize physical mixture by means of PXRD, thermal analysis by DTA/DSC, HSM, SEM and FTIR to analyze the possibility of intermolecular interactions between AS - AQ. With the effort of uncovering, it is expected to be a scientific foundation in problem-solving design and manufacturing processes of dosage formulations fixed combination artesunate - amodiaquine HCl.

MATERIALS AND METHODS

Materials

This study was performed at 2012. Preparation of the sample, FT IR analysis, HSM analysis and TG-DSC analysis were conducted in ITB Bandung. DTA analysis, PXRD analysis were conducted in Airlangga University Surabaya and ITS Surabaya. SEM analysis was done in Central Research of Geology Bandung. Artesunate was purchased from Haryana, India with bacth no. AS/M-001/07-08, while amodiaquine hydrochlortide USP was purchased from Mangalam Drugs & Organics Ltd, Mumbai, India with batch no. AMH – 113003. All of the solvent was purchased from Merck Chemical Indonesia without any purification.

Observation of habit and a mixture of two components

Each of artesunate (AS) and amodiaquine hydrochloride (AQ) was made to saturated solution in various solvents (methanol, ethanol, n - hexane, chloroform, distilled water), then each glass dripped on different objects and allowed to crystallize again at room temperature along with the evaporation of the solvent. Saturated solution of physical mixture ekimolar AS and AQ dripped on glass objects, allowed to crystallize. The crystallization process and the crystal habit observed by polarizing microscope and digital camera.

Preparation of the physical mixture of Artesunate and Amodiaquine HCl

Raw materials artesunate and amodiaquine hydrochloride weighed and mixed in a molar ratio = (0:10), (1:9), (2:8), (7:3), (5:5), (3:7), (2:8), (1:9), (10:0). Physical mixture homogenized by stirring the two materials in a mortar with zalf cart.

Hot Stages Method

Contact method performed under a polarizing microscope equipped with a desk heater electrik (Hot Stage). A specific number of AQ was put on the object glass and covered, then heated to melt, and allowed to crystallize again. AS powder was put on the side of the border the cover glass. The system is heated until the entire AS will be melt, move and contact with the surface of the AQ crystal. Contact area (contact zone) that occurs between solids and fused AQ –AS was observed for growing of a new crystal under a polarizing microscope at 200x magnification and recorded with a digital camera (6).

Cold Contact Method

Saturated solution of AQ in the solvent dripped on glass objects, waiting to crystallize. After that the same amount of AS saturated solution dripped on the edge of AQ recrystallization region. Crystallization behavior observed at the contact area between the crystal AQ with a solution of saturated AS. Physical interaction was observed with the polarizing microscope at 200x magnification and recorded with a digital camera (8).

DTA Thermal Analysis

Each materials and binary mixture of various molar ratio of drugs were prepared for DTA analyzing. Heating was performed in the range 30-300°C at scan rate 10°C/min. Thermogram of DTA results were used to construct of phase diagram of AS-AQ binary mixture.

Powder X-Ray Diffractometry (PXRD) Analysis

The X-ray diffraction patterns were obtained with X-ray diffraction analysis of the powder sample at room temperature by using a Rigaku diffractometer type Rint-2500. Measurement conditions as follows: the target metals Cu, Kα filter, voltage 40 kV, current 40 mA, the analysis performed at 2 theta range of 5-35°. The sample is placed on the sample holder and leveled to prevent particle orientation during sample preparation.

Scanning Electron Microscopy (SEM) analysis

Powder sample was placed on the sample holder and coated with gold aluminum with a thickness of 10 nm. Samples were then observed various magnification SEM instrument (JEOL, Japan). Voltage was set at 20 kV and 12 mA current.

Fourier Transform Infrared (FTIR) Spectroscopy

About 1% dispersion powder samples in potassium bromide (KBr) was made by mixing the mass with KBr. Infrared spectrum obtained with an infrared spectrophotometer in the range of wave numbers 400 - 4000 cm-1.

RESULTS AND DISCUSSION

Early identification to unravel the physics of the interaction between the two components was done by two methods, namely heat Kofler contact method and the method of crystallization reactions (2,8,19).

Thermal contact method was first introduced by Lehman and Kofler (2). This method is a simple technique to identify the phase behavior in a binary system. In this method, one component (which has a higher melting point that AQ) was melted and allowed to solidify again (recrystallization), the second component AS (lower melting point) was placed on the other side of the glass object, heated using a heater (hot stage) which associated with a polarization microscope. At the time of the second component (AS) melting, molten phase component of the AS will diffuse into the solid component of AQ and AQ dissolve most solids in the contact zone between the binary system AQ and AS. Samples were allowed to solidify (recrystallization) at room temperature. After the second component (AS and AQ) solidified, the contact zone was observed back in the polarizing microscope (6).

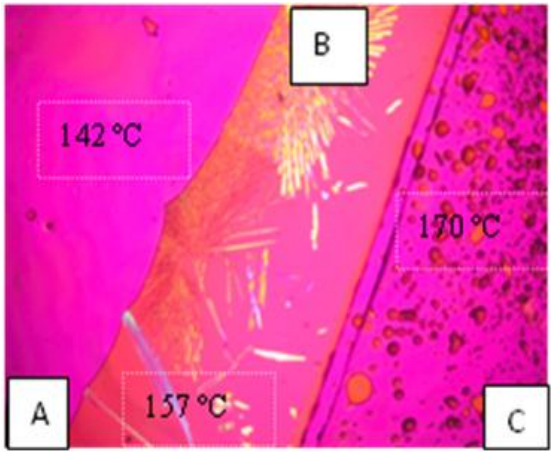

Fig. 1: It shows HSM photomicroscope A)AS area, B) contact zona, C) AQ area

Fig. 1 shown side A is the result of the AS and the melt recrystallization C is melt recrystallization AQ. Zone B is the contact zone between the AS and AQ solids. At the beginning of the formation of the contact zone, have not observed any new crystal habit, but are still in the liquid phase (amorphous). After settling in a certain time, begin to form a new crystal growth habit in zone B-shaped needle (needle shaped habit). Contact method of sample preparation was heated again, then AS solid phase melt at 142 ° C, contact zone melt at157 ° C and AQ solid phase melt at 170°C. The difference in crystal habit and thermal behavior of solids indicates the interaction between the two components (2, 6, 19). There are three types of solids interaction when observed from the thermal behavior of the molten phase mixture of two components, namely: i) conglomerates (eutectic) where both components still exist on a separate crystalline zone, ii) solid solution in which the two components mixed in a homogeneous solid phase, iii) cocrystal or molecular compounds which are formed in the solid phase mixing zone that has different properties of the two components of (6).

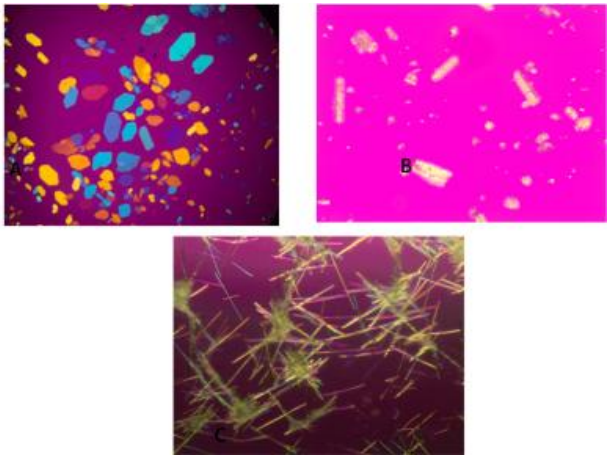

Fig. 2: Photomicroscopes of cold contact method, A) AS in methanol, B) AQ in methanol, C) AS-AQ binary mixture in methanol

Crystallization reaction method is also simple method for observing the identification between the two components, especially the drug compounds which unstable on heating (8). Each component have good solubility in the methanol solvent. Each component is dissolved in the methanol solvent, until it reaches a state of supersaturation.

AS components and a single AQ dripped on glass object and allowed experienced recrystallization and crystal habit observed in a single state with a polarizing microscope equipped with a digital camera.

To observe the interaction between the AS and AQ binary systems, super-saturated solution of both components dropped on the two sides of the glass object, then the two solutions will come into contact with one another, and left for some time at room temperature

New crystal growth was observed under a polarizing microscope. Figure 2 shows the crystal habit AS and AQ results recrystallization from methanol and crystal habit results of co-crystallization of both components AS-AQ from the same solvent. Some of the factors that determine the transformation of the solid phase is the nucleation rate, crystal growth rate and distribution of nucleation sites (nucleation sites). Nucleation rate and the growth rate will depend on the state of the supersaturated driving force for the co-crystallization of two solid components from both component molten phase and dissolved phase (8). To evaluate the results of the contact methods (hot and cold contac, analysis by DTA and the transition temperature data plotted in the phase diagram. AS-AQ phase diagram made by plotting the melting point (endothermic peak) obtained from thermal analysis DTA with the molar fraction of binary mixture AS-AQ on composition 0:1; 1:9; 2:8; 3:7; 5:5; 7:3; 8:2; 9:1 and 1: 0.

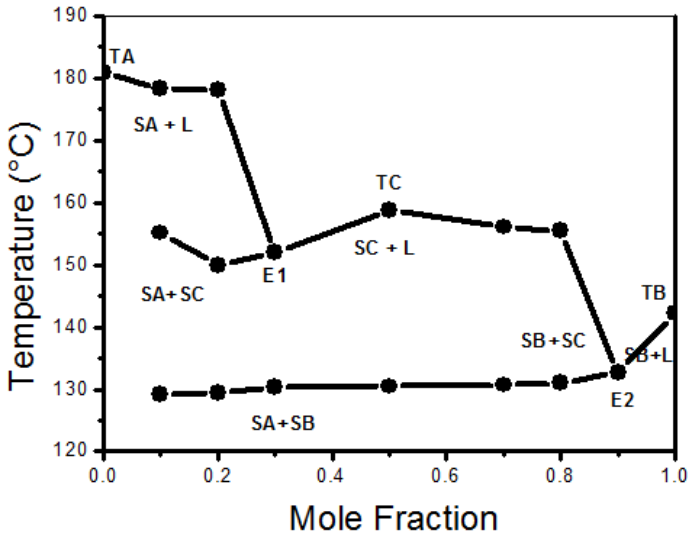

Fig. 3: TA=Melting temperature of AQ TB= Melting temperature of AS TC=Melting temperature of Cocrystal, E1,E2=Eutectic point,SA=Solid of component AQ, SB=Solid of component AS, SC=Solid of component Cocrystal, L= Liquid

Figure 3 is a graphic picture of the results of a typical phase diagram between two solid components that interact through the formation of molecular compounds (cocrystal) obtained at a mole fraction of 5:5. Endothermic peak is obtained at a temperature of 158.9 ° C, interpreted as a cocrystal melting point (MP). There are two points eutecticum E1 and E2 at a temperature of 150 ° C and 132.7 ° C. AQ melting point will decrease with the addition of AS and reaches a minimum value at the first eutecticum point (E1) 150 ° C (mole fraction 2:8). From the point of liquidus curve E1 rise due to the addition of more AS and led to increase melting point of the mixture and reach its maximum value at the point of TC. At this point they are in balance with the solid phase liquid phase, where the maximum temperature is congruent compound or AS-AQ cocrystal (158.9 ° C) on the mole fraction 5:5. Further, the addition of AS will cause a decrease in binary mixture melting point to the minimum point E2 (132.7 ° C) at a mole fraction of 9:1. According to figures we can conclude that there is equilibrium between the solid phase with the liquid phase at the same mole fraction composition. This suggests the formation of molecular compounds AS-AQ (cocrystal phase) as a consequence of the two components of the binary solids system.

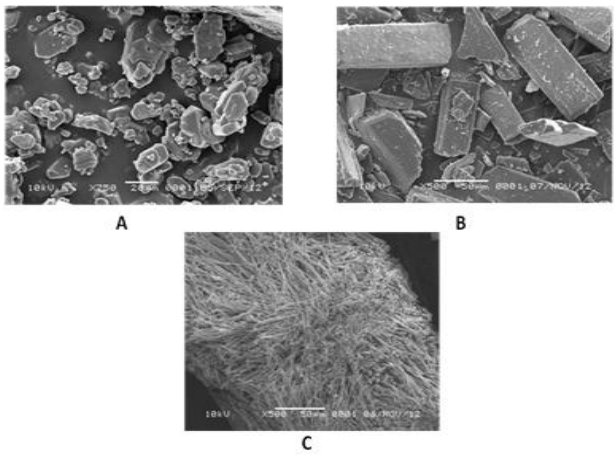

Fig. 4: SEM Microphotograph A) AS raw materials (750x) B) AQ raw materials (500x) C) AS-AQ Cocrystal in methanol solvent (500x)

Figure 4 shows the microscopic analysis with a scanning electron microscope (SEM). AS raw materials, AQ and the solid results of the AS-AQ interaction method cocrystalisasi reaction of methanol. The solid reaction products (AS-AQ cocrystal) showed the different crystal habit and particle sizes compare to each components. The solid interaction (AS-AQ cocrystal) results indicate needle-shaped crystal habit.

To verify the interaction between two solid components of AS – AQ, analysis of X-ray powder diffraction and differential thermal analysis scanning calorimetry (DSC) were done. X-ray diffractogram and DSC thermogram of cocrystal AS-AQ compared with each single component and the physical mixture of both components without treatment (Figures 5 and 6). X-ray powder diffraction is a powerful method for the characterization of the interaction between the two components (24). If the new crystalline phase is formed from the interaction between the two components it will be observed clearly from the X-ray diffractogram.

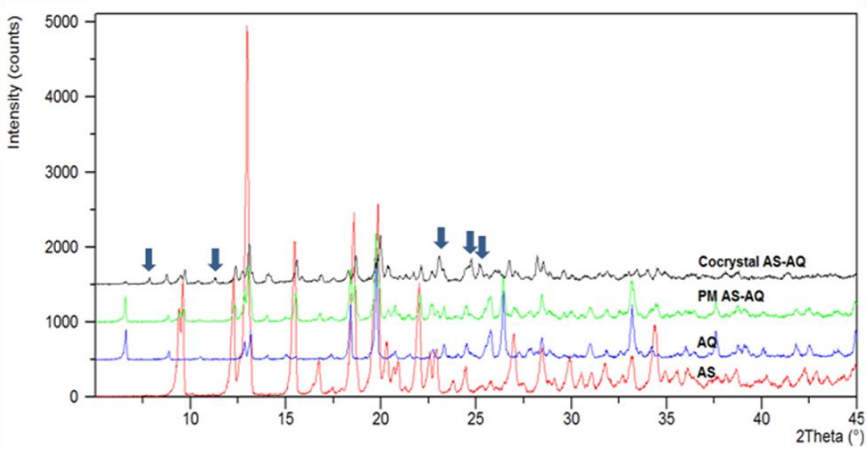

Fig. 5: PXRD difractrogram A) AS B) AQ C) Physical Mixture (PM) AS-AQ (1:1 ekimolar) D) AS-AQ Cocrystal

Figure 5 shows the X-ray powder diffractogram of AS-AQ cocrystal compared to the single component and physical mixture of both components without treatment. PXRD pattern of AS – AQ cocrystal (Fig. 5 D) significantly different from the pattern of diffraction AS, AQ and physical mixture of AS-AQ. The diffraction pattern of each component crystalline solids exhibit properties of a single compound. While the diffraction pattern of physical mixture of AS-AQ showed all the typical peak of AS interference and AQ, indicating only happens superimposition of the two components. Cocrystal diffraction pattern of AS-AQ showed some new interference peaks typical at 2θ : 8.77; 12.77; 23.11; 25.28 and 25.57. This indicates that the physical interaction between AS – AQ had formed, and formation result of a new crystalline phase, commonly called cocrystalin phase (molecular compounds or molecular complexes) in materials science.

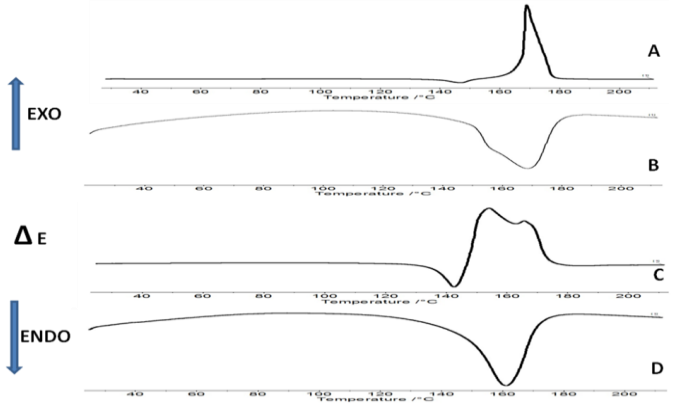

Fig. 6: DSC thermogram A) AS B) AQ C) physical mixture AS-AQ (ekimolar) D) AS-AQ cocrystal

DSC thermal analysis useful for characterizing interactions among multiple components of the solid ingredients. Thermal analysis was used to evaluate changes in thermodynamic properties that occur when the material supplied heat energy. Changes that can be observed is the process of melting, desolvasi, recrystallization and solid phase transformations indicated by an endothermic or exothermic peaks at thermogram. DSC thermogram showed AS solids endothermic peak at 142.2 ° C (Fig.6A), while the AQ has a pad endothermic peak 166.8 ° C.(Fig.6B). DSC thermogram of physical mixture ekimolar (Fig. 6C) shows three endothermic peak at 130.5 ° C; 150.5 ° C (eutectic mixture melting point) and 158.9 ° C, which is the result of solids interaction formed from the AS-AQ (cocrystal AS-AQ melting point). DSC thermogram data for AS-AQ crystal showed an endothermic peak at 160.4 ° C. This indicates that both the solid component is transformed into a new crystalline phase of AS-AQ known as cocrystal phase (molecular compounds). PXRD analysis results showing the formation of a new interference with different physical mixture and some shifts in the FTIR spectrum strengthens the case of AS-AQ cocrystal forming.

CONCLUSION

Verification of physical interaction with microscopic method (hot stages contact and cold contact methods and SEM micrograph), crystallographic methods (PXRD), thermal methods (DTA / DSC), spectroscopic methods (FTIR) indicated the formation of the solid phase cocrystalin between Artesunate and Amodiaquine (AS-AQ cocrystal).

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