Solubility Improvement of Antimalaria Drug through Co-Crystalization with Malic Acid

Gozali Dolih[1]*, Alatas Fikri[2] and Sugandi Rani[2]
1. Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, INDONESIA
2. Department of Pharmaceutical Technology, Faculty of Pharmacy, Universitas Jenderal Achmad Yani, Jl. Terusan Jenderal Sudirman, Cimahi, INDONESIA
*dolih.gozali@unpad.ac.id

Abstract

Pyrimethamine (PYR) is a biopharmaceutics classification system class II drug that has low solubility and high permeability, so its absorption rate is determined by the dissolution rate. The purpose of this study was to determine the effect of cocrystal PYR with malic acid (MAL) as co-crystal former (coformer)PYR-malic acid (PYR-MAL) binary mixture and to investigate its influence on the solubility and dissolution rate of PYR. The formation of co-crystals with a equimolar ratio is prepared with solvent drop grinding (SDG) method with acetone:water as solvents. PYR-MAL was characterized by powder X-ray diffraction (PXRD), Fourier transform infrared (FTIR) spectroscopy and Polarized microscopy methods. PYR-MAL is having solubility and dissolution rate higher than the active pharmaceutical ingredient (API). The powder X-ray diffraction results show the crystallinity of PYR changing to more amorphous indicating the salt formation.

Fourier transforms infrared spectroscopy (FTIR) spectra of PYR-MAL binary mixture showing the vibration peaks of an imine group on the pyridine ring of PYR and carbonyl group on the PYR is not detected. On the other hand, the polarizing microscope images show the crystal habit of PYR-MAL binary mixture after recrystallized from methanol contrast to crystal habit of its pure components. The solubility in water of PYR-MAL binary mixture is higher than pure PYR. The amount of PYR released from PYR-MAL binary mixture in pH 4.5 and 6.8 buffer solutions is faster than from pure PYR. A binary mixture of PYR and MAL may improve the solubility and dissolution rate of PIR.

Keywords: Co-crystal, malic acid, pyrimethamine, solvent drop grinding.

Introduction

Malaria is a health problem in many countries around the world. According to WHO (World Health Organization), there are estimated 207 million cases of malaria in 2012, which caused 627,000 deaths in 102 countries with malaria transmission. Indonesia is a malaria endemic area., although the program has been implemented and the eradication of malaria since 1959, until now the morbidity and mortality rate is still high[1]. Indonesia's Ministry of Health said the prevalence of malaria in Indonesia is still high, reaching 417.819 positive cases in 2012. 70 percent of malaria cases are found in eastern Indonesia, especially in Papua, West Papua, Maluku, North Maluku, Sulawesi and Nusa Tenggara.

Pyrimethamine is one of the water insoluble drugs with a low dissolution rate because of low bioavailability. Pyrimethamine is an essential drug in the treatment of falciparum malaria disease that has been resistant to chloroquine. In addition, it was recently discovered that pyrimethamine can be used as an anti-HIV and toxoplasmosis[2].

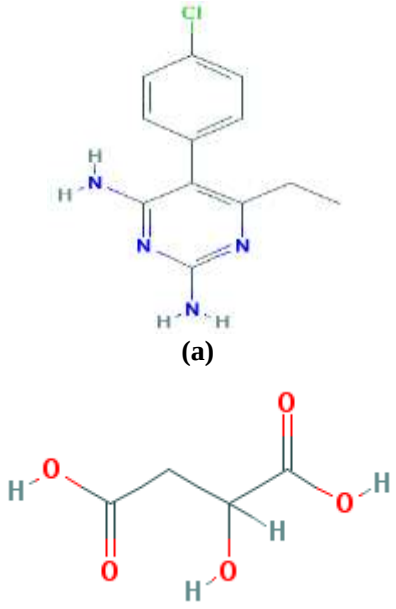
The efforts to increase the solubility of PYR have previously been reported, among others, solid dispersion[3]and the formation of inclusion complex. Today co-crystals are the preferred method of the pharmaceutical industry for two main reasons. First, the physicochemical properties of pharmaceutically active ingredients can be altered without altering their pharmacological activity. Second, the shelf life of pharmaceutical active ingredient can be prolonged by co-crystallizing. As with the formation of solid dispersions and inclusion complexes with cyclodextrins, the formation of co-crystals may increase solubility and dissolution rate of pharmaceutically active ingredients[4].

Modification of solid forms of drugs through the formation of salts or co-crystals is a very good technique to increase the solubility of some drugs[5-7]. Co-crystal is a new solid form that consists of a single crystalline phase of several components in a certain stoichiometric ratio, where different types of molecules interact with hydrogen bonds or other non-covalent bonds[8]. The pyridine ring in their chemical structure PYR as shown in fig. 1a allows the proton transfer with carboxylic acids to form a salt. Furthermore, N of the pyridine ring may also act as a hydrogen bond acceptor, allowing the formation of co-crystal when it binds to the hydroxyl group of carboxylic acids.

Malic acid (MAL) is a dicarboxylic acid that is safe to use orally and it is widely employed as an excipient in salt or co-crystal formation. MAL (fig. 1b) can improve the solubility of several drugs through the formation of salt[9,10] and co-crystals.[11-13]

This study is conducted to determine the effect of pyrimethamine co-crystalline formation with malic acid as co-former. The resulting complex is expected to have greater

solubility than pure PYR. Provision of mechanical energy such as grinding in a solid binary mixture with or without the addition of solvents can lead to interactions, among others, an eutectic mixture, salt, or co-crystal formation. The purpose of this research is mainly to prepare a binary mixture of PYR-MAL and to investigate its effect on the solubility and dissolution rate of PYR.



(a)

(b)

Fig. 1: Chemical structure of (a) pyrimethamine and (b) malic acid.

Material and Methods

PYR was obtained from Jintan Xinda-China. FUM was purchased from Sigma-Aldrich, Singapore (>99%). All reagents and solvents were purchased from Merck, Indonesia, without any further purification.

Phase solubility test of PYR in MAL solution: Approximately, 50 mg of PYR was put into each vial containing numerous concentrations of PYR solution in water (15-50 mM) and shaken on an orbital shaker at room temperature. The samples in the vials were filtered after 24 hours and then, the clear filtrate was analyzed with by ultraviolet (UV) spectrophotometry method at a wavelength of 272 nm. The phase solubility curve was constructed by plotting the solubility of PYR to the various concentrations of MAL.

Preparation of PYR - MAL binary mixture by wet grinding method: A binary mixture of PYR-MAL was prepared by milling an equimolar ratio of PYR-MAL physical mixture in the mortar using a pestle with the addition of two drops of methanol for five minutes. The grinding result was dried and ready to be characterized.

Characterization by X-ray powder diffraction method: The amount of 200 mg for each sample (PYR-MAL binary mixture, PYR and MAL) was placed in a glass container, the sample surface was flattened and then the X-ray powder diffraction pattern was observed using a Philips PW-1710 X-ray diffraction system over a range of angles 2θ=5-35°, the speed of 2°/minutes, a voltage of 40 kV and a current of 30 mA. Then, the powder X-ray diffraction pattern of PYR-MAL binary mixture was compared with the diffraction pattern of pure PYR and MAL.

The characterization of PYR-MAL crystal habit by polarizing microscope: As much as 2 mg of PYR-MAL physical mixture was placed on the glass slide, add two drops of methanol, cover with a glass lid and allow to recrystallize. The crystal habit after recrystallization was observed using an Olympus BX-53 polarizing microscope equipped with an Optilab Advanced Plus camera and compared with crystal habit of its pure components after recrystallized from the same solvent.

Solubility test: Solubility test was conducted on the binary mixture of the PYR-MAL and pure PYR in water at room temperature. A certain number of samples was inserted into the vial containing 10 mL of water and shaken on an orbital shaker. After 24 hours, the sample was filtered and then the filtrate of the sample was diluted and analyzed with a UV spectrophotometer at a wavelength of 272 nm.

***In vitro* dissolution test:** The *in vitro* dissolution test was carried out on the PYR-MAL binary mixture and pure LOR in 900 mL of pH 1.2, 4.5 and 6.8 buffer solutions at 37±0.5°C using a type II apparatus with 50 rotations/minutes (rpm) of speed for 120 minutes. 10 mL of sample was taken after 5, 10, 15, 30, 60, 90 and 120 minutes and replaced with the same volume of the buffer solution. Each sample was analyzed by the UV spectrophotometer.

Results and Discussion

Phase solubility test: The formation of the salt or co-crystal can be predicted by knowing the type of phase solubility curve. According to Higuchi and Connors, phase solubility of a substance in the solution at various concentrations of other substances consisting AL, AP, AN, BS and BL types.

The phase solubility curve of in various concentrations of MAL solution is presented in fig. 2. The solubility of PYR at 15, 20, 30, 35, 45 and 50 mM concentrations of MAL solutions was 2.3, 3.0, 14.6, 15.0, 6.9, and1.9 µM respectively., therefore, the type of phase solubility curve is BS. In this type, the solubility of PYR increased sharply in concentrations range of 15-30 mM of MAL solution due to the formation of dissolved new solid form (salt or co-crystal). At concentrations of 30-35 mM, the saturation condition began to occur as a result of reaching solubility product constant (Ksp) of PYR-MAL co-crystal.

Consequently, the solubility of PYR in the concentration range was fixed. Conversely, the increasing of PYR concentration up to 50 mM caused a decline in MAL solubility, therefore, the precipitating of salt or co-crystal

was formed. These results express that in between PYR and MAL an interaction occurred. There are two possibilities of the interaction, the first is the transfer of a proton from MAL to PYR to create the salt and the second is the formation of hydrogen bonds between the PYR and MAL to assemble the co-crystal.

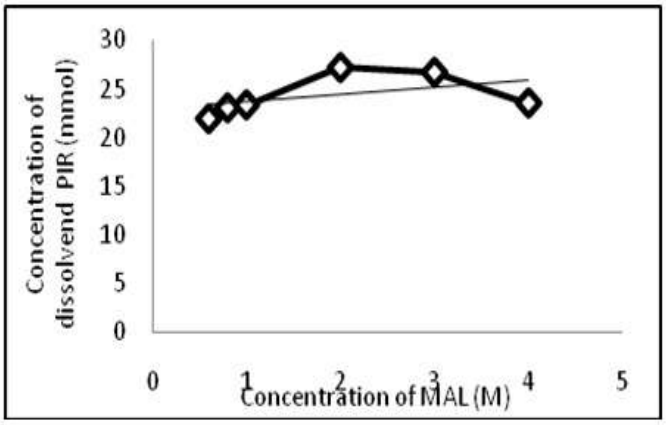


Fig. 2: Phase solubility curve of pyrimethamine in various concentrations of malic acid

Characterization by powder X-ray diffraction method: The formation of co-crystals can be characterized by X-ray powder diffraction (fig. 3). Different X-ray diffraction patterns from the scouring of pharmaceutical and co-former active ingredients may be an indication that a new solid has been formed[23]. X-ray diffraction pattern of PYR-MAL co-crystalline powder differs from pure PYR. The peak of the PYR at an angle of 18.5 θ and the MAL at an angle of 20 θ is lost and a new peak appears at an angle of 2θ of 23 after an SDG of 1: 1. This indicates that the co-crystals formed are not part of the PYR or MAL. The peak formed from the PYR-MAL co-crystals has a lower percentage of crystallinity, which can lead to increased solubility of the co-crystals compared to the pure PYR, because the higher is the crystallinity percentage, the lower is the water solubility.

Characterization by FTIR method: Infrared spectrophotometer (figure, 4) may be used to detect the formation of co-crystals, particularly for the detection of carboxylic acids used as co-former and OH-N hydrogen bonds formed between acids and bases[15]. PIR has an intense peak at 3117 cm^{-1} wave number which is tenuous N-H_2, 1558.5 cm^{-1} which is a split C = C aromatic and 1280.73 cm^{-1} which is a C-H substitution of aromatics. MAL has an intense peak at 3590 cm^{-1} wave number which is O-H, 3082,25 cm^{-1} which is C-H from alkene compound and 1050-1300 cm^{-1} which is C-O. The shift of PIR-MAL infrared spray of crystalline infra-red indicates the formation of supramolecular bonds. There is a new peak at 3390 cm^{-1} wavelength which is O-H loose from the carboxylic acid hydrogen bonds and 3317.56 cm^{-1} which is a N-H stretch.

The characterization of PYR-MAL crystal habit by polarizing microscope: The polarization microscope is used to view the crystal habit of PYR, MAL and PYR-MAL crystals (figure 5). Changes in crystal habitat from cubic-shaped PYR and MAL shaped like prism occur after cold contact of PYR and MAL with the addition of acetone: water (1: 1) producing crystals with a feather-like shape. Changes in crystal habit are an indication of the formation of PYR-MAL co-crystals.

Solubility test: The PYR-MAL crystals (1: 2) were tested for the solubility of the phases using several variations of MAL concentrations of 0.6; 0.8; 1.0; 2.0; 3.0 and 4.0 M. The solubility of the co-crystalline phase indicates a decrease in the concentration of the soluble PIR, the higher concentration of the MAL showed results of lower dissolved PYR concentrations. This happens because the co-crystals already contain MAL with enough concentration to increase the solubility. If the MAL is added more, there will be a decrease of the soluble PYR concentration because the previous PYR has reached saturation point

***In vitro* dissolution test:** Dissolution test is an important factor in drug quality control[16]. PIR-MAL and PIR co-crystalline dissolution tests were performed using various solvent media (fig. 6) in buffer hydrochloride solution pH 1,2, pH 4.5 acetate buffer solution and phosphate buffer solution pH 6.8. The purpose of using various pH is to simulate the pH in the body, in the stomach with pH 1.9 in the empty state and pH 3-5 when there is food and small intestine with pH 4-8. The highest dissolution rate is in the buffer pH 6.8 media buffer because the acidic MAL will make the pH of the media down to acid so that the basic PYR will be ionized on the acidic medium.

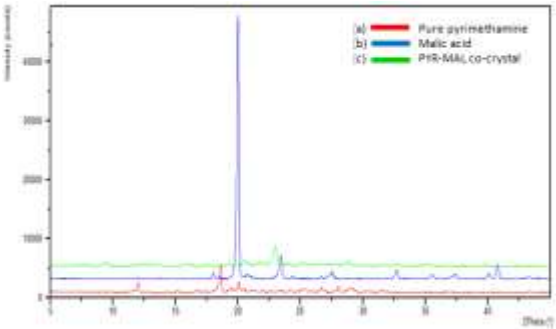


Fig. 3: Powder X-ray diffraction pattern of (a) pyrimethamine (PYR), (b) malic acid and (c) PYR-MAL by solid drop grinding.

Ionized substances have high solubility in water; phosphate buffer solution pH 6.8 has a pH that is not much different from water but ionized substances cannot be absorbed membrane by passive diffusion because passive diffusion can only absorb substances that are not ionized. Molecules cannot pass through the membrane channel because the strong polarity of the ionized form will inhibit the transmembrane diffusion process. Only the unionized and fat-soluble active substance fraction can pass through the membrane by passive diffusion. But for substances that have low solubility in water with complete absorption, such as PYR, the most decisive step is their solubility[4].

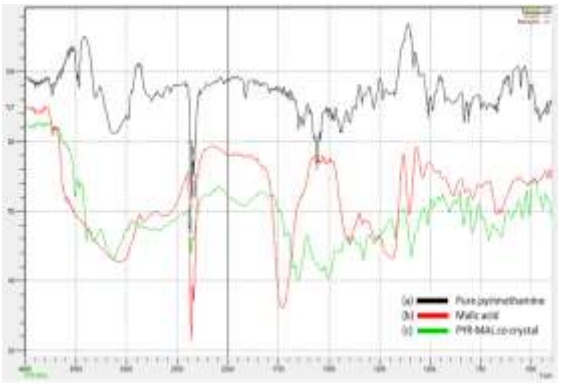
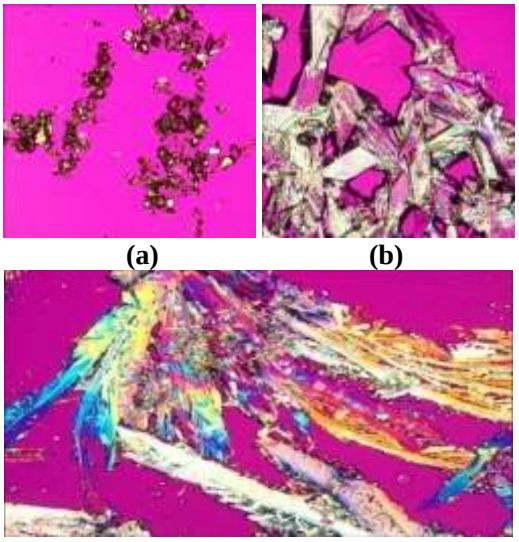


Fig. 4: Fourier transforms infrared spectroscopy of pyrimethamine (PYR), malic acid (MAL) and PYR-MAL.



(a) (b)

(c)

Fig. 5: Polarizing microscope photo of (a) pyrimethamine (PYR), (b) malic acid and (c) Pyrimethamine -Malic acid co-crystal (PYR-MAL) after recrystallized by aceton-water solvent

Conclusion

A binary mixture of PYR-MAL had been prepared by the wet grinding method and characterized by construction of the phase solubility, powder X-ray diffraction, FTIR spectroscopy and microscopy methods. The phase solubility curve of PYR numerous concentrations of MAL solution is BS type and consequently, it is supposedly assembled to a co-crystal between PYR and MAL. Although there is no difference in the powder X-ray diffraction pattern between the PYR-MAL binary mixture and its pure constituents, the intensity of this binary mixture is lower than its pure components.

The characterization results show the differences in the FTIR spectra and crystal habit between the PYR-MAL binary mixture and their pure components. The PYR-MAL binary mixture may improve the solubility of PYR in water and the dissolution rate in pH 6.8, 4.5 and 1.2 buffer solutions. The enhancement of the solubility and the dissolution rate of PYR may be due to co-crystal formation.

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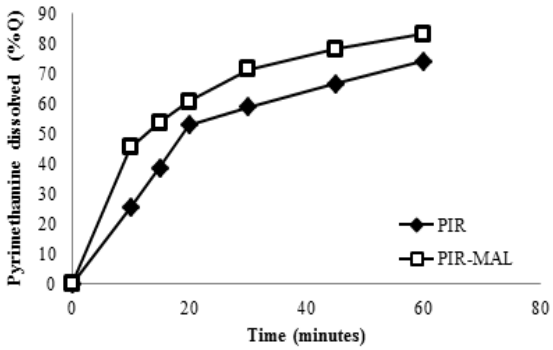
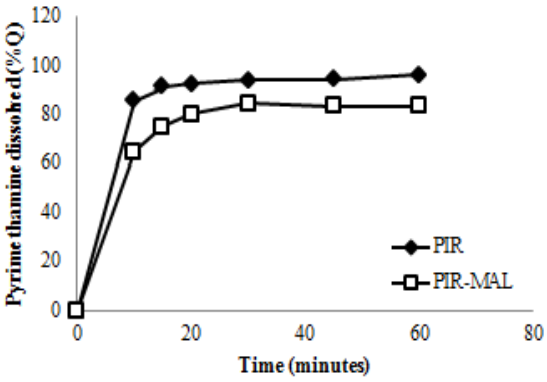
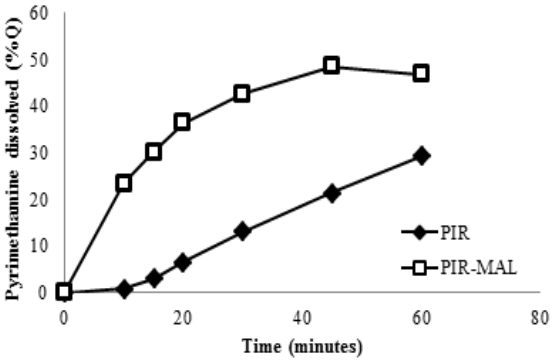


(a). HCL buffer pH 1.2



(b). Acetate buffer pH 4.5



(c) Phosphate buffer pH 6.8

Fig. 6: The dissolution profile of pure pyrimethamine (PYR) and PYR- MAL binary mixture in pH (a) 1.2, (b) 4.5 and (c) 6.8.

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