Malaysian Journal of Pharmaceutical Sciences,
Vol. 5, No. 1, 45–57 (2007)

SOLID STATE INTERACTION BETWEEN AMOXICILLIN TRIHYDRATE AND POTASSIUM CLAVULANATE

ILMA NUGRAHANI*, SUKMADJAJA ASYARIE, SUNDANI NURONO SOEWANDHI AND SLAMET IBRAHIM
School of Pharmacy, Bandung Institute of Technology,
Ganesha 10, Bandung, Indonesia

The aim of this research was to evaluate solid state interaction between amoxicillin trihydrate and potassium clavulanate. The interaction was observed by Differential Scanning Calorimeter (DSC), X-Ray Powder Diffractometer (XRPD), Fourier Transforms Infra Red (FTIR) and Scanning Electron Microscope (SEM). Mixtures of amoxicillin trihydrate and potassium clavulanate were prepared in molar ratios of 0:10, 1:9, 2:8, 3:7, 4:6; 5:5; 6:4; 7:3; 8:2; 9:1; 10:0 and analyzed by DSC to obtain the thermal profile and a phase diagram. From this phase diagram, the molar ratio point of interaction was determined. XRPD analysis was performed to check the type of physical interaction occurred and FTIR was conducted to predict the chemical mechanism of interaction. Thermo profile obtained by DSC analysis of the binary systems showed that endothermic curves of molar fractions of 1:9 – 5:5 overlapped at 201°C. On the other hand, the diffractogram obtained from Powder X-Ray analysis was very similar with that of amoxicillin trihydrate alone. FTIR spectrum of binary system in the molar ratio of 5:5 showed the loss of hydrate spectra from amoxicillin trihydrate. We conclude that the interaction involved strong hydrogen bonding between hydrates of amoxicillin with potassium clavulanate which produced a co-crystal system like a solid dispersion.

Keywords: *Amoxicillin-clavulanate, Solid state interaction, DSC, XRPD, FTIR, SEM*

INTRODUCTION

Pharmaceutical formulations consisting of two or more active ingredients have attracted much interest because they have been shown to have synergistic curative effects or decreased side effects (Freifeld, Marchigiani and Walsh 1999; Kern *et al.* 1999). However, incompatibilities between active ingredients or ingredients-excipients may result in toxic or no clinical effects (Eyjolfsson 1998; Blanco-Fuente 2004). These drug incompatibilities are categorized as physical or chemical interactions that may occur during manufacturing and storage as well as while mixing ingredients. They are sometimes manifested by precipitation or color changes. Occasionally, *in vitro* beta lactam antibiotic interactions occurred without any observable change but can

* Corresponding author: Ilma Nugrahani, e-mail: Ilma_nugrahani@yahoo.com

be determined quantitatively by determining their excess thermodynamic properties in beta lactam binary system solution (Jain *et al*. 2000; Chadha, Kashid and Jain 2003). Molar enthalpies of amoxicillin and ampicillin solutions in various pH values showed significant differences with the theoretical calculation of the molar enthalpies value (Jain *et al.* 2000). Chadha, Kashid and Jain (2003) observed similar phenomenon in amoxicillin-clavulanate solution binary system. The evidence of the interaction could be predicted based on the similarity of their structures (Vednere 1990).

Amoxicillin trihydrate–potassium clavulanate is a beta lactam antibiotic combination that has been used worldwide to treat upper respiratory tract infection and pneumonia. Clavulanate does not have antibiotic activity but it inhibits beta-lactamase, an enzyme which hydrolyzes beta lactam ring of amoxicillin trihydrate (Storm, Conley and Roush 2003). This antibiotic combination is available in various solid dosage forms. Unfortunately, there are wide variabilities in the quality of solid dosage forms, especially in their dissolution and pharmacokinetics profile (Vree, Dammers and Exler 2003). The high variability of the solid dosage forms characteristics was thought to be related to the physicochemical interactions. Preliminary studies using cold contact methods have shown that amoxicillin-clavulanate combination has resulted in a physicochemical solid state interaction (Nugrahani *et al.* 2007).

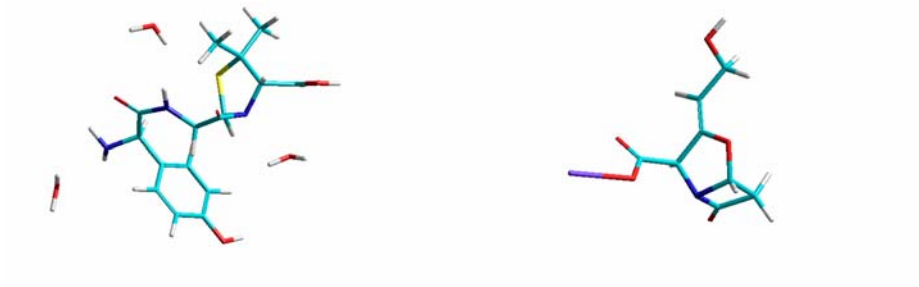
The purpose of this study was to identify the type of interaction and to predict the type of physicochemical interaction using DSC, XRPD, FTIR and SEM

METHODS

Materials and Apparatus

Amoxicillin trihydrate (ex. Sandoz, China, batch no. 476Z5H), Potassium clavulanate (ex. Fermic, Mexico, batch no. CKA – 2967), Siever (Retsch AS-200, Germany), Mettler digital milligrams balance (Mettler M3, Germany), X-Ray Diffractometer /XRD (Minifex 96012A26, Japan), Oven (Retsch, Germany), Mortar, HyperChem Professional Release 7.5 Software, DSC (Perkin Elmer, DSC-6, USA), FTIR (Jeoul FT/IR-

4200/type-A, Japan), SEM (Jeoul, Japan) were utilized in this study. Modeling of the 3-D structure [Fig. 1(a) and (b)] was done using HyperChem Professional Release 7.5.



(a) **(b)**

Fig. 1(a): Structure of amoxicillin trihydrate structure with 3 water crystals and beta lactam ring. **(b)** Potassium clavulanate – beta lactam ring structure.

Experiments

Mixtures of amoxicillin trihydrate - potassium clavulanate were prepared in various molar ratios, which were analyzed by DSC, XRPD, and FTIR, and SEM to identify the interaction (Brittain 2001; Cartensen 2001). Amoxicillin trihydrate and potassium clavulanate were sieved with 100 mesh sieve and filled into 10 vials with 1 g each to give different mixtures of 1:9; 2:8; 3:7; 4:6; 5:5; 6:4; 7:3; 8:2; 9:1; 10:0 (amoxicillin alone). In a separate vial, potassium clavulanate was filled and prepared as (0:10) mixture. All of the mixtures was homogenized manually (for 5 min) and then analyzed using DSC. Approximately 2 to 5 mg of each sample was heated in open aluminium pan from 30 to 350°C at a scanning rate of 10°C/min under a stream of nitrogen gas.

Based on DSC data, a phase diagram was constructed and the extreme point of the curve was determined as the molecular interaction ratio balance. The mixture (5:5) was filled into four separate vials and then these were treated as follows: (a) kept fresh in the refrigerator until it was analyzed; (b) stored in open vial at ambient temperature for four hours; (c) ground for 15 min by mortar and; (d) heated at 90°C for 15 min in a sealed vial. In another vial, potassium clavulanate alone stored at ambient temperature for four hours and then was analyzed as

counterpart. All samples were analyzed by XRPD under the following conditions; target/filter (monochromator) Cu, voltage 40 kV, current 30 mA, receiving slit 0.2 inches. The data were collected in the continuous scan mode using a step size of 0.5 degree/min. The scan range was 5˚ to 60˚.

FTIR spectroscopy was performed on Fourier – transformed infrared spectrophotometer Jasco - 4200 type A (Japan). The samples (i.e. amoxicillin trihydrate, potassium clavulanate, and their mixtures) were ground and mixed thoroughly with potassium bromide at 1:5 (sample:KBr) ratio, respectively. The KBr disc were prepared by compressing the powders at 20 psi for three minutes on KBr-press. The spectra were scanned over wave number range of 4500 to 500 cm^{-1}.

RESULTS AND DISCUSSION

Jain *et al.* (2000) have reported studies on physical mixtures of beta lactam antibiotic solutions at different pH using solution calorimetry. Incompatibilities between amoxicillin–clavulanate in the liquid phase have also been reported (Chadha, Kashid and Jain 2003). While many of these incompatibility studies have been conducted in the solution state, in this study we tried to determine the interaction in the solid state using crystallographic techniques.

Amoxicillin trihydrate and potassium clavulanate have similar structures (Drugbank 2006) [Fig. 1(a) and (b)]. The similarities of their molecular and crystal structures suggest an increase likelihood of interaction between the two compounds (Vednere 1990). In addition, the two compounds have a big difference in *pka*, which makes the mixture prone to chemical acid-base reaction (Florence and Salole 1994; Roth and Fenner 2000; Stahly 2007).

The main method used to determine physical interactions is thermal analysis. DSC analysis is most useful to characterize and analyze changes in properties of pharmaceutical materials such as polymorphism, phase transformation and compound instability (Brittain 2001). In this experiment, DSC analysis was conducted to determine the thermal profile of both compounds and the binary system interaction (Liu 2000). Figure 2(a) shows the amoxicillin trihydrate thermal profile while potassium clavulanate thermal profile was shown in Figure 2(e).

The thermal profiles in Figures 2(b) – (d) show that the exothermic curve of amoxicillin trihydrate moved towards exothermic curve of potassium clavulanate and increased steadily from 181.9°C to 201°C.

The DSC data indicates that there was a physical interaction between amoxicillin trihydrate and potassium clavulanate in solid phase similar to that of physical interaction in the liquid phase (Chadha, Kashid and Jain 2003). The thermal profiles show the exothermic curves of amoxicillin and potassium clavulanate overlapped at molar ratios of 1:9, 2:8, 3:7, 4:6 and 5:5. This important phenomenon has not been previously reported. This overlap indicates the occurrence of co-recrystallization followed by co-oxidation between amoxicillin trihydrate and potassium clavulanate, because the compounds have similar energy lattice (Brittain 1999; Blanco-Fuente 2004). The data is represented by 5:5 molar ratio thermal profile in Fig. 2(d). At molar ratios of 6:4 to 9:1, the thermal profiles show two separate exothermic curves indicating the existence of two components [Fig. 2(b) and (c)]. So, it could be predicted that the change of thermal profile occurred at molar ratio of 5:5 indicating a molar balance of the physical interaction.

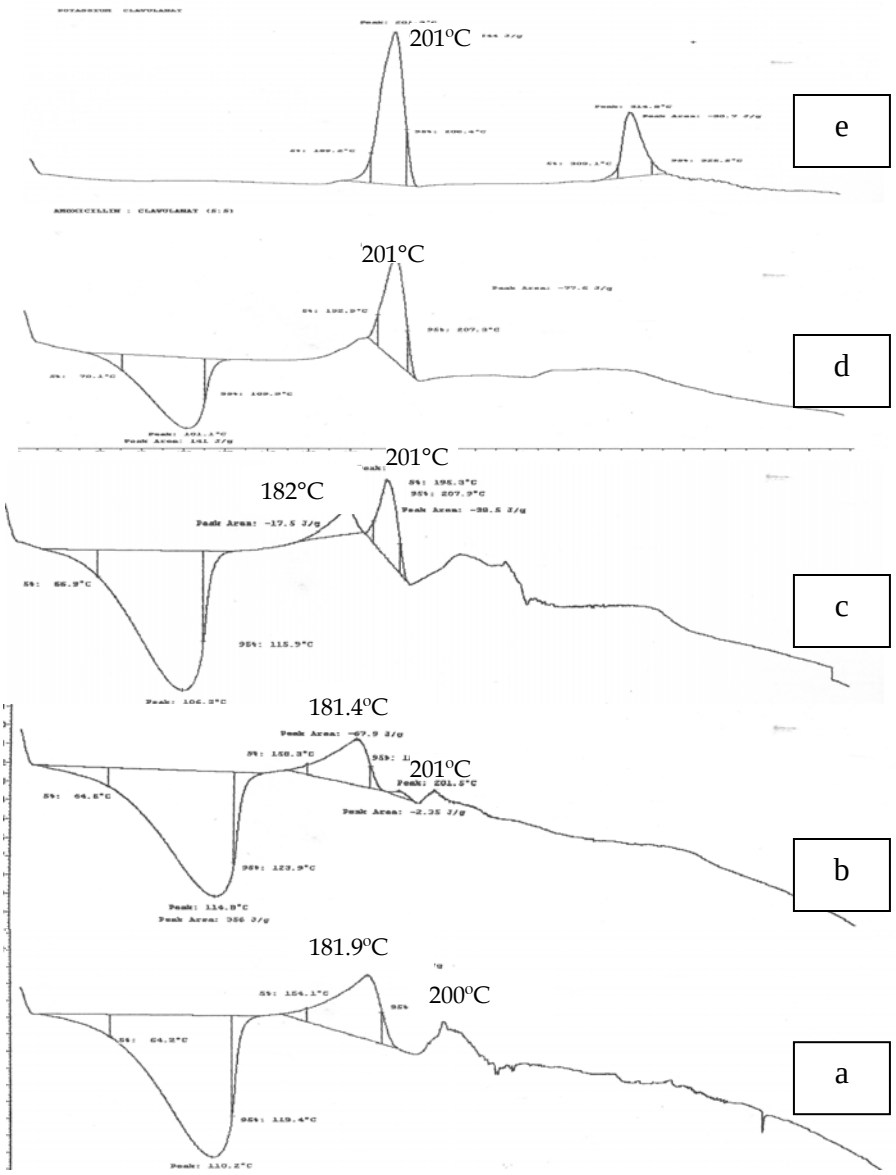


Fig. 2: Thermal profiles and exothermic transition peaks of: amoxicillin trihydrate alone (a); amoxicillin trihydrate-potassium clavulanate (AC) in molar ratios = 1:9 (b); 7:3 (c); 5:5 (d); and potassium clavulanate alone (e).

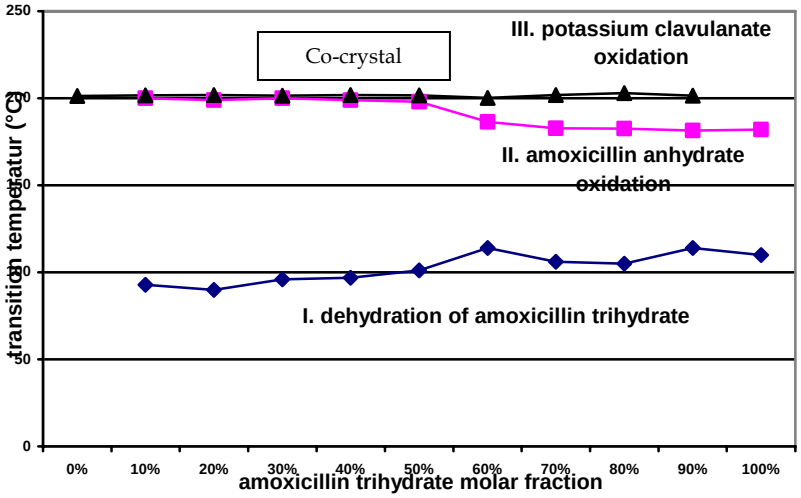


Fig. 3: Profiles of amoxicillin trihydrate weight fraction in the amoxicillin trihydrate–potassium clavulanate versus transition temperature.

The phase diagram of amoxicillin trihydrate–potassium clavulanate binary system is shown in Figure 3. There were no changes on the endothermic transition temperature of clavulanate and amoxicillin in all mixture compositions above 5:5 molar ratios. There was a loss of hydrate crystal from amoxicillin trihydrate, which is indicated by the appearance of endothermic curve at 50°C to 114°C [Fig. 2(a) and (d)]. The endothermic transition temperature decreased proportionally with increasing proportion of potassium clavulanate. We predict that the phenomenon was related to the formation of bonding between potassium clavulanate and the hydrates of amoxicillin.

XRPD, FTIR, and SEM were performed to identify the type and to determine the mechanism of interaction (Liu 2000; Cartensen 2001). XRPD analysis was carried out to characterize the solid state mixture before and after treatment. Figure 4 show diffractograms of amoxicillin trihydrate [Fig. 4(a)], potassium clavulanate [Fig. 4(b)], potassium clavulanate after storing at ambient temperature for four hours [Fig. 4(c)], amoxicillin trihydrate-potassium clavulanate (AC) in molar ratio of 5:5 and stored at ambient temperature for four hours [4(d)], ground AC mixtures of 5:5 molar ratio [4(e)], and after heating at 90°C for 15 min [4(f)]. These treatments were done to simulate interactions which may

occur during solid dosage forms manufacturing and storing (Liu 2000; Cartensen 2001; Umeda *et al.* 2007). The diffractograms of these mixtures before and after the treatment process were similar to the diffractogram of amoxicillin trihydrate as a single component. Amoxicillin is predicted to be located inside of the binary co-crystal, while potassium clavulanate is located on the surface of the system. During storing, grinding or heating process, potassium clavulanate disperses on the amoxicillin crystal surface and binds with the hydrates of amoxicillin.

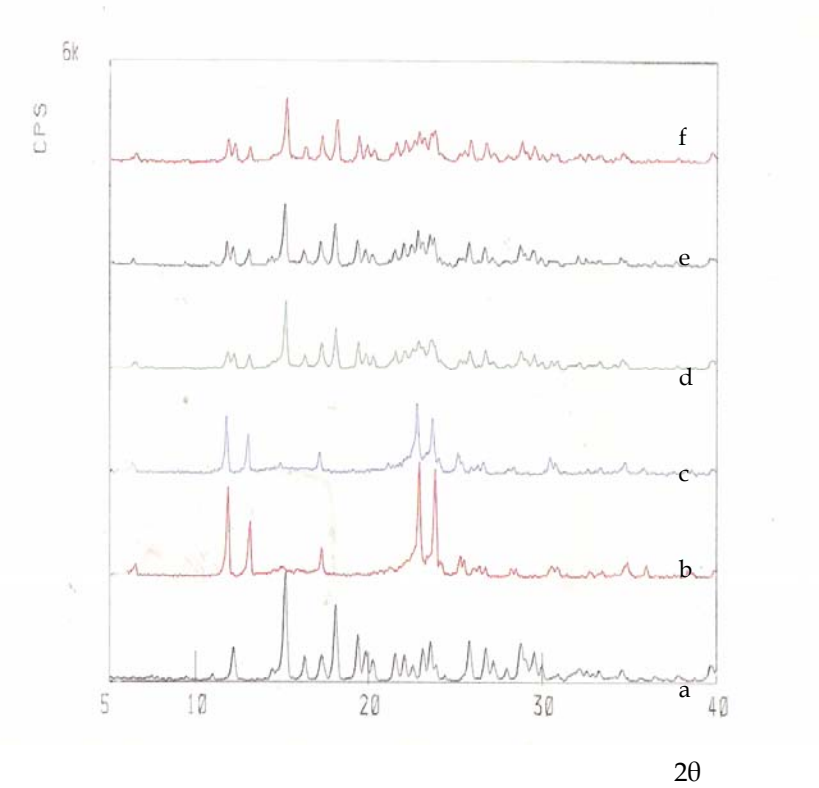


Fig. 4: Diffractograms of amoxicillin trihydrate (a); potassium clavulanate (b); potassium clavulanate stored at ambient temperature for four hours (c); amoxicillin trihydrate-potassium clavulanate (AC) in molar ratio of 5:5 stored at ambient temperature for four hours (d); ground AC(5:5) (e); AC(5:5) heated (f).

The loss of hydrate crystal from amoxicillin trihydrate is indicated by the appearance of endothermic curve at 50°C to 114°C. This may suggest that the interaction is related to the formation of hydrogen

bonding between potassium clavulanate and the hydrates of amoxicillin. To examine this, FTIR was conducted on the binary system (ie. using the ground and heated mixture). The results showed that the hydrate crystal spectra of amoxicillin trihydrate at 3300 nm were lost (Fig. 5).

Fig. 5: FTIR spectrum of: amoxicillin trihydrate (a), potassium clavulanate (b), and ground amoxicillin trihydrate-potassium clavulanate in molar ratio 5:5 (c).

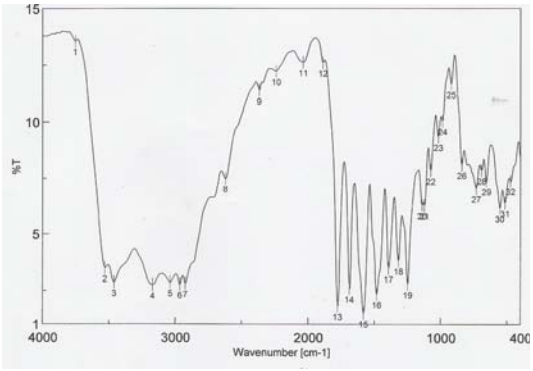


Fig. 5(a): FTIR spectra of amoxicillin trihydrate.

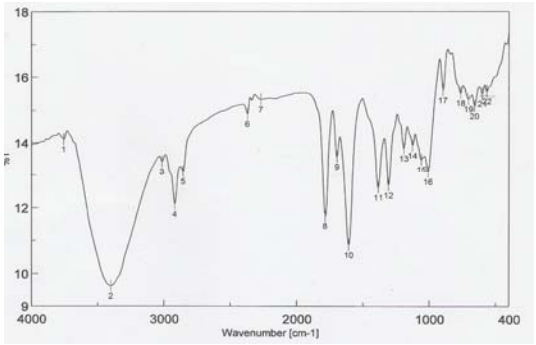


Fig. 5(b): FTIR spectra of potassium clavulanate.

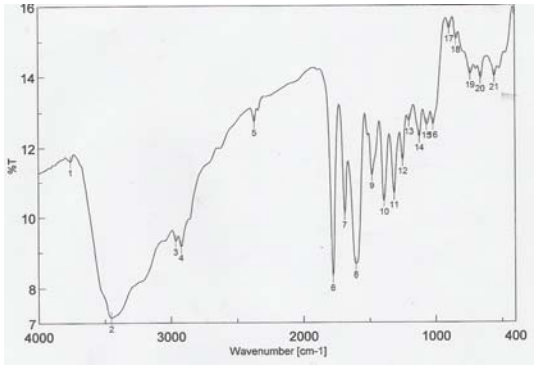


Fig. 5(c): FTIR spectra of ground mixture of amoxicillin trihydrate-potassium clavulanate 5:5.

Interestingly, the FTIR spectrum of the 5:5 binary system was similar to that of amoxicillin trihydrate alone but without the hydrates spectra. Figures 5(a), 5(b), and 5(c) show an increase in the spectra at 1080 and 800 nm of the 5:5 binary system, which were the –NH and C=O spectra. This data support the prediction that there is a formation of hydrogen bonding between –NH/-C=O site of potassium clavulanate with the hydrates of amoxicillin (Watson 2001).

Finally, SEM was conducted to get a visualization of the co-crystal binary system. Figure 6(a), 6(b) and 6(c) show potassium clavulanate, amoxicillin trihydrate, and the ground mixture of amoxicillin-potassium clavulanate in ratio of 5:5, respectively. Figure 6(a) indicates that potassium clavulanate is amorphous. This visual data was confirmed with DSC and XRPD data, which showed that potassium clavulanate is less crystalline than amoxicillin trihydrate. Figure 6(c) shows the binary system (ground mixture) that formed amoxicillin-potassium clavulanate co-crystal where amoxicillin crystal was covered with layers of amorphous clavulanate. The XRPD beam was not diffracted by clavulanate because of its amorphous structure. Instead, it was diffracted by amoxicillin crystal itself.

Our data have shown that amoxicillin trihydrate and potassium clavulanate could interact physically and form a solid dispersion/co-crystal in all molar ratios. The interaction is due to formation of hydrogen bonding between potassium clavulanate and hydrates of amoxicillin. This phenomenon was similar with that described by Bettinetti (1999) about hydrogen bonding between trimetoprim-

sulfomethoxazole. Similarly, Umeda *et al*. (2007) reported the molecular complex formation between indomethacin and lidocaine by a non-covalent bonding. The implication of this physical interaction could alter the pharmaceutical character, such as dissolution profile, stability, pharmacokinetic profile (Goldberg, Gibaldi and Kanig 1966).

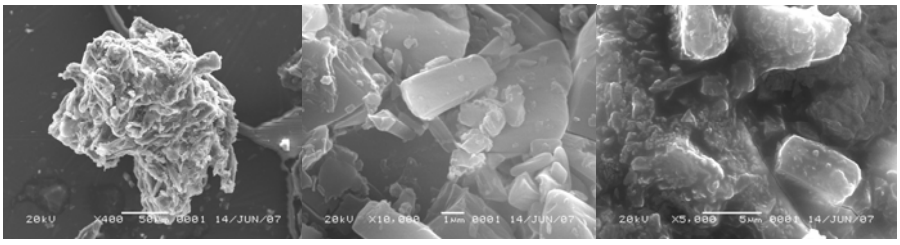


Fig. 6: SEM of potassium clavulanate (a); amoxicillin trihydrate (b); co-crystal (c).

CONCLUSION

Amoxicillin–clavulanate binary system has been shown to interact in the solid state and formed a co-crystal system at a molar ratio of 5:5. The interaction mechanism was related to the formation of hydrogen bonding between –NH and C=O with the hydrates of amoxicillin trihydrate.

ACKNOWLEDGEMENTS

The authors acknowledge the Directorate General of High Education of Indonesia for the research grant, and wish to thank PT Tempo Scan Pacific, Cikarang, Bekasi, Indonesia for the material and DSC analysis.

REFERENCES

BETTINETTI, G. (1999) Structure and Solid State a Chemistry of Anhydrous and Hydrated Crystal Form of Trimethoprim-Sulfamethoxypyridazine 1:1 Molecular Complex, *Journal of Pharmaceutical Sciences*, 89: 478–489.

BLANCO-FUENTE, H. B. (2004) Characterization of cyclodextrin-carbopol interactions by DSC and FTIR, *Journal of Thermal Analysis & Calorimetry*, 77: 403–411.

BRITTAIN, H. (1999) *Polymorphism in Pharmaceutical Solids*, pp. 227–278, pp. 331–361 (New York: Marcel Dekker).

BRITTAIN, H. (2001) *X-Ray Diffraction III: Pharmaceutical Applications of X-Ray Powder Diffraction, Spectroscopy*, 16(7), pp. 14–18, http://www.Spectroscopyonline.com. (25 April 2006)

CARTENSEN, J. T. (2001) *Advanced Pharmaceutical Solids*, pp. 169–189 (London: Taylor & Francis).

CHADHA, R., KASHID, N. & JAIN, D. V. S. (2003) Microcalorimetric studies to determine the enthalpy of solution of diclofenac sodium, paracetamol and their binary mixtures at 310.15 K, *Journal of Pharmaceutical and Biomedical Analysis*, 30: 1515–1522.

DRUGBANK (2006) *Amoxicillin and Potassium Clavulanate*, http://www.redpoll. pharmacy.ualberta.ca/drugbank. (25 April 2006).

EYJOLFSSON, R. (1998) Lisinopril-lactose incompability, *Drug Development and Industrial Pharmacy*, 24: 797–798.

FLORENCE, A. T. & SALOLE, E. G. (1994) *Formulation Factors in Adverse Reactions*, pp. 40–51 (London: Wright).

FREIFELD, A., MARCHIGIANI, D. & WALSH, T. (1999) A double-blind comparison of empirical oral and intravenous antibiotic therapy for low-risk febrile patients with neutropenia during cancer chemotherapy, *New England Journal of Medicine*, 341: 305–311.

GOLDBERG, A., GIBALDI, M. & KANIG, J. (1966) Increasing Dissolution Rates and Gastrointestinal Absorption of Drug via Solid Solution and Eutectic Mixtures II, Experimental Evaluation of Eutectic Mixtures Urea Acetaminophen System, *Journal of Pharmaceutical Sciences*, 55: 482.

JAIN, D. V. S., KASHID, N., KAPOOR, S. & CHADHA, R. (2000) Enthalpies of solution of ampicillin, amoxycillin and their binary mixtures at 310.15 K, *International Journal of Pharmaceutics*, 201: 1–6.

KERN, W. V., COMETTA, A., DE BOCK, R., LANGENAEKEN, J., PAESMANS, M. & GAYA, H. (1999) Oral versus Intravenous Empirical Antimicrobial Therapy for Fever in Patients with Granulocytopenia Who Are Receiving Cancer Chemotherapy, *New England Journal Medicinal*, 341: 312–318.

LIU, R. (2000) *Water-Insoluble Drug Formulation*, pp. 495—501, pp. 125–135 (London: Taylor & Francis).

NUGRAHANI, I., IBRAHIM, S., SOEWANDHI, S. N. & ASYARIE, S. (2007) Cold Contact Methods for Detected Amoxicillin-Clavulanate Interaction, ICCS Seminar, 27–29th May, Yogyakarta, Indonesia.

ROTH, V. H. J. & FENNER, H. (2000) *Arzneistoffe, Struktur – Bioreaktivitaat – Wikungsbezogene Eigenschaften*, p. 11, (Verlag Stuttgart: Deutscher Apotheker).

STORM, K. H, CONLEY, C. P. & ROUSH, J. A. (2003) Amoxicillin and potassium clavulanate dosage form, *United States Patent: 6.756.057*, http://www.patentstorm.us/patents/6756057, (25th February 2006).

STAHLY, G. P. (2007) Diversity in Single- and Multiple-Component Crystals. The Search for and Prevalence of Polymorphs and Cocrystals, *Crystal Growth & Design*, 7: 1007–1026.

UMEDA, Y., TOSHIRO, F., TAKAYUKI, F., TOYOFUMI, S., MIZUE, M. & KAZUO, T. (2007) Molecular Complex Consisting of Two Typical External Medicines: Intermolecular Interaction between Indometachin and Lidocaine, *Chemical and Pharmaceutical Bulletin*, 55: 832–836.

VREE, T. B., DAMMERS, E. & EXLER, P. S. (2003) Identical pattern of highly variable absorption of clavulanic acid from four different oral formulations of co-amoxiclav in healthy subjects, *Journal of Antimicrobial Chemotherapy*, 51: 373–378.

VEDNERE, K. M. (1990) Co-precipitates and Melts, IN: SWARBRICK, J. & BOYLE, J.C. (Eds.). *Encyclopedia in Pharmaceutical Technology 3*, pp. 337–350 (New York: Marcel Dekker).

WATSON, D. G. (2001) *Pharmaceutical Analysis*, p. 105 (London: Churchill Livingstone).