COCRYSTAL OF ASCORBIC ACID WITH SUCROSE AND ERYTHRITOL

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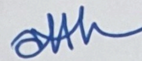

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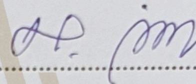

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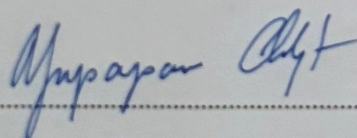

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กระบวนการตกผลึกร่วมเป็นกระบวนการที่ได้รับความนิยมอย่างแพร่หลายในอุตสาหกรรมยาและอาหารเพราะกระบวนการนี้ผลิตได้ง่าย ขยายกำลังการผลิตได้ และคุ้มค่าต้นทุน อีกทั้งใช้พัฒนาคุณสมบัติของสารให้ดียิ่งขึ้นกว่าเดิมเช่นเพิ่มความเสถียร ความสามารถในการไหล สมบัติทางฟิสิกส์เคมี และปรับปรุงรสชาติ เป็นต้น ในงานวิจัยนี้สนใจที่ใช้กระบวนการตกลึกร่วมในการเพิ่มความเสถียรของกรดแอสคอร์บิกหรืออีกชื่อที่เรารู้จักกันอย่างแพร่หลายคือวิตามินซี โดยนำวิตามินซีมาตกผลึกร่วมกับน้ำตาลซูโครส หรือที่เรารู้จักกันคือน้ำตาลทราย ซึ่งลดปริมาณน้ำตาลทรายโดยการผสมกับน้ำตาลอิริทริทอลทำการหาอัดตาราส่วนของน้ำตาลทรายและอิริทริทอลที่เหมาะสม อีกทั้งผู้ป่วยเบาหวานที่มักขาดวิตามินซีสามารถบริโภคได้อีกด้วย สำหรับวิธีการเตรียมโคคริสตัลจากการตกผลึกจากสารละลายเป็นพื้นฐาน (Solution based cocrystal preparation method) โดยทำการผสมน้ำตาลทรายและอิริทริทอลเข้าด้วยกันแล้วละลายน้ำตาลที่ผสมกันแล้วด้วยน้ำที่อุณหภูมิ 100 องศาเซลเซียส เพื่อให้ผสมกันได้ดี แล้วลดอุณหภูมิให้เหลือ 60 องศาเซลเซียสทันทีป้องกันการเสื่อมสภาพของวิตามินซีที่จะใส่ในขั้นตอนต่อไป ขั้นตอนนี้เป็นการเริ่มต้นกระบวนการตกผลึกร่วม จากนั้นใส่วิตามินซีลงไปทำการกวนอย่างต่อเนื่องจนได้ผลิตภัณฑ์ ผลิตภัณฑ์ที่ได้จากกระบวนการตกผลึกร่วมถูกเรียกว่าโคคริสตัล แล้วนำโคคริสตัลที่ได้ไปอบเพื่อไล่ความชื้นแล้วเก็บรักษาที่อุณหภูมิต่ำในที่ที่ไม่มีความชื้นเพื่อนำไปศึกษาในขั้นตอนต่อไป

ทำการยืนยันโคคริสตัลโดยเทคนิค FTIR และ XRD โดยทั้งสองเทคนิคยืนยันว่าเกิดโคคริสตัลขึ้นจริง อีกทั้งผล FTIR และ XRD ของทุกๆ อัตราส่วน ไม่มีความแตกต่างกัน ดังนั้นจึงทำการหาอัตราส่วนที่เหมาะสมในการบริโภคแต่ละวัน พบว่าอัตราส่วนที่เหมาะสมคืออัตราส่วนระหว่างน้ำตาลซูโครสและอิริทริทอล 2:3 จากนั้นทำการวิเคราะห์เพื่อดูลักษณะทางสัณฐานวิทยา (Morphology) โดย SEM เปรียบเทียบกับน้ำตาลทราย น้ำตาลอิริทริทอล วิตามินซีและโคคริสตัลพบว่าโคคริสตัลมีการรวมตัวอย่างเห็นได้ชัดได้ทำการศึกษาหาอัตราการละลายพบว่าโคคริสตัลละลายเร็วขึ้นกว่าน้ำตาลและวิตามินซีแต่ยังน้อยกว่าน้ำตาลอิริทริทอล อีกทั้งทำการหาความสามารถในการละลายพบว่า โคคริสตัลละลายได้ดีกว่าน้ำตาลอิริทริทอลและวิตามินซีแต่น้อยกว่าน้ำตาลทราย ใช้เทคนิค HPLC ในการศึกษาเพื่อยืนยันว่ามีวิตามินซีในโคคริสตัล พบว่าเกิดพีคของวิตามินซีเกิดขึ้นในช่วง 3.5-5 นาที ซึ่งสามารถยืนยันได้ว่าในโคคริสตัลมีวิตามินซีจริงๆ ศึกษาความสามารถในการต้านอนุมูลอิสระของโคอนุมูลอิสระได้สูงถึง 86.12% ในขณะที่วิตามินซีสามารถต้านอนุมูลอิสระได้ 87.38% ซึ่ง

มีค่าใกล้เคียงกันอย่างมาก การศึกษาความเสถียรโดยทำการทดลองเก็บโคคริสตัลและวิตามินซีไว้ในตู้อบ 40 องศาเซลเซียส ไม่มีแสงและความชื้นเป็นเวลา 1 เดือน พบว่าโคคริสตัลมีความเสถียรมากกว่าวิตามินซี เมื่อนำโคคริสตัลที่ผลิตได้ไปเปรียบเทียบความสามารถในการต้านอนุมูลอิสระกับเครื่องดื่มที่เป็นที่นิยมในประเทศไทย 2 ชนิด โดยเครื่องดื่ม 2 ชนิดนี้มีปริมาณวิตามินซีในเครื่องดื่ม 200 % พบว่า เครื่องดื่มชนิดที่ 1 และ 2 มีความสามารถในการต้านอนุมูลอิสระ 55.52 กับ 17.98% ตามลำดับดับ ในขณะที่โคคริสตัลสามารถต้านอนุมูลอิสระได้ถึง 86.12%

ในงานวิจัยนี้ได้ทำการศึกษาหาปริมาณน้ำอิสระ พบว่าโคคริสตัลให้ปริมาณน้ำอิสระเพียง 0.483 ซึ่งน้อยกว่าน้ำตาลทราย อิริทริทอล และวิตามินซี ค่าที่ได้น้อยกว่า 0.6 คือเป็นค่าที่ปลอดภัยในอาหาร หาคุณสมบัติในการดูดความชื้นของโคคริสตัลโดยใช้เทคนิค Hygroscopicity เพื่อหาข้อกำหนดในการบรรจุภัณฑ์ของผลิตภัณฑ์พบว่าโคคริสตัลมีการดูดความชื้นมากที่สุด จึงแนะนำให้จัดเก็บในที่ ๆ ไม่มีความชื้น

สาขาวิชา วิศวกรรมเคมี

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ลายเซ็นนักศึกษา.....นรมน.....

ลายเซ็นอาจารย์ที่ปรึกษา..........

NORRAMON SATHANASAOWAPHAK: COCRYSTAL OF ASCORBIC ACID WITH SUCROSE AND ERYTHRITOL

THESIS ADVISOR: ASSOC. PROF. LEK WANTHA, Ph.D., 87 PP.

Keyword: CO-CRYSTALLIZATION/ASCORBIC ACID/ERYTHRITOL/SUCROSE

Co-crystallization is a widely popular in the pharmaceutical and food industries because this is easy to perform, readily scalable and cost-effective. It can improve important properties such as stability, flowability, physicochemical characteristics and taste. This study focuses on using co-crystallization to enhance the stability of ascorbic acid or know as vitamin C. Ascorbic acid was co-crystallized with sucrose (table sugar), and part of the sucrose was replaced with erythritol to determine a suitable sucrose–erythritol ratio, taking into account dietary considerations, for example for people with diabetes. A solution-based method was employed to prepare the cocrystal. Sucrose and erythritol were mixed and dissolved in deionized water at 100 °C under continuous stirring. The solution was then cooled to 60 °C to prevent degradation of ascorbic acid. At this stage, ascorbic acid was added and stirring was continued until the cocrystal formed. The product was collected, dried in a hot-air oven to remove residual moisture, and stored at a low temperature (4–6 °C) in a dry environment for further study.

FTIR and XRD were used to confirm the formation of the cocrystal, and both techniques supported its successful preparation. The diffraction and spectral patterns were essentially the same across all tested sucrose–erythritol ratios. A 2:3 sucrose-to-erythritol ratio was selected as the working composition, as it was considered reasonable for daily dietary intake and had the lowest of water activity. SEM was employed to study the morphology, and the images revealed clear agglomeration in the cocrystal compared with pure sucrose, erythritol, and ascorbic acid. In terms of dissolution in water, the cocrystal exhibited a faster dissolution rate than sucrose and ascorbic acid, although it was still slower than erythritol. Regarding solubility, the cocrystal was more soluble than ascorbic acid and erythritol, but less soluble than sucrose. HPLC was used to confirm the including of ascorbic acid in cocrystal and it

confirmed the inclusion. DPPH radical scavenging activity was used to determine antioxidant capacity of cocrystal by dissolving DPPH in ethanol. DPPH radical scavenging activity of cocrystal was 86.12% while DPPH radical scavenging activity of ascorbic acid was 87.38%, which had similar value. Stability was studied by storing the cocrystal and ascorbic acid in an oven at 40°C, without light and moisture, for a month. Cocrystal had more stable than ascorbic acid. In addition, the antioxidant capacity of the cocrystal was compared with that of two popular vitamin C–fortified beverages in Thailand (type 1 and type 2), each labeled as containing 200 % of the recommended daily intake of ascorbic acid. The DPPH radical-scavenging activities of beverage type 1 and type 2 were 55.52 % and 17.98 %, respectively, both significantly lower than that of the cocrystal. These results indicate that the cocrystal retained antioxidant capacity more effectively than the two commercial beverages tested.

Cocrystal had a water activity value of 0.48, which was lower than sucrose, erythritol and ascorbic acid. Moreover, this value was below 0.6, a threshold generally considered safe for food products. Hygroscopicity testing was conducted to assess the packaging requirements of the cocrystal. The results showed that the cocrystal absorbed the highest amount of moisture among the samples; therefore, it should be stored in a dry, moisture-free environment.

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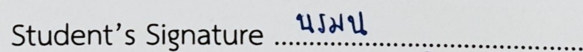
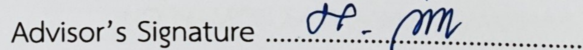
School of Chemical Engineering Student's Signature 

Academic Year 2025 Advisor's Signature 

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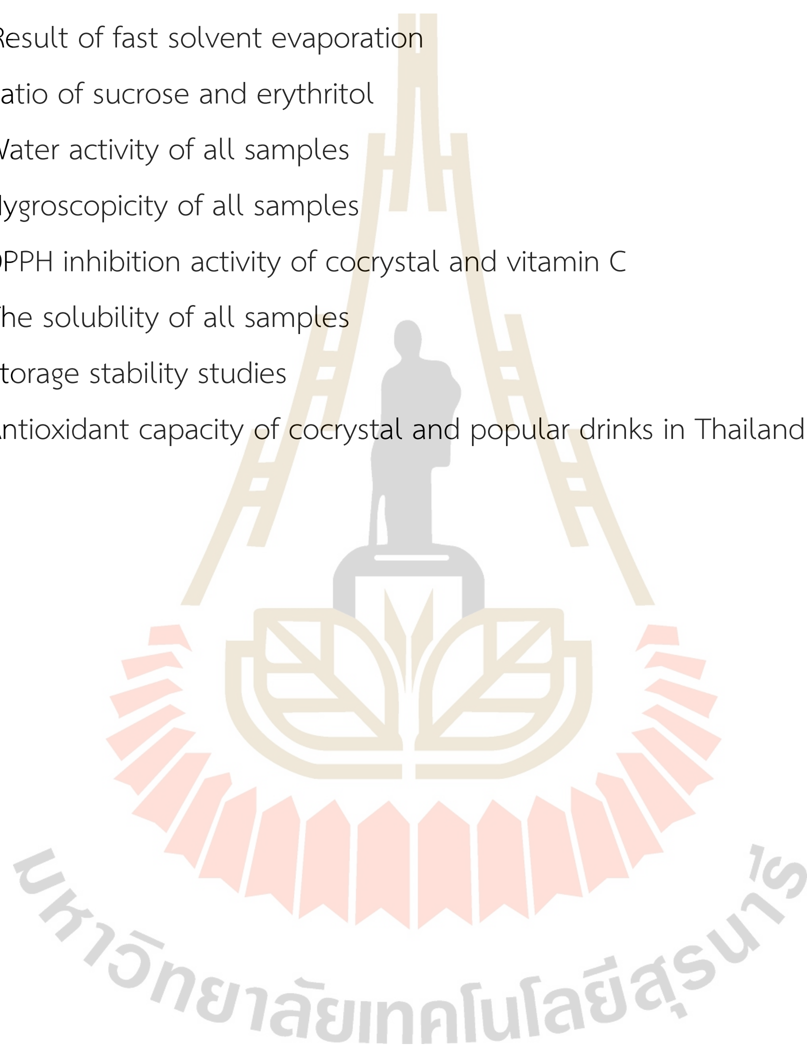

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CHAPTER I

INTRODUCTION

1.1 Background problem and significance of the study

Co-crystallization occurs when two or more substances crystallize together to form a new product with enhanced physicochemical properties, such as a higher melting point, increased stability, improved bioavailability, enhanced dissolution, and greater solubility (Schultheiss & Newman, 2009). It is now widely used in the pharmaceutical and food industries because the process is simple, economical, and cost-effective. Extensive research has focused on co-crystallization with sucrose, since sucrose is water-soluble and readily crystallizes into a solid (Chezanoglou & Goula, 2021). In the food industry, sucrose has been co-crystallized with carotenoids extracted from carrots, which act as antioxidants (Kaur, Elsayed, Subramanian, & Singh, 2021). Beyond its role as a sweetener, sucrose can also function as a stabilizing co-former for antioxidant compounds. For example, co-crystallization with sucrose has been shown to preserve catechins from tea, curcumin from turmeric, and antioxidant compounds from yerba mate tea (Wang et al., 2022). Vitamin B12 has also been successfully co-crystallized with sucrose, extending its shelf life up to 23 months by protecting it from light and heat degradation (Bajaj & Singhal, 2021). Ascorbic acid (vitamin C) is another water-soluble vitamin of particular interest. Given its instability and importance for human health, this study focused on developing a co-crystal of ascorbic acid with sucrose to improve its stability.

Ascorbic acid (vitamin C) is a water-soluble vitamin that cannot be synthesized by the human body and therefore must be obtained from the diet. It provides multiple health benefits, including supporting immune function and lowering the risk of cardiovascular disease through improved cholesterol regulation. In addition, ascorbic acid enhances skin blood circulation, strengthens collagen, and functions as a potent antioxidant. Deficiency of ascorbic acid is of particular concern in individuals with diabetes, who often exhibit reduced vitamin C levels. Supplementation can

mitigate oxidative stress, vascular inflammation, hyperglycemia, and insulin dysregulation, as well as abnormalities in triglycerides, LDL cholesterol, and other lipid parameters (Sathienluckana, 2017). Adequate intake may also help prevent complications such as cardiovascular disease and kidney failure. Severe deficiency results in scurvy, a condition now rare in children but still potentially fatal if untreated. Characteristic symptoms include swollen gums, easy bruising, fever, bone pain, and impaired mobility.

This research aims to investigate the co-crystallization of sucrose with ascorbic acid. However, too much sucrose is not suitable for people with diabetes. Therefore, sucrose was reduced and replaced with erythritol, which is a sugar alcohol. Erythritol is a noncaloric sweetener that is rapidly absorbed in the small intestine and excreted in the urine without structural changes. In this way, the cocrystal can provide both sweetness and the benefits of ascorbic acid, and it can also be consumed by people with diabetes.

1.2 Objective

1.2.1 To study the co-crystallization process of ascorbic acid with sucrose and erythritol, focusing on the preparation method and characterization of the obtained cocrystal.

1.2.2 To evaluate whether co-crystallization can improve the physicochemical stability of ascorbic acid, including its solubility, dissolution rate, antioxidant activity, and storage stability, compared with pure ascorbic acid and the individual co-formers.

1.3 Scopes

1.3.1 This research investigates the co-crystallization of sucrose with ascorbic acid using a solution-based preparation method. The process requires supersaturation of the target compound and co-former, with nucleation initiated through cooling crystallization.

1.3.2 The study covers cocrystal characterization, physicochemical properties, quantification of ascorbic acid, and the evaluation of its stability and antioxidant activity.

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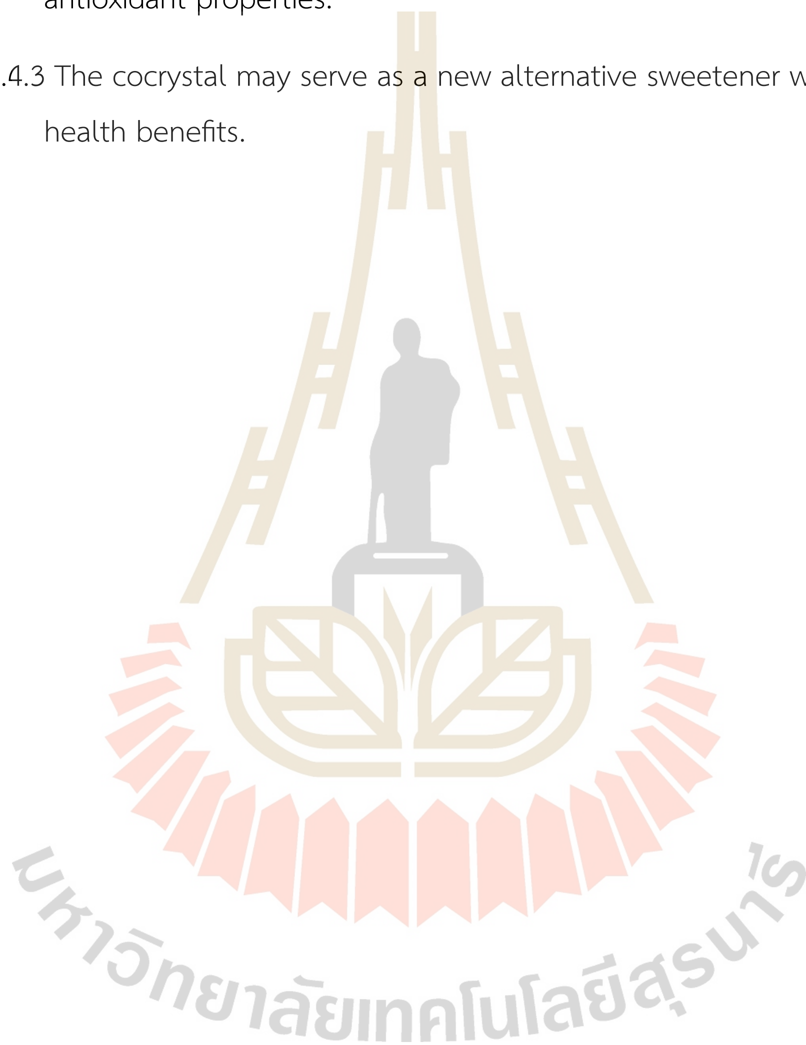
1.4 Expected Benefits of Research

1.4.1 The production of co-crystallized products composed of sucrose, erythritol and ascorbic acid will be achieved.

1.4.2 The obtained cocrystal is expected to exhibit both sweetening and antioxidant properties.

1.4.3 The cocrystal may serve as a new alternative sweetener with added health benefits.



CHAPTER II

THEORY AND LITERATURE REVIEWS

2.1 Co-crystallization

Co-crystallization is a cocrystal production process in which crystals containing two or more molecules in a specific stoichiometric ratio are formed within the lattice. Various molecular species involved in cocrystal formation are referred to as coformers. The coformer molecules are primarily bonded by non-covalent interactions such as hydrogen or halogen bonds. Among pharmaceutical and nutraceutical compounds, cocrystals are chosen to improve the physicochemical properties, such as dissolution rate, bioavailability, and aqueous solubility, compared to single-component crystals. Co-crystallization improves physicochemical properties, such as the stability, particle size, powder flowability, taste, hygroscopicity, solubility, and compatibility, of active pharmaceutical ingredients (APIs), which are critical attributes that impact the therapeutic effectiveness and manufacturing cost of solid dosage forms (Pawar, Saha, Nandan, & Parambil, 2021).

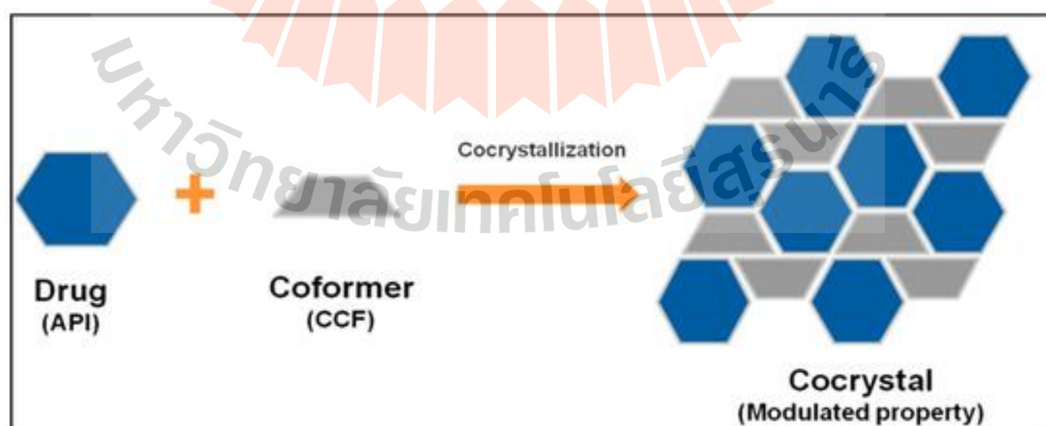


Figure 2.1 Typical representation of preparation of cocrystal (Thakuria & Sarma, 2018).

2.1.1 Co-crystallization Methods

2.1.1.1 Solid-state co-crystal preparation

Solid-state methods involve mixing and processing the active pharmaceutical ingredient (API) with the coformer in their powder or crystalline forms to generate cocrystals. In some cases, partial wetting of the samples may be required to facilitate the process.

2.1.1.1.1 Contact formation of the co-crystal

This method involves the spontaneous formation of a co-crystal by gently mixing the active pharmaceutical ingredient (API) with the co-former without the application of external mechanical force.

2.1.1.1.2 Solid-state grinding methods of co-crystal preparation

There are two widely used solid-state grinding approaches for co-crystal preparation: dry (or neat) grinding (DG) and liquid-assisted grinding (LAG). Both rely on mechanochemical principles. These techniques are simple, environmentally friendly, and provide reliable results, often superior to those obtained by solution-based methods.

- Dry grinding (DG)

This technique involves applying mechanical force, either manually with a pestle and mortar or using an automatic ball mill, to combine the active pharmaceutical ingredient (API) with the co-former. It is typically carried out at room temperature. Despite its simplicity and effectiveness, dry grinding can sometimes result in incomplete conversion to the co-crystal or lead to the formation of unstable amorphous phases due to crystal defects.

- Liquid-assisted grinding (LAG)

This technique involves grinding the active pharmaceutical ingredient (API) with the co-former in the presence of a small amount of solvents. The solvent acts as a catalyst, thereby accelerating and promoting co-crystal formation.

2.1.1.1.3. Extrusion

Extrusion using a twin-screw extruder is a relatively new method in which the starting materials are simultaneously mixed and pressed through a die under controlled conditions. The process is conducted below the melting temperature of any component in the mixture.

2.1.1.2. Solution-based cocrystal preparation

In solution-based methods, supersaturation of the API and the co-former are required to initiate nucleation, which is subsequently followed by crystal growth.

2.1.1.2.1. Evaporative method of co-crystallization

The evaporative method is an effective approach for producing high-quality single crystals suitable for single-crystal X-ray diffraction and is therefore widely applied in co-crystal preparation. Supersaturation occurs as the solvent evaporates, reducing the solution volume and thereby increasing the concentration of the co-crystallizing components. Although a higher evaporation rate accelerates co-crystallization, it is often associated with the formation of unstable or metastable crystals. Consequently, slow solvent evaporation is generally recommended.

2.1.1.2.2. Cooling crystallization (CC)

In cooling crystallization, the supersaturation required for co-crystal growth is achieved by gradually lowering the temperature of the solution. As the temperature decreases, the solubility of both co-formers is reduced, thereby increasing the driving force for precipitation and promoting subsequent cocrystal growth of the API.

2.1.1.3. Supercritical fluid methods

Supercritical carbon dioxide (CO_2) can be employed as a versatile medium for co-crystal production. Owing to its tunable density and solvent

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properties, CO_2 in the supercritical state provides a non-toxic and environmentally friendly alternative to conventional organic solvents. Depending on its application, supercritical CO_2can act as a solvent, a medium for rapid expansion, or an antisolvent, enabling various approaches to co-crystallization. These methods include co-crystallization with supercritical solvent, rapid expansion of supercritical solvents (RESS), and supercritical antisolvent co-crystallization (SAC), each with distinct mechanisms and advantages.

2.1.1.3.1. Co-crystallization with supercritical solvent

This technique exploits the solvent properties of supercritical CO as a non-toxic alternative to conventional organic solvents. The API and the co-former are suspended as slurry in liquid or supercritical CO_2. The thermodynamic conditions of CO_2 are regulated to optimize its density and solvating power, thereby enabling effective control of the co-crystallization process. Enhanced mass transfer by convection, promoted through agitation of the slurry, accelerates co-crystal formation. As a result, complete co-crystallization can be achieved, yielding a highly pure product.

2.1.1.3.2. Rapid Expansion of Supercritical Solvents (RESS)

In this method, a supercritical fluid—typically CO_2 is saturated with both the API and the co-former. The saturated solution is then rapidly depressurized through a nozzle into a drying chamber at atmospheric pressure. A major limitation of this technique is that it requires both the API and co-former to be soluble in supercritical CO_2. This condition is rarely met, as most pharmaceutical compounds exhibit low solubility in this medium.

2.1.1.3.3. Supercritical Antisolvent Co-crystallization (SAC)

This method uses supercritical CO_2 as an antisolvent in the co-crystallization process. It requires that both the API and the co-former have low solubility in supercritical CO2, allowing them to precipitate simultaneously as a

cocrystal. Within the crystallization vessel, supercritical CO_2 diffuses into the organic solvent, causing volume expansion and reducing the solvent's solvation power. This shift in solubility conditions induces the joint precipitation of the API and co-former, resulting in the formation of a single co-crystalline phase.

2.1.2. Miscellaneous cocrystal preparation

This technique, known as laser irradiation, employs a high-power CO_2laser to irradiate powdered blends of co-crystal formers, thereby inducing recrystallization into a co-crystal structure. It is hypothesized that the molecular rearrangement of the co-formers, along with nucleation, takes place in the vapor phase, triggered by the localized heating effects of the laser.

2.1.2.1 Freeze-drying

Lyophilization, also known as freeze-drying, has been widely used for the storage of various products, including pharmaceuticals. In this process, the material solution is first frozen, and then the surrounding pressure is reduced to allow the frozen water to sublimate directly from solid to gas phase. This method has also shown potential for the preparation of solid-state co-crystal forms.

2.1.2.2 Electrochemically induced Co-crystallization

Electrochemically induced co-crystallization is a versatile technique that employs electrochemical methods to promote cocrystal formation. The process involves creating a favorable environment, for example, by adjusting the pH to neutral and generating localized forces that facilitate nucleation and crystal growth.

2.1.2.3 Resonant acoustic mixing

Resonant acoustic mixing for cocrystal preparation relies on the transfer of mechanical energy to a wetted powder mixture containing the drug and its co-former. This energy promotes intimate mixing between the components, thereby increasing the likelihood of cocrystal formation.

2.1.2.4 Spray drying

Spray drying is a widely used technique for producing dry powders from solutions, emulsions, and suspensions. This advanced technology enables precise environmental control and utilizes hot air to evaporate the solvent rapidly. Compared to other solution-based co-crystallization methods, spray drying is faster and can yield purer co-crystals. As a result, it is considered one of the most preferred methods for both co-crystal preparation and scale-up.

2.1.2.5 Electrospray technology

This method involves liquid atomization driven by electrical forces, where the liquid is dispensed through a capillary nozzle under a high electric potential. The electric field then disperses the liquid into fine, highly charged droplets. Upon drying, the resulting particles are collected using an electrostatically charged powder collector.

2.2 Sucrose

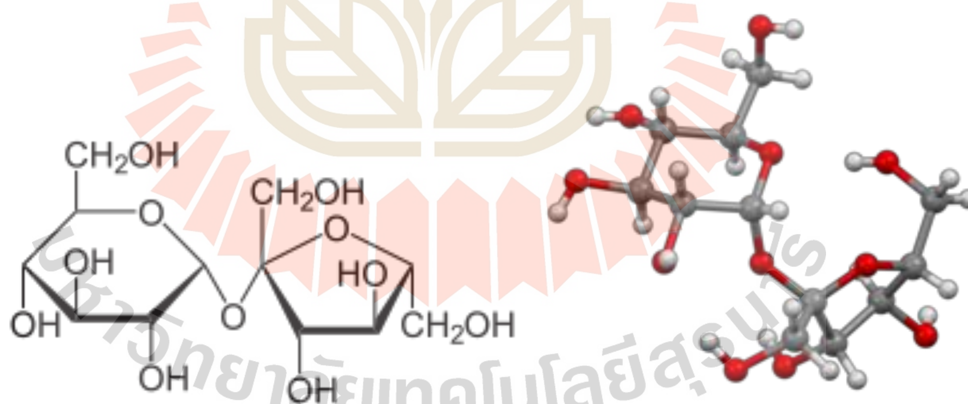

Figure 2.2 Chemical structure of sucrose.

The chemical structure of sucrose is shown in Figure 2.2. Sucrose is a sugar commonly known as table sugar, used as a sweetener, and widely found in many plants and fruits around the world. However, the raw materials used for commercials sugar production are sugarcane and beet root. Sucrose is a disaccharide, which consisting of two monosaccharides: fructose and glucose, linked by glycosidic bonds,

and have the molecular formula $C_{12}H_{22}O_{11}$. It is a non-reducing sugar because it lacks no functional groups left in the molecule (Pornchalermpong & Rattanapanont, 2010).

Sucrose is hydrolyzes to fructose and glucose by the enzyme sucrase. Approximately 10-25 % of fructose is converted to glucose in the upper gastrointestinal tract. Monosaccharides are absorbed and transported to the liver through the portal vein, where they are subsequently distributed to various tissues throughout the body. Other body tissues use glucose via the bloodstream; it is the primary form of carbohydrate used to provide energy to tissues. Excessive consumption of sucrose can negatively impact the body, potentially leading to conditions such as tooth decay, elevated triglyceride levels, diabetes, and persistent hyperactivity in children (Anderson, 2003). Therefore, in this research, the amount of sucrose was reduced by adding erythritol, a sugar-based polyol. This will be discussed in the next topic.

Sucrose is commonly used as the primary carrier in the co-crystallization process, where the active ingredient is incorporated within the crystal conglomerates. Various food compounds—such as honey, yerba mate, catechin, curcumin, carotenoids, and butterfly pea flower extracts—have been successfully co-crystallized with sucrose. Co-crystallization within a sucrose matrix is a relatively simple and cost-effective method that provides an alternative approach for handling, protecting, and preserving active components in powdered form. Numerous studies have reported that this technique improves the stability of encapsulated active ingredients; when heat-sensitive compounds are embedded in the porous crystal structure, they are effectively protected from degradation.

According to Mathlouthi and Genotelle (1998), the crystallization of sucrose from a supersaturated solution involves two main steps:

(i) Diffusion of sucrose molecules from the bulk solution to the crystal–solution interface.

(ii) Incorporation of sucrose molecules into the crystal lattice following the release of their hydration water. The crystallization rate is affected by several

parameters, most notably the temperature at which crystallization is initiated, the moisture content, and the presence of additional ingredients.

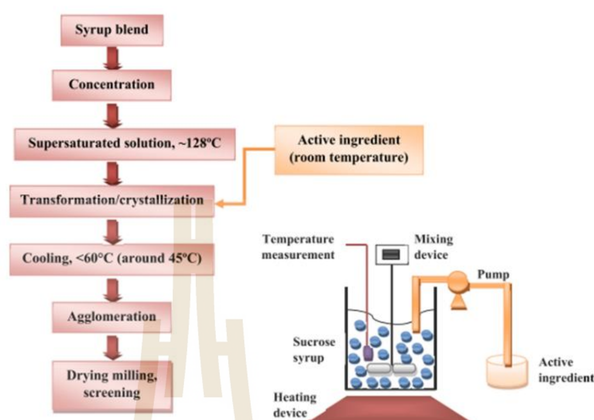

Figure 2.3 Steps and equipment of co-crystallization with sucrose (Evangelos & Athanasia, 2021).

An example of co-crystallization with sugar is illustrated in Figure 2.3. First, the mixture is heated above 130 °C under continuous stirring. These conditions prevent sucrose caramelization and prepare the system for subsequent crystal formation. Heating is continued to concentrate the solution by evaporation, during which the Brix value of the sucrose syrup increases from 70 to 75 and finally reaches 90–95. Once heating is stopped, stirring is maintained until the mixture cools to ambient temperature. At this stage, the initial concentration and volatility of hydrophobic active ingredients also play a crucial role in determining the outcome. Second, the active ingredient is rapidly and carefully incorporated into the sucrose matrix at room temperature, a critical step for ensuring uniform distribution within the crystal network. Third, the mixture is cooled quickly to below 60 °C; this step is essential for effective encapsulation and for inhibiting powder crystallization, since delayed cooling may increase.

2.3 Erythritol

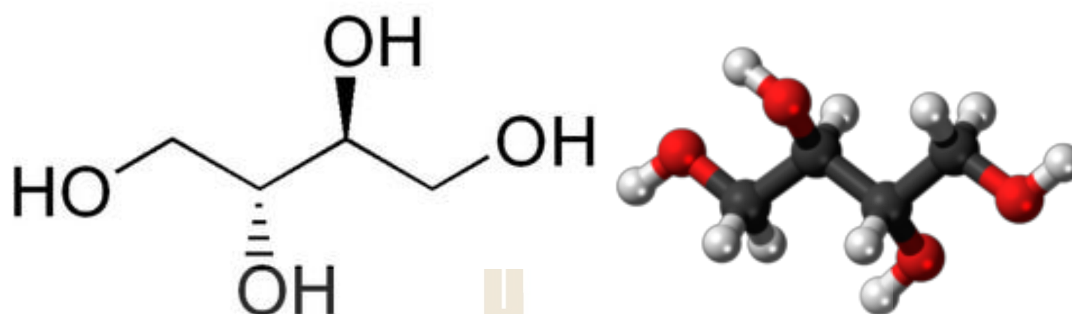

Figure 2.4 Chemical structure of erythritol.

Erythritol is a sugar alcohol (or polyol) that is a non-energy sweetener. The chemical structure of erythritol is shown in Figure 2.4. Erythritol is found in a variety of fruits, including melon, watermelon, pears, and grapes as well as in fermented foods such as cheese, and soy sauce (Mazi & Stanhope, 2023). Compared to sugar, erythritol is 60–70% as sweet as table sugar. Erythritol has a low glycemic index value and gives 0.2 kcal per gram of energy. Therefore, erythritol is suitable for individuals with diabetes or those aiming to manage their body weight. Like other sugar alcohols, erythritol is resistant to metabolism by oral bacteria that break down sugars and starches into acids. This bacterial acid production can lead to enamel erosion and tooth decay. Thereby, erythritol is considered a tooth-friendly sweetener and is recognized by the American Dental Association. Erythritol intake should be less than 1000 mg/kg of body weight, as excessive consumption may cause diarrhea (Moriconi et al., 2020). Nowadays, erythritol is commonly produced through yeast fermentation, as this method is cost-effective for large-scale commercial production. It utilizes various substrates, including glucose, fructose, xylose, sucrose, cellulose, and glycerol (Rzechonek, 2018).

Sugar alcohols are absorbed in the small intestine by passive diffusion. Erythritol, due to its small molecular size and low molecular weight—with only four carbon atoms—is absorbed into the bloodstream more rapidly and efficiently than larger sugar alcohols. Once absorbed, the majority of erythritol remains unmetabolized and is excreted unchanged in the urine (Mazi, & Stanhope, 2023).

2.3.1 Health effect (Boesten et al., 2015)

2.3.1.1 Dental health

Oral bacteria doesn’t ferment erythritol, so it does not produce acids that can damage tooth enamel. As a result, erythritol helps reduce the risk of tooth decay. Additionally, it helps maintain a neutral pH level in the mouth, creating an environment that is unfavorable for the growth of bacteria responsible for cavities.

2.3.1.2 Endothelial protective effects

Erythritol has been shown to protect against endothelial cell death caused by high sugar levels and oxidative stressors such as peroxynitrite.

However, consuming large amounts of sugar alcohol may negatively affect the digestive system, potentially leading to diarrhea. This side effect occurs because sugar alcohols are not absorbed in the stomach and are only partially absorbed in the small intestine. The unabsorbed portion reaches the colon, where it is fermented by bacteria, resulting in the production of gas. This fermentation process can result in gas buildup, bloating, indigestion, and diarrhea if consumed in excessive amounts (Hattakosol, 2021).

2.4 Vitamin C (Ascorbic acid)

Vitamin C has a chemical name, ascorbic acid. The chemical structure of vitamin C is shown in Figure 2.5. It is a water-soluble vitamin that our body cannot synthesize on its own. Vitamin C intake can be increased by consuming a variety of fruits and vegetables. Foods rich in vitamin C include oranges, mangoes, kiwis, pineapples, yellow bell peppers, broccoli, lemons, and other fruits and vegetables.

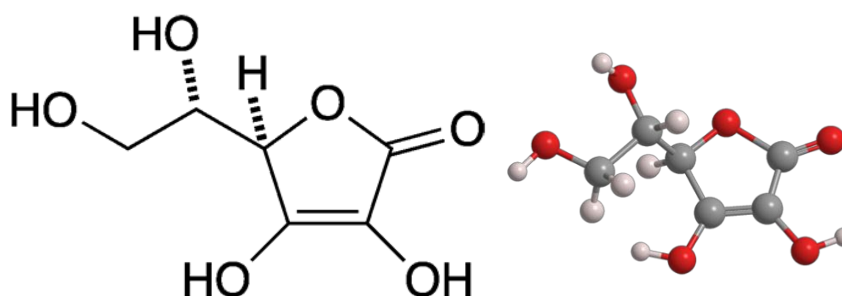

Figure 2.5 Chemical structure of vitamin C or ascorbic acid.

Although ascorbic acid is a water-soluble, organic solvent-insoluble substance, it is unstable when dissolved in water. It is oxidized to dehydroascorbic acid (DAA) and further decomposed into 2,3-diketogulonic acid (DKGA), a biologically inactive compound, as shown in Figure 2.6 (Ratniyom, Boonphadung & Unnanantn, 2017).

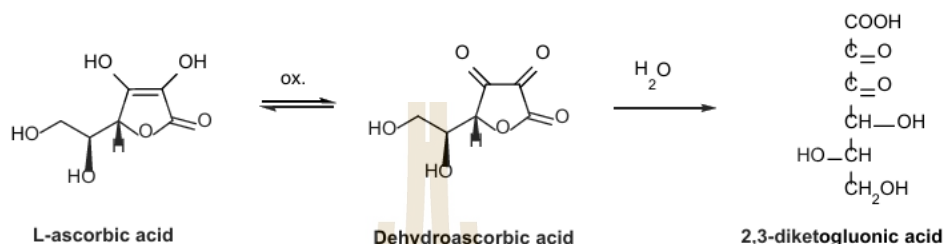

Figure 2.6 Degradation pathways of L-ascorbic acid in aqueous solution (Lee et al., 2004).

2.4.1 Mechanism and pharmacodynamics of vitamin C

Vitamin C in food exists in two forms, both of which can be utilized by the human body: ascorbic acid and dehydroascorbic acid. Ascorbic acid is a six-carbon compound biosynthetically derived from glucose. It typically appears as white crystals and has a sour taste. Vitamin C can be oxidized to dehydroascorbic acid, a molecule that is more chemically reactive and sensitive to further degradation in the body.Ascorbic acid functions as a reducing agent, serving as an electron donor in many biochemical reactions. This redox property underlies several important physiological roles of vitamin C (Figure 2.7):

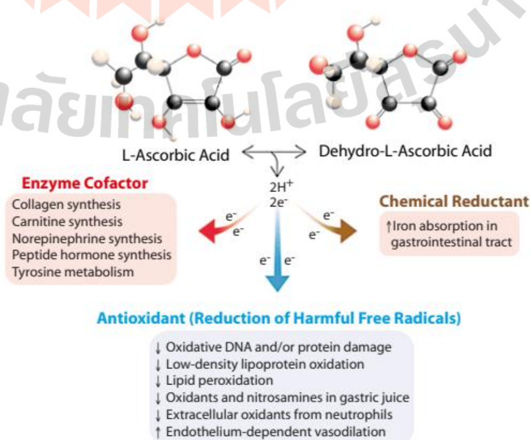

Figure 2.7 The effect of vitamin C on various body systems (Thanompong, 2017).

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First, by donating electrons to numerous enzymes, vitamin C promotes enzymatic activities involved in the biosynthesis of collagen, carnitine, and norepinephrine. Second, it acts as an antioxidant, counteracting free radicals and thereby protecting cells from oxidative damage and apoptosis. This antioxidant activity also reduces the oxidation of low-density lipoprotein (LDL), decreases the formation of carcinogenic nitrosamines, and protects endothelial cells, all of which contribute to cardiovascular health. Third, vitamin C enhances the absorption of non-heme iron in the gastrointestinal tract. Fourth, it strengthens the immune system by stimulating the proliferation of T-lymphocytes in response to infection and inflammation.

Vitamin C decomposes the fastest in the vitamin group. especially very sensitive to oxygen (sensitive to oxidation). It is also easily degraded in heat, light, humidity, heavy metals and in alkaline environments. Currently, researchers are attempting to synthesize vitamin C as a dietary supplement. To compensate for the loss of natural vitamins.

2.4.2 Negative effects of vitamin C

When high doses of vitamin C are used, adverse symptoms may occur, including diarrhea, and flatulence caused by excessive vitamin C intake. In addition, the saturation of absorption makes the unabsorbed vitamin C more abundant, which can cause adverse reactions in the gastrointestinal tract when consumed with food.

Additionally, high doses of vitamin C may cause kidney stones in certain patients. Iron overload has occurred in some patients, such as those with thalassemia major or hemochromatosis; therefore, caution should be exercised when using vitamin C in this group of patients.

2.4.3 Vitamin C Deficiency

Vitamin C deficiency can occur in patients with inadequate vitamin C diets or in high-risk groups such as the elderly, chronic smokers, and those with chronic diseases such as diabetes, chronic renal failure, and cancer A lack of vitamin C increases the risk of scurvy and can affect connective tissue, leading to bleeding, and making wounds more susceptible to infection in patients with wounds. The

ability to heal wounds may be reduced to slow wound healing. The early symptoms of vitamin C deficiency are often non-specific, including fatigue, muscle weakness, shortness of breath, and limb pain.

2.4.4 Medical benefits of vitamin C

High doses of vitamin C have been studied. It is clinically useful in the treatment and prevention of various diseases, including common cold, cancer, cardiovascular disease and diabetes mellitus.

2.4.4.1 Common cold

Pauling L concluded from the clinical study that the use of high doses of vitamin C can prevent and treat the common cold. It is believed to be due to its ability to stimulate T-lymphocyte cells in response to infection, and viruses, and inhibit inflammation caused by these infections.

2.4.4.2 Cancer

From its antioxidant effects, inhibitory properties against nitrosoamines, and immune system stimulating effects. It is believed that vitamin C intake may help prevent cancer. Epidemiological studies have shown that intakes of fruits and vegetables with a vitamin C content of 200 mg/day or more are associated with a lower incidence of oral, esophageal, colon and lung cancers. However, it is still unable to summarize the benefits received to come from vitamin C alone, because such benefits may be attributed to other substances. These benefits may also be due to other compounds present in fruits and vegetables

2.4.4.3 Cardiovascular disease

By decreasing the oxidation of LDL lipids, preventing the destruction of endothelial cells, and exerting antioxidant activity, vitamin C may have beneficial effects on the cardiovascular system. Therefore, taking vitamin C may help reduce the risk of cardiovascular disease.

2.4.5 Determining vitamin C in foods

2.4.5.1 Titration method

Titration is a quantitative analytical method that involves the gradual addition of a solution with a known concentration, known as the titrant, into a second solution containing the analyte. This process continues until the

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equivalence point is achieved—when the two substances have reacted in exact stoichiometric proportions. In cases where one of the substances is inherently colored, the equivalence point may be observed visually through a change or disappearance of color. When both solutions are colorless, an appropriate chemical indicator is often used, which changes color near the equivalent point. Alternatively, a titration curve can be plotted to determine the equivalence point. The amount of titrant used(n) is calculated by multiplying its volume(V) by its known concentration(C).

$$n(\text{mol}) = C(\text{mol/L}) \times V(\text{L})$$

and the amount of titrant can be used in the usual stoichiometric calculation to determine the amount of analyte (Ed Vitz).

This technique measures the concentration of vitamin C in a sample through a redox titration involving iodine. Ascorbic acid, commonly known as vitamin C, functions as a crucial antioxidant required by the human body (refer to supplementary notes). During the titration process, iodine is gradually introduced, leading to the oxidation of ascorbic acid into dehydroascorbic acid, while the iodine itself undergoes reduction to form iodide ions.

$$\text{Ascorbic acid} + I_2 \longrightarrow 2\,I^- + 2H^+ + \text{Dehydroascorbic acid}$$

As long as ascorbic acid remains in the solution, it reduces any iodine produced to iodide immediately. When the ascorbic acid is fully oxidized, any additional iodine is no longer consumed and instead reacts with the starch indicator, resulting in the appearance of a blue-black complex. This color change indicates endpoint of the titration. The method is applicable to a variety of samples, including vitamin C tablets, both fresh and processed fruit juices, as well as sold fruits and vegetables.

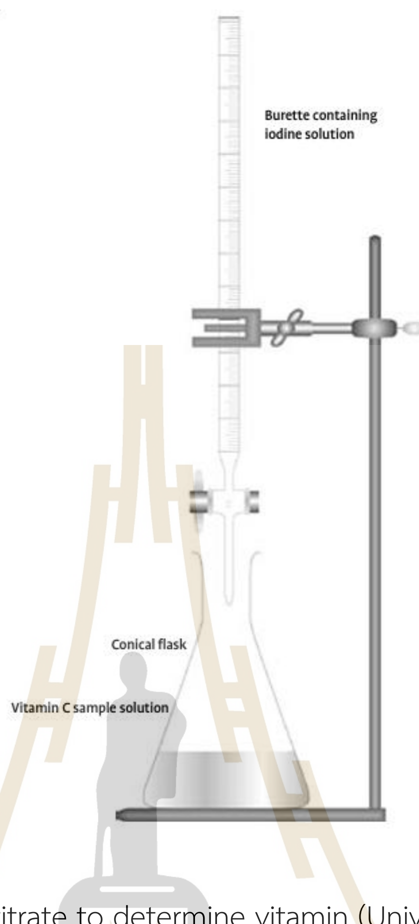


Figure 2.8 How to titrate to determine vitamin (University of Canterbury, College of Science, n.d.).

2.4.5.2 High Performance Liquid Chromatography (HPLC)

HPLC stands for High Performance Liquid Chromatography. In this technique, "chromatography" refers to the process used to separate components in a mixture, while the term "chromatogram" describes the output or visual result of that process.

The device used to perform the separation is known as a "chromatograph." Chromatography systems rely on several advanced components, including specialized columns for molecular separation and high-performance pumps that maintain a consistent solvent flow. As technology evolved, the method known as High Performance Liquid Chromatography began to be commonly abbreviated as "LC". More recently, Ultra High Performance Liquid Chromatography (UHPLC), which enables faster analyses, has seen increasing use. HPLC is suitable only for substances that can be dissolved in a solvent. It works by separating the components of a liquid

sample, enabling both qualitative and quantitative identification of the substances present and their respective concentrations (Shimadzu Corporation, n.d.).

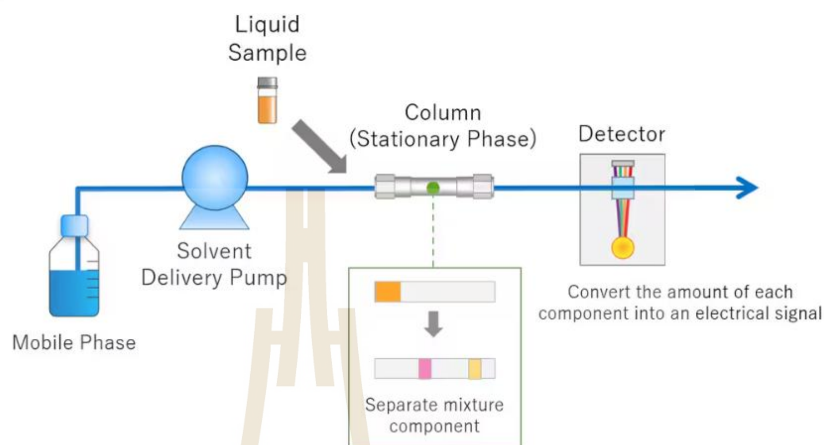


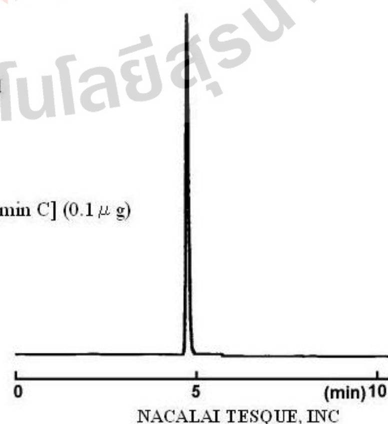
Figure 2.9 Shows a basic overview of the HPLC process (Shimadzu Corporation, n.d.).

In HPLC analysis, the solvent responsible for transporting the sample components is known as the mobile phase. This phase is pumped through the separation column—also called the stationary phase—at a constant flow rate regulated by a solvent delivery system. A specific volume of the sample is introduced into the column, where its components are separated based on their interactions with the stationary phase. As the individual compounds exit the column, they pass through a detector, which identifies and quantifies each one.

COSMOSIL Application Data

Column: $5C_{18}$-PAQ
Column size: 4.6mmI.D.-250mm
Mobile phase: 20mmol/l Phosphoric Acid
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV245nm, 0.16AUFS

Sample: *L*(+)-Ascorbic Acid [Vitamin C] (0.1 μ g)



AP-0372

Figure 2.10 COSMOSIL application data. (Nacalai Tesque, Inc., n.d.).

Figure 2.10 shows type of column, size column, mobile phase, flow rate, temperature and detection for using to determine ascorbic acid and area which peak of ascorbic acid occurred.

2.4.5.3 Ultraviolet–visible spectroscopy

UV-Visible (UV-Vis) spectroscopy is a method used to analyze how much ultraviolet or visible light a sample absorbs or transmits at specific wavelengths, typically by comparing its absorption or transmission to that of a reference or blank. The absorption characteristics depend on the sample's composition, which can offer insight into the substances present and their concentrations. Because this technique is based on the interaction of light with matter, it is essential to begin by understanding the fundamental properties of light.

Light possesses energy that is inversely related to its wavelength—meaning that shorter wavelengths correspond to higher energy levels, while longer wavelengths carry less energy. When light interacts with a substance, a specific amount of energy is required to excite electrons to higher energy states. This excitation appears as light absorption. The exact energy needed depends on the electron's bonding environment, which varies across different substances.

As a result, each substance absorbs light at characteristic wavelengths. The human eye can detect visible light ranging from roughly 380 nm (violet) to 780 nm (red). Ultraviolet (UV) light, by contrast, has shorter wavelengths, extending down to about 100 nm. Because each compound absorbs light differently across this range, UV-Vis spectroscopy uses these wavelength-dependent absorption patterns to identify and quantify substances by observing their absorbance peaks (Justin, 2023).

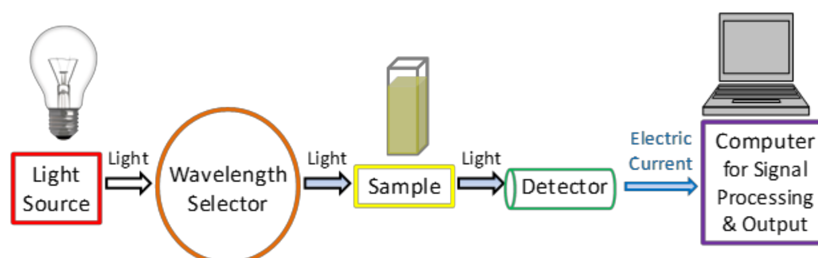


Figure 2.11 A simplified schematic of the main components in a UV-Vis spectrophotometer (Justin, 2023).

UV spectrophotometry is commonly employed to analyze ascorbic acid due to its simplicity and the fact that vitamin C readily absorbs ultraviolet light. This technique can be applied to various sample types, including vitamin C tablets, fresh or packaged fruit juices, and solid fruits and vegetables. In this procedure, bromine water is added to oxidize ascorbic acid into dehydroascorbic acid. Subsequently, 2,4-dinitrophenylhydrazine reacts with the oxidized product in a coupling reaction, which is carried out at 37°C for 3 hours. After the reaction period, concentrated sulfuric acid (85% H_2SO_4) is added, resulting in the formation of a colored complex. The absorbance of this complex is then measured at a wavelength of 491 nm (Desai, 2019).

There are several methods used to quantify vitamin C. The three techniques mentioned above are the most commonly used.

2.5 DPPH

The DPPH method, originally introduced by Blois in 1958, is widely used to assess antioxidant activity by employing the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH; $C_{18}H_{12}N_5O_6$, M = 394.33). This essay evaluates the ability of antioxidants to neutralize the radical by donating a hydrogen atom, which reduces the nitrogen-centered odd electron in DPPH to form a stable hydrazine compound (Kedare & Singh, 2011). The molecular structure of DPPH is illustrated in Figure 2.12.

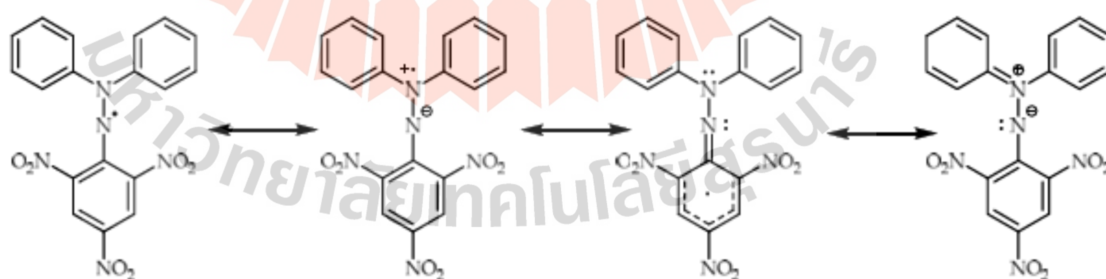


Figure 2.12 The chemical structures of a 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Gulcin & Alwasel, 2023).

DPPH is a stable radical in the solvents such as methanol or ethanol. This solution is purple, exhibiting good light absorption at wavelengths of 515-517 nanometers (nm). DPPH● reacts with antioxidants (AH) or with radical species (R●).

DPPH• + AH-------------DPPH-H + A•

DPPH• + R•-------------- DPPH-R

When DPPH• reacts with an antioxidant, the color of the purple solution changes to yellow [19]. Antioxidant activity was expressed as the percentage of radical scavenging activity of DPPH free radical scavenging activity of calculated using the following equation:

$$Antioxidant\ activity\ (\%) = 100\ \times \frac{Abs_{conctrol}^{517nm} - Abs_{sample}^{517nm}}{Abs_{control}^{517nm}}$$

Where $Abs_{control}$ is the absorbance of the control (without the sample) and Abs_{sample} is the absorbance of the sample.

2.6 Water Activity (Patil, 2022)

In food systems, water exists in two primary forms: free water and bound water. Bound water is tightly associated with food components such as carbohydrates and proteins, rendering it unavailable for biological activities. In contrast, free water is accessible and supports biochemical reactions and microbial growth.

When discussing food safety and preservation, the term moisture content often arises. Moisture content represents the total amount of water in a food product, encompassing both free and bound water. However, controlling water activity—rather than total moisture—is key to ensuring a product's shelf stability. Simply put, water activity refers to the fraction of water in a food that is not bound and is therefore available to support microbial growth and to facilitate chemical or biochemical reactions. Water activity values range from 0 (completely dry) to 1.0 (pure water). Scientifically, it is defined as the ratio of the vapor pressure of water in a food item to the vapor pressure of pure water at the same temperature.

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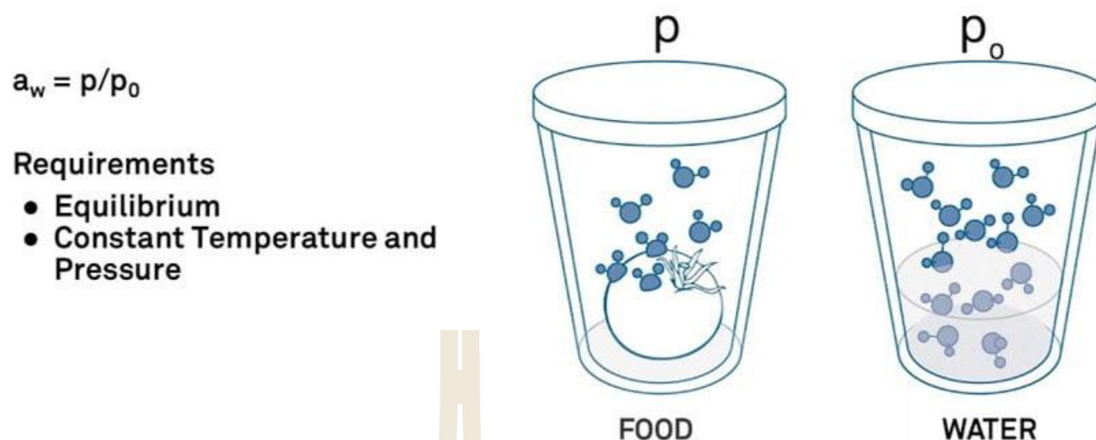

Figure 2.13 How to calculate water activity (a_w) (Neonics Co. Ltd., 2021).

A_w is the water activity

P is the vapour pressure of water in food

P_0 is the vapour pressure of pure water at the same temperature

Water activity plays a crucial role in determining the safety and stability of food products by influencing microbial growth as well as the chemical, biochemical, and physical properties of these products. In the context of food safety, microbial spoilage is a major concern, particularly in high-moisture or perishable foods. Microorganisms such as bacteria, yeasts, and molds require a certain level of free water to grow. Water activity helps to indicate whether that minimum threshold—known as the limiting water activity level—has been met. Below this level, microbial growth is inhibited. Each type of microorganism has a specific optimal water activity required for its development, making water activity a key parameter in food preservation strategies.

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a_w	Effects on microorganisms	Examples of foods
1.00–0.95	Some yeasts, Gram-negative rods, bacterial spores	Fresh foods, foods containing 40% sucrose or 7% salt (canned foods, cheeses, several sausages, bread, etc.)
0.95–0.91	Most cocci, bacilli, lactobacilli, some molds	Foods containing 50% sucrose or 15% salt (mayonnaise, hard cheeses, jams, etc.)
0.94	Growth and toxin production by *C. botulinum*	Sausages
0.91–0.88	Most yeasts	Foods containing 65% sucrose or 15% salt (fruit jams, margarine, fruit juice concentrates, some hard cheeses, etc.)
0.86	Growth of *S. aureus*	Flour, cakes, rice, beans
0.86–0.80	Most molds	Foods containing 15–20% water (fruit cake, sweetened condensed milk, salted meat, salami, etc.)
0.80–0.75	Halophilic bacteria	Foods with 26% salt or very high sugar content (salted fish, molasses, jam, etc.)
0.75–0.65	Xerophilic molds	Foods containing less than 10% water (dates, figs, rolled oats, nuts, etc.)
0.68	Limit for fungi	Dry milk, peanut butter
0.65–0.60	Osmophilic yeasts	Confectionary products, dried fruits containing 15–20% water, honey
<0.60	No microbial growth	Dried milk, instant coffee, dried egg, spices, crackers, flour, cereals, and so on

Figure 2.14 Water activity levels of different foods and inactivation of microorganisms (Erkmen & Bozoglu, 2016).

Figure 2.14 illustrates the water activity levels of various foods and the corresponding inactivation thresholds for microorganisms. Generally, the higher the water activity, the greater the risk of spoilage. Among microorganisms, bacteria require higher water activity levels to grow compared to fungi, such as yeasts and molds. Notably, no microbial growth occurs below a water activity of 0.60, making this a critical threshold for ensuring food stability and safety.

2.7 Hygroscopicity

Hygroscopicity is a physical property that describes a material's ability to absorb and release water molecules from its environment. Some substances may even swell or deform upon exposure to moisture, making this property valuable for applicationssuch as environmental sensors or moisture-responsive actuators (Riza, Go, Maier, Harun & Anas, 2020). In the field of food science, hygroscopicity plays a crucial role, as it directly affects the stability, texture, and shelf-life of food products. A thorough understanding of this characteristic is essential for predicting ingredient

behavior under various environmental conditions, thereby informing processes like drying, storage, and formulation of food items (Fiveable Inc., n.d.).

2.8 Literature Review

(Fayed, et al., 2022) studied the co-crystallization of Lopinavir with menthol. Lopinavir is an antiviral, anti-parasitic drug that has been recently used to treat COVID-19. However, it is poorly absorbed due to its low solubility. Therefore, this research was to increase the dissolution and bioavailability of Lopinavir through co-crystallization with menthol. Menthol can increase the permeability of biological membrane to drug, it has been shown to form eutectic mixtures or cocrystals. From DSC analysis, the optimal ratio for Lopinavir-menthol cocrystals was 1:2. When comparing between Lopinavir-menthol cocrystals at a ratio of 1:2 with pure lopinavir an increase in dissolution was observed from 24.96% to 91.43%. Lopinavir showed incomplete absorption from the duodenum and jejuno-iliac regions, but cocrystals of Lopinavir-menthol showed improved absorption.

(Pagire, et al., 2017) studied the thermodynamics of carbamazepine-saccharin cocrystal polymorphs. Polymorphism is a phenomenon in which some substances exhibit multiple crystal forms. Polymorphism in APIs is important for the potential of crystalline forms to affect the quality, efficacy, and safety of the final pharmaceutical product. In each form of crystal, there will be different potentials. The work aimed to characterize the thermodynamic relationship between the dimorphic cocrystals and the FI and FII forms of carbamazepine (CBZ) and saccharin (SAC) molecules. The FI form was produced by preparing cocrystals using a slow evaporative solution crystallization method. The solvent used was ethanol and the FII form was obtained by the preparing of cocrystals via a rotary (fast) evaporation technique employing a solvent mixture of 62.5% methanol and 37.5% ethanol. Both methods used the 1:1 ratio of carbamazepine (CBZ) and saccharin (SAC). From DSC analysis, it was found that FI had a melting point of 172.45 °C and FII had a melting point of 164.7 °C. Thermodynamic examination suggests that if there is a higher melting point, there should also be a higher heat of fusion value, according to Burger and Ramberger's law. FI and FII had heat of fusion values of 121.1 and 110.3 J/g, respectively. From the

solubility analysis and the results of Van't Hoff plots, it was found that FI is a stable form and FII is a metastable form. Both forms exhibit monotropic morphology and neither form undergoes a phase change during heating. However, FII can be transformed into FI during conversion using a solution as an intermediate which may affect further development.

(Lin, Hsu and Lin, 2013) studied the co-crystallization of theophylline with citric acid. Theophylline is a methylxanthine drug. It is used to treat respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma. However, theophylline has low water solubility. Theophylline can be neutral, positive and negative, depending on the strength of the acid / base of the organic solvents. In this work, real-time co-crystalline formation between theophylline (TP) and citric acid (CA) was investigated using DSC-FTIR micro spectroscopy. Cocrystals were prepared by slow solvent evaporation (Physical mixture) and a neat co-grinding process (Ground mixture). From the physical mixture analysis, endothermic peaks of TP and CA were observed at 273 and 157 °C, respectively. When analyzing the TP-CA crystals, endothermic peaks were observed at 149, 173 and 264 °C. At 149 °C the aggregates of CA and TP crystals were observed. The dissolved CA combines with TP to form an aggregate crystal, leading to a peak at 173 °C, the melting point of TP-CA crystals, and at 264 °C, the melting point of TP. After CA decomposition, the ground mixture has similar peaks to the physical mixture. Under storage conditions, TP-CA cocrystals obtained from the ground mixture were more stable than the physical mixture at 25 °C /75% RH and 55 °C /75% RH over 90 days.

(Salem, et al., 2021) studied the solvent dependent cocrystal of 4-aminosalicylic acid and sulfamethazine. Polymorphic cocrystals were obtained by using different solvents in which 4-aminosalicylic acid used in the treatment of tuberculosis was co-crystallized with sulfamethazine. It is a broad class of antimicrobials used to inhibit the growth of pathogenic microorganisms. To select suitable solvents, Hansen Solubility Parameters (HSPs) were used to identify hydrogen bonding dependent solvents from which eight solvents with varying HSPs were selected. The crystals were prepared by solvent evaporation at two different temperatures 23 °C and 55 °C, respectively. It was found that in each solvent at different temperatures, different crystalline forms were obtained. The crystalline stability test showed that all co-

crystal polymorphs remained unchanged after storage at room temperature and ambient pressure for six months. The polymorphic forms obtained from different solvents are summarized in Table 2.1.

Table 2.1. Result of fast solvent evaporation (Salem, et al., 2021).

Solvent	**Temp (°C)**	**Form**
Ethanol	55	III
	23	I
2-Propanol	55	Mixed phases + III
	23	I
Methyl acetate	55	II
	23	Mixed phases
Ethyl acetate	55	II
	23	Mixed phases
Acetone	55	II
	23	II
Isopropyl acetate	55	Mixed phases
	23	I
Acetonitrile	55	II
	23	I
Methanol	55	II
	23	II

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(Wang, Zhou, Yu and Mei, 2014) investigated the stabilization of vitamin D_3 through conformationally selective co-crystallization. Vitamin D_3 is a highly unstable compound that rarely exists in a solid form. Therefore, vitamin D_3 is generally unstable in the solid state. In their study, vitamin D_3 was co-crystallized with cholesterol (1a) and cholestanol (1b) by slowly evaporating a supersaturated solution of these compounds. It was found that 1a was a colorless prism-shaped, while 1b was a thin prism-shaped crystal. When calculating the crystal densities of both 1a and 1b, they

were comparatively higher than pure vitamin D_3. As analyzed by XRPD, 1a and 1b had higher melting points than pure vitamin D_3. In the TG/DTA analysis, the crystals decomposed at 200°C. Then it was tested for stability, with storage at 40 °C/75% RH for six months, and exposure lighting at 5000 lx for ten days. After two days, vitamin D3 changed from a white to a brown powder in both conditions, but the cocrystals remained unchanged. After six months, both cocrystals were unchanged. It was found that the cocrystals could be stored for two years.

(Wang, et a., 2016) investigated drug–drug co-crystallization for the development of stable vitamin formulations. Combining the two drugs has advantages in reducing prescription numbers, lowering prices, and increasing patient compliance. Vitamin D is a fat-soluble vitamin. A lack of vitamin D is not only negatively affects bone health but also increases the risk of both acute and chronic diseases including infectious diseases, autoimmune disease and cardiovascular disease, as well as infertility, unwanted pregnancy and delivery. Vitamin D is sensitive to oxygen, heat and light. Vitamin D is rarely found in solid form because in general vitamin D is unstable in the solid state. Vitamins D_2 and D_3 play important roles in the human body. In this study, vitamin D_2 and D_3 were co-crystallized to form polymorphs A and B. Crystalline form A was obtained by complete crystallization, evaporation 1, using acetonitrile or methanol. It is a solvent at room temperature. Form B is crystallized by the rapid cooling method. Acetonitrile is used as a solvent at room temperature. DSC analysis revealed that the melting points of crystalline forms A and B were 97 and 103 °C, respectively which are higher than that of vitamin D_3. TGA analysis, showed that degradation began at 200 °C for A and 170 °C for B, respectively. The stability was evaluated under storage at 40 °C/75% RH for six months and under light exposure at 5000 lx for ten days. After two days, vitamin D_3 changed from white to brown under both conditions, whereas form A remained unchanged. Form B showed a 69.4% decrease in experimental values after one month storage at 40 °C/75% RH and 8.4% after ten days of light exposure. However, exhibited no change throughout the test period.

(Nik, et al., 2019) investigate antioxidant and physicochemical properties of microencapsulated bioactive compounds in Securigera securidaca (L.) seed extracted by co-crystallization. Securigera securidaca seeds contain phenolic compounds that

have high antioxidant properties. This plant has the advantage of being able to grow at any temperature and humidity on Earth. In this research, the co-crystallization of sucrose and Securigera securidaca (L.) seed extract was studied. From being tested for antioxidant activity, it was found that the total phenolic content was as high as 198.27±0.04 mg of gallic acid/100 g of co-crystallized material, and the DPPH inhibiting activity was 56.74±1.49%. The crystalline sugar content was only 1.80±0.01% and co-crystallization exhibited low moisture content and good flowability.

(Marpaung, et al., 2020) studied the co- crystallization of butterfly pea (Clitoria ternatea) flower extract with sucrose to produce a powdered drink. Butterfly pea flowers are widely found in Southwest Asia and possess various health benefits, including antioxidant, anti-diabetic, anti-inflammatory and anti-cancer properties. These benefits as mainly attributed to the bioactive, compound anthocyanin, however, it is quite sensitive to heat. In this study, butterfly pea flowers extract was co-crystallized with sugar by vacuum evaporation method and used to prepare beverages. The stability of anthocyanin was evaluated by storing the sample at different temperatures (27, 40 and 50 °C). It was found that as the temperature increased, the stability of anthocyanin decreased to 27.99, 16.53 and 9.81 days, respectively.

(Bajaj and Singhal, 2021) studied the stability of vitamin B_{12} by co-crystallization. Vitamin B_{12} is very important for the body. A lack of vitamin B_{12} can cause disorders in the body such as those affecting the nervous system, anemia, dementia and endocrine disorders, however, vitamin B_{12} is quite sensitive to environmental factors. Therefore, it was co-crystallized with sucrose. In this research, various hydrocolloids, including guar gum, gum acacia, and starch Hi-Cap 100 were used as emulsifiers. Gum acacia was found to have the highest retention of vitamin B_{12} in sugar crystals. In the retention capacity test, it was found that it can be stored for
up to 23 months at 25 °C/33% RH.

(Queiroz, et al., 2022) studied the co-crystallization of sucrose-soluble fiber matrix. Dietary fiber is often under consumed even though it plays an important
role in supporting digestive health. In this research, sucrose was mixed with erythritol, a sugar alcohol beneficial to the body, and then co-crystallized with fibersol-2, a soluble dietary fiber with prebiotic properties. The resulting co-crystals exhibited low

moisture content and a water activity below 0.6, indicating the absence of mold or yeast growth. Based on physical and physicochemical analysis, the cocrystals behavior showed stable crystal behavior with no detectable structural change.

(Wang, et al., 2022) studied the incorporation of catechin or curcumin into co-crystallized sucrose. Catechin (hydrophilic) and curcumin (hydrophobic) both possess antioxidant properties. In this study, catechin and curcumin are pre-emulsified with soy protein to enhance their stability and solubility, followed by co-crystallization with sucrose. The antioxidant capacity was evaluated using the assay. It was found that sucrose co-crystallized with catechin and co-crystallized with curcumin exhibited high DPPH scavenging activity. The obtained cocrystals were then incorporated into the meatballs, which are known to generate large amount of free radicals. The effects of protein oxidation in meatballs containing cocrystals were analyzed. It was found that both types of cocrystals effectively inhibited carbonyl formation, indicating a protective effect against protein oxidation.

(Kaur, et al., 2021) investigated the co-crystallization of carotenoids with sucrose. The carotenoids in carrots can act as antioxidants. However, they are quite sensitive to heat and light. In this research, sucrose co-crystallized with carotenoids extracted from carrots. A concentration of 5 g of carotenoids per 100 ml of carrots was used, which represented the highest amount that could be crystallized in sugar syrup without affecting storage stability of the products. According to the analysis, the cocrystal exhibited low moisture content and hygroscopicity while showing high retention efficiency. The encapsulation efficiency reached 75-78% when the cocrystal samples were treated at elevated temperature. Only a slight color loss was observed, attributed to the natural degradation of pigments.

(Quast, et al., 2020) studied the co-crystallization of honey with sucrose. Honey can be difficult to store, as it often tends to crystallize within its packaging. In this research, honey derived from nine different floral sources was co-crystallized with sucrose. It was found that all samples exhibited water activity value below 0.6 indicating that the obtained crystals were dry and free of moisture. As a result, they could be stored without the risk of mold and yeast growth. However, based on the co-crystallization test, it was not recommended to store the samples at high temperature for extend periods because of thermal decomposition and undesirable

caramelization. The samples remained stable when stored at room temperature. Subsequenly, samples 2 and 4 were selected to be mixed into drinks for sensory evaluation. The result showed that samples were accepted by the participants.

(Jana and Singhal, 2023) investigated the co-crystallization of sucrose with Azadirachta indica extract (commonly known as Neem) and zinc sulfate to reduce bitterness and enhance nutritional value for food fortification. Zinc sulfate was added to mitigate the bitterness of neem while improving nutritional properties. The experiment showed that zinc sulfate effectively reduced the bitterness of neem juice. The polyphenol content of the co-crystallized sugar cubes, evaluated over 75 days of storage, decreased with increasing in temperature (5/25/35 °C) and relative humidity (33/63/93%).

Co-crystallization offers numerous advantages. It can improve the physicochemical properties of active pharmaceutical ingredients (APIs), such as stability, taste and solubility. Several studies have also reported that co-crystallization can enhance other properties. For instance, sugars, addition to providing sweetness, can act as antioxidants and serves as carriers for vitamins. Co-crystallization is particularly attractive because it is cost-effective, easy to produce and scalable. The present study aimed to co-crystallize sucrose with ascorbic acid (vitamin C). Vitamin C is a water-soluble compound; however, it is sensitive to heat and easily oxidized by oxygen in the air. This research aims to increase the stability of vitamin C and produce antioxidant sugar. Vitamin C is particularly important for individuals with diabetes. People with diabetics often lack vitamin C, and the body cannot synthesize it. However, if there is too much sucrose, it is not suitable for people with diabetes. Therefore, sucrose was reduced, and erythritol was added to this sugar. This research used a solution-based cocrystal preparation method. This preparation required supersaturation of the target drug and co-former which was initiated during the nucleation process through cooling crystallization.

CHAPTER III

RESEARCH METHODOLOGY

This research focused on the co-crystallization of sucrose, erythritol and ascorbic acid (vitamin C) by using food-grade sucrose obtained from Khonburi Sugar in Thailand. The cocrystals were prepared using a solution-based method. In this method, supersaturation was generated by cooling, which subsequently initiated nucleation and crystal growth.

3.1 Research Methodology

1) Design the experimental plan and apparatus.

2) Characterize the physical properties of cocrystal.

3) Determine the optimal sucrose and erythritol ratio for the formulation.

4) Observation of the morphology of cocrystal.

5) Evaluate the physiochemical properties.

6) Assess the antioxidant of ascorbic acid in cocrystal.

7) Investigate the stability of cocrystal.

3.2 Location of Research

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1) Equipment Building 1 (F1) at Suranaree University of Technology, Nakhon Ratchasima.

2) Equipment Building 10 (F10) at Suranaree University of Technology, Nakhon Ratchasima.

3) Equipment Building 14 (F14) at Suranaree University of Technology, Nakhon Ratchasima.

4) School of Engineering at Sugimoto Campus, Osaka Metropolitan University, Osaka, Japan.

3.3 Chemicals and Instrument

3.3.1 Material

1) Food-grade sucrose (Khonburi Sugar, Thailand)

2) Erythritol (Chemiphan Corporation Co., Ltd., Thailand)

3.3.2 Chemical

1) L-ascorbic acid (Kemaus, Australia)

2) DPPH (1,1-diphenyl-2-picrylhydrazyl) (Tokyo chemical industry, Japan)

3) Phosphoric Acid (FUJIFILM Wako Pure Chemical Corporation, Japan)

4) Ethanol (GR Grade) 99.9% (Duksan pure chemical, Korea)

5) Deionized water

3.3.3 Equipment

1) Magnetic stirrer with magnetic bar

2) Hotplate

3) Pipettes (1, and 5 ml)

4) Beakers (10, 25, 50, and 100 mL)

5) Jacketed reactors (50, and 100 mL)

6) Volumetric flask (100 mL)

7) Water bath

8) Graduated cylinder (10, and 25 mL)

9) Desiccator

10) Drying oven

11) Overhead stirrer

12) Vacuum pump

13) Buchner funnel and flask set

14) Membrane filter (0.2, and 0.45 µm)

15) Quartz and glass cuvette (3.5 mL)

16) Syringe (5, and 10 ml)

17) Refractometer

18) Mortar and pestle

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3.3.4 Measuring and analytical instruments

1) Cocrystal characterization.

- Scanning Electron Microscope (SEM)

2) Confirmation of cocrystal formation

- Fourier Transform Infrared Spectrometer (FTIR)
- X-ray Diffractometer (XRD)
- Thermogravimetric Analyzer (TG/DTA)

3) Determination of ascorbic acid content and stability

- High-performance liquid chromatography (HPLC)
- DPPH radical scavenging assay

4) Determination of moisture related properties

- Water activity meter
- Hygroscopicity test

3.4 Co-crystallization of sucrose and erythritol to determine the optimal ratio.

1) Sucrose (1 mol) was mixed with erythritol (1 mol) and dissolved in deionize (DI) water. The ratio between mixed sugars and water was maintained at 6:1 (g/mL).

2) The solution was preheated in a jacketed reactor at 100 °C, under continuous stirring for 30 minutes.

3) After heating, agitation was continued at room temperature until dry agglomerates were formed.

4) The obtained cocrystals were packed in amber zip-lock bags and stored at 4-6 °C under dry conditions for further analysis.

5) Step 1 to 4 were repeated while varying the ratio of sucrose and erythritol, as shown in Table 3.1.

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Table 3.1 Molar ratio of sucrose and erythritol used in the co-crystallization experiments.

Sucrose (%)	Erythritol (%)
66.7	33.3
60	40
50	50
40	60
33.3	66.7

3.5 Co-crystallization of sucrose, erythritol and ascorbic acid.

1) Sucrose (1 mol) was mixed with erythritol (1 mol) and dissolved in DI water. The ratio between mixed sugars and water maintained at 6:1 (g/mL).
2) The solution was preheated in a jacketed reactor at 100 °C under continuous stirring for 30 minutes.
3) The mixture was then continuously stirred, while the temperature reduced to 60 °C.
4) The appearance of slight turbidity in the sucrose-erythritol syrup; indicated the onset of the crystallization process. At this stage, 1.5 g of ascorbic acid was added to the system.
5) Cooling gel pads were then placed around jacketed reactor to further decrease the temperature. Agitation was continued at room temperature until agglomerates were formed.
6) The obtained cocrystals were packed in amber zip-lock bag and stored at 4-6 °C under dry conditions for subsequent analysis.

3.6 Crystal Characterization

3.6.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (FTIR) was employed to analyze the functional groups on the sample surface by observing the energy absorbed and transmitted when exposed to infrared radiation. The characteristic peaks appearing at specific vibrational frequencies or wavenumbers (cm^{-1}) correspond to the

functional groups of the compound. The analysis was performed using a Bruker Tensor 27 FTIR with an Attenuated Total Reflectance (ATR) accessory, and a KBr sample holder, as shown in Figure 3.1. The sample powder was placed on the KBr holder, and spectra were recorded in the wave-number range of 4000-400 cm^{-1} with a resolution of 2 cm^{-1}.

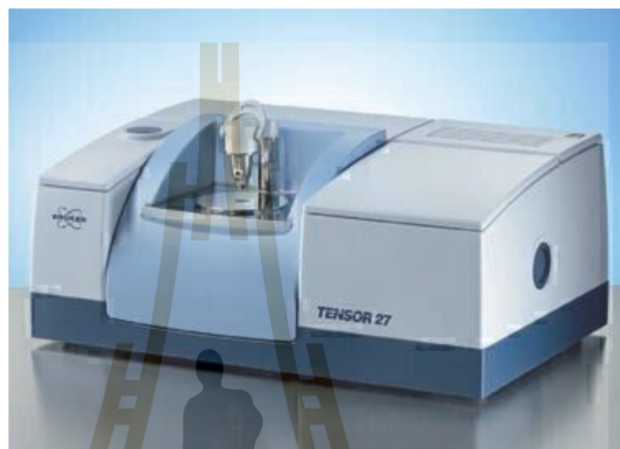

Figure 3.1 Bruker Tensor 27 FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) accessory.

(Ref: https://www.labmate-online.com/news/chromatography/1/bruker-optics-gmbh-co-kg/new-platinum-atr/9173)

3.6.2 Powder x-ray Diffractometer (XRD)

The powder X-ray diffractometer (PXRD) is an analytical instrument used for studying the structural characteristics of materials, through non-destructive analytical technique. It allows both qualitative and quantitative investigation of the crystal structure, and atomic arrangement within molecular compounds. The sample was ground into a fine powder using mortar and pestle, then placed in a sample holder for PXRD analysis. PXRD equipment used in this study is shown in Figure 3.2

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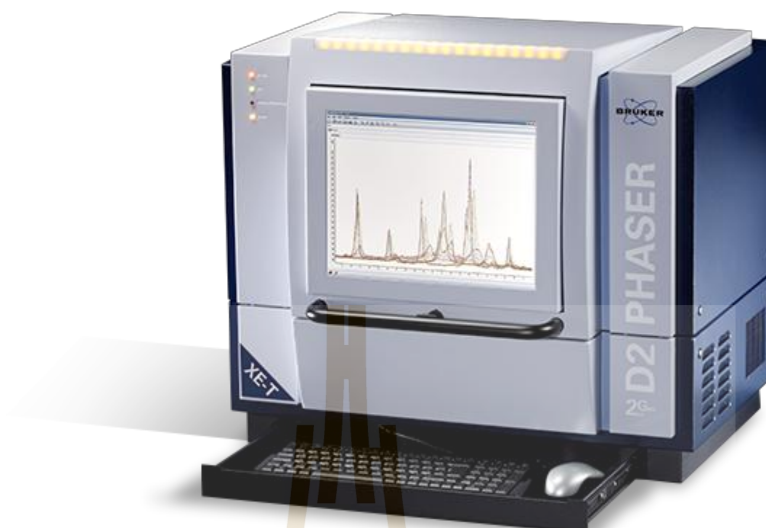

Figure 3.2 Powder X-ray Diffraction (PXRD) equipment (D2 PHASER)
(Ref: https://www.bruker.com/ja/products-and-solutions/diffractometers-and-x-ray-microscopes/x-ray-diffractometers/d2-phaser.html)

3.6.3 Thermogravimetric Analysis (TGA)

The thermal behavior of the cocrystals was examined using a thermogravimetric analyzer (Rigaku Thermo plus TG 8120). Samples of 2-5 mg were placed in aluminum pans, while an empty pan was used as a reference. Samples were heated from 25 °C to 250 °C at heating rate was of 10 °C/min under a nitrogen atmosphere. TGA instrument used in this study is shown in Figure 3.3.

Figure 3.3. Rigaku Thermo Plus TG 8120 Thermogravimetric Analyzer (TGA).
(Ref: https://www.ebay.com/itm/156614569339)

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3.6.4 Scanning Electron Microscope (SEM)

Scanning Electron Microscopy (SEM) was used to examine the morphology of co-crystallized products. This technique employs a focused beam of electrons that interacts with the surface of the sample, generating a variety of signals that are subsequently converted into high-resolution images. The analysis procedure consisted of sample preparation and imaging steps as follows:

1) Cut the carbon tape into small pieces and attach them to the stube. Lebel the sample number.
2) Spread the cocrystals on the carbon tape attached to the stube. Remove excess powder using a rubber dust blower.
3) Coat the sample with gold to prevent charging and ensure good electrical conductivity, which is essential for obtaining clear images.
4) Insert the coated sample into the analysis chamber of the SEM (JEOL JSM-6010LV), and imaging was performed at an accelerating voltage of 10 kV. The SEM instrument used in this study is shown in Figure 3.4.

Figure 3.4 SEM JEOL JSM-6010LV

(Ref: https://analytical.sut.ac.th/eq_page.php?id=1004)

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3.7 Physicochemical property of Cocrystal

3.7.1 Water Activity

Figure 3.5 Water Activity Meter

(Ref: https://os4u.com.bd/product/benchtop-water-activity-meter-price-in-bangladesh-aqualab-pre)

The water activity meter used in this study is shown in Figure 3.5.

1) Place the sample into the bowl of water activity meter, filling approximately half of the bowl.
2) Put the bowl into the water activity meter by rotating the load button.
3) Start the measurement; the water activity meter automatically determines the water activity of sample at room temperature.
4) When the reading is complete, the water activity value is displayed. Remove the sample bowl from the instrument
5) Repeat the measurement for another samples.

3.8.2 Hygroscopicity

1) Prepare a saturated sodium chloride (NaCl), which maintains a relative humidity of 75%.
2) Place the saturated NaCl solution into a sealed container.
3) Allow the saturated NaCl solution to equilibrate the humidity inside the container.

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4) Weigh the co-crystal, sucrose, and erythritol samples, and record their initial weights.
5) Place the samples into the sealed container prepared in step (3).
6) Weigh the cocrystal, sucrose, and erythritol again after 7 days.
7) Calculate hygroscopicity from the equation below

$$\% \text{ Hygroscopicity} = (W_t - W_0) \times 100 / W_0 \qquad \text{Eq (1)}$$

Where W_t is final weight of the sample.

W_0 is beginning weight of the sample.

3.8 Antioxidant Activity of Cocrystal and Ascorbic Acid

3.8.1 Determination of DPPH radical scavenging activity.

1) Weigh 0.0394 g of DPPH powder to prepare the solution.
2) Dissolve the DPPH powder in ethanol to prepare a 0.1 mM solution (1000 mL).
3) Store the DPPH solution into the dark to prevent light-induced degradation.
4) Mix 2 mL of the DPPH solution with 2 mL of the ascorbic acid solution.
5) Dissolve 1 g of cocrystal in 10 mL of DI water.
6) Mix 2 mL of the DPPH solution with 2 mL of the cocrystal solution.
7) Incubate the mixtures from steps 4) and 6) at room temperature for 30 minutes.
8) After incubation, measure the absorbance at 517 nm using a UV-vis spectrophotometer (Figure 3.6).
9) Calculate % DPPH radical scavenging activity by following equation:

$$DPPH\ radical\ scavenging\ activity\ \% = \frac{A_c - A_s}{A_c} \times 100 \qquad \text{Eq (2)}$$

Where A_c is the absorbance of control DPPH (Blank).

A_s is the absorbance of the sample.

Figure 3.6 UV-Vis Spectrophotometer (SP-UV 200)

(Ref:

http://www.labsciences.com.my/index.php?ws=showproducts&products_id=4797885)

3.8.2 Ascorbic acid analysis by HPLC

1) Prepare 0.02 M phosphoric acid to be used as the mobile phase.
2) Use a $5C_{18}$-PAQ column for separation.
3) Set the flow rate to 1.0 mL/min.
4) Set the UV detector wavelength to 245 nm.
5) Prepare ascorbic acid standard solution with concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mg/mL.
6) Inject each standard solution into HPLC system.
7) Record the chromatograms and determine the peak areas to construct a calibration curve, as shown in Figure 3.7.

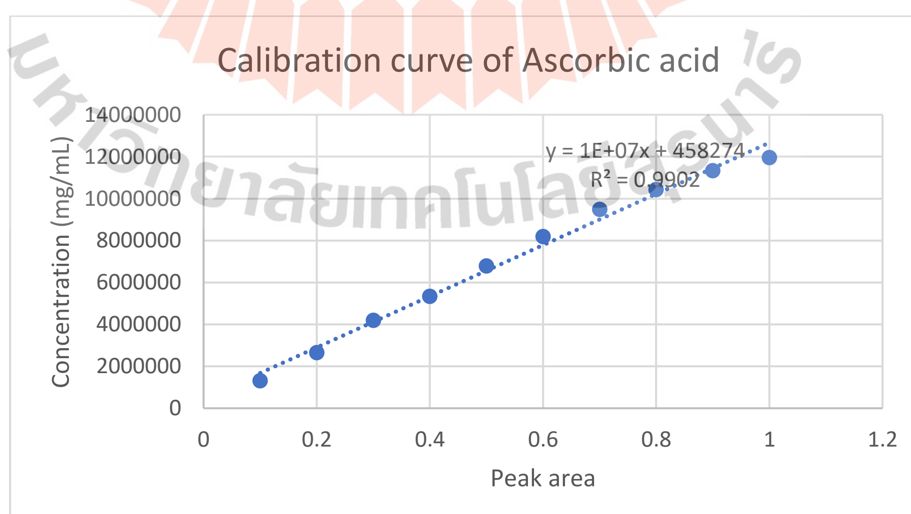

Figure 3.7 Calibration curve of ascorbic acid.

8) Weigh 10 mg of the cocrystals sample.

9) Dissolve the weighed samples in 1 mL of DI water.

10) Inject the cocrystals solution into the HPLC system.

11) Record the chromatogram and determine the peak area.

12) Determine the concentration of ascorbic acid using the calibration curve shown in Figure 3.7

Figure 3.8 High-performance liquid chromatography (HPLC) system.

3.9 Dissolution rate

1) Add 5 g of the sample into 50 mL of distilled water maintained at 35 °C

2) Stir continuously using a magnetic stirrer at 270 rpm.

3) Withdraw aliquots at different time intervals.

4) The dissolved sample in the solution was determined using a refractometer (IMS, MSDR-P2-102) until the refractive index reached equilibrium.

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Figure 3.9 Refractometer (IMS, MSDR-P2-102)
(Ref: https://www.ims-refractometer.com/Portable-Brix-Digital-Refractometer-pd783661098.html).

3.10 Solubility of cocrystal (Using Gravimetric Analysis)

1) Maintain the temperature at 37 °C.
2) Add the cocrystal sample to 10 mL of DI water and stir with a magnetic stirrer until further dissolution occurs.
3) Filter the solution using vacuum pump, with a Büchner funnel and flask set.
4) Evaporate the filtrate at 50 °C until only solid residue remains.
5) Weigh the dried residue.

3.11 Stability Test

1) The cocrystal and pure ascorbic acid samples were stored in an oven maintained 25 and 40 °C.
2) The storage conditions were free from light and humidity.
3) The samples were tested by DPPH radical scavenging activity every week for one month, following the same procedure as described in Section 3.9.1.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter presents and discusses the result of co-crystallization process involving sucrose, erythritol, and ascorbic acid (vitamin C). After co-crystallization, the obtained product was characterized for its morphology by Scanning Electron Microscope (SEM) and confirmed as a cocrystal through Fourier Transform Infrared Spectroscopy (FTIR), Powder X-ray Diffraction (PXRD) and Thermogravimetric Analysis (TGA). TGA is also used to determine the melting point of the cocrystal. The water activity and hygroscopicity of the co-crystallized products were measured to evaluate its physicochemical properties. The antioxidant capacities and stability of cocrystal were assessed using the DPPH radical scavenging activity assay. In addition, the dissolution rate was determined by using a refractometer, and the solubility of cocrystal was evaluated by gravimetric analysis.

4.1 Crystal Characterization

4.1.1 FTIR analysis

Fourier Transform Infrared (FTIR) spectroscopy was employed to investigate the chemical structure of the cocrystals, focusing on variations in the absorbance bands of functional groups (Kaur et al., 2021). Figure 4.1 shows the FTIR spectra of all sucrose-erythritol ratios, as well as pure, sucrose and erythritol. Figure 4.1 (a) displays the FTIR spectra of sucrose-erythritol at different ratios, all of which similar vibrational bands. However, differences in absorbance intensity were observed, which can be attributed to variations in the concentration of sucrose and erythritol. This observation is consistent with the Beer–Lambert law, "which states that absorbance is directly proportional to the concentration of the absorbing species and the optical path length of the sample" (Li et al., 2022). No significant difference were detected among the variosmolar ratios of sucrose and erythritol. Therefore, one ratio of sucrose-erythritol

(1:1) was selected for comparison with pure sucrose and erythritol as shown in Figure 4.1 (b).

Figure 4.1 (b) presents the FTIR spectra of sucrose, erythritol, and 1:1 sucrose-erythritol cocrystal. Sucrose displayed characteristic –OH stretching bands associated with hydrogen bonding at 3562, 3384 and 3333 cm^{-1}. The band between 3000 and 2800 cm^{-1} corresponded to C-H stretching vibration. The region between 1500 and 800 cm^{-1}, known as the fingerprint region, was consistent with previous reports (Sritham & Gunasekaran, 2017; Queiroz et al., 2022 ;NIST Chemistry Webbook).

Erythritol displayed an –OH stretching band associated with hydrogen bonding at 3227 cm^{-1}. C-H stretching vibrations were group observed at 2980-2900 cm^{-1} in agreement with Yang et al., (2004). The peaks at 1078 cm^{-1} and 1051 cm^{-1} corresponded to -OH groups of primary and secondary alcohol, respectively, which are key functional sites involved in the co-crystal formation (Queiroz et al., 2022).

In the 1:1 sucrose–erythritol cocrystal, the absorption band of the –OH group at 3562 cm^{-1} exhibited noticeable shift compared with pure sucrose, indicating a modification in hydrogen-bonding interaction. Similarly, the erythritol at 1078 cm^{-1} and 1051 cm^{-1} shifted to 1066 cm^{-1} and 1046 cm^{-1} in 1:1 cocrystal, indicating hydrogen bond formation between sucrose and erythritol (Sun et al., 2025).

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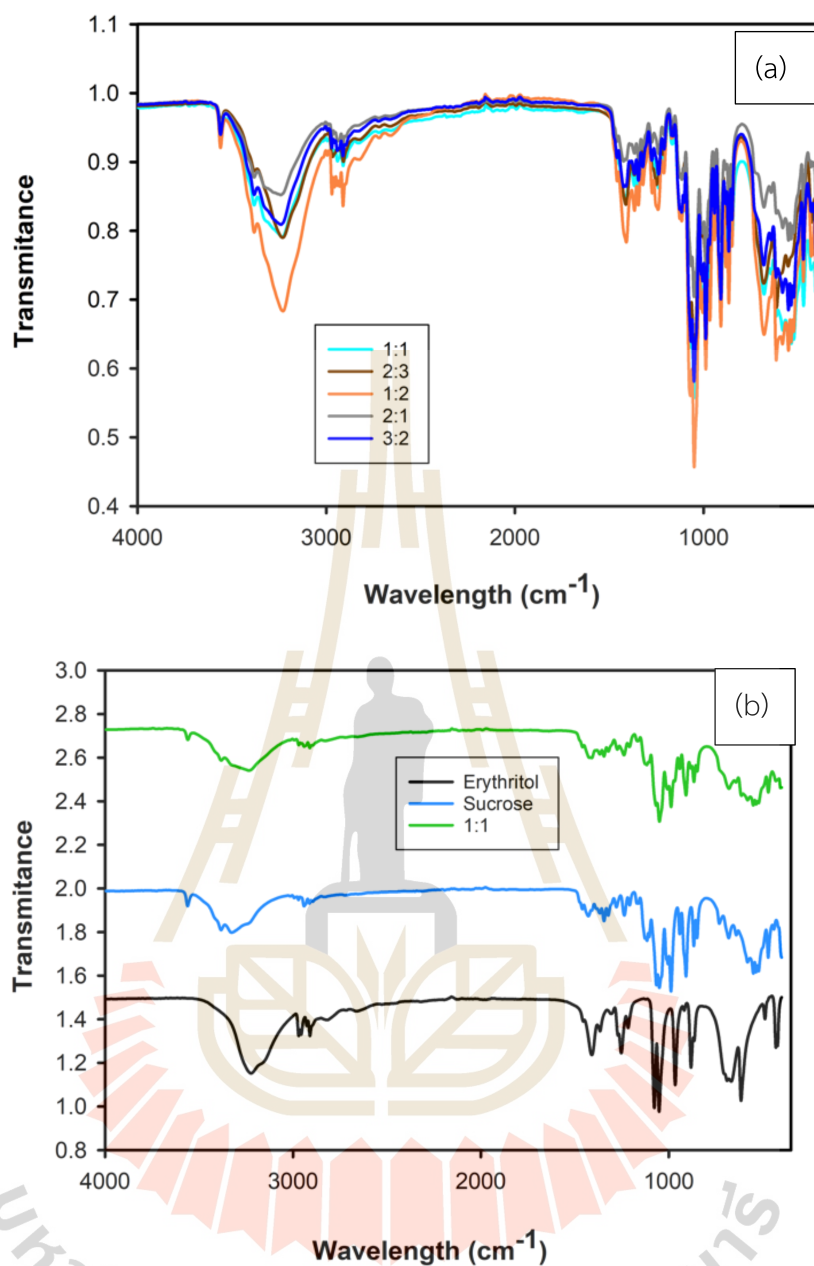

Figure 4.1 FTIR spectra of (a) sucrose-erythritol cocrystal of all ratios and (b) sucrose, erythritol, and 1:1 (sucrose: erythritol cocrystal).

Figure 4.2 shows the FTIR spectra of sucrose–erythritol–ascorbic acid cocrystal at different ratios, as well as pure ascorbic acid, the 1:1 (sucrose-erythritol cocrystal), and the 1:1 C (sucrose–erythritol–ascorbic acid) cocrystal. Figure 4.2 (a) presents the FTIR spectra of sucrose–erythritol–ascorbic acid cocrystal at all ratios which exhibited the similar vibrational band. However, differences in absorbance intensity, were observed among the ratios, which can be attributed to variations in the concentrations

of sucrose and erythritol, consistent with the Beer–Lambert law. Therefore, sucrose–erythritol–ascorbic acid cocrystal was selected for comparison with pure ascorbic acid and sucrose–erythritol as show in Figure 4.2 (b), to observe change in the FTIR spectra.

In Figure 4.2 (b), the FTIR spectra of ascorbic acid displays characteristic –OH stretching vibrations at 3524, 3406, 3309 and 3219 cm^{-1} consistent with the results reported by (Bichara et al., 2011). Ascorbic acid also exhibits a stretching vibration of the C=C double bond at 1653 cm^{-1} which agrees with the findings of (Umer et al., 2014). In addition, a characteristic absorption band at 1751 cm^{-1}, corresponding to the C=O stretching vibrations, consistent with the report by Sreeja et al., (2014). Figure 4.2 (b), 1:1 C exhibits vibrational bands clearly distinct from those of pure ascorbic acid. When comparing the spectra of 1:1 C and 1:1, distinct vibrational bands of 1:1 C were observed at 1760 cm^{-1} and 1660 cm^{-1}, corresponding to the stretching vibrations of C=O and vibration of C=C double bond, respectively, which are also presented in ascorbic acid. However, they were shifted from original position due to the presence of hydrogen bond formation between sucrose, erythritol and ascorbic acid (Indraa et al., 2019). It was confirmed that the co-crystallization process did not change the chemical structure of samples encapsulated with ascorbic acid (Wang et al., 2022).

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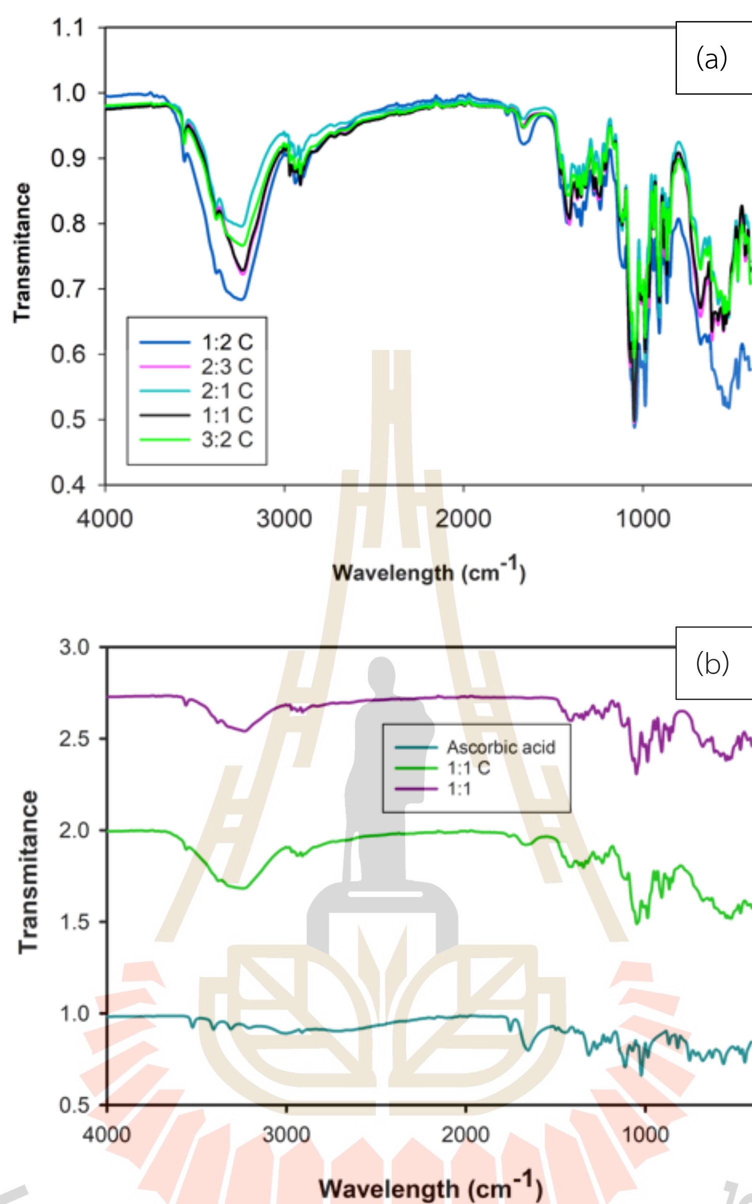

Figure 4.2 FTIR spectra of (a) sucrose-erythritol-ascorbic acid cocrystal of all ratios, and (b) ascorbic acid, sucrose-erythritol cocrystal and sucrose-erythritol-ascorbic acid cocrystal.

4.1.2 XRD analysis

Figure 4.3 shows the powder X-ray diffraction (PXRD) patterns of sucrose, erythritol, ascorbic acid, 2:3 (sucrose–erythritol cocrystal) and 2:3 C (sucrose–erythritol–ascorbic acid cocrystal). Sucrose displayed characteristic peaks at 2θ values of 8.70 °, 12.11°, 17.06°, 19.38°, 19.91°, 25.13°, and 25.60° which similar that reported by Wongwiwat & Wattanachant, 2014; Queiroz et al, 2022 and Li et al., 2009. Erythritol

showed dominant peaks at 2θ values of 14.18°, 19.12°, 20.08°, 24.29°, 29.30°, 32.05° and 41.93° that similar that found by Li et al., 2009; Hou et al., 2024 and Yan et al., 2021. Ascorbic acid exhibited high intensity peaks at 2θ values of 10.42°, 15.68°, 19.89°, 25.40°, 30.04° and 34.64°, which similar that reported by Palma-Rodríguez et al., 2018 and Ebnalwaled et al., 2022.

PXRD analysis of the 2:3 (sucrose–erythritol) and 2:3 C (sucrose–erythritol–ascorbic acid) cocrystals, in comparison with sucrose, erythritol, and ascorbic acid, demonstrated that the diffraction patterns of both cocrystals were significantly different from those of the individual compounds. The 2:3 sample exhibited characteristic crystalline peaks at 2θ values of 13.61° and 15.12° which were not observed in any of the individual compound. 2:3 C presented the characteristic crystalline peaks at 2θ values of 11.85°, 25.08°, and 50.97°. This individual peak in the diffractogram could suggest the interaction between sucrose, erythritol and ascorbic acid to form cocrystal after co-crystallization process. From the PXRD result, sucrose exhibited higher intensity peak than both of cocrystal. Therefore, both of cocrystal had lower crystallinity than sucrose which similar that reported by Kaur et al., 2021 and Queiroz et al., 2022.

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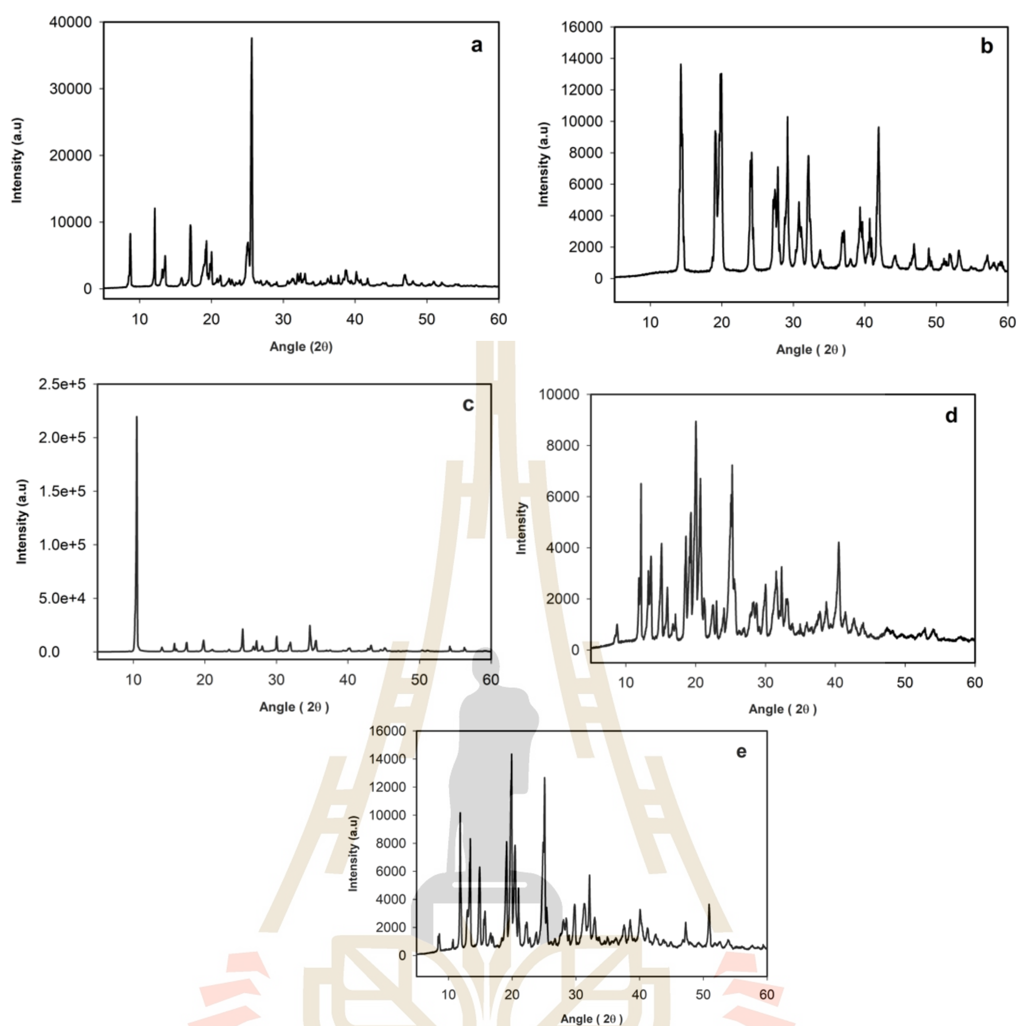

Figure 4.3 Powder X-ray diffraction patterns of (a) sucrose, (b) erythritol, (c) ascorbic acid, (d) sucrose–erythritol cocrystal and (e) sucrose–erythritol–ascorbic acid cocrystal.

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4.1.3 DTA analysis

Differential thermal analysis (DTA) was used to investigate the melting behavior of the prepared cocrystals. The melting point served as evidence of cocrystal formation, since the melting temperature of cocrystals generally differs from that of their individual components (Karagianni, Malamatari & Kachrimanis, 2018). According to Roos et al., 2012, sucrose shows a melting point of 185 °C, while erythritol and ascorbic acid display melting points of 121 °C and approximately 190–192 °C, respectively, as reported by the American Chemical Society.

As shown in Figure 4.4, the DTA curve of cocrystals displayed a distinct endothermic peak at 112.21 °C, which is significantly lower than those of the starting materials. This observation is consistent with the findings of Schultheiss & Newman, 2009, who reported that in a survey of 50 co-crystalline samples, 19 samples (39%) showed melting points lower than either the active pharmaceutical ingredient (API) or the coformer. Such a reduction in melting point is often associated with improved solubility and bioavailability, which are particularly advantageous in pharmaceutical applications (Stahly, 2007).

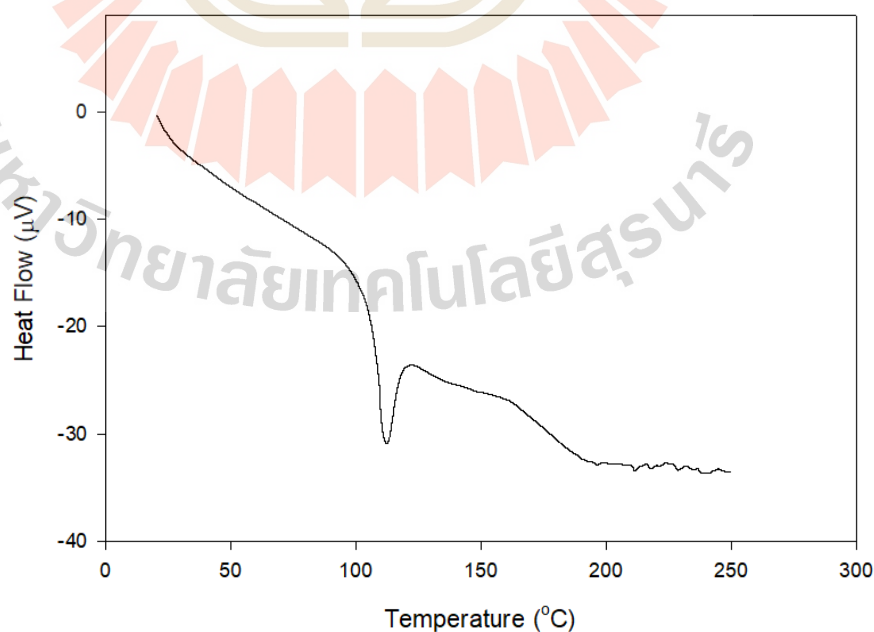

Figure 4.4 TG/DTA thermogram of the cocrystals.

4.1.4 SEM analysis

The morphology of the samples was examined using scanning electron microscopy (SEM) to observe the surface characteristics and particle structure (Chezanoglou & Goula, 2021). Figure 4.5 shows SEM micrographs of sucrose, erythritol, ascorbic acid, the 2:3 cocrystal (sucrose–erythritol), and 2:3C cocrystal (sucrose–erythritol–ascorbic acid). Sucrose and erythritol showed well-defined crystalline structures with sharp, distinct edges, consistent with previous reports by Queiroz et al., 2022. In contrast, ascorbic acid displayed an amorphous-like morphology, lacking well-defined crystalline edges, as previously described by Burgess, 2025, though some edge features were still observable. The SEM images of both the 2:3 cocrystal and 2:3C cocrystal revealed rough, aggregated surface morphologies resembling agglomerates. These cocrystals did not exhibit the clear, sharp edges seen in the individual components, which agrees reported in previous studies by Baja et al., 2021; Kaur et al., 2021; Wang et al., 2022 and Queiroz et al., 2022.

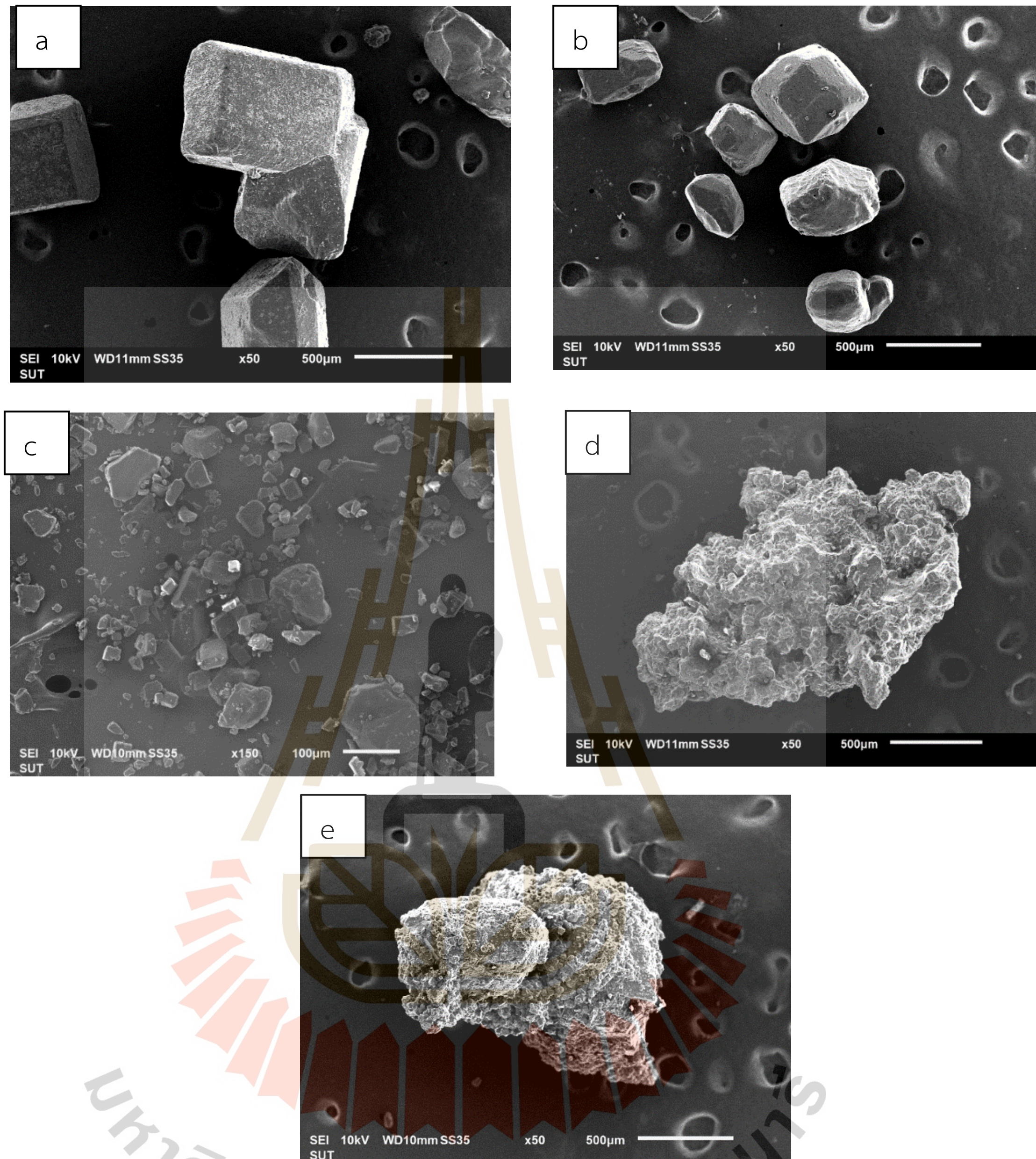

Figure 4.5 SEM micrographs of (a) sucrose, (b) erythritol, (c) ascorbic acid, (d) 2:3 cocrystal, and (e) 2:3 C cocrystal.

4.2 Physicochemical Property of Cocrystal

4.2.1 Water activity

Table 4.1 shows the water activity value of sucrose, erythritol, ascorbic acid, 2:3 cocrystals and 2:3 C cocrystal. Among the individual component, all showed the water activity value was higher than 0.6. For safe food stage, the water activity should

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be below 0.6 as microbial growth does not occur (Erkmen & Bozoglu, 2016) therefore the food can be safe. In 2:3 C cocrystal between sucrose and erythritol and ascorbic acid showed that the water activity was lowest. In all cocrystals between sucrose, erythritol and ascorbic acid, the water activity was lower than 0.6. This reduce can be attributed intermolecular interactions formed during co-crystallization, which reduce the likelihood of water molecules inserting themselves into the crystal lattice. As a result, water is less likely to exist in a high-energy, free state, leading to a decrease in water activity. Therefore 2:3 C was selected.

Table 4.1 Water activity of all samples.

Material	a_w
Sucrose	0.673 ± 0.00014
Erythritol	0.706 ± 0.00034
Ascorbic acid	0.720 ± 0.00008
2:3 (sucrose-erythritol)	0.65 ± 0.00002
2:3 C (sucrose-erythritol- ascorbic acid)	0.483 ± 0.00000
1:1 C	0.51 ± 0.00006
1:2 C	0.4985 ± 0.0002
2:1 C	0.584 ± 0.00018
3:2 C	0.576 ± 0.00022

4.2.2 Hygroscopicity

Hygroscopicity is an important factor in determining the packaging requirements of the product (Anbarasan et al., 2018). Table 4.2 presents the final weight of samples after 1 week in a sealed container containing sodium chloride (NaCl), which maintained a relative humidity of 75%. The 2:3 C cocrystal showed the highest hygroscopicity value. This is because the in cocrystal had a high surface capacity for moisture adsorption due to the presence of hydrophilic functional group (–OH) in the component molecules. Moreover, ascorbic acid is highly hydrophilic, meaning it is the ability to attract moisture from the air, especially when in its powder

form (Rowe et al., 2009). From the SEM result, cocrystal showed revealed rough, aggregated surface morphologies resembling agglomerates which cocrystal can absorb a high moisture. Therefore, cocrystal exhibitrd very high hygroscopicity. This research recommended that 2:3C cocrystal should be stored in a humidity-free environment.

Table 4.2 Hygroscopicity of all samples.

Material	Hygroscopicity (%)
Sucrose	0.112 ± 0.02414
Erythritol	0.085 ± 0.00025
2:3	0.174 ± 0.00042
2:3 C	25.964 ± 7.61281

4.3 Antioxidant of Cocrystal and Ascorbic Acid Analysis

4.3.1 Antioxidant of cocrystal

This research used DPPH radical scavenging activity assay to determine the antioxidant capacity of ascorbic acid and the cocrystal. This method, as reported by Alamu et al., 2017 utilizes DPPH radical scavenging activity to determine the antioxidant of ascorbic acid. Table 4.3 shows DPPH radical scavenging activity of the cocrystal and ascorbic acid which exhibited 86.225 and 87.276 %, respectively. The antioxidant capacity of the co-crystal was mainly attributed to the ascorbic acid incorporated during the co-crystallization process. The DPPH radical scavenging activity of the cocrystal was almost equal to that of ascorbic acid. Therefore, the cocrystal obtained through co-crystallization process can serve as both a sweeter and a compound with antioxidant capacity.

Table 4.3 DPPH inhibition activity of cocrystal and ascorbic acid.

Materials	**DPPH radical scavenging activity (%)**
Cocrystals	86.225 ± 0.033
Ascorbic acid	87.276 ± 0.033

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4.3.2 Ascorbic acid analysis by HPLC

Figure 4.6 presents the HPLC chromatogram of the 10 mg/mL cocrystal solution. As sucrose and erythritol did not exhibit absorbance at 245 nm, the observed chromatographic peak was attributed exclusively to ascorbic acid. The ascorbic acid peak appeared between 3.4 and 4.1 minutes, which was consistent with the retention time reported by Nagy et al., 2017. The concentration of ascorbic acid in the 10 mg/mL cocrystal solution was determined to be 0.201 ± 0.00029 mg/mL, indicating that the cocrystals contained 2.01 ± 0.029% ascorbic acid by weight. This corresponds to 93.8 ± 0.019% of the total theoretical ascorbic acid content within the cocrystals. The successful detection of ascorbic acid by HPLC confirmed its presence in the cocrystals, indicating that ascorbic acid remained intact after the co-crystallization process.

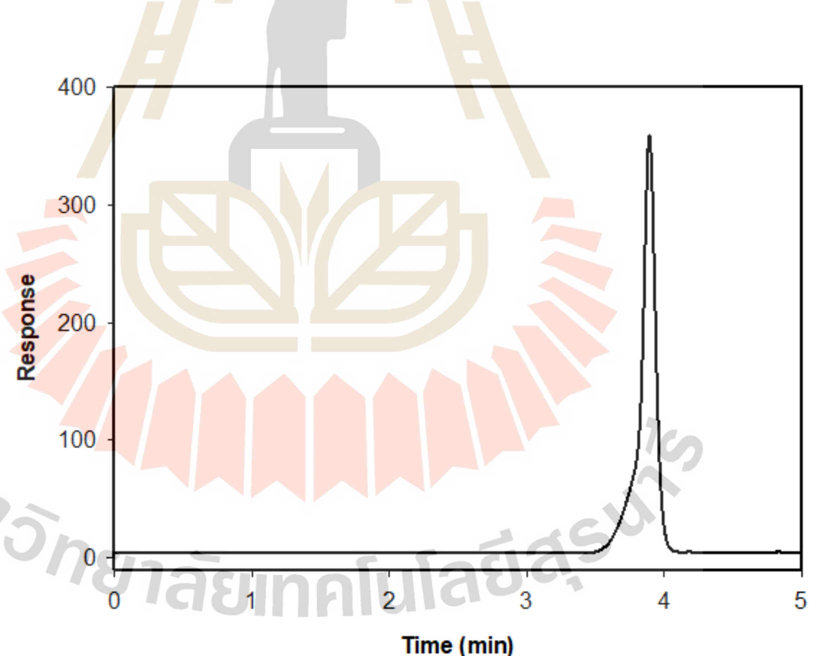

Figure 4.6 HPLC chromatogram of 10 mg/mL in cocrystal solution.

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4.4 Dissolution rate and solubility

4.4.1 Dissolution rate

Figure 4.7 shows the change in the concentration of erythritol, sucrose, ascorbic acid and cocrystal during dissolution in water at 35 °C. The initial slope of each curve represents the dissolution rate (Queiroz et al., 2022). Erythritol exhibited the fastest dissolution, since its polyol structure contain multiple hydroxyl groups (–OH) that form strong hydrogen bonds with water. Sucrose and the cocrystals displayed the similar in dissolution behavior, while ascorbic acid showed the slowest increase in concentration.

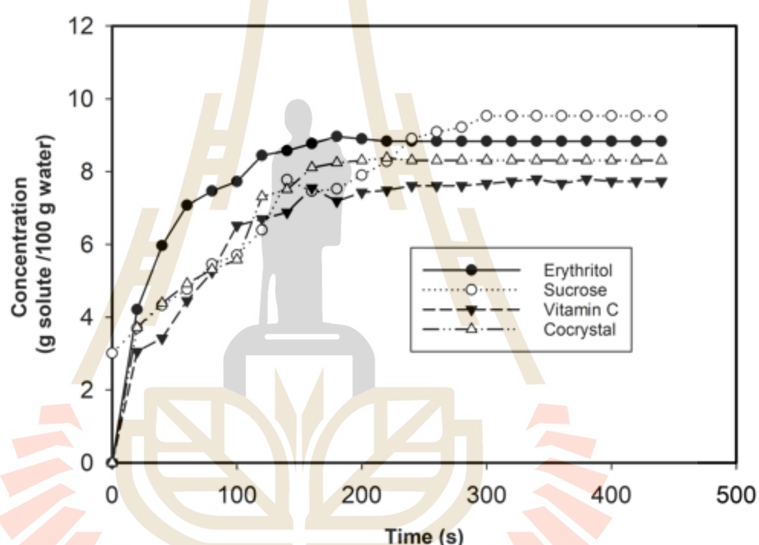

Figure 4.7 Concentration change during dissolution of erythritol, sucrose, ascorbic acid, and the co-crystal in water at 35 °C.

4.4.2 Solubility

The solubility of the cocrystal in water was determined at 37 °C corresponding to the human body temperature. Table 4.4 presents the solubility of sucrose, erythritol, ascorbic acid and the cocrystal at 37 °C. The solubility of sucrose at 37 °C was 2.34 g/mL (Hagen et al., 2019). The solubility of erythritol at 37 °C was 1.0 g/mL (Zumbe, Lee& Storey, 2001). The solubility of ascorbic acid at 37 °C was 0.47 g/mL (Nemdili et al., 2022). The solubility of cocrystal was determined to be 1.692 g/mL which lies between those sucrose, erythritol, and ascorbic acid. This difference attributed to altered intermolecular interactions after co-crystallization of sucrose,

erythritol, and ascorbic acid (Newman & Schultheiss, 2009). The solubility of the cocrystal was higher than that of ascorbic acid, indicating the cocrystal was able to dissolve in body fluid, making it easy to absorb and work effectively in the body (Bichara et al., 2011).

Therefore, the cocrystal is expected to exhibit greater bioavailability than pure ascorbic acid enabling more efficient uptake and biological activity in the human body.

Table 4.4 The solubility of all samples.

Material	Solubility (g/ml)
Sucrose	2.34
Erythritol	1
Ascorbic acid	0.47
Cocrystal (2:3 C)	1.692

4.5 Stability testing

The DPPH radical scavenging activity was assessed to evaluate the storage stability of the samples. The final decomposition product of ascorbic acid is 2,3-diketogulonic acid, which lacks biological activity (Ratniyom et al., 2017). Consequently, DPPH cannot directly detect 2,3-diketogulonic acid, as this compound does not exhibit a significant antioxidant effect. Table 4.5 presents the results of the storage stability study of the cocrystal and ascorbic acid after one month under different temperature and controlled humidity (no moisture). After one month 25 °C, DPPH radical scavenging activity of the cocrystal was 82.124 %, whereas that of ascorbic acid was 78.759 %. When stored at 40 °C after one month, the cocrystal retained 80.967 % of its DPPH radical scavenging activity, while ascorbic acid showed a markedly lower value of 64.984 %, indicating noticed a significant difference in thermal stability between two samples. The DPPH radical scavenging activity at 25 °C was higher than at 40 °C since ascorbic acid decomposes more rapidly at elevated temperatures (Essodolom et al., 2020). From these result, it can conclude that the

co-crystallization process improved the thermal stability of ascorbic acid. However, cocrystals are not recommended to be stored at high temperatures, as prolonged exposure to heat may compromise their stability and structural integrity.

Table 4.5 Storage stability studies of samples at different temperatures.

Materials	DPPH radical scavenging activity (%)	DPPH radical scavenging activity (%) after 1 month at 25 °C	DPPH radical scavenging activity (%) after 1 month at 40 °C
Cocrystals	86.225 ± 0.0332	82.124 ± 0.232	80.967 ± 0.033
Ascorbic acid	87.276 ± 0.0332	78.759 ± 0.033	64.984 ± 0.398

4.6 Antioxidant of cocrystal testing and comparing with the popular ascorbic acid beverages in Thailand.

Table 4.6 presents a comparative analysis of DPPH radical scavenging activity (%) between the cocrystal dissolved in pure water and two commercially available beverages in Thailand that are marketed as containing 200% of the recommended daily intake of ascorbic acid. The cocrystal demonstrated a DPPH radical scavenging activity of 86.23%, whereas ascorbic acid drink No. 1 and No. 2 showed 55.52% and 17.98%, respectively. These results indicate that the cocrystal possesses a significantly higher antioxidant capacity than selected commercial beverages. However, the antioxidant capacity of the commercial drink may have decreased due to storing vitamin C in a dissolved form, temperature fluctuations during transportation, or light-sensitive packaging. This finding suggests that the developed cocrystal in powder form has the potential to serve as a novel alternative sweetener suitable for general consumption while simultaneously providing the health benefits of ascorbic acid.

Table 4.6 Antioxidant capacity of cocrystal and popular ascorbic acid beverages in Thailand.

Materials	DPPH radical scavenging activity (%)
Cocrystals	86.225 ± 0.033
Drink with ascorbic acid No. 1	55.521
Drink with ascorbic acid No. 2	17.981

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusions

This study successfully achieved in the co-crystallization of sucrose, erythritol and ascorbic acid using a solution based cocrystal preparation method. During the co-crystallization process, the mix solution of sucrose–erythritol solution was rapidly cooled to 60 °C to prevent ascorbic acid degradation at high temperature, which was confirmed from HPLC analysis. The FTIR and PXRD analysis of all sucrose–erythritol ratios revealed similar characteristic diffraction and vibrational peak patterns. Therefore the 2:3 (sucrose: erythritol) ratio was selected for further investigation, as it was considered the most suitable for daily consumption, including for individuals with diabetes or people who need to control their body weight also can consume. The morphology of cocrystal were rugged, clusters agglomerates without well-defined crystalline edge observed in sucrose, erythritol and ascorbic acid.

The inclusion of ascorbic acid in cocrystal successfully confirmed by HPLC, showing ascorbic acid peak at 3.4 – 4.1 minutes. The concentration of ascorbic acid in 10 mg/mL of cocrystal solution was 0.22 mg/mL (2.18%), confirming that ascorbic acid remained intact after the co-crystallization process.

The antioxidant capacity test using the DPPH radical scavenging activity showed that cocrystal exhibited 86.12% activity, comparable to that of pure ascorbic acid (87.38%), indicating the cocrystal retained antioxidant activity similar to ascorbic acid. When compared with two popular ascorbic acid beverages in Thailand, the cocrystal showed that cocrystal also had higher DPPH radical scavenging activity than both. The storage stability testing for one month under moisture-free and light-free conditions at 25 and 40 °C, revealed that the cocrystal was more stable than ascorbic acid, with better preservation at 25 °C.

The water activity of cocrystal decreased to 0.483, which was lower than that of sucrose, erythritol and ascorbic acid and below the 0.6 threshold that inhibit microbial

growth. However, the cocrystal exhibited very high hygroscopicity, it was the ability to attract moisture from the air.

In summary, the cocrystal demonstrated desirable properties, including sweetness, antioxidant activity, benefit of ascorbic acid and low water activity. However, due to its high hygroscopicity, proper storage is essential. It is recommended that the cocrystal be stored in light-resistant, moisture-proof and tightly sealed packaging with silica gel, preferably at low temperature to prolong shelf life. Therefore, this study hopes the developed cocrystal will be the alternative sugar in the future which can be sweetener and had antioxidant property which will be used in food and beverages for daily consumption.

5.2 Recommendations

1) If ascorbic acid is mixed with hydrocolloid before co-crystallization, it will be increasing the stability of ascorbic acid.
2) Investigating release kinetics of this co-crystallized product under various gastrointestinal conditions. This will help calculate the efficiency of cocrystal in the body.
3) Conduct a taste test on a sample group to test the flavor and efficiency of ascorbic acid.

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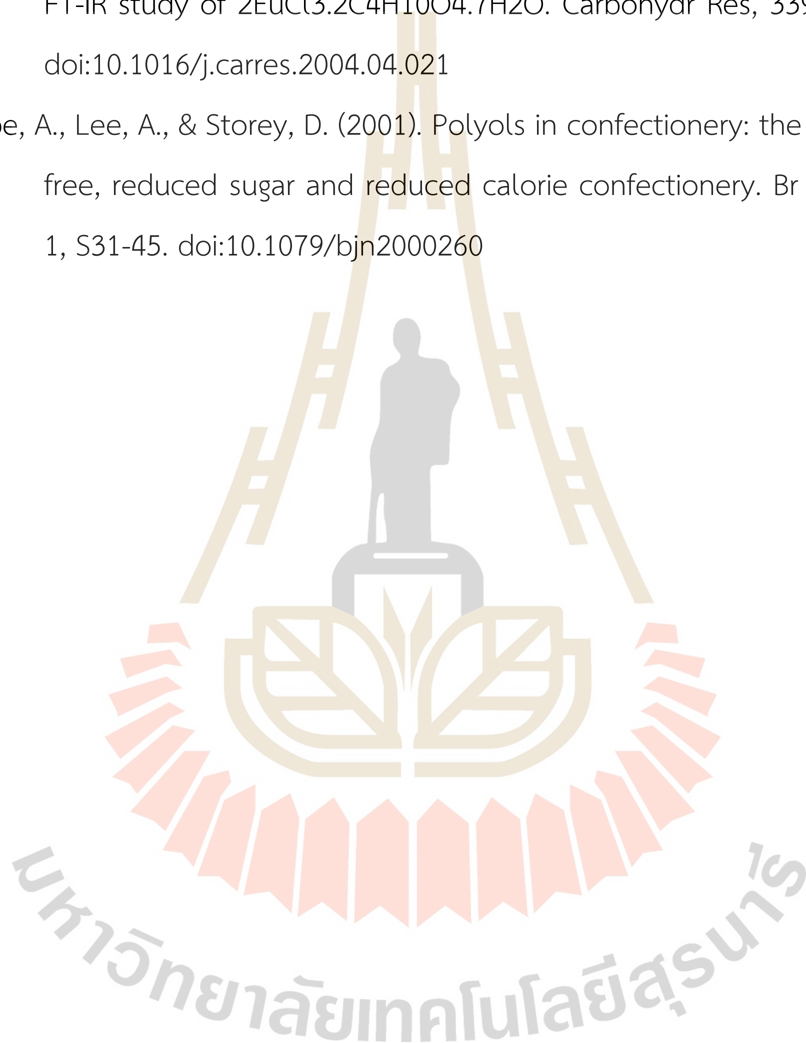

APPENDIX

SUPPLEMENTARY INFORMATION

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APPENDIX A

A.1 SEM images of sucrose-erythritol

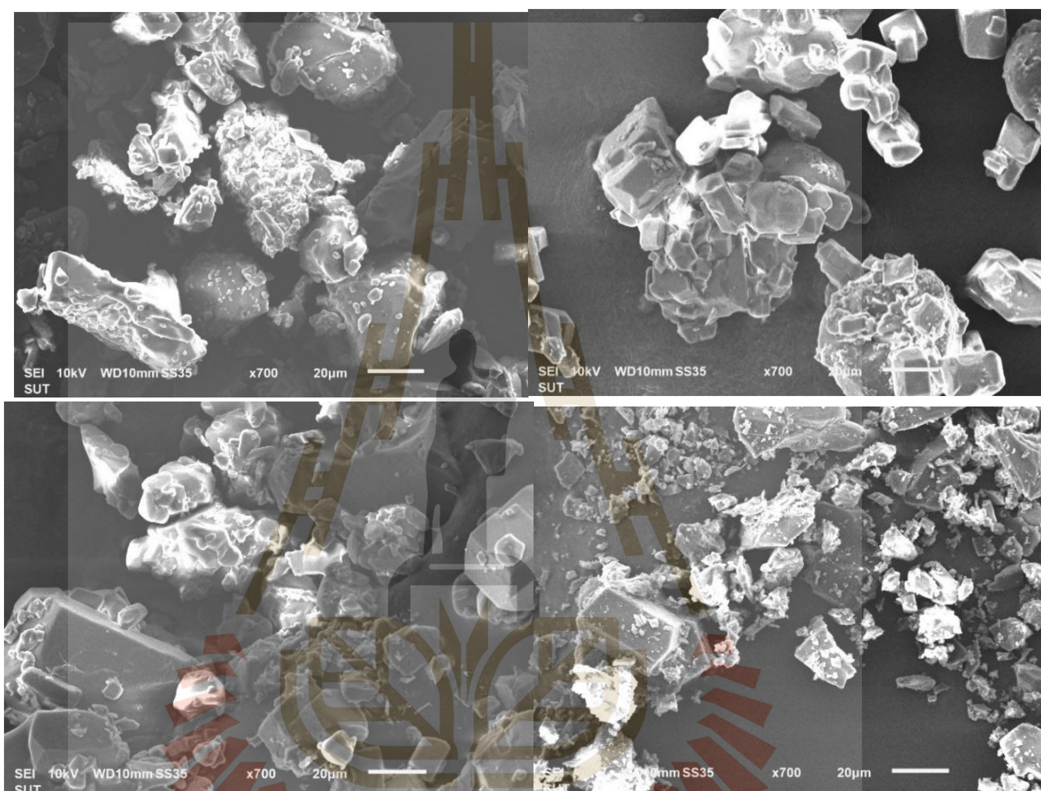

Figure A1. SEM images of sucrose-erythritol a. 1:1, b. 1:2, c. 2:1 and d. 3:2

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A.2 SEM images of sucrose-erythritol-ascorbic acid

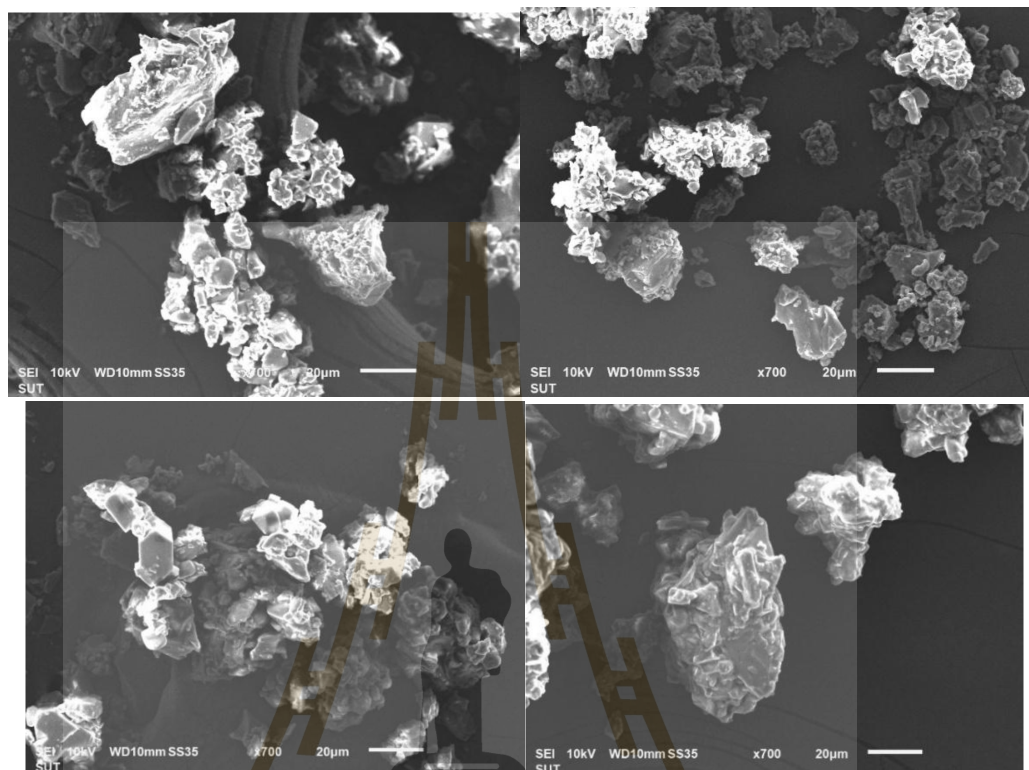

Figure A2. SEM images of cocrystal a. 1:1 C, b. 1:2 C, c. 2:1 C and d. 3:2 C.

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A3. XRD patterns of sucrose-erythritol.

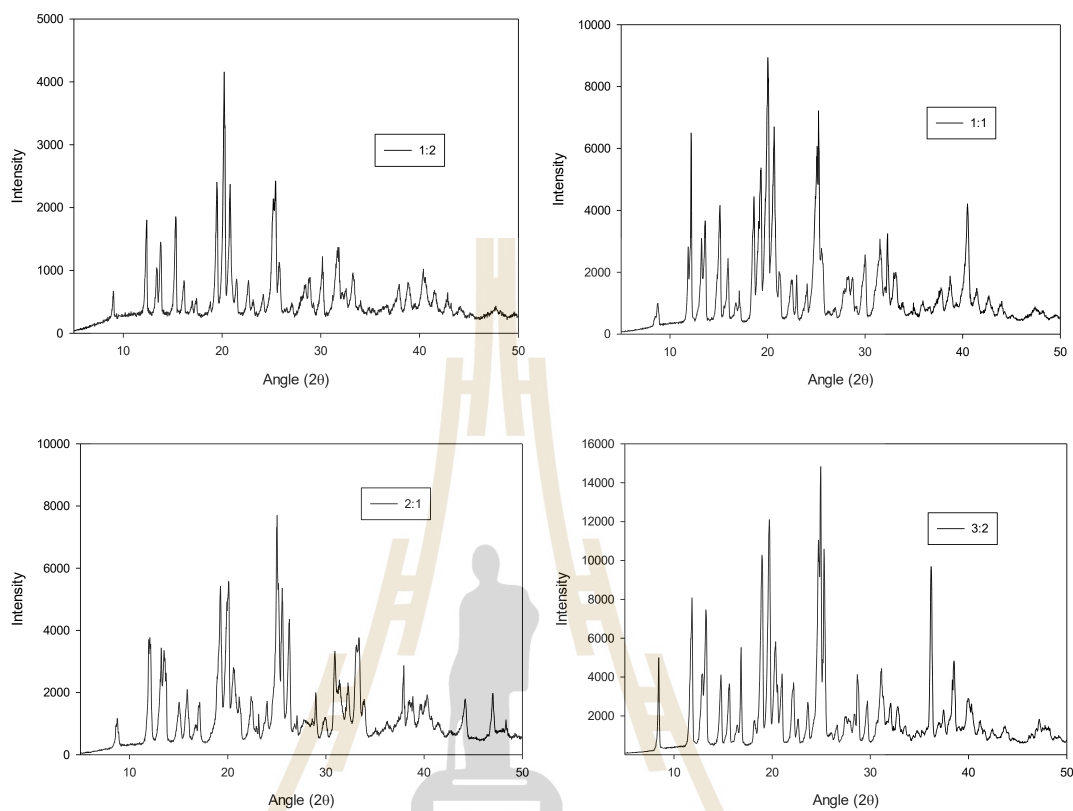

Fig A3. X-ray diffraction patterns of sucrose-erythritol 1:2, 1:1, 2:1 and 3:2.

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A4. Calibration curve of concentration and refractive index (RI) at 25 °C

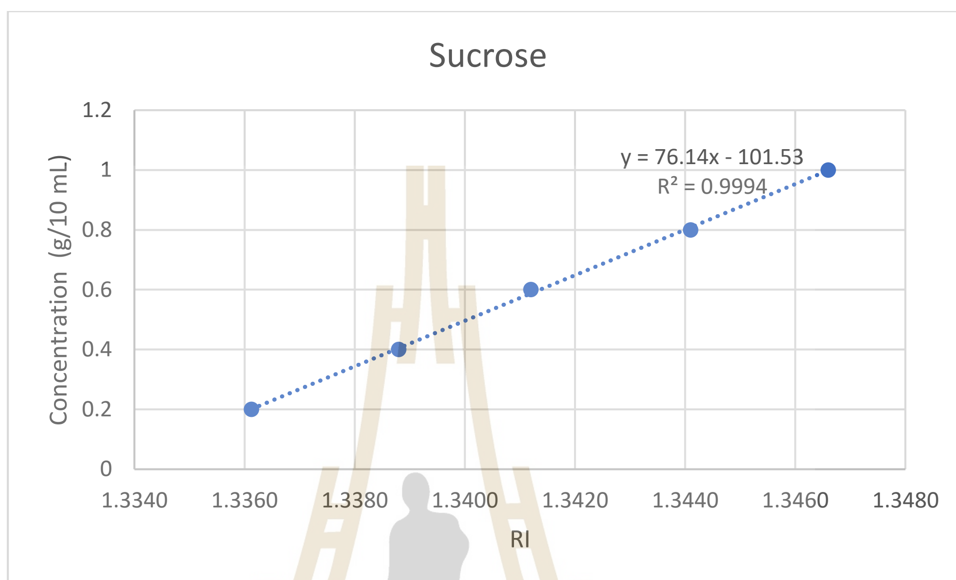

Fig A4. Concentration and refractive index of sucrose at 25 °C.

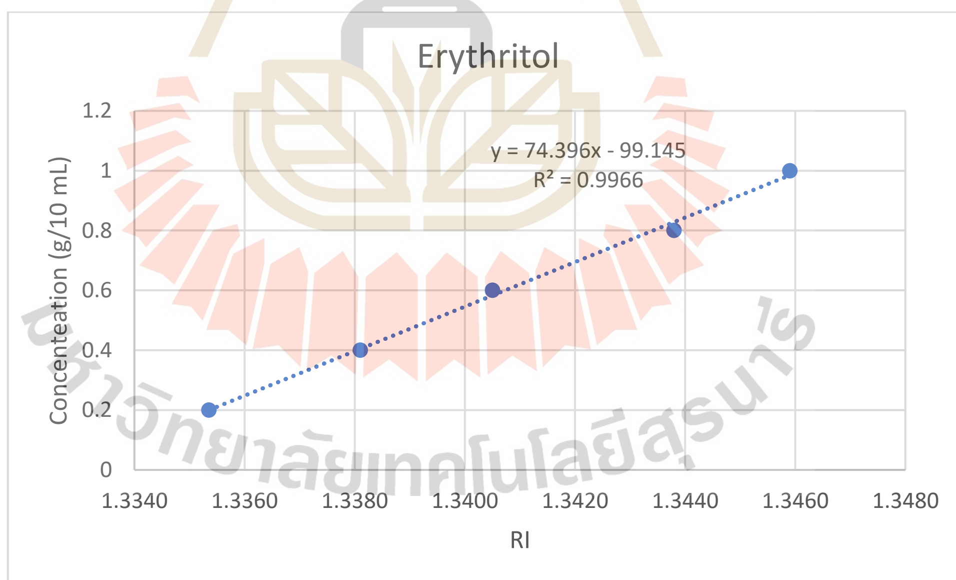

Fig A5. Concentration and refractive index of erythritol at 25 °C.

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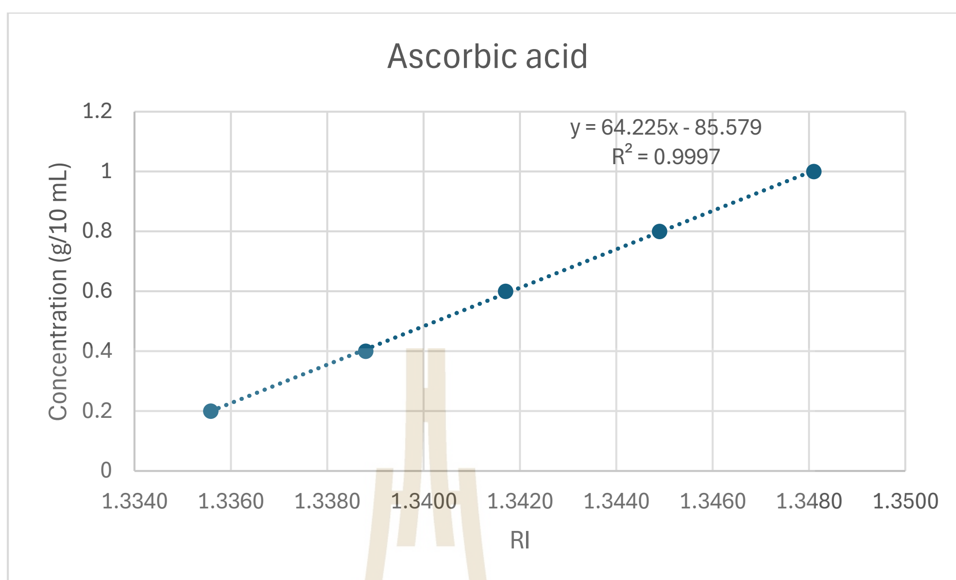

Fig A6. Concentration and refractive index of ascorbic acid at 25 °C.

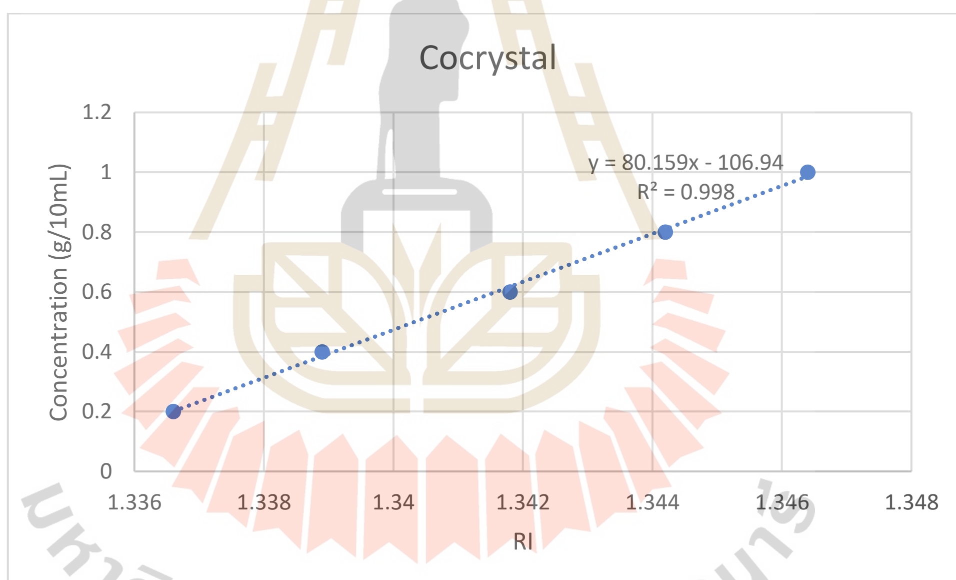

Fig A7. Concentration and refractive index of cocrystal at 25 °C.

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A4. Calibration curve of concentration and refractive index (RI) at 35 °C.

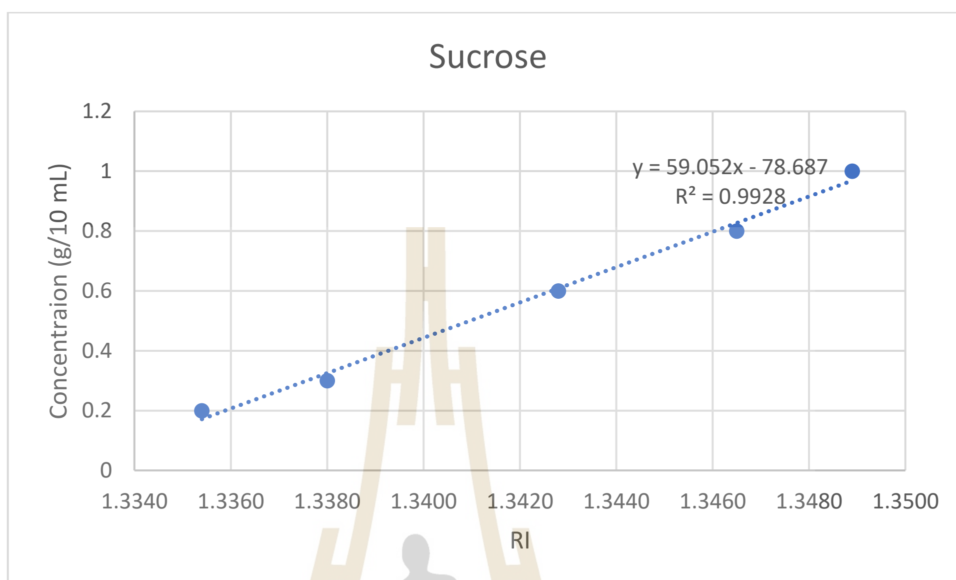

Fig A8. Concentration and refractive index of sucrose at 35 °C.

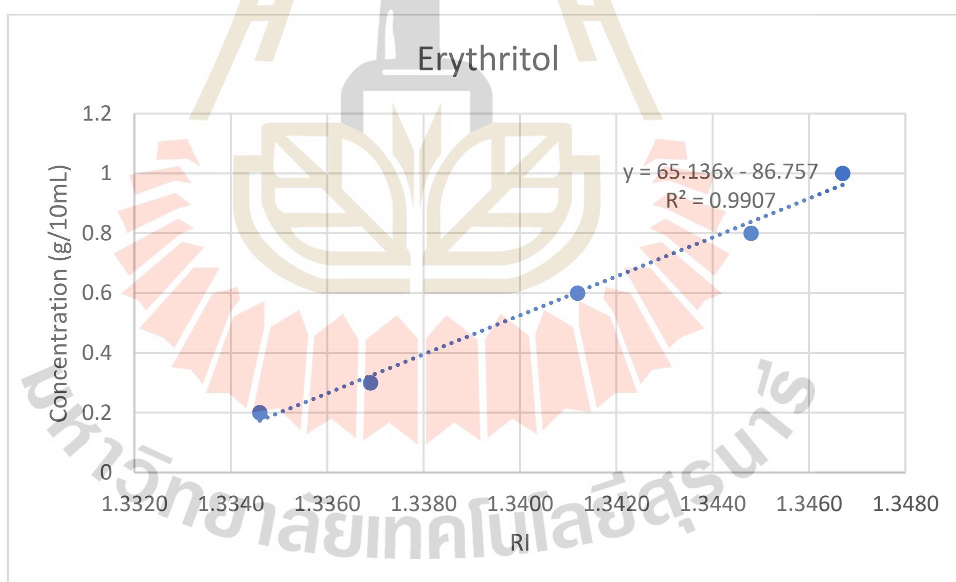

Fig A9. Concentration and refractive index of erythritol at 35 °C.

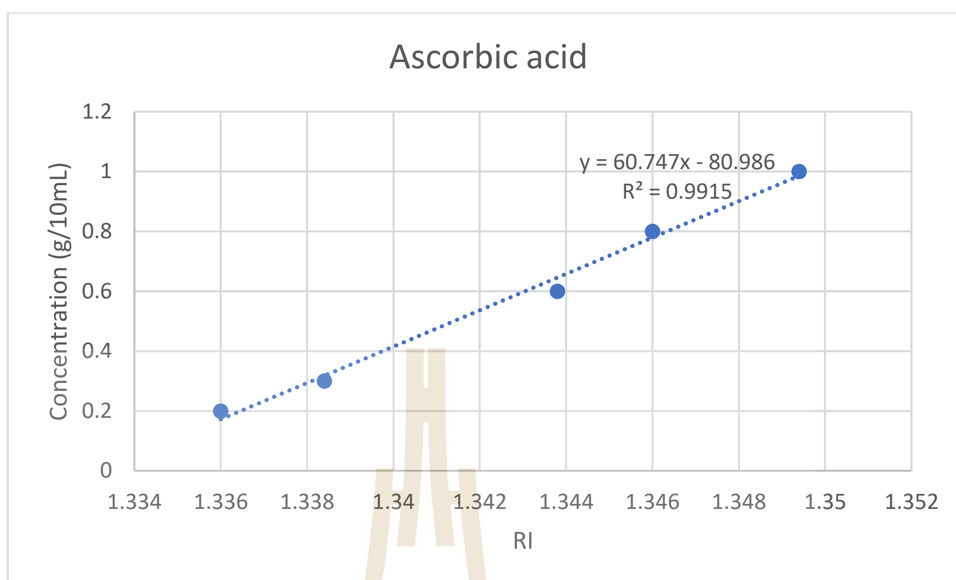

Fig A10. Concentration and refractive index of ascorbic acid at 35 °C.

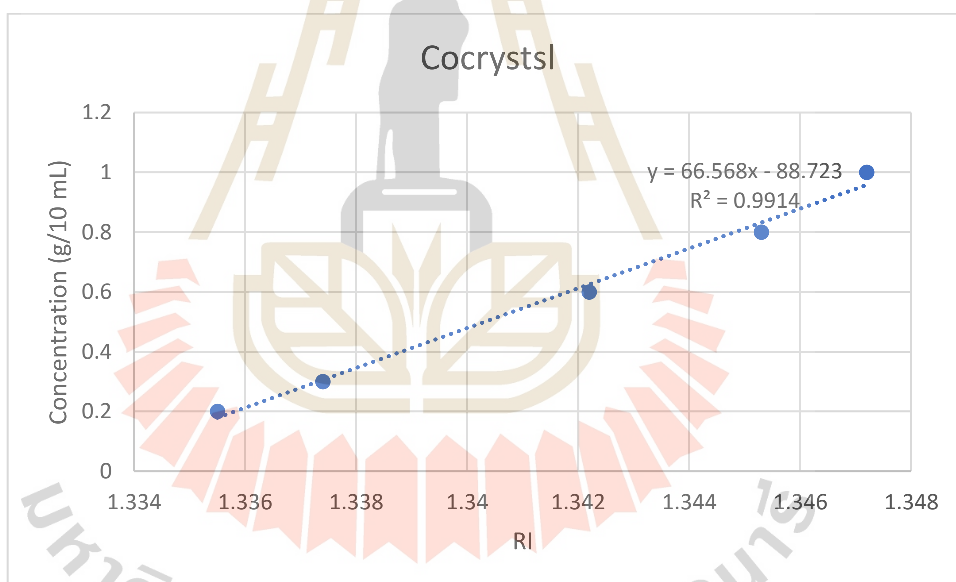

Fig A11. Concentration and refractive index of cocrystal at 35 °C.

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A3. Calibration curve of ascorbic acid in HPLC analysis.

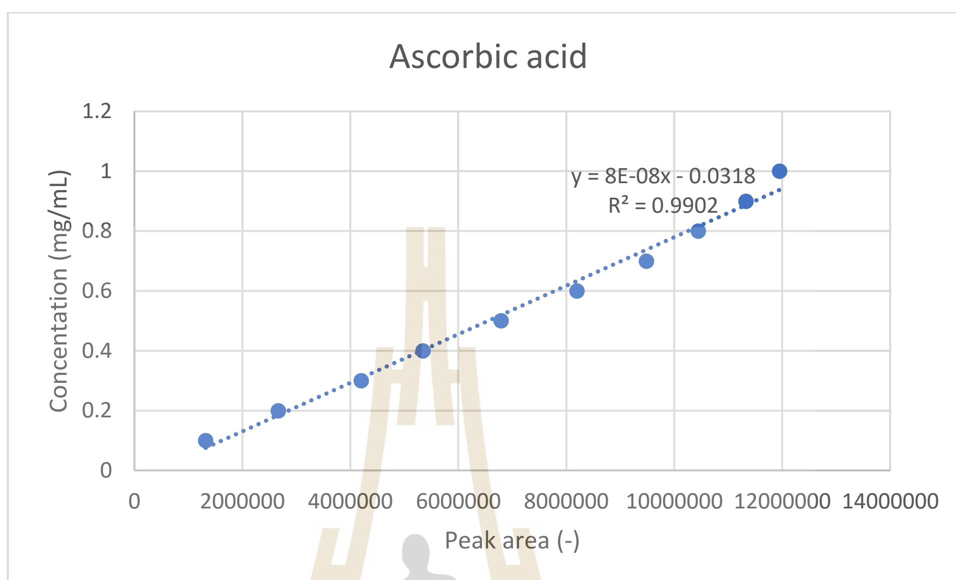

Fig A12. Concentration and peak area of ascorbic acid .

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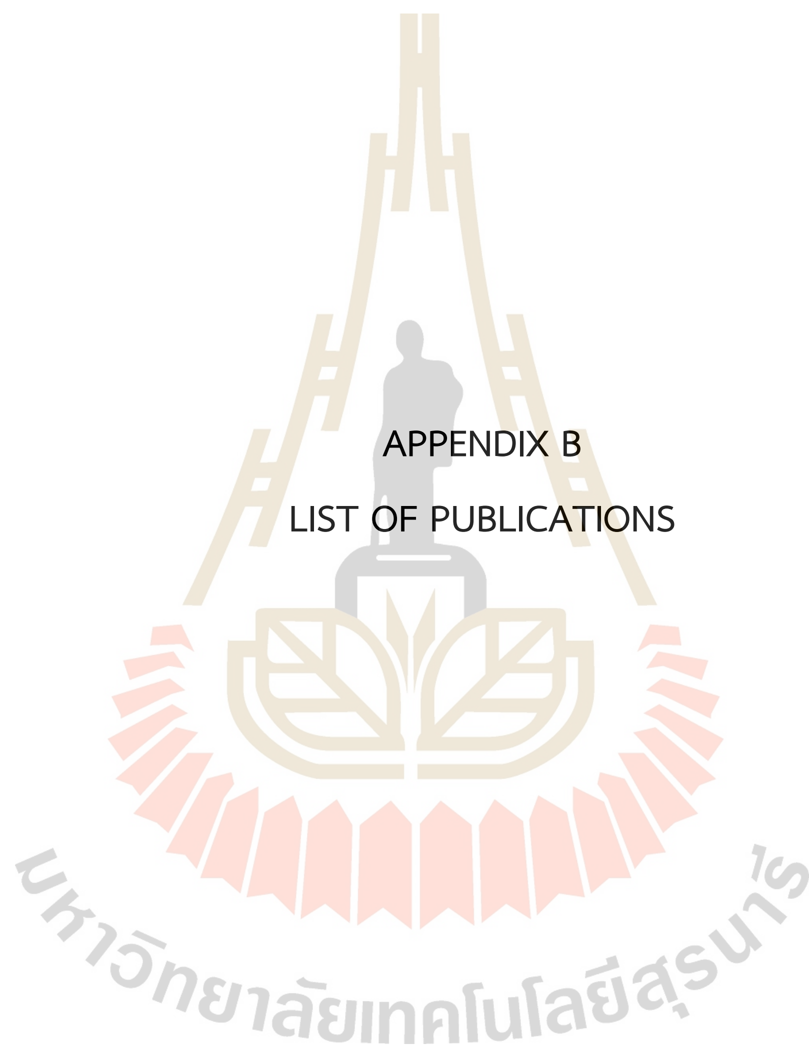

APPENDIX B

LIST OF PUBLICATIONS

B.1 List of publications

Norramon Sathanasaowaphak, and Lek Wantha (2025). Antioxidant Sugar from Co-crystallization of Sucrose, Erythritol and Vitamin C. The 34th Thai Institute of Chemical Engineering and Applied Chemistry Conference (2025), Pattaya, Thailand, May 20-21, 2025, organized by Department of Chemical Engineering, Faculty of Engineering, Khonkaen University. (Oral presentation)

Antioxidant sugar from co-crystallization of sucrose, erythritol and vitamin C

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ABSTRACT

Co-crystallization is a process of producing cocrystals. The co-crystals obtained through co-crystallization exhibited improved properties, such as increased solubility, stability, and flowability. Erythritol is a sugar alcohol used as a low-calorie sweetener. In this study, sucrose, erythritol, and vitamin C (L-ascorbic acid) were co-crystallized to determine a suitable molar ratio between sucrose and erythritol, which can be sweetening and suitable with diabetic patient and delivering vitamin C benefits. The physical properties of the co-crystals were analyzed using XRD, FTIR, and SEM. From FTIR result, all of molar ratio was the same. Therefore, the 2:3 was selected because this ratio is reasonable for daily consumption. Antioxidant activity of co-crystals was determined by DPPH inhibition activity. An antioxidant activity of co-crystals was 86.225%, while that of pure vitamin C was 87.276%. Therefore, Co-crystals can be a sweetener and an antioxidant.

Keywords: Co-crystallization, Vitamin C, Erythritol, Antioxidant.

1. INTRODUCTION

Thailand is the second largest sugar exporter in the world that reported by Shahbandeh [1]. Sugar has another name, "sucrose". Sugar is used everywhere in food, drink, candy, and more, and is consumed by people every day. Sugar is a sweetener, but excessive consumption can lead to various health issues such as diabetes, obesity, cardiovascular disease, and hyperlipidemia [2].

Co-crystallization is a co-crystal production process which crystals containing two or more molecules in a specific stoichiometric ratio within the lattice. Various molecular species involved in the co-crystal formation is called the coformer. The coformer molecules are primarily bonded by non-covalent interactions such as hydrogen or halogen bonds [3]. Many research used sucrose to co-crystallize with another active pharmaceutical ingredient (API) such as vitamin B12 [4], carotenoid in carrots [5], catechin or curcumin from plant that has antioxidant property [6], etc. In this research co-crystallized sucrose with vitamin C (L-ascorbic acid). Cocrystals obtained from co-crystallization can provide sweetness and the benefit of vitamin C. In diabetes who often lack vitamin C. Vitamin C can help reduce free radicals, inflammation of blood vessels, blood sugar insulin, hormone levels, triglyceride levels, LDL cholesterol and lipid levels. It also helps prevent complications such as heart disease, kidney failure, etc [7]. Vitamin C should be consumed, which is why this research combined erythritol with sucrose to reduce the amount of sucrose. As reported by [8], they co-crystallized sucrose, erythritol, and fibersol-2 for making a sugar that help the digestive system.

Erythritol is a sugar alcohol, also known as polyols, that used to add to sugar free products. Erythritol is about 60-80% as sweet as sugar. Erythritol should be consumed in amounts less than 1000 mg/kg of body weight of erythritol; otherwise, it may cause diarrhea [9]. However, erythritol is generally considered safe in people with diabetes and who want to control their body weight. After cocrystallization obtained co-crystals. Co-crystals can be a sweetening, delivering vitamin C benefits which everyone can consume, also suitable in diabetes.

2. EXPERIMENTAL

2.1 Materials

Food grade sucrose was purchased from Khonburi Sugar Co., Ltd. Erythritol was purchased from Chemiphan Corporation Co., Ltd. L-ascorbic acid was purchased from Kemaus Co., Ltd. DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Chemical Express Co., Ltd.

2.2 Preparation of co-crystals

Sucrose and erythritol were mixed in a jacket crystallizer in a molar ratio of 1:1 and dissolving in distilled water. The ratio between mixed sugar and water was 6:1 g/ml. This was heated at 100oC using a stirring speed of 300 rpm until the solution was clear and then cooled down at 65 °C. When the slight turbidity occurred, stirring was continued, and 1.5 g of

L-ascorbic acid was added, indicated the beginning of co-crystallization process. The mixture was then immediately cooled by covering the crystallizer with cooling gel until the co-crystals formed. The obtained co-crystals were dried at 40 ºC for 1 day in oven. The co-crystals were packed in amber zip bag and stored at 4-6 ºC in a low-humidity environment for analysis. The experiment was then repeated by varying the variables according to Table 1.

Table 1. Molar ratio of sucrose and erythritol

Sucrose	Erythritol
1	1
1	2
2	1
2	3
3	2

2.3 XRD analysis of co-crystals

The crystalline structure of co-crystals was determined using X-ray diffractometer (D2, PHASER, Bruker). The scanning was conducted at 40kV with a radiation wavelength of 40 mA in the 2θ range between 5º and 60º.

2.4 FTIR analysis of co-crystals

The co-crystals were scanned by using Fourier Transform Infrared Spectroscopy (Tensor 27, Germany). The spectra were recorded in transmission mode from 400-4000 cm-1 at a resolution of 4 cm-1, 64 scans. The obtained spectra were analysed by using Sigma plot.

2.5 Structural morphology of co-crystal

Structural morphology of co-crystal samples was measuring using a scanning electron microscope (SEM JOEL, JSM-6010LV).

2.6 Antioxidant of co-crystals

Preparing 0.1 mmol of DPPH (1,1-diphenyl-2-picrylhydryl) ethanol solution. 1 g of co-crystals was dissolved in 10 ml of distilled water. 2 ml of dissolution was mixed with 2 ml of DPPH ethanol solution. The absorbance of the mixture was measured at 517 nm after incubating for 40 min in dark at room temperature. Antioxidant activity of the co-crystals was expressed as the percentage of radical scavenging activity of DPPH free radical, calculated using the following equation:

$$Antioxidant\ activity\ (\%) = 100 \times \frac{Abs_{control}^{517nm} - Abs_{sample}^{517nm}}{Abs_{control}^{517nm}}$$

where Abscontrol is the absorbance of the control (without the sample) and Abssample is the absorbance of the sample.

3. RESULTS AND DISCUSSION

3.1 X-ray diffractograms (XRD)

Figure 1 shows powder x-ray diffraction (PXRD) pattern of sucrose, erythritol, l-ascorbic acid (vitamin C) and, 2:3 C (co-crystal). PXRD was used to identify a new crystalline phase since characteristic powder pattern arises from its unique internal crystal structure [10]. From this figure presents the difference between diffraction pattern of cocrystal with single compound. Co-crystals displays the characteristic crystalline peak at 11.85º, 25.08º, and 50.82º. This individual peak in the diffractogram could suggest the interaction between sucrose, erythritol and l-ascorbic acid to form co-crystal. From the PXRD result, sucrose has higher intensity peak than co-crystals.

3.2 Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy is used to analyze changes in hydrogen bonding because the formation of a solid phase can be directly correlated with changes in the vibrational frequencies of functional groups [9]. Figure 2 displays the FTIR spectra of co-crystals at all ratios. All co-crystal ratios exhibited the same vibrational bands. Therefore, the 2:3 C ratio was selected, as it is considered reasonable for daily consumption.

Figure 3 shows the FTIR spectra of sucrose, erythritol, l-ascorbic acid and co-crystal. It was found that all samples had different change in vibrational frequencies of their functional group. Sucrose, erythritol and l-ascorbic acid showed similar and typical as displayed in the spectral database for organic compounds (SDBS) [11]. FTIR spectra of co-crystal presented appearance C=O and C=C at 1758 cm-1 and 1660 cm-1, respectively. In l-ascorbic acid, the C=O and C=C bonds showed peaks at 1753 cm^{-1} and 1655 cm^{-1}, respectively, in similar areas. This shift occurred due to the formation of hydrogen bonds. In FTIR spectra of co-crystal at 3600-3000 cm-1, several overlapping spectral bands corresponding to sucrose, erythritol, and L-ascorbic acid were observed in all the individual material. This revels that the co-crystallization did not change the chemical structure of the materials [12].

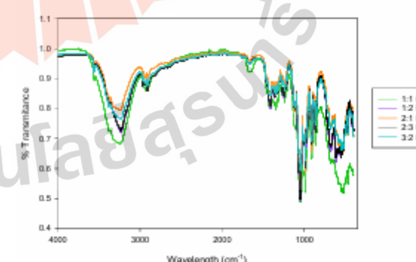

Fig 2. FTIR spectra of all ratios of co-crystals

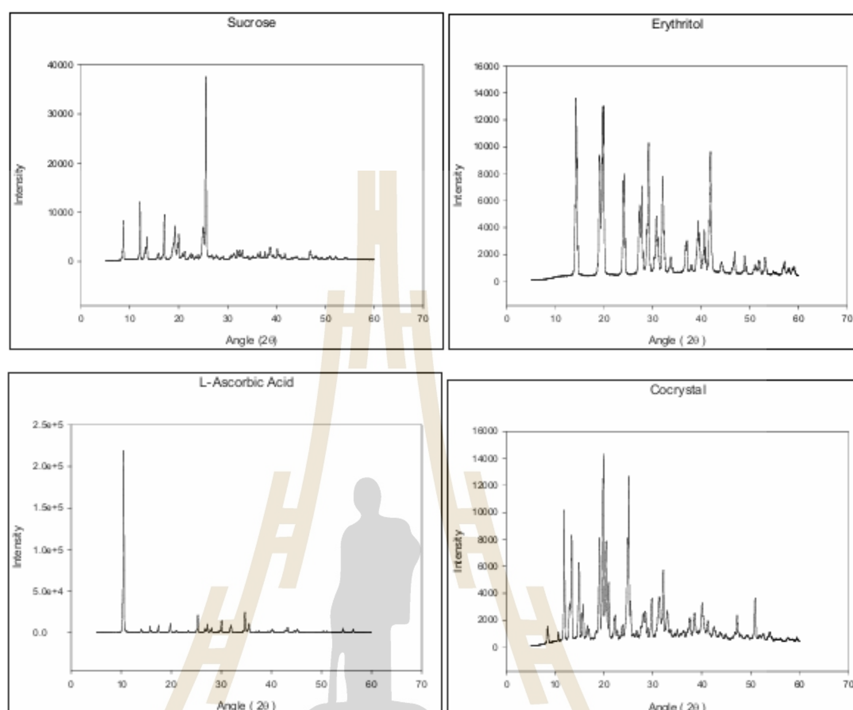

Fig 1. Powder X-ray diffraction pattern of sucrose, erythritol, l-ascorbic acid (vitamin C), and co-crystals

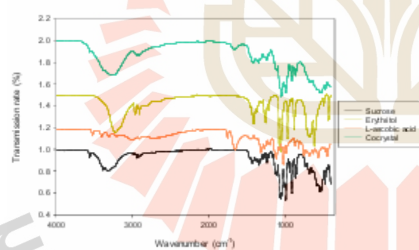

Fig 3. FTIR spectra of sucrose, erythritol, l-ascorbic acid and co-crystal

3.3 Structural morphology

The structural morphology of single components and co-crystals was examined using a scanning electron microscope (SEM) to study their structure. Figure 4 presents SEM micrographs of sucrose, erythritol, l-ascorbic acid and co-crystals. In SEM co-crystals displayed agglomeration on the surface, which was different from that of each individual component. SEM of each single component presented well- defined crystal structures especially sucrose and erythritol as reported by [13] and [14]. L-ascorbic acid showed SEM micrographs similar as reported by [15].

3.4 Antioxidant of cocrystals

Antioxidant of vitamin C were determined by DPPH radical scavenging activity which was used to measure antioxidant activity, as previously reported in [5]. Table 2 presents antioxidant properties of co-crystal, vitamin C and sucrose. Co-crystals exhibited 86.225% radical scavenging activity against free radicals, which was nearly equal to the antioxidant capacity of vitamin C (87.276%). In contrast, pure sucrose showed no antioxidant activity. Therefore, the co-crystals can serve as both a sweetening agent and a carrier for the antioxidant benefits of vitamin C.

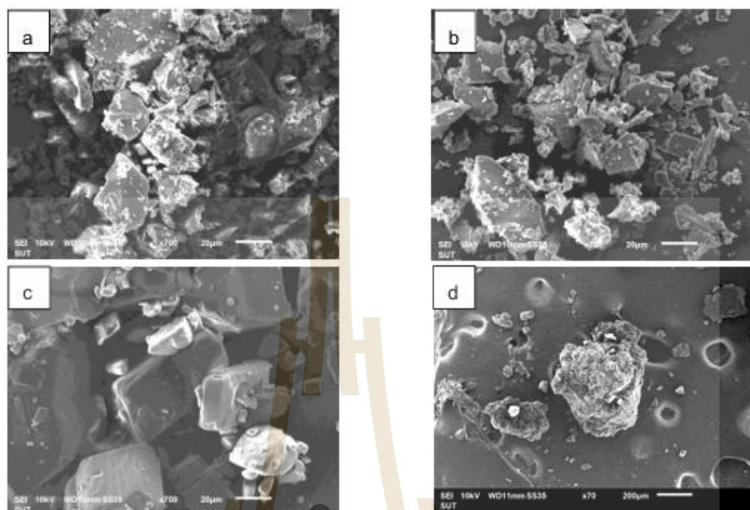

Fig 4. SEM micrographs of (a) sucrose, (b) erythritol, (c) l-ascorbic acid and (d) co-crystal

Table 2. DPPH radical scavenging activity of co-crystal and vitamin C.

Materials	DPPH radical scavenging activity (%)
Co-crystals	86.225 ± 0.0332
Vitamin C	87.276 ± 0.0332

4. CONCLUSION

This study successfully produced co-crystals that can be a sweetening and delivery vitamin C benefits. The FTIR and XRD results confirmed the formation of a new crystalline phase. SEM of co-crystals presented agglomeration on a surface, which was different from each single component. Antioxidant of cocrystals was confirmed by DPPH radical scavenging activity. The co-crystals exhibited 86.225% radical scavenging activity against free radicals.

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BIOGRAPHY

Miss Norramon Sathanasaowaphak was born on August 2, 1999, in Nakhon Ratchasima, Thailand. She completed her secondary level education at Mari Vithaya school, Nakhon Ratchasima, in the academic year 2018. She pursued a bachelor's degree in chemical engineering at King Mongkut's University of Technology Thonburi, Bangkok and graduated in the academic year 2022. Her undergraduate research focused on studying the development of thermoresponsive hydrogel for light modulator application. As part of her studies, she completed an internship at NMB-Minebea Thai Ltd. She continued her education by pursuing a master's degree in mechanical and process system engineering (chemical engineering) at Suranaree University of Technology, starting in 2022. During her master's research, she focused on co-crystallization process between sucrose, erythritol and ascorbic acid. she has presented her research at various international conferences, delivering oral presentations at the 34th Thai Institute of Chemical Engineering and Applied Chemistry Conference (2025), Pattaya, Thailand, May 20-21, 2025. Furthermore, she was a teaching assistant, contributing to knowledge enhancement and research development. Additionally, she had the opportunity to be an exchange student at Osaka Metropolitan University, Osaka, Japan.

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