Effect of Solvent on the Complex between α-Mangostin and β-Cyclodextrin

Pichthida Jittamaro, Uracha Rangsardthong Ruktanonchai, Sarunya Phunpee, and Apinan Soottitantawat

***Abstract*—**The complex between α-mangostin and β-cyclodextrin can be formed in a little amount due to the α-mangostin is in solid state and its characteristic of soluble is low. So, this research fills up alcohol to enhance solubility of α-mangostin to be formed complex. The experiment used ethanol and methanol to be solvent. The condition has resident time at 48 hrs and 25°c. HPLC is used to find quantity of α-mangostin. The result shows the alcohols can make more free α-mangostin. In addition, the molecular size of alcohols affect to the complex. Ethanol can increase ternary complex while binary complex decreases. In the other hand, methanol which has smaller molecular size can make less amount of ternary complex and does not affect binary complex because the molecular size is not fit.

***Keywords*—** β-cyclodextrin, Inclusion complex, α-mangostin, ternary complex

I. Introduction

α-MANGOSTIN ($C_{24}H_{26}O_6$) is yellow substance that is extracted from xanthone found in pericarp, whole fruit, bark and leaves of *Garcinia mangostana Linn* [1]. Its molecular weight is 410.46 $gmol^{-1}$. There are a lot of researches about its benefits such as α-mangostin which is extracted by ethyl acetate, can inhibit *Propionibacterium* acnes that is dislike oxygen bacteria and growth in hair[2]. 20μmol α-mangostin which dissolves in DMSO can inhibit cancer cell [3]. Moreover, α-mangostin can inhibit *Vancomycin Resistant Enterococci* bacteria [4]. It demonstrates antioxidant, antitumoral, anti-inflammatory, antiallergy, antibacterial, antifungal and antiviral activities so it can be applied to pharmaceutical industries. Encapsulation is one of technology that enhances performance of drug. However, α-mangostin is not easy to change into complex form because it is solid state at room temperature and poor water soluble.

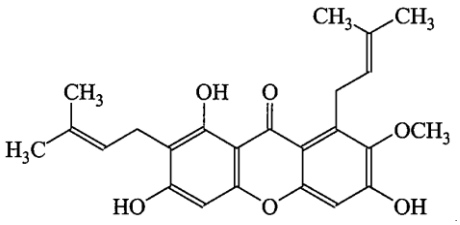

Fig. 1 α-Mangostin structure [5]

Cyclodextrin (CDs) is widely used in food and pharmaceutical industries. It is cyclic oligosaccharide of α-D-Glucose by moltodextrin glucanotransferase enzyme. It can be classified by number of glucose monomer. α-Cyclodextrin, β-cyclodextrin, γ-cyclodextrin include with glucose 6,7 and 8 units respectively. The structure of CDs has cavity that its property is hydrophobia and the outside surface is hydrophilic. Because of its characteristic, CDs can improve the solubility and bioavailability of insoluble compounds [6].

The complex is formed between host and guest molecules. Host molecule, called "ligand", is residence for guest molecule [7]. This research, β-cyclodextrin is host whereas α-mangostin is guest. The formation can be binary and ternary complex. The binary complex has only one kind of guest molecule in host molecule whereas ternary complex has more than one kind of guest molecule [8]. This research studies the complexation between α-mangostin and β-cyclodextrin but it is difficult to be formed. Solvent is one of interesting support for forming the complex.

II. Materials and Methods

A. Materials

α-Mangostin (α-mg) with an average molecular weight (Mw) of 410.46 $gmol^{-1}$ with purchased from GuanzhouHonsea Sunshine Bio Science & Technology Co., Ltd in China. β-cyclodextrin (β-CD) was obtained from Wacker Chemical AG (Germany). Methanol, ethanol were purchased from carlo Erba (Italy). Water used for all experiments was purified water obtained from a milliQ Plus (Millipore, Schwalbach, Germany).

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B. Binary inclusion of α-mg with β-CD

β-CD (5.0 g) was dissolved in DI water (100 mL). The solution of β-CD was heated up to 70°c and stirred at 200 rpm. Add α-mg 41.0 mg (20 mmol) in 12-ml vials. The β-CD solution was varied from 0-10 mmol in each vial. Then, the vials were sonicated for 15 minutes. After that they were shaken at 25°c 250 rpm for 48 hours.

C. Ternary inclusion of α-mg, solvent and β-CD

β-CD (5.0 g) was dissolved in DI water (100 mL). The solution of β-CD was heated up to 70°c and stirred at 200 rpm. Add α-mg 2.05 g in 150 ml of ethanol. The β-CD solution were varied from 0-10 mmol in each vial. The α-mg solution 2 ml (20 mmol of α-mg, 40%EtOH) was added to vials. Then, the vials were sonicated for 15 minutes. After that they were shaken at 25°c 250 rpm for 48 hours. The concentration of ethanol was varied from 10-40%. Then, all steps are repeated with methanol.

D. Characterization

The total concentration of α-mg was measured by HPLC. The C18 column (thermo scientific) (2.1x250 mm, 5 μm) is used in this research. Mobile phase is 1% (v/v) acetic acid and methanol in ratio 1:9. Flow rate is 1 ml/min. The sample rate is 10 μL. The analysis time is 12 minutes.

III. Methods

In this study, the mixture was filtered by 0.45 μm nylon filter membrane to separate insoluble α-mg from the solution. The concentration of inclusion was determined by Ping model, used to explain the forming of inclusion complex of insoluble drug [9], [10]. *D* is insoluble drug, *L* is ligand and *C* is solvent.

The result of HPLC shows the total concentration of drug that dissolves in solution as in (1).

$$[D_{tot}] = [D] + [DL] + [DLC]. \quad (1)$$

Where $[D_{tot}]$ is the total concentration of drug, $[D]$ is the free drug that is dissolve in solvent but it is not inclusion with ligand, $[DL]$ is the binary complex between drug and ligand, $[DLC]$ is the ternary complex that is combination of drug, ligand and solvent. However, we cannot find each term from HPLC but we use the equation to find the relationship of experimental data and calculate the constant variable for solving term of binary and ternary inclusion.

A. Determination of the α-mg concentration in solvent

The solubility of nonpolar solutes on solvent concentration is described in exponential form as in (2).

$$[D] = [D_u] \times 10^{\sigma[C]}. \quad (2)$$

This equation can reform into log-linear form as in (3).

$$\log[D] = \log[D_u] + \sigma[C]. \quad (3)$$

Where $[D]$ is the total soluble drug concentration, $[D_u]$ is the intrinsic drug solubility, $[C]$ is solvent concentration, σ is the solvent solubilizing power for the solute.

B. Determination of the α-mg concentration with β-CD in binary inclusion term [DL]

The concentration of binary complex [DL] between nonpolar solutes and ligand is described in (4).

$$[DL] = [D_u] + K_b[D_u][L]. \quad (4)$$

The equation 4 can reform into linear form through the origin point as in (5).

$$[DL] - [D_u] = K_b[D_u][L]. \quad (5)$$

Where $[DL]$ is the total soluble drug concentration, $[L]$ is ligand concentration, K_b is binary complexation constant.

C. Determination of complex of α-mg, solvent and β-CD in ternary inclusion term [DLC]

The concentration of ternary complex that has relationship with drug, ligand and solvent (ratio 1:1:1) can described from (1) into (6).

$$[D_{tot}] = [D_u]10^{\sigma[C]} + K_b[D_u][L] \times 10^{(\sigma-\rho_b)[C]} + K_t[D_u][L][C] \times 10^{(\sigma-\rho_t)[C]}. \quad (6)$$

Where K_b is binary complexation constant, ρ_b is solvent destabilizing power for the binary complexation and ρ_t is solvent destabilizing power for the ternary complexation. This equation reforms into linear form through the origin point as shown in (7).

$$[D_{tot}] - [D_u] \times 10^{\sigma[C]} = \left(K_b[D_u] \times 10^{(\sigma-\rho_b)[C]} + K_t[D_u][C] \times 10^{(\sigma-\rho_t)[C]}\right)[L]. \quad (7)$$

This equation is used to find ρ_b, ρ_t and K_t by solver with plotting graph from the HPLC result for calculating term of binary and ternary complex.

IV. Results and Discussion

A. Solubilization of α-mg in solvent

From the experimental data, the graph shows that the α-mg solubility increases linearly with β-CD concentration. Similarly to the ethanol concentration, it effects on solubility of α-mg as shown in figure 2 (a). However from figure 2 (b), the 40%EtOH is obviously high over 20%EtOH due to the ternary complexation.

The characteristic of methanol solution is similar to ethanol solution but the 40%MeOH does not form the ternary complex. So, the graph trend changes to be sequence of 10%, 20% and 40% as shown in figure 2 (c).

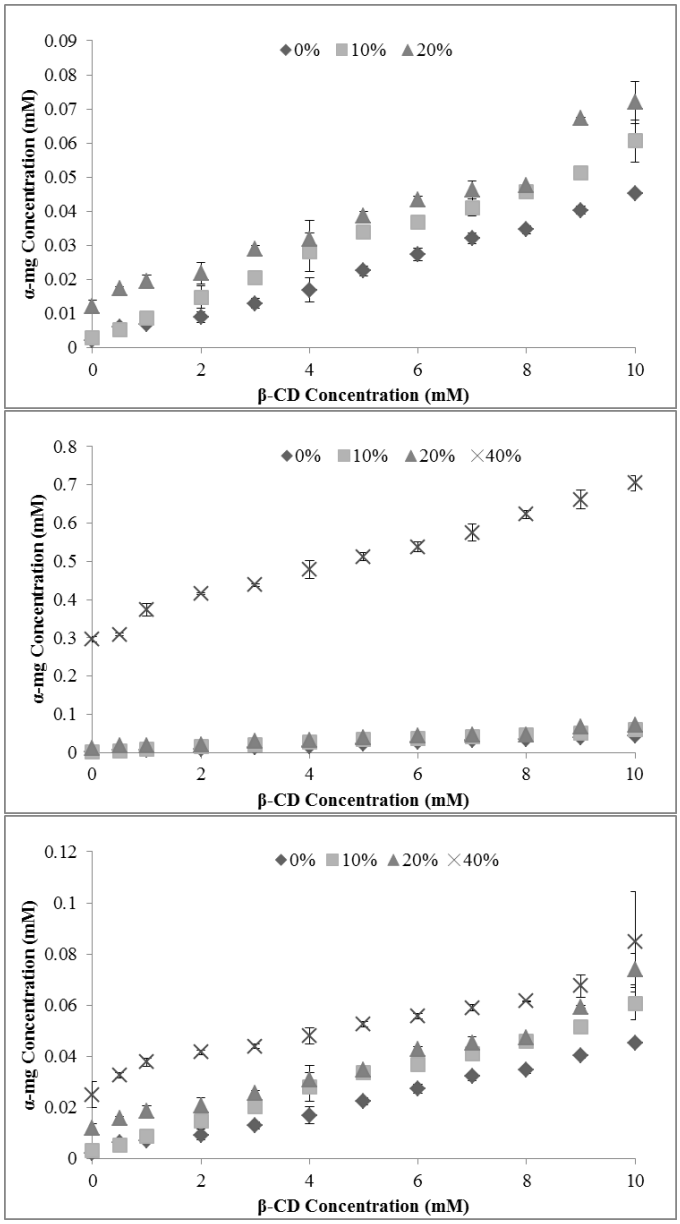


Fig. 2 Effect of solvent concentration on the α-mg solubility
(a) ethanol concentration 0-20%
(b) ethanol concentration 0-40%
(c) methanol concentration 0-40%

The intrinsic solubility of α-mg in water $[D_u]$ was determined to be 2.14e-6 mM. Figure 3 (a) shows the solvent solubilizing power (σ) in ethanol is 0.2837 M^{-1}. Figure 3 (b) shows the solvent solubilizing power (σ) in methanol is 0.164 M^{-1}.

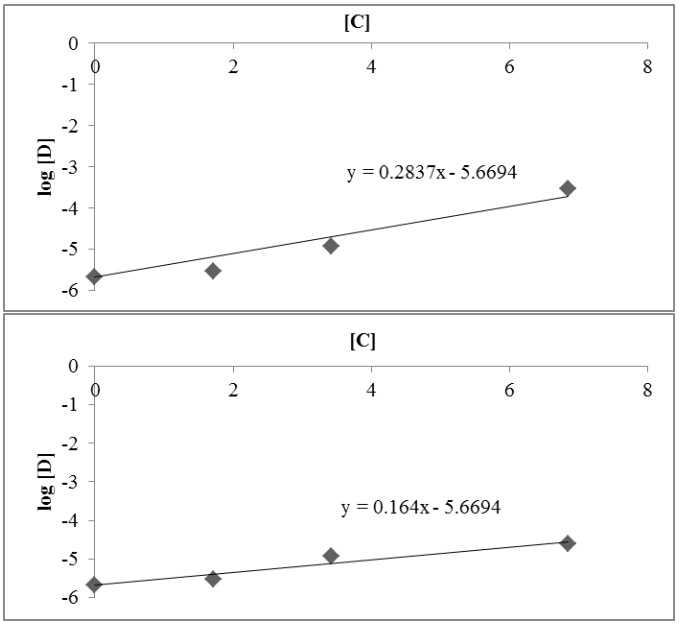


Fig. 3 Logarithm of α-mg solubility with solvent concentration
(a) ethanol (b) methanol

The experimental data in Figure 2 were used to solve K_b in (5) and ρ_b, ρ_t and K_t in (7). All parameter is listed into Table 1.

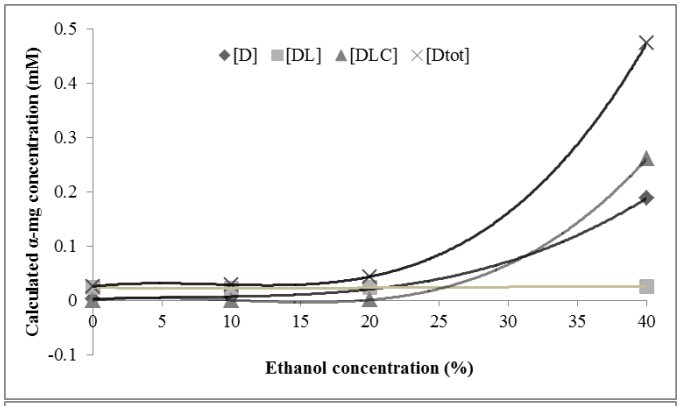
TABLE I
PARAMETERS OF THE SOLVENT SOLUTION

Symbol	Ethanol	Methanol
σ (M^{-1})	0.2837	0.164
K_b (M^{-1})	1961.78	1961.78
K_t (M^{-2})	43.207	0.00015
ρ_b (M^{-1})	0.3569	0.156
ρ_t (M^{-1})	0	1.614e-6

B. Determination of free α-mg, binary complex and ternary complex

The concentration of free α-mg, binary complex and ternary complex were plotted in Figure 4A and Figure 4B with β-CD concentration 5 mM. Figure 4A shows free α-mg increases together with ethanol concentration because α-mg dissolves in ethanol solvent. The binary complex slightly decreases while ternary complex increases upon ethanol concentration.

Figure 4 (b) shows free α-mg rises upon methanol concentration because α-mg is more solubility in methanol solvent. The binary complex changes in a little variation. In the other hand there is no ternary complex because of methanol molecular size is not fit the cavity of β-CD.



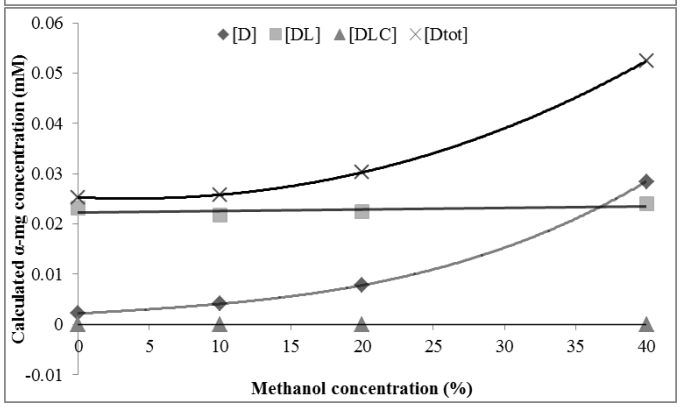
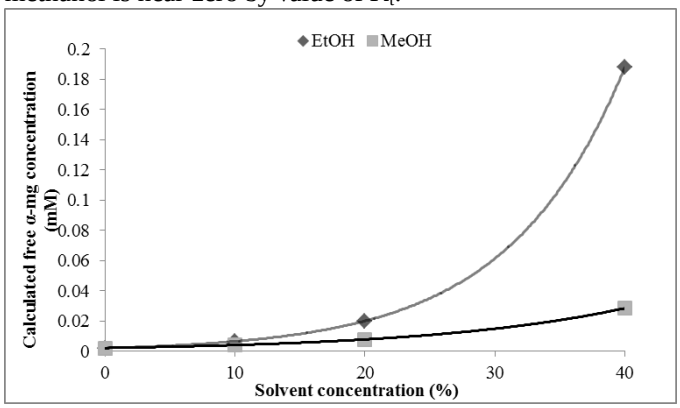
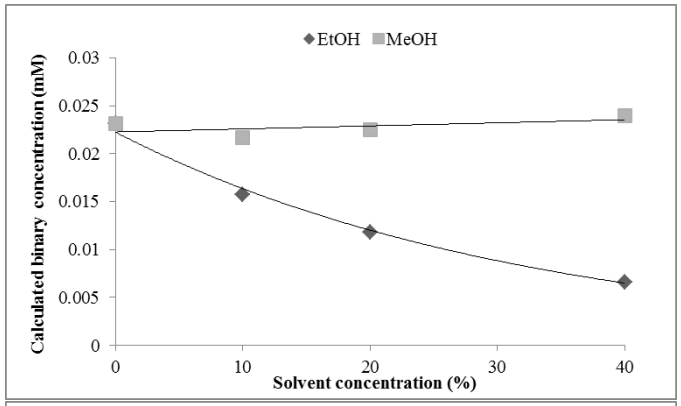


Fig. 4 Calculated α-mg solubility in 5 mM β-CD in different solvent concentration
(a) ethanol (b) methanol

Figure 5 (a) shows free α-mg with ethanol is more than free α-mg in methanol because σ of ethanol is higher than σ of methanol. The binary complex in ethanol significantly decreases due to $\rho_b>\sigma$ whereas methanol slightly increases because the difference of σ and ρ_b is small amount as shown in figure 5 (b). Figure 5 (c) shows ternary complex in ethanol rises with exponential trend whereas ternary complex in methanol is near zero by value of K_t.





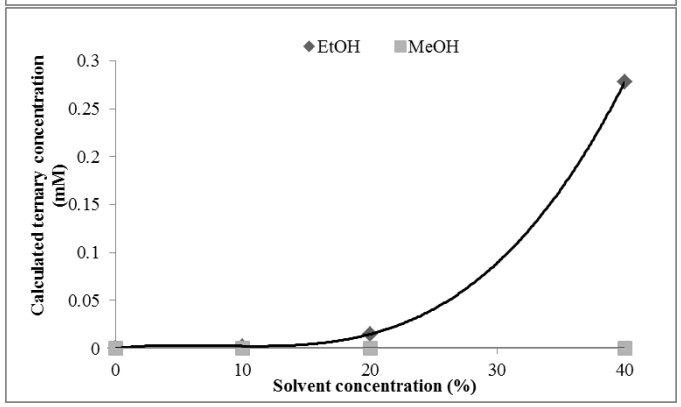


Fig. 5 Calculated α-mg solubility in 5 mM β-CD in different solvent concentration
(a) free α-mg (b) binary complex (c) ternary complex

V. Conclusion

This research finds out relationship of complex between a-mg, b-cd and alcohol solutions which can be described experimental data in term of mathematic. The free a-mg increases in exponential upon alcohol concentration. The ethanol solution can create more free a-mg than methanol solution which can be described in term of $10^{\sigma[C]}$. So, σ of ethanol is higher than methanol. For the binary complex between a-mg and b-cd solution, the result shows the more b-cd concentration, the more increasing of binary complex. The binary complex is not high because a-mg has low solubility in water. When adding ethanol, the binary complex will decrease which can be noticed in $\rho_b>\sigma$. Meanwhile, ternary complex will exponentially increase which can be described in $\rho_t<\sigma$. In the other hand, adding methanol does not affect to binary complex and it does not make much effect on ternary complex which can be seen in low value of K_t.

References

[1] L. M. R.-F. Jose Pedraza-Chaverri, Eva Guadalupe Nolasco-Amaya, Marisol Orazco-Ibarra, Omar Noel Medina-campos, Octavio Gonzalez-Cuahutencos, Isabel Riveri-Cruz, Rachel Mato, "ROS Scavenging Capacity and Neuroprotective Effect of α-Mangostin against 3-nitropropionic acid in Celebellar Granule Neurons," *Experimental and Toxicologic Pathology*, vol.61, pp. 491-501, 2009. http://dx.doi.org/10.1016/j.etp.2008.11.002

[2] Khanittha Udomsubpong, "Effect of Mangosteen Extract on *Propionibactirium* acnes,"*Natural Beauty and Health through Aesthetic Sciences (1st Decade of Aesthetic Sciences and Health And 50th Anniversary of KKU),* vol.1, pp. 245–249.

[3] Kenji Matsumoto, Yukihiro Akao, Emi Kobayashi, Kenji Ohguchi, Tetsuro Ito, Toshiyuki Tanaka, Munekazu Iinuma, Yoshinori Nozawa, "Induction of Apoptosis by Xanthones from Mangosteen in Human Leukemia Cell Lines," *J. Nat. Prod*, vol.66, pp. 1124–1127, 2003. http://dx.doi.org/10.1021/np020546u

[4] Yukihiro Akao, Nakagawa Y., Iinuma M., Nozawa T., "Anti-cancer effects of Xanthones from Pericarps of Mangosteen," *Int. J. Mol. Sci.*, Mar9(3): pp. 355-70, Mar. 2008.

[5] A. A. M. M. Y. Ibrahim, S. Mohan, N. M. Hashim, M.A. Abdulla, S.I. Abdelwahab, I.A. Arhab, L.Z. Ali, "α-Mangostin from Garcinia mangostana Linn: an updated review of its pharmaceutical properties," *Arabian journal of Chemistry*, 2014. http://dx.doi.org/10.1016/j.arabjc.2014.02.011

[6] J. B. Catherine L. Lawson, Peter M. Bruinenberg, Gert de Veries, Lubbert Dijkhuizen, Banke W. Dijkstra 1, "Maltodextrin-Dependent Crystallization of Cyclomaltodextrin Glucanotranferase from *Bacillus circulans*," *Journal of Molecular Biology*, vol.214, pp. 807-809, 1990. http://dx.doi.org/10.1016/0022-2836(90)90335-J

[7] E. M. M. D. Valle, "Cyclodextrins and Their Uses: a review," Process *Biochemistry*, vol.39, pp. 1009-1046, 2004.

[8] K. Takahashi, "Organic Reactions Mediated by Cyclodextrins," *Chem. Rev*., vol. 88, pp. 2013–2033, 1998. http://dx.doi.org/10.1021/cr9700235

[9] L. Z. A. S. H. Y. Ping Li, "Combined Effect of Cosolvent and Cyclodextrin on Solubilization of Nonpolar Drugs," *Journal of Pharmaceutical Sciences*. Vol.88, pp. 1107-1111, 1999. http://dx.doi.org/10.1021/js990159d

[10] P. L. Yan He, Samuel H. Yalkowsky, "Solubility of Fluasterone in Cosolvent/Cyclodextrin Combinations," *Internation Journal of Pharmaceutics*, vol.264, pp. 25–34, 2003. http://dx.doi.org/10.1016/S0378-5173(03)00389-2