Effect of Temperature on Cyclodextrin Production and Characterization of Paracetamol/Cyclodextrin Complexes

Jarunee Kaulpiboon PhD*,
Prakarn Rudeekulthamrong PhD**

** Department of Pre-Clinical Science (Biochemistry), Faculty of Medicine, Thammasat University, Pathumthanee, Thailand*
*** Department of Biochemistry, Phramongkutklao College of Medicine, Bangkok, Thailand*

Objective: *To compare the effect of different reaction temperatures on the cyclization and coupling reactions of the Toruzyme® CGTase influencing the yield of cyclodextrins (CDs) and to study the solubility of paracetamol with CDs.*
Material and Method: *Type and amount of CDs were analyzed by HPAEC-PAD. The stability constants for the inclusion complex formed between CDs and paracetamol were determined using the phase solubility method. The solubility of paracetamol with CDs was measured by UV-spectrophotometer at 240 nm.*
Results: *The result has shown that the reaction temperature has effect on the Toruzyme® CGTase reactions in production of CDs. The CDs yield after 30 min of incubation was higher at 60°C than at 80°C. The catalytic efficiency (k_{cat}/K_m) of this enzyme indicated the higher value of the cyclization reaction at 60°C compared to 80°C while the opposite was found for the coupling reaction. Paracetamol is used as an analgesic and antipyretic but it is poorly water-soluble drug. To improve the solubility of paracetamol, CDs obtained were used to study for paracetamol/CDs complexes. The phase-solubility diagrams of paracetamol with α-, β- and γ-CD were A_N type while that of paracetamol with maltosyl-β-CD (G_2-β-CD) complex was A_L type. The stability constants (K_c) for the inclusion complex of paracetamol with α-, β-, γ-CD and G_2-β-CD were 5.69, 16.75, 4.73 and 2,223.25 M^{-1}, respectively.*
Conclusion: *The optimum temperature for CDs production was at 60°C and the low solubility of paracetamol was significantly improved by complexation with CDs, where the enhancing effect was in the order of G_2-β-CD > β-CD > α-CD > γ-CD.*

Keyword: *Cyclodextrin, G_2-β-CD, Paracetamol, Phase solubility, Stability constant*

J Med Assoc Thai 2010; 93 (Suppl. 6): S160-S165
Full text. e-Journal: *http://www.mat.or.th/journal*

Cyclodextrin glycosyltransferase (CGTase, EC 2.4.1.19) is an enzyme involved in the conversion of starch to cyclodextrins (CDs). The enzyme is a member of the α-amylase family of glycosyl hydrolases (family 13)[1]. CGTase performs three transglycosylation reactions and a hydrolysis reaction. CDs (Cyclic α-1,4-glucans) are formed by an intramolecular transglycosylation reaction where the terminal 4-OH group of the intermediate acts as an acceptor (cyclization reaction). The reverse reaction also occurs where the CD ring is opened and the linear oligosaccharide is transferred to a linear glucan acceptor (coupling reaction). Linear α-1,4-glucan products are also formed in the third reaction, an inter-molecular transglycosylation where a linear oligosaccharide is transferred to another linear glucan acceptor (disproportionation reaction). In a hydrolysis reaction, a glucan is cleaved and the reducing end is transferred to water. The amounts and size distribution of CD formed by CGTase are strongly influenced by the combined effects of the three transglycosylation reactions, as well as by the hydrolytic activity of the enzyme.

The CDs are naturally produced by various CGTases and were found mainly to consist of six, seven, or eight glucose units, named α-, β-, and γ-CD (CD_6, CD_7, and CD_8), respectively. In addition to the native forms of CDs, there are still many types of CD

Correspondence to:
Rudeekulthamrong P, Department of Biochemistry, Phramongkutklao College of Medicine, Bangkok 10400, Thailand.
Phone: 0-2354-7797
E-mail: prakarn_r@hotmail.com

derivatives such as methyl-β-CD (M-β-CD) and hydroxypropyl-β-CD (HP-β-CD) that have been synthesized by chemical methods[2-4]. The molecular structures of CD resemble a hollow, truncated cone with a hydrophilic outside and hydrophobic inside[3]. Due to this structure, CD can form inclusion complexes with guest molecules. It is widely applied in many food and pharmaceutical industries. CDs have been extensively used for improving solubility[4-6], stability[7,8], and bioavailability[9,10] of drugs.

In this study, we compared the effect of different reaction temperatures on the cyclization and coupling reactions of the *Toruzyme®* CGTase from *Thermoanaerobacter* sp. in CDs production. Moreover, inclusion complexes of paracetamol with α-, β-, γ- and G_2-β-CD for improving the solubility of paracetamol were also investigated.

Material and Method

Materials and enzymes

The mixture of α-, β-, γ-CDs was synthesized by *Toruzyme®* CGTase, and separated and purified by Mokhtar MN[11]. G_2-β-CD (CD derivative), potato starch (molecular mass 296 kDa), BSA, methyl-α-D-glucose (MaDG), were purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany). Pea starch (degree of polymerization = 4000) was kindly provided by Emsland-Starke GmbH (Emlichheim, Germany). *Toruzyme®* CGTase 3.0 L was obtained from Novo (Nordisk A/S Bagsvaerd, Denmark).

CGTase assays and protein determination

Cyclization activity was determined as CD-forming activity by the phenolphthalein method[12]. CGTase (0.5 mg) was added to 0.6 ml of 1.5% (w/v) pea starch in 0.2 M potassium phosphate buffer (pH 6.0) containing 50% (v/v) DMSO. The reaction mixture was incubated for 30 min at various temperatures. The reaction was stopped by boiling for 10 min. An aliquot (0.5 ml) was incubated with 2.0 ml of a solution containing 1.0 ml of 4 mM phenolphthalein in ethanol, 4 ml of ethanol and 100 ml of 125 mM Na_2CO_3 in distilled water. The absorption was measured at 550 nm, and the amount of β-CD formed was calculated using a calibration curve. One unit of activity was defined as the amount of enzyme that produced 1 mmol of β-CD per min.

The coupling activity was determined by incubating β-CD as donor with 60 mM MαDG as glucosyl acceptor at various temperatures. The 50 mM potassium phosphate buffer, (pH 6.0) was added to obtain a total volume of 0.5 ml. The β-CD and MαDG were preincubated for 5 min at each temperature. The reaction was started by adding enzyme (0.5 mg). After 5 min, the reaction was stopped by boiling for 5 min. Subsequently, *Rhizopus* sp. glucoamylase (0.385 U) was added to convert linearized oligosaccharides to glucose at 40°C for 30 min. The released reducing sugars were determined with the dinitrosalicylic acid method[13]. One unit of activity was defined as the amount of enzyme that produced 1 mmol glucose per min.

All kinetic experiments were carried out at 60 and 80°C in potassium phosphate buffer pH 6.0. Lineweaver-Burk diagrams of the initial velocity against substrate concentration were plotted, and kinetic parameters were determined using enzfitter software (Biosoft, Cambridge, UK). Reaction time of 30 min for cyclization reaction and 5 min for coupling reaction was used in the Lineweaver-Burk experiments. By varying the reaction time with fixed substrate concentration, it was confirmed that the reaction velocity was linear at this time point. The protein concentrations were determined according to Bradford[14], using BSA as standard.

Synthesis of cyclodextrins

The CD synthesis reaction was performed using 2.5 ml of 1.5% (w/v) pea starch and 2 U/ml of CGTase in 0.2 M potassium phosphate buffer, pH 6.0. The incubation time was 30 min at 60°C and 80°C. The reaction was terminated by boiling the mixture for 10 min. Glucoamylase (3.85 U/ml) was added and the mixture was incubated for 24 h to convert the linear oligosaccharides to glucose. The CD product obtained was determined by HPAEC.

Analysis of cyclodextrins

High performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was carried out using a DX-600 system (Dionex Crop, Sunnydale, USA) to analyze and quantify the CD products. A Carbopac PA-100 analytical column (4 x 250 mm, Dionex Crop, Sunnydale, USA) was used. A sample (25 ml) was injected and eluted with a linear gradient of sodium nitrate (0-10 min, increasing from 0% to 4%; 10-12 min, 4%; 12-32 min, increasing from 4% to 8%; 32-48 min, increasing from 8% to 9%; 48-59 min, increasing from 9% to 18%; 59-79 min, increasing from 18% to 28%) in 150 mM NaOH containing 2% acetonitrile with a flow rate of 1 ml/min. The amounts of α-, β-, γ-CD were quantified by comparison with standard curves of authentic α-, β-, γ-CD samples.

Phase solubility study

The stability constant for the inclusion complex formed between various types of CD and paracetamol was determined using the phase solubility method which was carried out according to Higuchi and Conners[15]. Briefly, 20 mg of paracetamol was added to 1 ml solutions of CD, varying concentrations from 0-60 mM in distilled water and then shaken at 250 rpm for 1 h at 25°C. After that, the solutions were filtered through 0.45 μm membrane filter. The filtered solution was analyzed by UV spectrophotometer (Shimadzu Double Beam Spectrophotometer 1700) at 240 nm for concentration of paracetamol.

Results

Effect of different reaction temperatures on the cyclization and coupling activities of the Toruzyme® CGTase

To investigate the effect of the reaction temperature on CD production, the optimum temperature for the cyclization and coupling activities was determined. The *Toruzyme®* CGTase showed the optimum temperature for the cyclization and coupling reaction at 60°C and 80°C, respectively (Fig. 1). CD products obtained from using pea starch as substrate catalyzed by *Toruzyme®* CGTase at 60°C and 80°C were also confirmed by HPAEC (Fig. 2). The result showed clearly that the yield of CDs at 60°C was higher than at 80°C.

Effect of different temperatures on kinetic parameters of Toruzyme® CGTase

Kinetic parameters, Michaelis-Menten constant (K_m), turnover rate (k_{cat}) and catalytic efficiency (k_{cat}/K_m) for the cyclization and coupling reactions of *Toruzyme®* CGTase were determined and shown in Table 1. The affinity constants (K_m) of cyclization reaction at 60°C and 80°C were 0.36 and

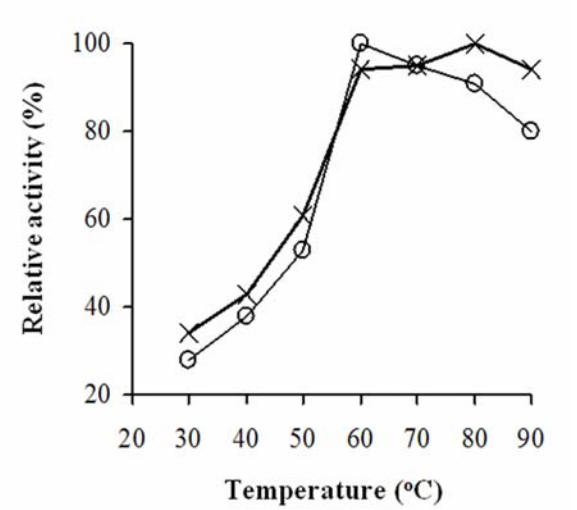

Fig. 1 Comparison of the cyclization (o) and coupling (x) activities of the *Toruzyme®* CGTase at different temperatures.

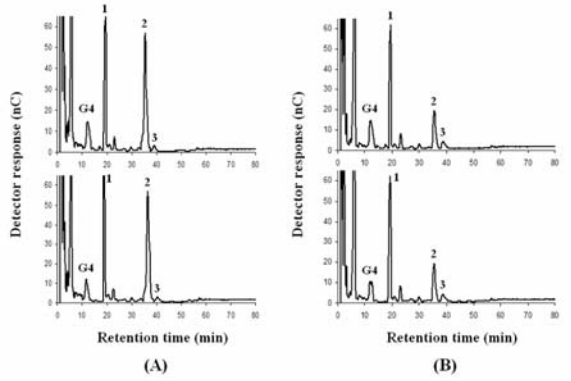

Fig. 2 Duplicate HPAEC analysis of cyclodextrins formed by *Toruzyme®* CGTase at 60°C (A) and 80°C (B) for 30 min. 1.5% Pea starch was incubated with 2 U CGTase. The number 1, 2 and 3 listed on each peak indicates as α-, β-, γ-CD, respectively. G4 indicates a glucose tetrasaccharide (0.1 mg/ml) added as an internal standard.

Table 1. Kinetic parameters of the β-CD cyclization reaction and the coupling reaction of β-CD with MaDG catalyzed by *Toruzyme®* CGTase from *Thermoanaerobacter* sp. at 60 and 80°C

CGTase reaction	60°C			80°C		
	K_m (mg/ml)	k_{cat} (s^{-1})	k_{cat}/K_m $(s^{-1})(mg/ml)^{-1}$	K_m (mg/ml)	k_{cat} (s^{-1})	k_{cat}/K_m $(s^{-1})(mg/ml)^{-1}$
Cyclization	0.36 ± 0.04	62.14 ± 0.09	1.73×10^2	0.41 ± 0.02	31.70 ± 0.10	7.73×10^1
Coupling	1.81 ± 0.11	337.38 ± 10.93	1.86×10^2	1.54 ± 0.09	427.35 ± 11.90	2.78×10^2

0.41 mg starch/ml, respectively while those of the coupling reaction were 1.81 and 1.54 mg β-CD/ml for at 60°C and 80°C. The *Toruzyme*® CGTase showed the k_{cat}/K_m for the cyclization reaction was higher at 60°C than at 80°C; in contrast the k_{cat}/K_m for the coupling reaction was higher at 80°C than at 60°C.

Phase solubility study

The phase solubility diagrams for the inclusion complex between various types of CD and paracetamol were presented in Fig. 3. The plot showed the solubility of the drug increased linearly as a function of G_2-β-CD concentration. It is clearly observed that the solubility diagram of paracetamol and G_2-β-CD can be classified as the A_L type[15]. For α-, β-, γ-CD, the solubility diagrams of paracetamol revealed hyperbolic curve which was classified as the A_N type.

Discussion

The optimum temperature for the cyclization and coupling activity of the *Toruzyme*® CGTase was determined at 60°C and 80°C, respectively. The result showed clearly that the yield of CDs at 60°C was higher than at 80°C by HPAEC. These data are useful for choosing the operating temperature for CD synthesis by avoiding the temperature at which CD could be highly degraded in a coupling reaction[16,17]. In addition, we also found that the optimum temperature for the cyclization and coupling activity depended on the type of microorganism. Qi et al[17] reported that *Bacillus macerans* CGTase showed optimum temperature for the cyclization and coupling reactions at 60°C and 50°C, respectively. In contrast, CGTase from *Paenibacillus* sp. A11 showed optimum temperature for the cyclization and coupling reactions at 40°C and 60°C[18]. Similar to *Paenibacillus* sp. A11, the optimum temperature for the coupling reaction of *Toruzyme*® was at higher temperature than the cyclization reaction.

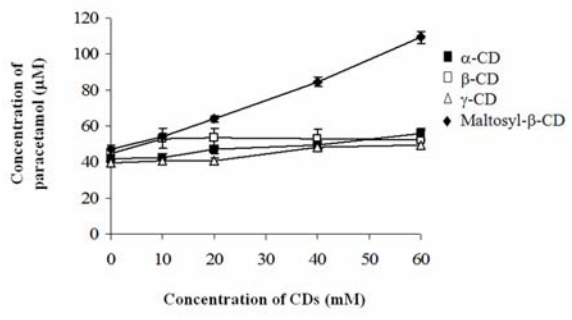

Fig. 3 Phase solubility diagrams of paracetamol with various types of CD. Data are shown as the mean ± SD (n = 3)

In Table 1, the *Toruzyme*® CGTase shows an increase of k_{cat}/K_m value for the cyclization reaction at 60°C higher than that at 80°C. For coupling reaction, a higher k_{cat}/K_m value was obtained with a reaction temperature of 80°C compared to 60°C. With the K_m values, the *Toruzyme*® CGTase indicated the stronger binding on starch to form β-CD for the cyclization reaction at 60°C with K_m value of 0.36 mg starch/ml compared to K_m value of 0.41 mg/ml at 80°C. The coupling reaction also indicated that the stronger binding on β-CD to degrade to linear-maltooligosaccaride was found at 80°C with K_m value of 1.54 mg β-CD/ml compared to K_m value at 60°C. However, the changes of K_m, k_{cat} and k_{cat}/K_m values are probably caused by several factors[19]. For this work, kinetic parameter values changed had directly effect from choosing the optimum temperature for β-CD formation. The easier accessibility of substrate to the active site of the β-CD-*Toruzyme*® CGTase at optimum temperature could cause changes in the binding affinity and turnover rate of the enzyme in the better way.

Fig. 3 shows the phase solubility diagrams of paracetamol with CDs in water at 25°C. The solubility diagrams of paracetamol with α-, β-, γ-CD showed A_N type where the solubility of the drug increases as hyperbolic curve. For paracetamol with G_2-β-CD, the diagram showed A_L type curve where the solubility of the drug increases linearly with CD concentration. The solubilizing ability of paracetamol increased in the order of G_2-β-CD > β-CD > α-CD > γ-CD with K_c of 2,223.25, 16.75, 5.69 and 4.73 and M^{-1}, respectively. Using CD derivatives such as HP-β-CD in drug application was firstly reported by Abe et al[20]. HP-β-CD could enhance the aqueous solubility of lipophilic penetration enhancers and oleic acid, and improved the stability of oleic acid against the oxidative degradation through the inclusion complexation. Nevertheless, this complexation is particularly useful for the design of aqueous nasal formulations of peptide and protein drugs. In the year 2007, Talegaonkar et al[21] found that the solubility of paracetamol was increased 6-folds (K_c = 416.4 M^{-1}) of normal solubility by complexation with HP-β-CD while complexation of paracetamol with G_2-β-CD was studied firstly in this report. For other CDs, Reddy et al[22] found that the aqueous solubility and dissolution rate of celecoxib could be increased by inclusion complexation with β-CD. These results suggest that CDs and their derivatives can be used as drug carrier for enhancing bioavailability of drugs. In the clinical study of paracetamol, CDs can be used

instead of organic solvents: methanol, ethanol, dimethylformamide, ethylene dichloride, acetone, ethyl acetate for drug solubility. This advantage of CDs helps to decrease risks on liver and target organs damage from organic solvent [23].

Conclusion

In conclusion, the β-CD forming cyclization reaction showed the highest rate at 60°C while the coupling reaction resulting in the CD degradation was found the highest rate at 80°C. Therefore, the optimum temperature of incubation at 60°C should be chosen to obtain the maximum CDs yield using in process of paracetamol/CD complexation. With drug application, phase solubility profiles indicated that the solubility of paracetamol was increased in the presence of CDs and derivative. In this study, G_2-β-CD was the most suitable for the drug-CD inclusion complex.

Acknowledgement

The authors are thankful to Prof. Dr. Zimmermann W and Dr. Mokhtar MN, Leipzig University, Germany for providing CDs. This work was partially supported by the research fellowship of the Alexander von Humboldt Foundation, Germany.

References

1. Henrissat B, Davies G. Structural and sequence-based classification of glycoside hydrolases. Curr Opin Struct Biol 1997; 7: 637-44.
2. Bowen RL, Schumacher GE, Carey CM, Giuseppetti AA, Flynn KM, Guttman CM. Synthesis of polymerizable cyclodextrin derivatives for use in adhesion-promoting monomer formulations. J Res Natl Inst Stand Technol 2009; 114: 1-9.
3. Lindner K, Saenger W. Topography of cyclodextrin inclusion complexes. XVI. Cyclic system of hydrogen bonds: structure of α-cyclodextrin hexahydrate, form (II): comparison with form (I) Acta Cryst 1982; 38: 203-10.
4. Veiga H, Teixeira-Dias JJC, Kedzierewicz F, Sousa A, Mamcent P. Inclusion complexation of tolbutamide with β-cyclodextrin and hydroxypropyl-β-cyclodextrin. Int J Pharm 1996; 129: 63-71.
5. Becket G, Schep LJ, Tan MY. Improvement of the *in vitro* dissolution of praziquantal by complextion with α-, β- and γ-cyclodextrins. Int J Pharm 1999; 179: 65-71.
6. Cappello B, Carmingnani C, Iervolino M, Immacolata M, Rotonda L, Saettone MF. Solubilization of tropicamide by hydroxypropyl-β-cyclodextrin and water soluble polymers: *in vitro/in vivo* studies. Int J Pharm 2001; 213: 75-81.
7. Lin HS, Chean CS, Ng YY, Chan SY, Ho PC. 2-Hydroxypropyl-beta-cyclodextrin increases aqueous solubility and photostability of all-trans-retinoic acid. J Clin Pharm Ther 2000; 25: 265-9.
8. Liu X, Lin HS, Thenmozhiyal JC, Chan SY and Ho PC. Inclusion of acitretin into cyclodextrins: phase solubility, photostability study and physicochemical characterization. J Pharm Sci 2003; 92: 2449-57.
9. Wong JW, Yuen KH. Improved bioavilability of artemisnin through inclusion complexation with β- and γ- cyclodextrins. Int J Pharm 2001; 227: 177-85.
10. Liu X, Lin HS, Chan SY, Ho PC. Biopharmaceutics of β-cyclodextrin derivative-based formulations of acitretin in sprague-dawley rats. J Pharm Sci 2004; 93: 805-15.
11. Mokhtar MN, Lauckner KU, Zimmermann W. Process for the preparation of cyclodextrins. European Patent Application EP 09153819.9; 2009.
12. Goel A, Nene SN. Modifications in the phenolphthalein method for spectrophotometric estimation of beta-cyclodextrin. Starch D/Starke 1995; 47: 399-400.
13. Bernfeld P. Amylases α and β. Methods Enzymol 1955; 1: 149-50.
14. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-54.
15. Higuchi T, Connors KA. Phase solubility techniques. In: Reilley CN, editor. Advances in analytical chemistry and instrumentation. Vol. 4. New York: Interscience; 1965: 117-212.
16. Van der Veen BA, van Alebeek GJ, Uitdehaag JC, Dijkstra BW, Dijkhuizen L. The three transglycosylation reactions catalyzed by cyclodextrin glycosyltransferase from *Bacillus circulans* (strain 251) proceed via different kinetic mechanisms. Eur J Biochem 2000; 267: 658-65.
17. Qi Q, She X, Endo T, Zimmermann W. Effect of the reaction temperature on the transglycosylation reactions catalyzed by the cyclodextrin glucanotransferase from *Bacillus macerans* for the synthesis of large-ring cyclodextrins. Tetrahedron 2004; 60: 799-806.
18. Kaulpiboon J, Pongsawasdi P. Zimmerman W. Altered product specificity of a cyclodextrin glycosyltransferase by molecular imprinting with cyclomaltododecaose. J Mol Recognit 2010 Jan

29. [PMID: 20119970].
19. Hu J, Li S, Liu B. Properties of immobilized pepsin on modified PMMA microspheres. Biotechnol J 2006; 1: 75-9.
20. Abe K, Sato H, Irie T, Uekama K, Kuriki T, Manako T, Saita M. Use of hydroxypropyl- β-cyclodextrin in nasal peptide formulations involving lipophilic penetration enhancers. In: The 7th international cyclodextrin symposium, Tokyo, Japan, April 25-28, 1994.
21. Talegaonkar S, Khan YK, Khar RK, Ahmad FJ, Khan ZI. Development and characterization of paracetamol complexes with hydroxypropyl-β-cyclodextrin. IJPR 2007; 6: 95-99.
22. Reddy MN, Rehana T, Ramakrishna S, Chowdary KP, Diwan PV. β-Cyclodextrin complexes of celecoxib: molecular-modeling, characterization, and dissolution studies. AAPS Pharm Sci 2004; 6: E7.
23. International Programme on Chemical Safety. Poisons Information Monograph: Paracetamol (PIM 396) [database on the Internet]. 1998 [cited 2009 Apr 7]. Available from: http://www.lhl.uab.edu: 17292/pages/pims.html

การศึกษาผลของอุณหภูมิต่อการผลิตไซโคลเดกซ์ทริน และลักษณะสมบัติของสารประกอบเชิงซ้อนระหว่างพาราเซตามอลกับไซโคลเดกซ์ทริน

จารุณี ควรพิบูลย์, ประกานต์ ฤดีกุลธำรง

วัตถุประสงค์*: เพื่อเปรียบเทียบผลกระทบของอุณหภูมิต่อปฏิกิริยาการสร้าง และการสลายไซโคลเดกซ์ทรินโดยเอนไซม์ Toruzyme® CGTase และเพื่อปรับปรุงความสามารถในการละลายน้ำของพาราเซตามอลด้วยไซโคลเดกซ์ทริน*
วัสดุและวิธีการ*: วิเคราะห์ชนิด และปริมาณของไซโคลเดกซ์ทรินโดยเครื่อง HPAEC-PAD และคำนวณค่าคงที่ของสารประกอบเชิงซ้อนที่เกิดขึ้นระหว่างพาราเซตามอลกับไซโคลเดกซ์ทรินชนิดต่าง ๆ โดยวิธี phase solubility สำหรับค่าการละลายน้ำของพาราเซตามอลวัดที่ความยาวคลื่น 240 นาโนเมตร โดยเครื่องสเปกโตรโฟโตมิเตอร์*
ผลการศึกษา*: พบว่าอุณหภูมิในการทำปฏิกิริยามีผลต่อการทำงานของ เอนไซม์ Toruzyme® CGTase ในการผลิตไซโคลเดกซ์ทรินชนิดต่าง ๆ โดยปริมาณของไซโคลเดกซ์ทรินที่ผลิตที่อุณหภูมิ 60 องศาเซลเซียส มีปริมาณมากกว่าการผลิตที่อุณหภูมิ 80 องศาเซลเซียส และค่าสัมประสิทธ์ในการเร่งปฏิกิริยาของเอนไซม์แสดงให้เห็นว่าปฏิกิริยาการสร้างไซโคลเดกซ์ทรินเกิดที่อุณหภูมิ 60 องศาเซลเซียสมากกว่าที่อุณหภูมิ 80 องศาเซลเซียส ในขณะที่อุณหภูมิของปฏิกิริยาการสลาย เป็นไปในทางตรงข้าม และด้วยเหตุที่พาราเซตามอลซึ่งเป็นยาแก้ปวดลดไข้ มีการละลายน้ำได้ยาก เพื่อปรับปรุงการละลายของพาราเซตามอล ได้นำผลผลิตไซโคลเดกซ์ทรินที่สังเคราะห์ได้ด้วยเอนไซม์ มาศึกษาผลของสารประกอบเชิงซ้อนระหว่างพาราเซตามอลกับไซโคลเดกซ์ทรินชนิดต่าง ๆ กราฟแสดงรูปแบบการละลายของสารประกอบเชิงซ้อนระหว่างพาราเซตามอลกับแอลฟา- บีตา- และแกมม่า-ไซโคลเดกซ์ทรินเป็นชนิด A_N ในขณะที่รูปแบบการละลายของสารประกอบเชิงซ้อนระหว่างพาราเซตามอลกับมอลโตซิล-บีตา-ไซโคลเดกซ์ทรินเป็นชนิด A_L โดยมีค่าคงที่ K_c ของสารประกอบเชิงซ้อนระหว่างพาราเซตามอลกับมอลโตซิล-บีตา-บีตา- แอลฟา- และแกมม่า- ไซโคลเดกซ์ทรินเท่ากับ 2,223.25, 16.75, 5.69 และ 4.73 โมล่าร์$^{-1}$ ตามลำดับ*
สรุป*: อุณหภูมิที่เหมาะสมในการผลิตไซโคลเดกซ์ทรินคือ อุณหภูมิ 60 องศาเซลเซียส และการเกิดสารประกอบเชิงซ้อนกับไซโคลเดกซ์ทรินชนิดต่าง ๆ ช่วยเพิ่มการละลายของพาราเซตามอลได้อย่างมีนัยสำคัญ เรียงลำดับจากมากไปน้อยดังนี้ มอลโตซิล-บีตา- บีตา- แอลฟา- และแกมม่า-ไซโคลเดกซ์ทริน*