INVESTIGATION OF THERMODYNAMIC PROPERTIES OF ASCORBIC ACID CRYSTAL IN DIFFERENT SOLVENTS USING EXPERIMENTAL AND SIMULATIONS METHODS

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Thesis submitted in fulfillment of the requirements
for the award of the degree of
Master of Engineering (Chemical)

Faculty of Chemical and Natural Resources Engineering

UNIVERSITI MALAYSIA PAHANG

APRIL 2019

ACKNOWLEDGEMENTS

Praises to Allah S.W.T. for His blessings that has enabled me to complete this humble thesis. I would like to thank my supervisor, an iron woman that never stop to encourage me to finish this journey. Thank you Dr. Fatmawati binti Adam for your continuous advice and support, during my ups and downs. I also would like to thank Dr. Mohd. Rushdi bin Abu Bakar as my co-supervisor for your time and patients during completion of this thesis and two journals. My warm thanks and gratitude also goes to simulation team for on-going discussion, ideas and support.

To all laboratory and administration staff in the Faculty of Chemical and Natural Resources Engineering, thank you for making my studies in UMP as a very pleasant experiences. Thank you for all your help to make it possible for me to complete my work.

I am no one without my biggest support system, my family members. Thank you Mommy, Hajah Jahyah binti Ali, late father Abah, Haji Hassan bin Mohamed, and my beloved siblings. Thank you. I do hope this small achievement will inspire my nephews and nieces to study harder and never give up on their dreams.

Special thanks to a friend of mine, Dr. Raihana Zahirah Edroz for assuring me that I can complete this study despite many challenges that coming my way. You help me to go through this technically and emotionally.

I would like also to thank all the researchers that I have cited, without the information from their previous research, this research cannot be made possible.

ABSTRAK

Bidang kristal kimia dikenalpasti sebagai salah satu faktor penting dalam kajian termokimia bagi process penghasilan kristal yang akhirnya menghasilkan kristal bahan farmaseutical aktif. Penyelidikan ini mengkaji kelarutan asid askorbik menggunakan kaedah "gravimetric" di dalam empat pelarut berpolar iaitu air, metanol, etanol dan 2-propanol. Ujikaji menunjukkan bahawa kelarutan asid askorbik di dalam air sangat tinggi, ini menunjukkan ia mudah terlarut di dalam pelarut berpolar tinggi. Kelarutan asid askorbik mula menurun apabila dilarutkan ke dalam pelarut yang berpolar rendah mengikut urutan metanol > etanol > 2-propanol. Kelarutan asid askorbik juga boleh diramal dengan menggunakan perisian simulasi seperti Conductor-like Screening Model – Realistic Solvent (COSMO-RS). COSMO-RS mengunakan nilai tenaga bebas Gibbs *(ΔG)* yang diperolehi daripada hasil ujikaji kelarutan. Δ*G* digunakan sebagai asas pengiraan untuk menentukan jenis dan kekuatan interaksi antara molekul seperti tenaga ikatan hidrogen (E_{HB}), tenaga ikatan Van der Waals (E_{vdW}) dan juga tenaga interaksi elektrostatik (E_{misfit}). Hasil daripada simulasi COSMO-RS didapati bahawa ikatan hidrogen lebih kuat berbanding ikatan antara molekul yang lain. Tenaga ikatan hidrogen yang kuat memberikan arah ketika pembentukan kristal. Selain interaksi di antara molekul, kualiti thermokimia lain yang dibincangkan dalam tesis ini ialah entalpi, entropi dan Δ*G*. Kualiti thermokimia ini diperolehi daripada plot van't Hoff. Tesis ini bakal menerangkan kaitan antara kualiti termokimia dan pembentukan pelbagai bentuk asid askorbik hasil daripada proses pembentukan kristal daripada pelbagai pelarut polar. Bentuk dan tabiat kristal dikaji melalui analisa mikroskopi menggunakan mikroskop dan Scanning Electron Microscope (SEM). Seterusnya, kualiti kristal dikaji melalui "powder x-ray diffraction" (PXRD) dan "x-ray diffraction" bagi kristal tunggal (SCXRD). Akhir sekali, asid askorbik daripada pelbagai pelarut dianalisa menggunakan "thermal gravimetric analysis" (TGA) dan "differential scanning calorimeter" (DSC). Kajian ini menunjukkan bahawa pelarut berpolar tinggi berinteraksi lebih kuat dengan asid askorbik berbanding pelarut berpolar rendah. Interaksi antara molekul yang kuat memberi kesan kepada kelarutan asid askorbik. Ia juga mempengaruhi pembentukan bentuk kristal kerana interaksi antara molekul memberi arah pembentukan kristal. Interaksi antara molekul yang kuat membentuk kristal yang mempunyai bentuk prism, manakala interaksi antara molekul yang lemah menjadikan pembentukan kristal tidak seimbang, lantas membentuk kristal yang lebih kecil. Polariti pelarut yang digunakan mempengaruhi bentuk Kristal yang terhasil namun tidak membentuk polymorph baru,

ABSTRACT

Crystal chemistry is identified as an important key factor to be explored in relation to the thermodynamic of crystallization solution to lead the formation of active pharmaceutical ingredient (API) crystals. In this study, solubility of ascorbic acid was determined by gravimetric method in four different polar protic solvents; water, methanol, ethanol and 2-propanol. It shows that ascorbic acid is highly soluble in high polar molecule (water) and the solubility was decreased with the reduction of solvent polarity (methanol > ethanol> 2-propanol). The solubility of ascorbic acid crystal was also predicted using Conductor-like Screening Model – Realistic Solvent (COSMO-RS) approach. In this computational analysis, the generated Gibbs free energy (ΔG) values is based on the solubility that were experimentally obtained to simulate main intermolecular forces like hydrogen bond, Van der Waals forces and electrostatic forces. Hydrogen bond forces is found to be stronger compared to other intermolecular forces which give directionality to crystal formation. The other thermodynamic properties of ascorbic acid in different solvents was analysed using van't Hoff plot. Crystallization is likely to occur at a more thermodynamically favorable condition in ΔG is low. This thermochemistry information provides an insight on the relationship between the crystal habit and its habit across four different polar protic solvents. The habit of crystal was then observed and its crystal quality was characterized by powder X-ray diffraction (PXRD), single x-ray diffraction (SCXRD), light microscopy and scanning electron microscopy techniques. The thermal properties of the crystals were studied using results from thermal gravimetric analysis (TGA) and differential scanning calorimeter (DSC). The results showed that high polarity solvents formed stronger intermolecular forces during dissolution hence influence the solubility of ascorbic acid. Strong intermolecular forces are also observed to help to give directionality during crystallization process. Strong polarity and stronger hydrogen bond propensity solvent tend to form equidimensional crystal like prism while weaker polarity solvent showed smaller crystal formation. Solvent polarity changes the habit of ascorbic acid crystal but did form a new polymorph.

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LIST OF SYMBOLS

a, b, c	lattice constant of the unit cell (Å)
c	solution concentration (g/ 100 g solvent)
c*	saturated solution concentration (g/ 100 g solvent)
D	percentage deviation (%)
M	molecular weight (g/mol)
N	number of molecules
R	universal gas constant (8.314 $kJ.mol^{-1}.K^{-1}$)
T_{melt}	melting temperature
ΔH_f	enthalpy of fusion
R	gas constant
T	solution temperature
T_f	fusion temperature of the solute
γ	activity coefficient
x	mole fraction of the solute in the solution
$\mu_i$solid	chemical potential of the solute
$\mu_i$solution	chemical potential of the solution
ρ^{α} and ρ^{β}	density in different solution a and b
ΔG_{free}	Gibbs free energy
ΔG_{fus}	Gibbs energy of fusion
$mu_{pure(i)}$	solute in the solvent
$mu_{solv(i)}$	solute in the solvent
ΔS	entropy

LIST OF ABBREVIATIONS

API	Active Pharmaceutical Ingredient
COSMO-RS	Conductor-like Screening Model for Real Solvents
FDA	Food and Drug Administration
IPA	2-propanol
DSC	Differential Scanning Calorimeter
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
TGA	Thermal Gravimetric Analysis
XRD	X-Ray Diffraction
PXRD	Powder X-Ray Diffraction
SCXRD	Single Crystal X-Ray Diffraction
NRTL	Non-random two liquid model
UNIFAC	UNIQUAC Functional Group Activity Coefficient
QbD	Quality by Design
CNT	Classical Nucleation Theory

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The crystallization operation is a very critical process because it determines crystal properties such as crystal size, crystal morphology and crystal polymorphic form. Crystal properties influence the efficiency of the subsequent downstream processes, which for some active pharmaceutical ingredient (API), it may effects the crystal performance such as dissolution rate and solubility. During downstream processing, manufacturers commonly milled the API crystal prior to formulation step to obtain desired crystal size (Loh, Samanta and Sia, 2015). Design and development of approach that are able to control the size and shape of crystal as desired by downstream processing is important to improve the procesability and will be able to reduce material, money and time wastage. The improvement of API properties prior to downstream processing is in line with United States Food and Drug Administration (FDA) framework regarding Quality by Design (QbD) that emphasize understanding of product since development stage.

The quality of product from the crystallization process is typically depends on the nucleation and crystal growth, which related by thermodynamics properties and molecular recognition (Chanda et al., 2015; Vedantam and Ranade, 2013). Solubility data as one of thermochemistry properties are important because it may affect the whole process from the earliest stages of drug discovery, throughout the entire process development and up to product formulation. The use of an accurate computational method to predict the aqueous solubility could lead to cheaper and faster drugs development. The Conductor-like Screening Model for Real Solvents (COSMO-RS) polarization charge density and the thermodynamic properties as solubilities and

partition coefficients have been proven to be a robust tools for pharmaceutical applications ranging from drug design to drug development. These thermodynamic properties are determined by the structure of the molecule of interest where the size of the molecule, number of hydrogen donors and acceptors, and intermolecular interaction of the molecule are considered to be important factors.

The thermodynamic properties including intermolecular interactions of API crystal can be characterized by various methods like studies of its physical and morphology using light microscope, scanning electron microscope (SEM), and transmission electron microscopy (TEM). Identification of phases in crystal can be investigated using powder X-ray diffraction (PXRD) or single crystal X-ray diffraction (SCXRD) method and the thermal behavior can be analyzed using differential scanning calorimetry (DSC) and thermogravimetry analysis (TGA).

1.2 Problem Statement

Pharmaceutical industry is largest consumer of ascorbic acid. About 50% of the total production of ascorbic acid is used for vitamin preparations in the pharmaceutical industry. The rest is mainly applied as an additive to food and as feed to enhance product quality and stability. Ascorbic acid that is added to foods during processing or before packing protects color, aroma and nutrient content (Hancock and Viola, 2002; Peppenberger and Hohmann, 2010; Zorn and Czermak, n.d.). The processability of ascorbic acid could be improved by crystallization. The solubility of ascorbic acid is a very important parameter in the design and optimization of its crystallization processes. Solubility data gives not just information of solubility of one solute in a solvent, it may also provide thermochemical information such as Gibb's Free energy, enthalpy and entropy of dissolution which is important to lead understanding towards ascorbic acid crystal characteristic.

Due to the large number of possible solvents, the main drawback of experimental solvent screening is that the consumption of time and material. Several methods have been reported to predict the thermodynamic behavior of solute-solvent systems from molecular dynamics models to classical thermodynamic models, such as NRTL, Wilson or UNIFAC (Brittain, 2009; Ertl and Mannhold, 2007). The parameter sets of these models need to be determined from a large amount of experimental values.

The Conductor Like Screening Model for Realistic Solvation (COSMO-RS) is a quantum chemical and statistical method that was developed by Klamt and colleague over recent years (Eckert and Klamt, 2002; Klamt, 2011). This method, compared to previous model requires only small parameter set that does not varies for specific compounds. Consequently, the COSMO-RS model enables efficient prediction of a number of physicochemical properties including solubility, activity coefficient, intermolecular forces and satisfying quantitative predictions according to the chemical structure of compounds (Bouillot, Teychené, et al., 2011).

While most attention is given to study of ascorbic acid production and it's applications, several papers discussed on various characteristic of ascorbic acid and very few discuss on the crystallization aspect of ascorbic acid (Srinivasan and Devi, 2010). Ascorbic acid present in various crystal habit like cubic, prism, plate and needle. This phenomenon occurs widely among organic compounds, where several crystal packing possibilities might exist in variations due to intermolecular hydrogen bonding of functional groups (Cross et al., 2003). Figure 1.1 shows differences in ascorbic acid crystal habit based on the solvent used. But none of the author relates the change in habit with solvent polarity nor intermolecular forces during the crystal formation.

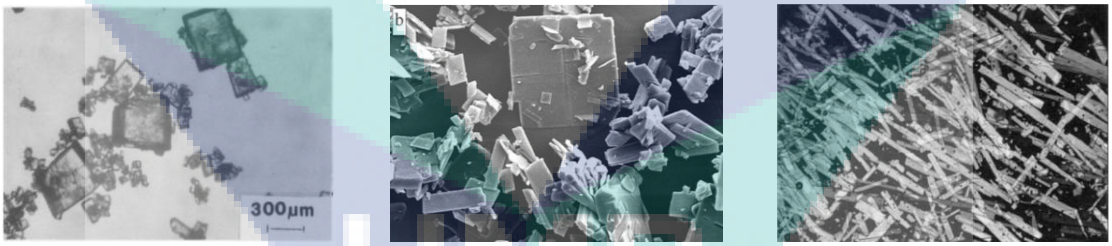

Figure 1.1: Multiple habit of ascorbic acid crystal grown in different solvents (a) water by Halász and Bodor (1993), (b) mixture water-methanol-ethanol by Wierzbowska, Matynia, et al., (2007) , (c) 2-propanol by Arslantas, Ermler, et al., (2004)

1.3 Objectives

This research aims to study the thermodynamic properties of ascorbic acid crystal in different solvents:

1. To study the effects of solvents on solubility and dissolution by experimental approach.

2. To study the effects of solvents on solubility, dissolution and intermolecular interactions by computer simulation.

3. To evaluate the physical properties such as crystal morphology, thermal properties, crystal purity and crystal profile of crystal from polar and polar protic solvents with relation to intermolecular forces.

1.4 Scope of Research

This research is limited to polar and polar protic solvents namely, water, ethanol, methanol, and 2-propanol. The solubility measurement by experiment was conducted using gravimetric method with temperature range in between 303 K to 323 K. The thermal properties of ascorbic acid only be evaluated using Van't Hoff plot.

The simulations was done using COSMO-RS. The molecular structure was obtained from CCDC before it was optimized by using TurboMole software. During data collection from COSMO-RS, no modification has been made to COSMO-RS coding and procedure. Intermolecular interaction obtained from COSMO-RS was further discussed and relate to ascorbic acid various habit.

The ascorbic acid crystals from different solvents was grown by evaporation method. The physical property of the crystal was characterized by optical microscopy analysis and Scanning Electron Microscopy (SEM). The crystallography analysis was characterized by Powder X-ray Diffraction Analysis (PXRD) and Single Crystal X-ray Diffraction Analysis (SCXRD) and the thermal analysis was conducted by Thermal Gravimetric Analysis (TGA) and Diffraction Scanning Calorimetry (DSC).

1.5 Significant of Study

In this work, the thermochemical information such as solubility, Gibb's Free energy, enthalpy of dissolution and entropy of dissolution are important parameters to understand ascorbic acid crystal is studied and evaluated. By understanding the factor that may influence the habit of ascorbic acid, it will open up to more possibility of ascorbic acid crystal modification. These criteria is important because it affects the physical and chemical properties of the final formulated product such as dissolution rate, solubility, and shelf-life (Abu Bakar et al., 2011; Chen et al., 2011).

It has been reported that the solubility of API are solvents dependent, however very few discussed this dependency in the light of intermolecular interaction especially from data obtain by COSMO-RS quantum mechanics simulation. This study compares the solubility of ascorbic acid in polar solvents using experiment and simulation data. The differences from these two methods were discussed. From this simulation, the molecular interactions that lead to different solubility can also be obtained and discussed. This is the first study that investigates the solubility of ascorbic acid and its intermolecular interactions with polar solvents using COSMO-RS (Hassan, 2019).

The crystals were also characterized by various analysis to study its morphology, crystallography and thermal decomposition. All the data and information gathered is discussed based on its intermolecular attractions which is yet still lacking in ascorbic acid crystal studies (citation).

1.6 Thesis Outline

This thesis is divided into 5 chapters, where Chapter 1 is as an introduction or background of the research works and objectives. Chapter 2 provides a review of recent works in pharmaceutical crystallization process which includes an application of molecular dynamics simulation COSMO-RS. In addition, fundamental aspects of pharmaceutical crystallization such as solubility, supersaturation, crystallization mechanism and ascorbic acid crystal characteristics are also presented in this chapter. The methodology of the research is covered in Chapter 3, giving a detailed explanation of materials, equipment and experimental procedures used in this study.

Chapter 4 is divided into a few section. Section 4.1 discuss the solubility of ascorbic acid in four polar solvent namely water, methanol, ethanol and 2-propanol. Then, from solubility data, van't Hoff plot is constructed and further thermochemistry properties of ascorbic acid crystal was discussed and the best solvents for dissolution and crystallization of ascorbic acid is identified. In Section 4.2 evaluate COSMO-RS model as a solvent screening tool and for prediction of ascorbic acid solubility in polar solvents. The relationship between solvent polarity and intermolecular forces is also explained. Section 4.3 presents the characterization techniques that were used and it's relation to intermolecular interaction of ascorbic acid crystal. The analysis are optical microscopy, SEM, DSC, TGA, PXRD and SCXRD. Finally, Chapter 5 states the conclusion of the findings and recommendations of further works.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter aims to review the fundamental aspects of the thermodynamic and kinetics of crystallization process starting from solubility, to its intermolecular interaction and its relation to crystal habit. Section 2.2 provides information regarding solubility theory and its importance towards pharmaceutical industry. In Section 2.3 and Section 2.4 described Gibb's free energy and intermolecular forces. Crystallization theory and crystals characterization were discussed in Section 2.5 to Section 2.7. Section 2.8 introduced ascorbic acid as very common API used in various industry and its crystal characteristic. The last sub chapter summarized Section 2.

2.2 Solubility

Solubility is defined as the maximum quantity of a solute that can be completely dissolved in a given amount of solvent (Yuchuan, David and Grant, 2007). It represents a fundamental concept in various fields of research such as pharmaceutical, chemistry, food science, and biological sciences. Solubility is even more important in the pharmaceutical field since it influence many major decision step as lead selection of screening parameters, bioavailability and drug delivery system. Due to its important characteristic, solubility data is also necessary for optimization of formula Moreover, solubility and solubility related properties can also provide knowledge regarding the structure of drugs and their range of possible intermolecular interactions. A comprehensive knowledge of solubility allows pharmaceutical researchers to develop an understanding of a drug substance starting from drug development stage.

2.2.1 Solvent Selection

Types of solvent play an important role in governing the solubility of solutes. Solvents can be classified into three types based on their intermolecular interactions. They are polar protic, dipolar protic and non-polar aprotic. Protic solvent is a solvent that has a hydrogen atom that is bound to an oxygen (-OH, hydroxyl group) or a nitrogen (NH, amine group). Therefore, protic solvent has high dipole moment that allows the solvent to donate the proton (H^+) easily. Polar protic solvents tend to form strong hydrogen bond with the neighboring molecules. On the other hand, dipolar protic solvents is suitable for dipolar and aprotic molecule. While the non-polar aprotic solvents have a weak dipole, is therefore interact based on weak van der Waals interactions rather than hydrogen bonds. This solvents most likely suitable for non-polar solutes.

Solvent shows a huge effect in crystallization process (Stoica et al., 2004). At molecular level, the solvent can selectively adsorb onto the crystals faces or interact with the solute cluster (Llinas and Goodman, 2008). A solvent that has stronger ability than solute to donate or accept proton may establish hydrogen bonding network with solute molecule, resulting in selective nucleation (Davey and Garside, 2002) that produce different polymorph.

Solvents also influence the final habit of the crystal. The ability of solvents to produce different crystal habit is due to solvent-solute interaction (Rasenack and Muller, 2002). This interaction may either inhibit or enhance the growth of certain crystal faces and subsequently altered growth kinetics. This process eventually caused changes to the final crystal habit.

The selection of a solvent that has too close chemical similarity must be avoided because their mutual solubility is probably high, hence the crystallization process will be uneconomical. Although several literature reported the solubility for some solutes, it is worth assessing the solubility of the targeted solute in the mother liquor, a supernatant of the slurry after crystallization from which it is crystallized. This is because the impurities can have a great impact on the solubility. For example, the Lovastatin's solubility is significantly greater in mother liquors than in a pure solvent (Nti-Gyabaah et al., 2008).

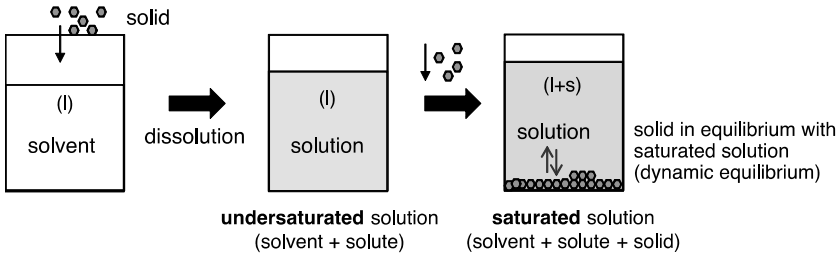

Figure 2.1: Solution equilibria
Source: Beckman (2013)

2.2.2 Solubility Determination

The solubility data can be obtained experimentally using polythermal or isothermal method. The polythermal method involves observation of complete dissolution of the solid phase when the mixture is subjected to slow heating (Mullin, 2001).

The isothermal method is used to determine of a maximum amount of solute that can dissolve in a solvent at a constant temperature with agitation for 4 to 24 hours (Mullin, 2001). Normally, a sample of the slurry is taken and filtered at room temperature for ease of handling. However, this may cause errors in the measurement if the temperature at which solubility is measured is not at the ambient temperature. Modification of this protocol such as by incorporating the filter into the solubility measurement flask to enable in-situ filtration can be performed.

In addition to experimental approaches, the solubility data can be predicted using Equation 2.1, modified van't Hoff equation (Mullin, 2001a):

$$ln\, x = \frac{\Delta H_f}{R}\left[\frac{1}{T_f} - \frac{1}{T}\right] - ln\, \gamma \qquad 2.1$$

x is the mole fraction of the solute in the solution, γ is the activity coefficient, ΔH_f is the molar enthalpy of fusion of the solute (J/mol), R with a value of 8.314 J/mol.K is the gas constant, T_f is the fusion temperature of the solute (K) and T is the solution temperature (K). The value of activity coefficient can be higher or less than unity depending on the presence of the heteroatomic groups of the solute, which may form strong or weak intermolecular interactions with the solvent (Kolár□ et al., 2002). The predictive thermodynamic models and quantum chemical calculation approach could be the best possible method for calculating the activity coefficient values.

Solubility is defined as the maximum quantity of solute that can dissolve in a certain quantity of solvent or solution at a specified temperature or pressure. It relies on temperature, system pressure and composition of the solution (Rasmuson, 2009). Solubility is very weakly dependent on pressure, thus the pressure of 1 atm can be generally applied in drug solubility (Ertl and Mannhold, 2007). Solvent and solute are the two main systems involved in the formation of crystal from a solution. The analysis for the solute can be done either gravimetrically (Abu Bakar et al., 2009) or using spectroscopy (Fujiwara et al., 2002). The chemical potential of the solute equals to the chemical potential of the species in solid phase is the main condition for equilibrium between solid solute and a solvent. This can be shown in Equation 2.2 where μ_i is defined as chemical potential:

$$\mu_i solid = \mu_i solution \qquad 2.2$$

Accurate solubility data is important in design, development and operation phases of crystallization process. The solubility data can be obtained experimentally using a technique known as the isothermal method as described by (Abdul Mudalip et al., 2013). The maximum amount of solute that can dissolve in a solvent at a constant temperature with agitation for a time period between 4 to 24 h is measured and analyzed (Abdul Mudalip et al., 2013).

2.2.3 Solubility Prediction by Thermodynamic Model

Reliable drug solubility prediction methods are important because in the early drug design and development phase, oftentimes only virtual compounds are considered by computational drug design techniques or very small amounts of the new drug candidates are synthesized by combinatorial chemistry methods (Ertl and Mannhold, 2007).

The solubility of drugs in various solvents is very important to evaluate the profiles of administration, distribution, metabolism, excretion, and toxicity of drugs. This data is crucial for drug development and manufacturing. Since the solubility of drugs plays a decisive role in the process of drug discovery, it is extremely useful if the solubility can be predicted (Ikeda et al., 2005).

A number of thermodynamics models such as Wilson equation (Gosh and Chopra, 1975), non-random two liquid (NRTL) equation (Renon and Prausnitz, 1968), UNIQUAC equation (Abrams and Prausnitz, 1975), NRTL segment activity coefficient (SAC) (Chen et al., 2006; Chen and Song, 2004), Van Laar equation (Peng, 2010), which were developed for predictions of the phase behavior of liquid-liquid system have been used to calculate the solid-liquid equilibrium behavior (Bouillot et al., 2011; Mota et al., 2009; Sheikholeslamzadeh and Rohani, 2012). Some of these thermodynamic models such as Wilson's model may not be very suitable for solubility prediction and solvent screening purposes because it requires many experimental data under different conditions (Kokitkar, Plocharczyk, and Chen, 2008).

Solubility can also be predicted using COSMO-RS for real solvents (Zhou et al., 2012). COSMO stands for Conductor like Screening Model. COSMO-RS is a fast and efficient methodology for predicting the thermodynamic properties of fluids and liquid mixtures that use a statistical thermodynamics approach based on the results of quantum chemical methods (QM) (Klamt and Eckert, 2000; Zhou et al., 2012). COSMO program is based on COSMO-RS theory of interacting molecular surface charges. It combines an electrostatic theory of locally interacting molecular surface descriptors with a statistical thermodynamic methodology.

COSMO RS will be used to explain the nature of the free energy of liquid solubility, which is the free energy of transfer of one molecule from its liquid or super-cooled liquid state to its state in a solvent (Klamt, 1995). For the prediction of ΔG_{fus}, a method that is able to handle the liquid and solid crystal of the compound on the same foundation because any change in the method would introduce a large error in the free energy differences of both states. However, for now, drug solubility prediction is inaccessible due to differences in methods for the treatment of the vibrational and motional degrees of freedom for liquid and fluid phase simulations.

Efficiency concerns may arise with respect to the time-consuming quantum chemical basis of COSMO-RS, but a highly efficient shortcut of COSMO-RS has already been developed in form of the COSMO fragment approach. COSMO-RS has been widely proven to be able to treat liquid-liquid and ionic liquid thermodynamics (Klamt et al., 2003; Zhou et al., 2012). The loopholes in this prediction are the free energy of fusion and acid dissociation energy.

COSMO-RS predictions rely much on the accuracy of the thermodynamic properties of solute and solvent of interest. The overall temperature dependency of solubility has two main inputs: the temperature dependency of the chemical potential of the solute in the liquid solution and the temperature dependency of the solute in the solid. This can be expressed by Equation 2.3:

$$log(x_{solub(i)}) = (mu_{pure(i)} - mu_{solv(i)} - \Delta G_{fus(i)})/(RTIn(10)) \qquad 2.3$$

The contribution of the liquid phase is described by the difference of the chemical potentials of the pure solute $mu_{pure(i)}$ and the solute in the solvent $mu_{solv(i)}$. The chemical potentials of the solute in the liquid are predicted by COSMO-RS. This liquid COSMO-RS prediction includes the temperature dependency of the chemical potential in the liquid.

In the expression above, the contribution of the solid phase is described by the free energy of fusion $\Delta G_{fus(i)}$. COSMO-RS, being a theory of liquids, cannot predict $\Delta G_{fus(i)}$ directly. Hence $\Delta G_{fus(i)}$ has to be estimated in some other way. $\Delta G_{fus(i)}$ has its own temperature dependency, which is independent and different from the temperature dependency of the liquid phase, which is clear from the physics of the solid state since the solid state is assumed to have a crystal-like order, while the liquid state is not in order at all.

This problem will occur regularly in compound classes that have a lot of hydroxyl groups such as sugar and in this studies, referring to ascorbic acid and all selected solvents. The hydroxyl groups cause high melting point on because they form many intramolecular hydrogen bonds and a low decomposition temperature because they increase reactivity so the decomposition often takes place before the melting point (Eckert, Diedenhofen, and Klamt, 2010; Klamt, 2005).

2.3 Gibb's Free Energy

The free energy of hydration $\Delta G_{g \to aq}$ and free energy of vaporization $\Delta G_{l \to g}$ can experimentally be obtained from Equation 2.4:

$$\Delta G_{free} = kTln\left(\frac{\rho^{\alpha}}{\rho^{\beta}}\right) \qquad 2.4$$

where ρ^{α} and ρ^{β} are the densities in the different phases. Since the chemical potential is difficult to calculate the free energy is usually related to enthalpy H and entropy S as per Equation 2.5:

$$\Delta G = \Delta H - T\Delta S \qquad 2.5$$

Enthalpy and entropy that are combined in a single equation is called Gibb's free energy. Gibb's free energy is an energy that is available to do work. It depicts the process of solubility reaction and gives us the insight on the energy usage. When ΔG is less than zero, the reaction is spontaneous and thermodynamically favored. The solute is expected to be more stable in solid phase and harder to dissolve (i.e. less soluble) when it has higher energy.

2.4 Intermolecular Forces

Intermolecular forces are forces of attraction that act between neighboring particles. Examples of intermolecular forces are dipole-dipole, dipole-induced-dipole, and hydrogen bond. The intensity of intermolecular forces influence physical and chemical properties of substances, including the boiling points of liquids, the melting points of solids, solubility and free energy. The stronger the attractive force in a substance, the higher the boiling point because more energy is required to overcome the forces. Intermolecular forces that exist between solute and solvent molecules may reduce the free energy of the solution, hence increase the solubility. In polar solutions, intermolecular forces may also lead to molecular orientation (Yuchuan and David, 2007), which it has the tendency to decrease the enthalpy and entropy of mixing. Prediction of solubility using the solution theory normally fails when both solute and solvent are polar. Polar molecules, will have a permanent dipole moment and interact with the oppositely charged portions or other molecules having permanent dipole moments. Intermolecular force varies in strength however, intermolecular forces are weaker than intramolecular forces.

Hydrogen bond occurs due to electrostatic attraction between partial positive charge on hydrogen atom and partial negative charge on opposite atom, commonly strong electronegative atom like fluorine, oxygen and nitrogen atom although in very

rare occasion, chlorine atom may also form weak hydrogen bonds. The specific site of attraction defines the direction of hydrogen bond, making it a very strong dipole-dipole attractive force. The relatively high directionality and strength of hydrogen bonds make the prediction and control of molecular orientation in organic solids practical and reliable (Tshepelevitsh, et al., 2013). The strength of hydrogen bond varies from around 40–50 kcal/mol for strong hydrogen bonds to few kcal/mol for weak ones (Grabowski, 2006). Strong hydrogen bonds have a strong contribution of covalent interaction, while weak ones are largely electrostatic. The strength of a hydrogen bond is influenced by its length and angle. Even a small deviation from the optimal length leads to a significant weakening of the hydrogen bond. The hydrogen bond is important to influence the structure arrangement of an organic crystal in their crystal lattice. Example is given in the case of a simple molecule of diacetamide, where it has two proton acceptors, one proton donor and three possible conformers, each of which could form two or three centred hydrogen bonded chains or dimers. Four of the hydrogen bonding possibilities are detected (Kuroda, Yokoyama, et al., 1978).

Van der Waals or London dispersion forces represent important intermolecular interactions by temporary dipoles created when electron locations are loopsided. The electrons are constantly orbiting the nucleus, and by chance, the electrons could be located close together (Silberberg, 2002). The nearest neighbor interactions determine the energetics of the arrangement. The uneven negativity of atoms in a molecule creates a temporary dipole. As there are more electrons in an atom and the shells are further away from the nucleus, these forces become stronger. The Van der Waals interaction is commonly influenced by the size and surface area of contact. Nonspecific van der Waals interactions particularly dispersion forces also contribute significantly to the solvent dependent (Reichardt and Welton, 2014). In case of 11α-hydroxy-16α, 17α-epoxyprogesterone (HEP) crystals, van der Waals is found to contribute to its crystal packing (Qiang, Yongli, and Ying, 2007) . Regardless of other interactions found within a complex, there will always be a contribution from van der Waals.

2.5 Crystallization

Crystals are solids made up by atoms, ions or molecules in a regular systematic pattern extending in all three spatial dimensions. The construction of atoms within crystals is mimicked in the crystals' forms of surface faces, edges and corners. Crystals

are often described from the form or appearance of the crystal. The forms is referred to coordinated system called Miller Indices. Crystals may also be represented from X-ray beam reflections of x-ray diffraction (XRD).

There are two major steps that took place, for crystallization process to take place. The first one is a nucleation process where the first atomic structure formed. The second steps is crystal growth where the stable nuclei grow into visible size crystal (Mullin, 2001a).

Crystallization is a chemical solid–liquid separation technique, in which mass transfer of a solute from the liquid solution to a pure solid crystalline phase occurs. It is the oldest technique for the production of pure crystal and is a critical process for the pharmaceutical industry. The vast majority of pharmaceuticals are manufactured in solid, generally crystalline, form. Crystallization is used to identify structure for use in drug design, to isolate chemical species from mixtures of reaction products, and to achieve consistent and controlled drug. Crystallization processes that are commonly practiced by the industry are crystal growth from melt, growth from solution, flux growth and hydrothermal synthesis (Series, 2010). The advantage of growth from solution is better crystal quality with reference to point defects, few dislocation density and low angle grain boundaries. By growing from the solution, the solvent evaporation method is the least expensive method (Ramukutty and Ramachandran, 2012) and simplest to be conducted. Crystal growth from solution is widely used for many applications in industry such as mass crystallization and production of API.

2.5.1 Phase Diagram

The relation between solute-solvent can be illustrated using phase diagram as Figure 2.2. This figure shows that the solubility curve is the thin line that separates a composition of solution from supersaturation and undersaturation. Those that lie below the solubility curve is undersaturated region. Thus, any point above the line is supersaturated. In supersaturated solution, the dissolved solid and solute are not in equilibrium, hence it is not a stable solution. In order for the solution to gain equilibrium, the nuclei shall be removed and grown into crystal.

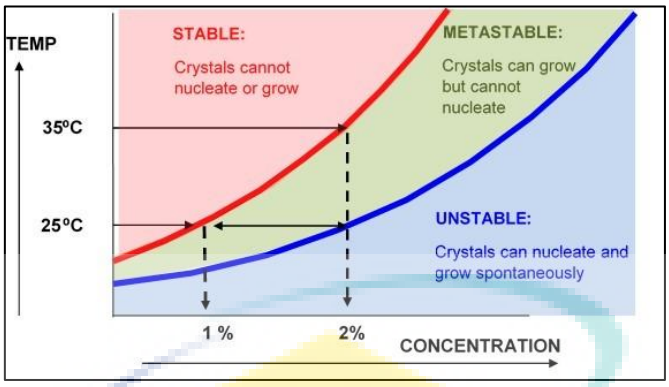

Figure 2.2: Typical solubility-supersaturation diagram is showing the undersaturated, metastable and labile zone.

Source: Mullin (2001, a)

2.5.2 Crystallization from Solution

Crystallization commonly starts from solution. Currently, there are many techniques to grow crystal from solution. They are slow evaporation, rapid evaporation, slow cooling, sublimation, and many others. In the cooling crystallization method, the higher the supersaturation of a solution, the higher the growth rate for different crystal faces will be. When the growth rate is high, some of the faces will disappear and the habit will become plate-like (Chen and Wang, 2000). Appropriate cooling rate may transform crystal habit. Crystallization from solution using slow evaporation technique mainly aims to isolate the crystal solid and purify a soluble solid (Hulliger, 1994).

2.5.3 Nucleation

Nucleation is the most significant phenomenon in determining the rate of formation of a new phase. A nucleus can be defined as the minimum amount of a new phase capable to exist by its own. The birth of these nuclei in an initially metastable phase is called nucleation, which is a key mechanism of first-order phase transition. The growth stage, which immediately follows the nucleation, is governed by the diffusion of particles, called the growth units, to the surface of the existing nuclei and their incorporation in the structure of the crystal lattice. There are two theories that govern the nucleation process which are Classical Nucleation Theory (CNT) and Two Steps Nucleation Theory as describe in Figure 2.3 (Erdemir, Lee and Myerson, 2009; Mullin, 2001b)

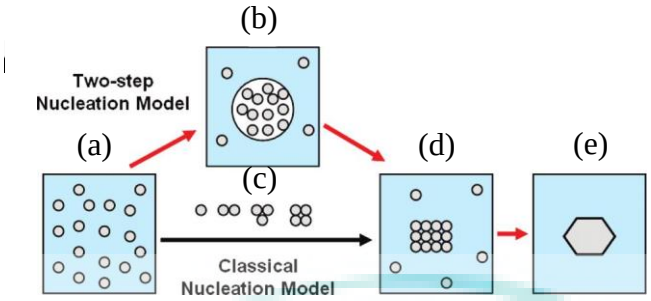

Figure 2.3: Alternative pathways leading from solution to solid crystal. (a) supersaturated solution. (b) high density liquid like cluster of solute molecule formed from supersaturated solution (a). (c) subcritical cluster of ordered solute molecules. (d) ordered crystalline nuclei (e) crystal solid.

Source: Erdemir et al (2009)

CNT describes nucleation as the free energy change required for cluster formation (ΔG) as sum of the free energy change for the phase transformation (ΔG_v) and the free energy change for the formation of a surface (ΔG_s) as describe in Equation 2.6.

$$\Delta G = \Delta G_s + \Delta G_v \qquad 2.6$$

In terms of crystallization from solution, ΔG_v explains the spontaneous tendency of a supersaturated solution to undergo deposition. Solid state is more stable than the liquid, therefore ΔG_v becomes negative and decreases the Gibbs free energy of the whole system. As a result, the growth of clusters depends on the competition between a decrease in ΔG_v, which favors growth, and an increase in ΔG_s, which favors dissolution. The positive surface free energy ΔG_s term dominates at small radii, which causes an increase in the total free energy change initially. Thus the smallest clusters in solution typically dissolve. As cluster size increases, total free energy goes through a maximum at a critical size, above which the total free energy decreases continuously and growth becomes energetically favorable, resulting in the formation of crystal nuclei and followed by crystal solid. The stronger the supersaturation, higher solubility, will produce smaller critical nuclei and ease the nucleation process (Veesler and Puel, 2015).

Two steps Nucleation Model on the other hand describe nucleation process as the formation of a highly disordered liquid droplet then followed by the formation of a crystalline nucleus inside the droplet beyond a certain critical size. Furthermore,

proximity to the critical point decreased the free energy barrier for crystallization and thus increased the nucleation rate (Erdemir et al., 2009). This model has been demonstrated by Savage and Dinsmore by proving that the amorphous cluster grow to ~30 particles before transforming to crystal solid very quickly (Savage and Dinsmore, 2009).

2.5.4 Crystal Growth by Evaporation

Crystal growth is a process where stable nuclei formed in the supersaturated solution begin to grow into larger crystals. Crystal nuclei and seeds provide a surface for crystal growth to occur. Crystal growth involves solute molecules attaching themselves to the surfaces of the crystal according to the crystalline structure. In short, this process can be divided into two major steps. The first step is the mass transport of the solute molecule from the supersaturated solution to the crystal surface either by diffusion, convection or a combination of both mechanisms. The second step is the integration of the solute material into the crystal lattice via adsorption process (Jones, 2002).

Figure 2.4 shows the three possible growth sites of the crystal surface; namely, flatty (F), step (S) and kink (K). This classification is made based on the maximum number of bonds established on the crystal surface. F site is a crystal face with one possible surface bond, while S and K are crystal surfaces with the possibility of two and three surface bonds, respectively (Hartman and Perdock, 1955).

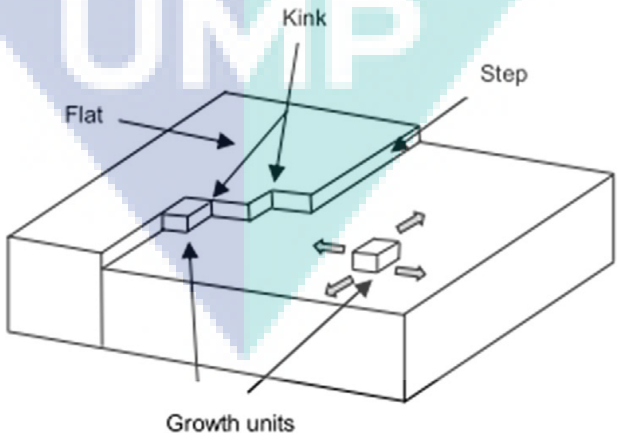

Figure 2.4: A three-dimensional crystal surface showing three type of growth sites. Source: Hartman (1955)

2.5.5 Crystal Habit and Shape

The habit of crystals is determined by the relative growth rate of the different faces. Different sites on the crystal surface would grow at different rates. Consequently, the produced crystals have varying appearances due to the difference in the relative areas of their faces, the lengths of the axes in the three dimensions and the angles between faces. This property of the crystals is termed morphology or habit. In certain crystal formation, there are some variations in crystal size and faces development while maintaining its interfacial angle. This is called a crystal habit (Mullin, 2001a), which is described as the ability of a crystal to exist in the same crystal system. Meanwhile, crystal shape is referred to actual geometrical features of the crystal such as elongation of one or another crystal face forming rods, flakes and needles. The definition of crystal habit is standardized, while the crystal shape may vary upon studies (Carlton, 2011). Figure 2.5 shows some of the typical habit of ascorbic acid crystals (Blagden and Davey, 1998).

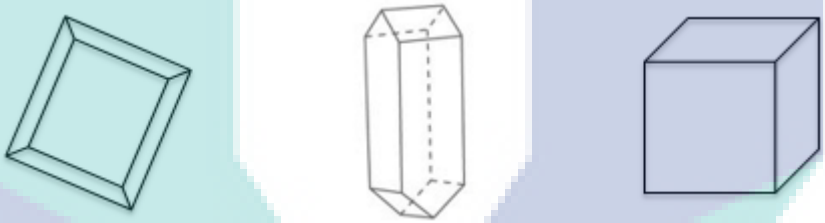

Figure 2.5: The common habit for ascorbic acid, prism and cubic.

The habit is dominated by those faces that have the lowest growth rate. The growth rate of a given face increases with supersaturation, but the exact relation may vary from face to face. Hence, crystal habit is determined by the internal structure and external influences on the crystal such as the growth rate, solvent used and the presence of impurities during the crystallization growth period (Perry, 1997). Solvents used during crystallization may influence the crystal habit due to the variation in intensity of the solute-solvent interaction (Beckmann, 2013; Tiwary, 2001). These interactions at various solute-solvent interfaces lead to changes in interfaces, changes in crystal growth kinetics, and enhancement or inhibition of growth at certain crystal faces.

Polarity of the solvent and the intermolecular interaction that leads to its preferential adsorption at selected faces of the solute are critical factors in determining the habit of a crystal solid. It is important in industry because different habits will

exhibit different product characteristics. Long, needle-like crystals tend to break easily during centrifugation and drying, whereas flat, plate-like crystals are difficult to wash during filtration or centrifugation, resulting in low filtration rates. Spherical crystals are desirable compared to cubical-shaped crystals because they have better flow ability and do not form cakes easily (Perry, 1997). If the final product is a suspension of crystals in a liquid, its rheology is very much dependent on the crystal's morphology (Rawlings et al., 1993).

Shape factors are a convenient mathematical way of describing the geometry of a crystal (Myerson and Ginde, 2002). If the size of a crystal is defined in terms of a characterization dimension *L*, two shape factors can be defined as the volume shape factor, α, and the area shape factor, β as referred to Equation 2.7 and 2.8 Values of shape factors for common materials and geometries are available in the literature (Mullin, 2001).

$$\alpha = \frac{V}{L} \qquad 2.7$$

$$\beta = \frac{A}{L2} \qquad 2.8$$

Different habits of an API can also be obtained using the same solvent by altering the process variables of crystallization, such as cosolvent, crystallizing solvent ratio, temperature of cosolvent and crystallizing solvent, and rate of cooling. A decrease in the initial supersaturation of solution due to increase in temperature of cosolvent or crystallizing solvent delays crystallization and therefore produces small crystals with uniform dimensional morphology. This seems to be due to slow and uniform deposition of the solvent molecules on the nuclei.

However, cooling rate does not likely affect the habit because of spontaneous precipitation of the drug. It only affects its external habit without changing its internal structure. The alteration in habit is caused by the interference with the uniform approach of crystallizing molecules to different faces of a crystal growth. Crystal growth may be hindered by adjacent crystals growing simultaneously or contacting container walls. As a result, the development of plane faces may be inhibited, leading to formation of a moderate parallel faces, platy (excessive development of parallel faces),

prismatic, acicular (inhibited width), or bladed (flattened acicular) crystal habit (Tiwary, 2001).

2.6 Miller Indices

Miller indices is used in crystallography to indicate specific directions and planes. It defines coefficients of imaginary planes in a crystal. These directions and planes could be in the form of crystals or lattices. The number of indices will match the dimension of the crystals.

Miller indices of the plane (hkl) is calculated by intercepting the plane with the axes along the primitive translation vectors a1, a2 and a3. Assume these intercepts as x, y, and z, so that x is fractional multiple of a1, y is a fractional multiple of a2 and z is a fractional multiple of a3. Therefore, x, y, and z can be measured in units a1, a2 and a3, respectively. Invert (x y z) into (1/x 1/y 1/z) and this set is reduced to the smallest integers by multiplying with a common factor. For example, if the plane intercepts x, y, and z in points 1, 3, and 1, the index of this plane will be (313).

There are 32 ways in which lattice points can be arranged in space. These non-translation elements are called point groups. There are 230 unique 3D structures known as habits that are generated when linear translations, glide plane and screw axis are applied to the point groups. These are called space groups. Figure 2.6 shows the common planes on Miller indices.

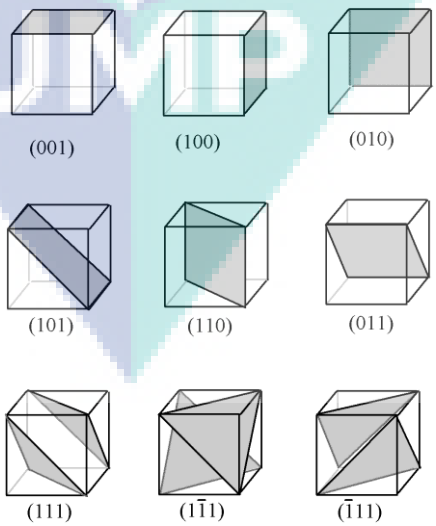

Figure 2.6: Common planes on Miller indices

The habit of a crystal depends on two factors. Firstly, the forms it exhibits and its class. Crystal class in turn encapsulates two additional kinds of information which is the type of symmetries the crystal has, and the axes, points they are relative to.

2.7 Crystal Characterization

There are number of experimental techniques used in the present investigation to characterize the properties of the grown single crystals such morphology, composition, structure, physical properties and crystal quality (Benz and Neumann, 2014) The materials characterization methods includes of imaging, x-ray diffraction, spectroscopy, specific thermal analysis, electrical and magnetic analysis, and optical techniques. The importance of crystal characterization is to study the quality of crystals and it defects.

2.7.1 Habit and Size

Direct observation of the crystal by microscopy is a fundamental method to study the crystals. At least four main types of microscopy techniques have been applied in pharmaceutical field. The techniques include polarized light microscopy (PLM), hot stage microscopy (HSM), atomic force microscopy (AFM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

An optical microscope creates a magnified image of a crystal with an objective lens and magnifies the image further more with an eyepiece to allow specimen observation by the naked eye which enables crystal size and crystal habit to be evaluated. The crystal specimens may be observed as aggregated particles, where the crystals are rather weakly attached to one another in a fairly loose structure (Holmbäck and Rasmuson, 1999).

SEM is a microscopy technique using an electron beam to scan the surface of a sample (Kuang et al., 2017). The focus beam of electrons interact with the atoms surface and generate signals that provide information about the samples morphology, surface topography and composition. The samples surface image are projected in vacuum in conventional SEM or in low vacuum in variable pressure of SEM. SEM can be applied to analyze both crystals and noncrystals at nanoscale level (Jegatheesan, Murugan, and Rajarajan, 2012).

2.7.2 X-ray Diffraction

XRD has been reported as an important tool for the study of polycrystalline material. (Brittain, 2009; Shankland, 2016). Example of information that can be extracted using XRD are identification different crystal forms, determination of crystal structure and determination of lattice parameter. XRD methods can be divided into two types: powder X-ray diffraction (PXRD) and single crystal X-ray diffraction (SCXRD).

PXRD analyzed a large number of randomly oriented crystals. It is a rapid analytical technique used for identification of a crystal material and able to provide information on unit cell dimensions. This method is simple and rapid to analyze the crystal structures of a material. Like a fingerprint of human being, the PXRD pattern of every crystal can be applied to analyze the crystal form change, crystallinity, state of crystal structure, and the presence of mixed crystal. The diffraction pattern is a spectrum of real space periodicities in a material. Atomic periodicities with long repeated distances cause diffraction at small angles, meanwhile, short repeated distances (as from small interplanar spacing) cause diffraction at high angles. Crystals with precise periodicities over long distances have sharp and clear diffraction peaks (Dinnebier and Billinge, 2008). It should be noted it is necessary to prevent the crystal structure change or defect during the sample preparation process such as the grinding and screening processes. Crystals with defects like impurities, dislocations, planar faults, internal strains, or small precipitates are less precisely periodic in their atomic arrangements but they still have distinct diffraction peaks. Their diffraction peaks are commonly wider and distorted (Fultz and Howe, 2008).

SCXRD analysis uses the phenomena of interference and diffraction of X-rays. This method is used to analyze crystal because it reveals primary information on molecular structure. It is also used to differentiate the chirality and packing of polymorphism (Hasegawa, 2012). Appropriate size and purity are required for the SCRXD analysis. Yet, it is challenging to acquire a single crystal with appropriate size and high purity for many API, which limit the application of this analysis.

2.7.3 Thermal Analysis

Thermal analysis is defined as a group of methods based on the determination of changes in chemical or physical properties of material as a function of temperature in a

controlled atmosphere. There are three main thermal analysis methods: differential scanning calorimetry (DSC), differential thermal analysis (DTA), and thermogravimetric analysis (TGA). Different crystal forms have different heat absorption/release behaviors during their heating/cooling process. Thermal analysis is to analyze the relationship between the physicochemical properties and temperature by controlling the temperature change. The produced thermal analysis curves can be applied to judge the similarities and differences of crystal forms of different drug/food crystals.

DSC is a thermoanalytical technique to measure the heat flow between the sample and the inert reference (commonly α-Al_2O_3) as a function of temperature and time. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. The principle of this technique is that compared with the inert reference, the sample requires more or less heat when the sample undergoes a physical transformation such as phase transitions. It can be applied to the observed fusion and crystallization events as well as glass transition temperature. It also can be applied for studies of oxidation, determination of melting temperature and heat capacity and other chemical reactions.

Thermogravimetric analysis (TGA) is a thermoanalytical technique based on the measurement of the weight loss of the material as a function of temperature. It is suitable for checking the loss of solvent in the crystal or the sublimation and decomposition process of a sample (Bernstein, 2002). TGA curves can be applied to speculate whether the crystals contain water or solvent, and therefore it can be used to distinguish water-free crystal structure and pseudo polymorphs. The method has several advantages such as simple operation, high sensitivity, reproducibility, and less sample amount. It is commonly used for the analysis of drug polymorph. But, it is generally combined with other instrumental analytical techniques such as XRD, and IR analysis because API polymorph is hard to determine.

TGA has been widely applied for studies of API crystals. Liu et al. synthesized hydroxy zinc phosphate particles for drug loading of epirubicin and used TGA to analyze the hydroxy zinc phosphate particles.

TGA curves showed the weight loss at the temperature range of 0-200 ℃ corresponding to the loss of physically adsorbed water, weight loss at the temperature range of 200 ℃ to 400 ℃ corresponding to the loss of lattice water, and weight loss at the temperature range of 400 ℃ to 600 ℃ corresponding to the gradual dehydroxylation of the particles. The TGA results also showed thermal stability of the particles obtained at higher reaction temperatures was better than that of the particles obtained at lower reaction temperatures.

DTA technique is similar to DSC technique except that it is to measure the temperature difference between the sample and the inert reference (commonly α-Al_2O_3) as a function of temperature. DTA curves can provide data on the transformation including glass transitions, crystallization, melting, sublimation, and etc. DTA is an important way to analyze the physical properties of materials because every kind of materials has their own unique differential thermal curves.

In this study, DSC and TGA are going to be used to access thermal decomposition of ascorbic acid crystal from different solvents. The stability of the crystalline phases obtained can be analyzed by DSC measurement (Mangin, Puel and Veesler, 2009).

2.8 Ascorbic Acid

The synthesis of ascorbic acid developed in 1934 by Reichstein and Grussner has remained the main method for the commercial production of vitamin C. Almost all industrial processes for ascorbic acid are derived from the Reichstein and Grussner process.

Ascorbic acid ($C_6H_8O_6$) is an active pharmaceutical ingredient (API) that is commonly used as antioxidant (Zumreoglukaran, 2006), pharmaceutical agent (Omar, 2006), cosmetic ingredient, and dietary supplement. The structural formula of ascorbic acid is shown in Figure 2.7. It is a polar organic molecule that contains four hydroxyls groups. Ascorbic acid is reported to melt at 191 °C, but Shi Jingyan and colleague reported that ascorbic acid's first decomposition step starts at 180 °C (Jingyan, Yuwen, Zhiyong and Cunxin, 2013). This is further observed in the existence of 4 hydroxyl groups (–OH) that makes ascorbic acid as a strong reducing agent.

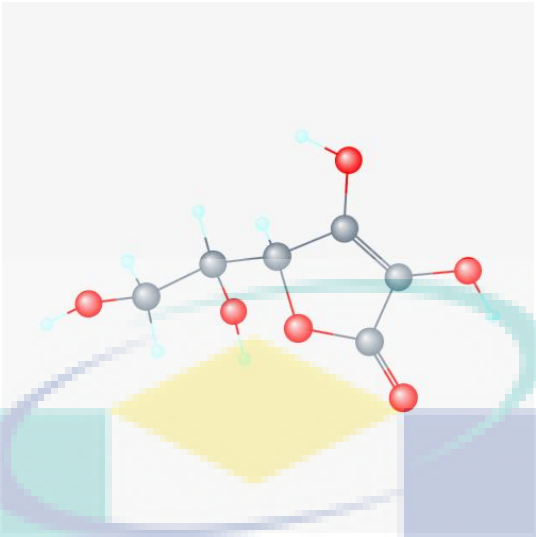

Figure 2.7: Molecular structure of ascorbic acid

Ascorbic acid has a very unique feature that shows an acidic nature without a carboxyl functioning group (–COOH). Instead, the acidity is based on its conjugate C=O, C=C and the lone pair of –OH group (Cotton, 2011). Ascorbic acid is a weak acid. Due to its weak acidic nature, it partially dissociates in water. Even though ascorbic acid is highly soluble in water, not all components of ascorbic acid will dissociate into hydronium ion [H_3O^+] in water. It is a mild acid in aqueous solution because of the ionization of the enolic –OH linked to C-3 (pKa = 4.25) gives a delocalized ascorbate anion. Due to the presence of multiple hydroxyl group (-OH), ascorbic acid commonly decomposes before it could reach its melting point (Jingyan et al., 2013).

Another characteristic of ascorbic acid is that it is a diprotic acid, which means it has two hydrogen atoms that are ready to be protonated. This is given by the Equation 2.9 to Equation 2.12:

$$H_2Asc\ (aq) \Leftrightarrow H^+(aq) + HA_{sc}{}^-(aq) \qquad 2.9$$

$$HA_{sc}{}^-(aq) \Leftrightarrow H^+(aq) + Asc^{2-}(aq) \qquad 2.10$$

$$K_{a1}(aq) = \frac{[H_3O^+][Hasc^-]}{[H_{2Asc}]} = 7.9 \times 10^{-5} \qquad 2.11$$

$$K_{a2}(aq) = \frac{[H_3O^+][Hasc^{2-}]}{[H_{Asc}{}^-]} = 1.6 \times 10^{-12} \qquad 2.12$$

Ascorbic acid, despite being very commonly used in human, animal and plants, there is still room for improvement and a need for further study. It is used in human as an antioxidant and also reported, as a cancer control supplement. Figure 2.8 shows the ascorbic acid skeletal diagram with hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) indicator.

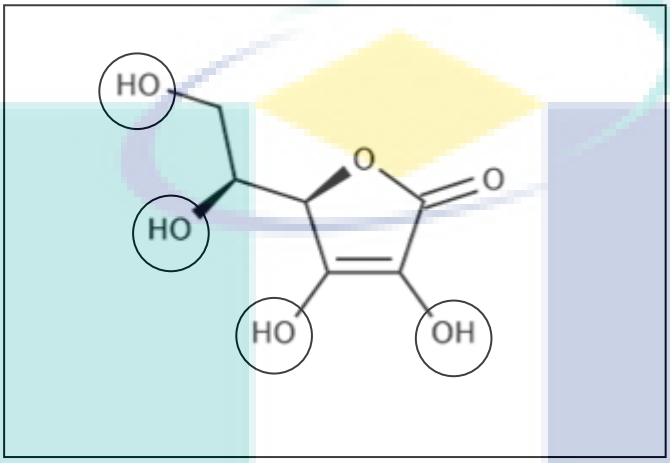

Figure 2.8: Ascorbic acid skeletal diagram with HBA and HBD indicator

2.8.1 Ascorbic Acid Crystal Habit

Crystal habit of an ascorbic acid is important to be understood because it plays an important role in determining not only the process ability of intended material, but also of its efficacy and performance in final product (York, 1983). Thus, many researchers study about this for its industrial improvement (Stoica et al., 2004).

Ascorbic acid molecules are tightly bound in the crystal network of eight intermolecular H-bonds per molecule (Milanesio, et al., 1997). The first ascorbic acid structure was determined using x-ray reflections (Hvoslef, 1968a, 1968b). The ascorbic acid crystal properties is summarized in Table 2.1.

The unit cell parameters comparison is tabulated is Table 2.2 and unit cell characteristic is shown in Figure 2.9. The Cambridge Structural Database maintained by the Cambridge Crystallographic Data Centre (CCDC) reported that ascorbic acid exists in two crystal habits, which are block and prism.

Table 2.1: Crystal properties of ascorbic acid

Properties	Value
Chemical formula	$C_6H_8O_6$
Formula weight	176 g/mole
Melting temperature (decomposition)	190°C
Crystal system	Monoclinic
Space group	$P2_1$

Table 2.2: Ascorbic acid unit cell parameters comparison by different researchers.

Crystal unit cell parameter	(Hvoslef, 1968)	(Jones, Davey and Cox, 2005)	(Srinivasan and Devi, 2010)
a (Å)	16.95	17.299	16.97
B (Å)	6.32	6.35	6.33
C (Å)	6.38	6.411	6.38
α (°)	N/A	N/A	90.00
β (°)	102 °30’	102 °11’	102 °32’
γ (°)	N/A	N/A	90.00

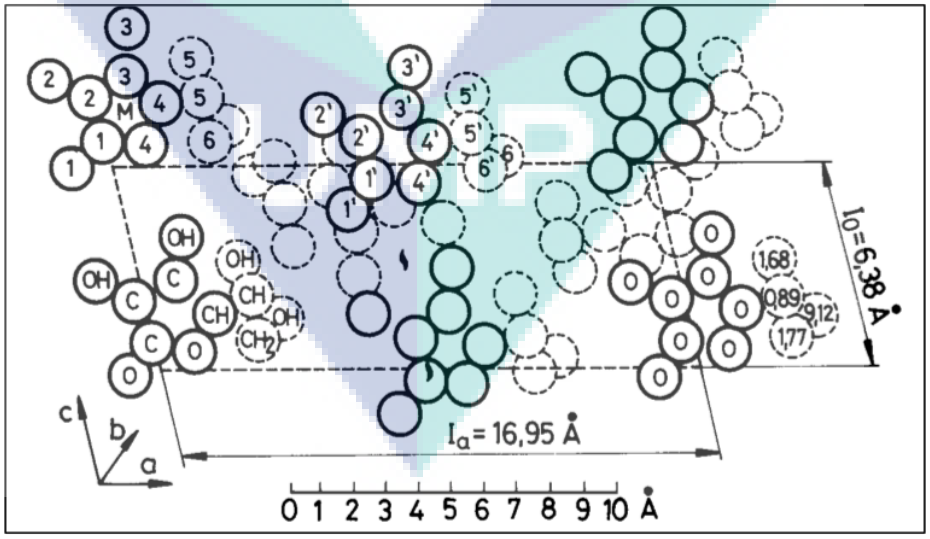

Figure 2.9: Unit cell characteristic of ascorbic acid

Source: Halász and Bodor (1993)

In 1968, Hvoslef reported the habit of ascorbic acid is prism (Hvoslef, 1968a, 1968b). The habit of ascorbic acid was also reported and found to be in needle form by preparing it from methanol. Ascorbic acid also found out that it forms into a plate when grown in water (Wierzbowska et al., 2008) and change into a needle form in methanol (Uesaka and Kobayashi, 2002), and acicular when crystal is obtained from mixture solvents (Srinivasan and Devi, 2010). Ascorbic acid crystal molecule arrangement in crystal lattice system is influenced by factors like type of solvents (Horst, Geertman and Rosmalen, 2001).

Crystal size of ascorbic acid vary from coarse to ultrafine powder, where it constitutes the major commercial product forms of the compound, followed by special coated and granulated forms. Sodium L-ascorbate is also produced in granular and powder forms. Limited production of other forms such as calcium ascorbate and ascorbyl palmitate depend on the demand of these products in specialty applications (Bauernfeind, 1982).

Ascorbic acid, despite been hugely consumed worldwide, its crystals are still not suitable for direct tableting due to their poorly compactible properties (Jagtap et al., 2012). Ascorbic acid crystal is unsuitable for direct tableting due to their poorly compactible properties. Kawashima et al. designed spherically agglomerated crystals of ascorbic acid with improved compactibility for direct tableting. Kawashima and colleague precipitated ascorbic acid crystals by a solvent change method, followed by agglomerations with the emulsion solvent diffusion or spherical agglomeration mechanism, depending on the solvent combination for crystallization. Kawashima and colleague precipitated ascorbic acid crystals by a solvent change method, followed by agglomerations with the emulsion solvent diffusion or spherical agglomeration mechanism, depending on the solvent combination for crystallization. Based on the results of the study, under static compression, the proper compact with a sufficient strength was effectively produced. After the improvement on the micromeritic properties such as flowability and packability for the spherically agglomerated crystals, the crystals of ascorbic acid were obtained via the spherical crystallization technique (Kawashima et al., 2003; Kawashima et al., 2002).

2.9 Summary

This review shows the importance of solubility studies in pharmaceuticals process. Solubility data can be obtain from gravimetric method and computational method like COSMO-RS. The solubility data not only reveal the ability of one compound to be soluble in one solvent, but it also helps to understand other thermochemistry properties such as enthalpy, entropy and intermolecular interactions. With relation to intermolecular forces like hydrogen bond and van der Waals interaction, a compound may form different habit or polymorph. With the help of characterization analysis such as microscopic analysis, thermal analysis and x-ray diffraction analysis, the importance of intermolecular forces to the formation of different ascorbic acid crystal habit can be studied more thoroughly.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter details out the methodology applied including materials, laboratory experimental methods and the computational method used in this study. This section introduce the chapter and give the flow of research works. In Section 3.2 materials used (ascorbic acid and solvents) were introduced. Section 3.3 describes solubility using gravimetric method. The following part Section 3.4 describes computational modelling methods using COSMO-RS and Turbomole. Lastly, Section 3.5 describes the methods used for solid-state characterization of crystals produced.

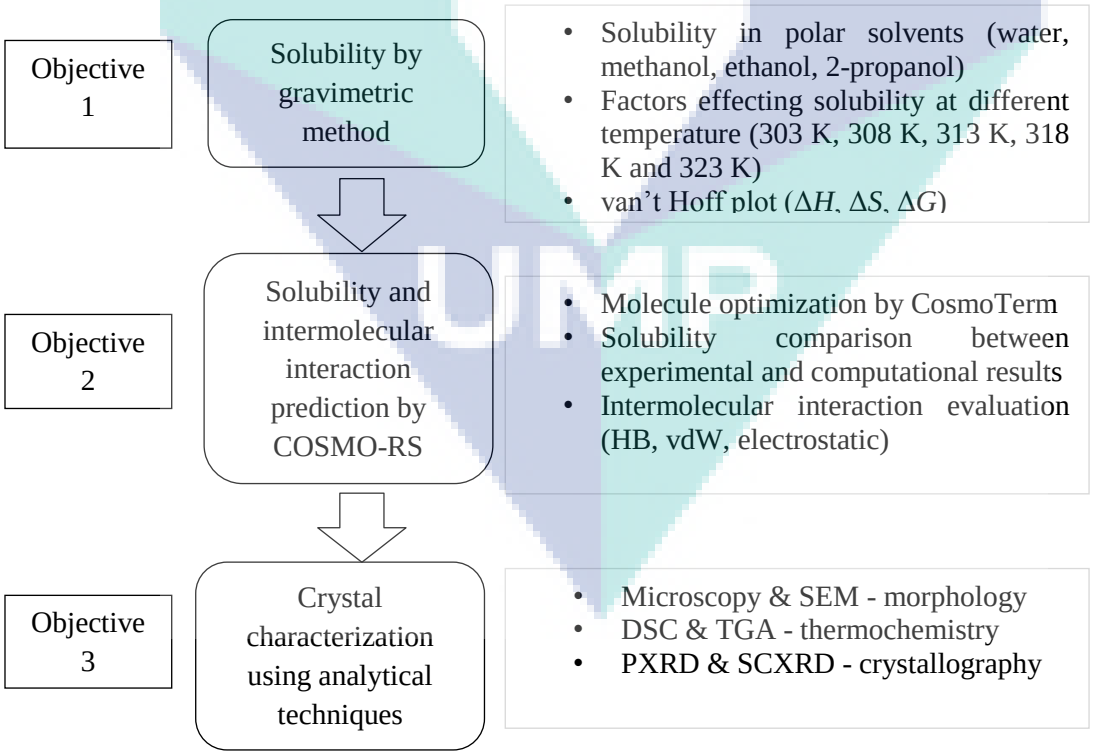

Figure 3.1: Flowchart of research work showing main activities

3.2 Material

In this work, (2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one ($C_6H_8O_6$, ascorbic acid, CAS No. 50-81-7) was used. It was supplied from Fisher Scientific (Loughborough, UK). The ascorbic acid used for this work is analytical grade. Ascorbic acid is a weak acid. Due to its weak acidic nature, it partially dissociates in water. Another characteristic of ascorbic acid is that it is a diprotic acid, which means it has two hydrogen atoms that are ready to be protonated. Ascorbic acid was used for solubility determination by experimental method and to produce crystal habits from different solvents.

The solvents selected are from polar and polar protic solvents family. Polar protic solvents tend to form strong hydrogen bond with the neighboring molecules. Solvents selected to be used in this works are common solvents in crystallization of ascorbic acid and other active pharmaceutical ingredient (API). Based on the FDA guidelines, these solvents are categorized as nontoxic in small residue (Hassan et al., 2019, FDA, 2018). The solvents used are water (H_2O), ethanol (CH_3OH), methanol (CH_3CH_2OH) and 2-propanol ($CH_3CHOHCH_3$). Ascorbic acid, methanol, ethanol and 2-propanol used were from Fisher Scientific with purity in between 98.00% – 99.99%. All materials were used as it is without any further purification. Table 3.1 shows the properties of materials used in current work such as boiling points, density, relative polarity and polarity of each solvent.

Table 3.1: Properties and characteristics of solvents used in ascorbic acid solubility studies and crystallization.

Solvent	**Boiling point (^oC)**	**Density (g/mL)**	**Relative polarity**	**Polarity**	**Molecular Dipole Moment, μ (Lide, 1995)**
Water	100	0.998	1	Polar	1.87
Methanol	64.6	0.791	0.762	Polar protic	1.72
Ethanol	78.5	0.789	0.654	Polar protic	1.69
2- propanol	82.4	0.785	0.546	Polar protic	1.56

3.3 Solubility Determination through Gravimetric Method

The solubility of ascorbic acid in four pure solvents has been determined gravimetrically at temperature range from 303 *K* to 323 *K*. The experiments were carried out in a 50 mL falcon tube using thermomixer as per Figure 3.2. The thermomixer is by Eppendorf Thermomixer Shaker (Eppendorf, Hamburg, Germany) at intended temperatures (303 *K*, 308 *K*, 313 *K*, 318 *K*, and 323 *K*) (Mudalip et al, 2013). The sample mass was determined by an electronic balance (Sartorius, GMBH). Agitation rate was set at 300 rpm (Wassvik et al., 2006). Mixing and maximum amount of solid material is not optimized as it will only affect the time taken for the solvent and solid to reach equilibrium concentration (Rasmuson, 2009).

For each measurement, an excess of known mass of ascorbic acid was added to a known mass of solvent. Then, the equilibrium cell was heated to the required temperature with continuous agitation at 300 rpm. After 24 h, the agitation was stopped. Then, the excess solid could be observed in the lower part of the equilibrium cell. The sample of the upper part of the solution was withdrawn with a suitable, warmed filtered pipet to a pre-weighed glass petri dish. The petri dish was weighed again to determine the mass of the sample. The petri dish is sealed with poked aluminum foil to let the solvent evaporate. Then, the petri dish was placed in desiccator to evaporate the solvent. After the evaporation of the solvent, the petri dish was dried until the weight of the petri dish became constant. Thus, the solid concentration of the sample (x_i) was able to be determined which can be referred to Equation 3.1.

$$Solubility = \frac{Mass\ of\ solute}{Mass\ of\ solvent\ used} \qquad 3.1$$

where, m_1, is the weight of solute and m_2, is the weight of solvent. M_1 and M_2 is molecular weight of the solute and solvent, respectively as shown in Equation 3.2.

$$x_i = \frac{\frac{m1}{M1}}{\frac{m1}{M1} + \frac{m2}{M2}} \qquad 3.2$$

Figure 3.2: Eppendorf 50 mL Thermomixer Shaker

3.3.1 Determination of Thermochemical Properties from Van't Hoff plot

The enthalpy and entropy of dissolution in respective solvents were determined using Van't Hoff plot by plotting ln x against 1/T (1/K). The enthalpy of dissolution ΔH_{diss} and entropy of dissolution ΔS_{diss} can be calculated from the slope and intercept of the fitting line. The value of enthalpy of dissolution, ΔH_{diss}, in J/mol, calculated from the graph's slope, indicated the amount of heat adsorbed or released during disaggregation and diffusion of ascorbic acid in each solvent used. The entropy of dissolution, ΔS_{diss}, in J/mol.K is obtained from the y-intercept described the disorder of the dissolution process as shown in Equation 3.3.

$$\ln x = -\frac{\Delta H_{diss}}{RT} + \frac{\Delta S_{diss}}{R} \qquad 3.3$$

The relation between ΔH_{diss}, and ΔS_{diss}, with the changes of Gibbs free energy, ΔG in different solvents is shown by Gibbs-Helmholtz, Equation 3.4.

$$\Delta G_{diss} = \Delta H_{diss} - T\Delta S_{diss} \qquad 3.4$$

3.4 Thermochemistry Properties Determination through COSMO-RS

COSMO-RS provides a convenient method to predict the solubility of a molecule in a variety of solvents using specific thermochemistry parameter that enables the software to calculate the solubility based on its charge density interaction between the solute and solvents. The important parameter that is required to do the solubility predictions are ΔH_{melt} and T_{melt}. However, as described earlier, ascorbic acid does not have an actual T_{melt}, thus the solubility prediction that is commonly used by many researchers are not applicable in ascorbic acid molecule. Instead, the comparison of ascorbic acid solubility was conducted based on experimental value obtained from thermogravimetric method.

3.4.1 Molecule Optimization

Prior to solubility prediction by COSMO-RS, the 3 dimension (3D) input file of all molecular structures used must be prepared. The input files for water, methanol, ethanol and 2-propanol were taken directly from the COSMO-RS database. However, the ascorbic acid structure was obtained from Cambridge Crystallographic Data Centre (CCDC, Cambridge, United Kingdom) database with the "mol" extension was exported to TurboMole (Karlsruhe, Germany) for optimization. The ascorbic acid molecular structure was optimized using TurboMole program package version 7.0 (COSMOlogic GmbH & Co. KG, Leverkusen, Germany) by applying Density Functional Theory (DFT) with Becke-Perdew triple valence electrons plus polarization function (BP-TZVP) level with the Resolution Identity (RI) approximation. RI approximation, which is also known as density fitting, is a method to reduce the computational load associated with large number of electron repulsion.

In total, there are 14 molecule structures obtained from CCDC. To set up molecular attribute, the Molecular Optimization (MO) that starts the vector menu was used to start Density Functional Theory (DFT) energy calculation. The molecular charge was set to zero (0) and no advance setting was selected. After the structure has been optimized, a single energy calculation has been established with basis set of Triple Zeta Valence Set with Polarization (TZVP). TZVP basis set was chosen as atomic attribute because ascorbic acid is an organic molecule (Schäfer, et al., 2000). The

flowchart in Figure 3.3 shows the steps for the optimization process. By the end of the optimization process, only one is used for the whole solubility prediction process.

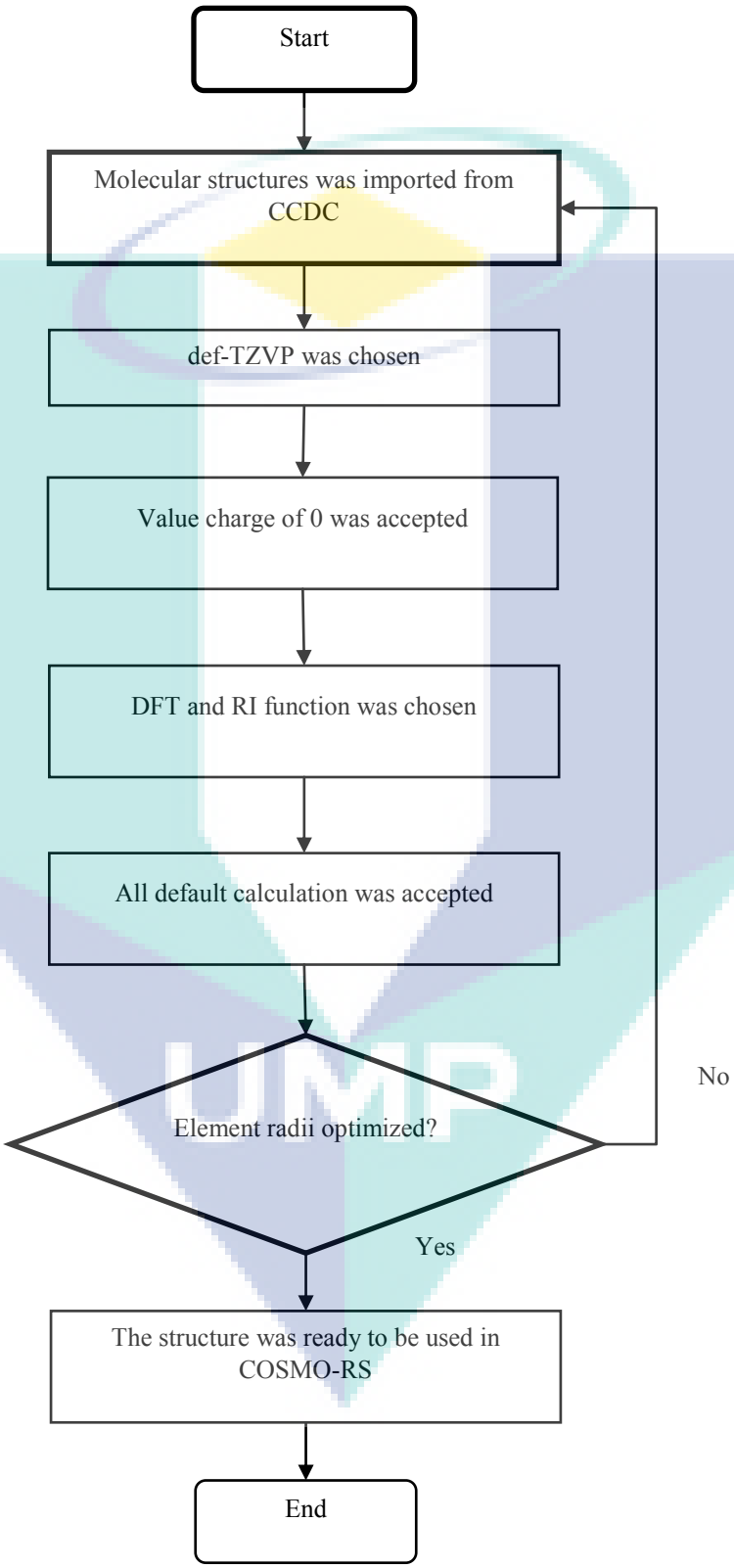

Figure 3.3: Turbomole setup and optimization procedure.

3.4.2 Solubility Prediction by COSMO-RS

The solubility predictions of ascorbic acid in different solvents for temperatures of 298 to 323 K using COSMO-RS were performed using COSMOthermX program (COSMOlogic GmbH & Co KG, Leverkusen, Germany). Conductor-like Screening Model for Real Solvents (COSMO-RS, COSMOlogic GmbH & Co. KG, Leverkusen, Germany) continuum solvation was applied to simulate a virtual conductor environment for ascorbic acid molecule and to evaluate the charge density (σ-profile) on the three dimensions surface molecule (Durand et al., 2011).

COSMO-RS is based on surface interaction model. Surface interaction model considers molecular charge densities rather than the interaction of groups which are obtained by molecular quantum chemical COSMO-RS calculations. The main features of COSMO-RS is probability distribution of screening charges for the molecule solvated in a perfect conductor material exhibiting infinite electrical conductivity or, equivalently, zero resistivity. σ-profile values has been proven to be excellent parameter to a predictive quantification of conductor hydrogen bond energy in COSMO-RS simulation. The statistical thermodynamics of the molecules interaction was used to calculate the interaction of the pair-wise interacting surface segments (σ , σ‘). The three main coulomb interactions evaluated are electrostatic energy (E_{misfit}), hydrogen bond (E_{HB}) and van der Waals (E_{vdW}). environment. A perfect conductor or perfect electric conductor (PEC) is an idealized environment.

The solubility prediction was conducted by filling in the details and information of the compound such as temperature and the T_{melt}. The simulation was conducted by selecting the optimized three dimension molecular structure from COSMO*therm*X database for the compound of interest and the solvents used. Then, the parameters such as temperature was inserted for the calculation. Once the details was filled in, “Run” button was clicked to initiate the calculation. The solubility prediction and its intermolecular interaction was ready to be analysed. The flowchart of this process is shown in Figure 3.4. The example of the interface is as shown in Figure 3.5.

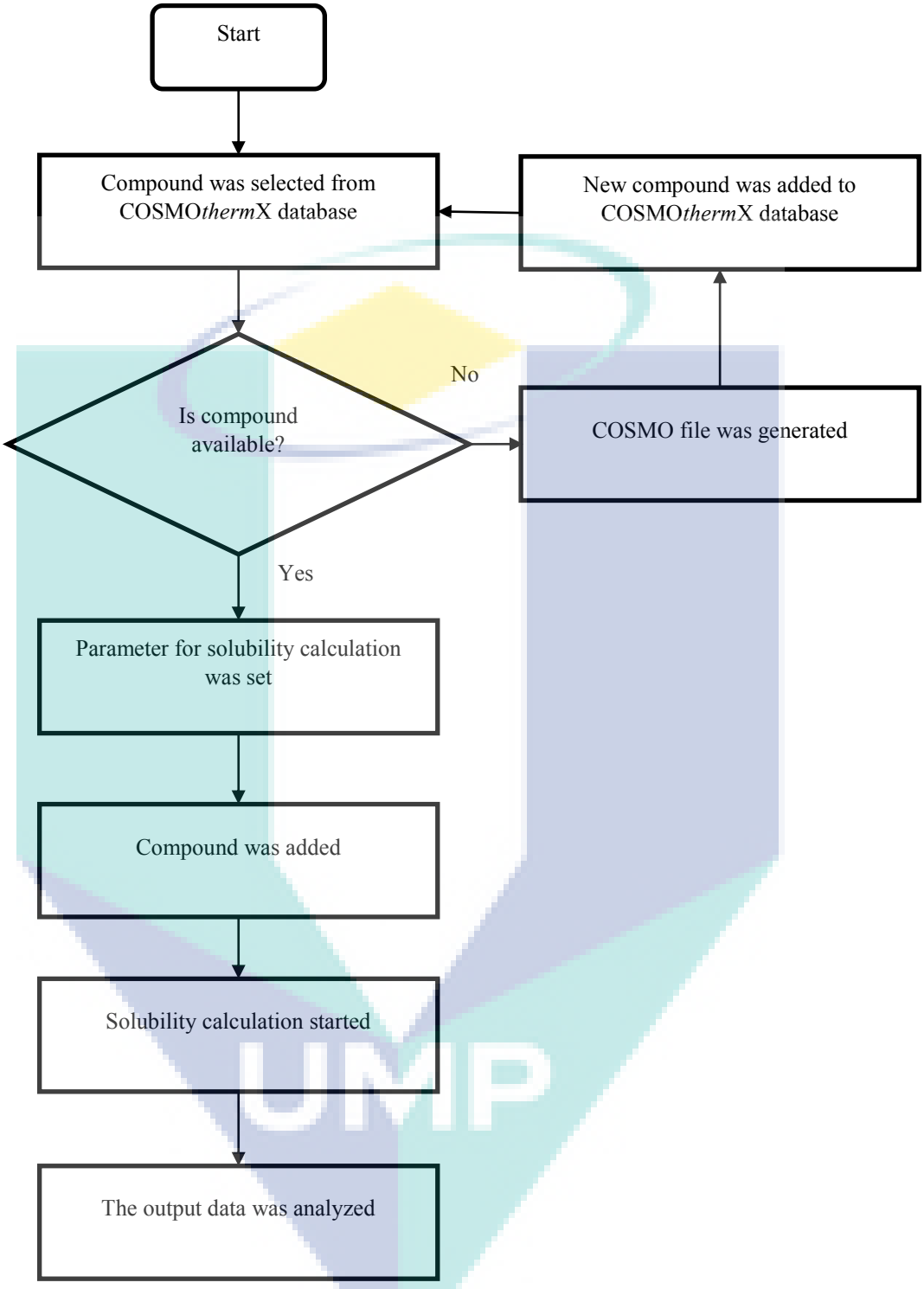

Figure 3.4: Flowchart of COSMO-RS setup and calculation procedure (Eckert, 2012).

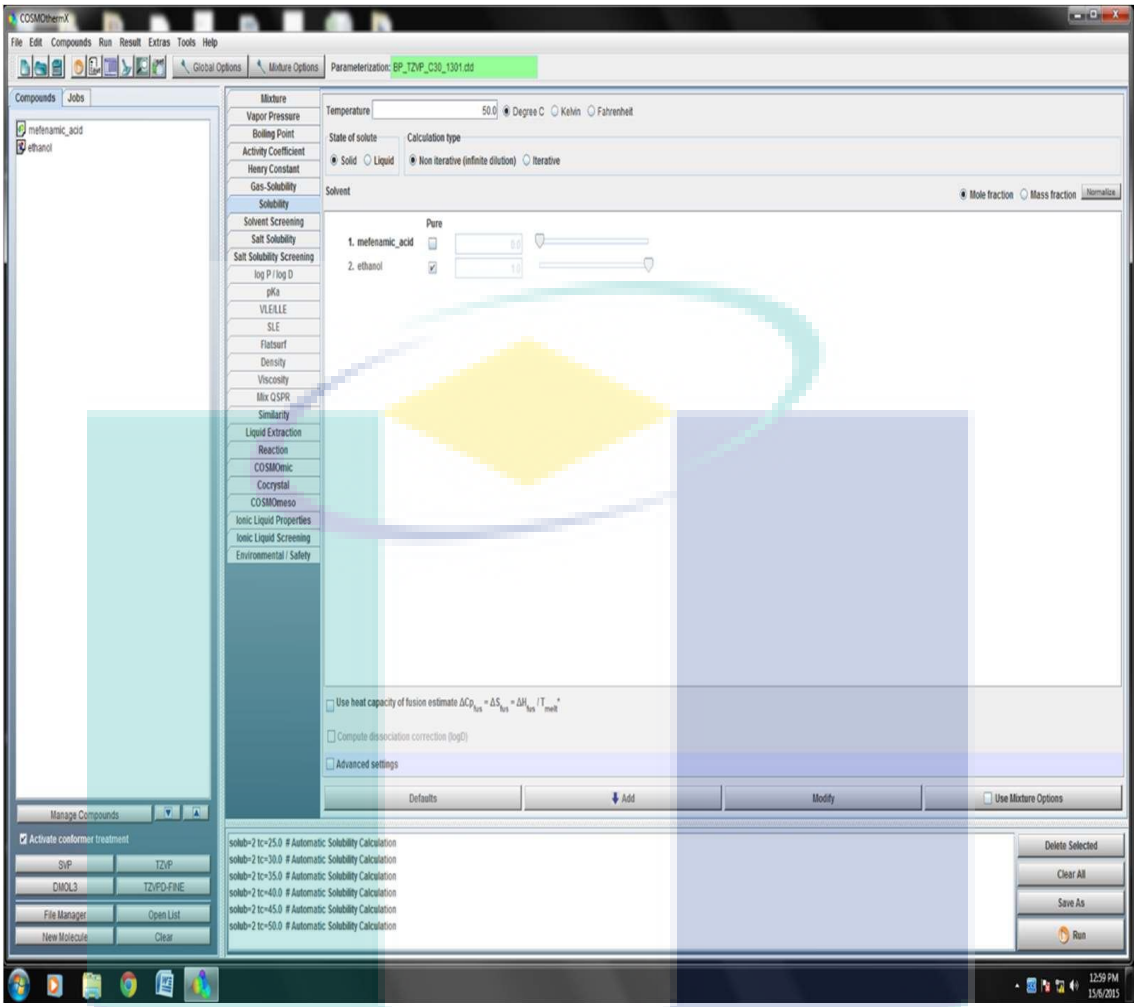

Figure 3.5: COSMO-RS interface

The solubility predictions were performed in COSMO*thermX* using solubility tool with BP/TZVP parameterization. The predictions utilized the iterative algorithm as shown in Equation 3.5:

$$log_{10}\left(x_j^{sol(n+1)}\right) = \left[\mu_j^{pure} - \mu_j^{S}\left(x_j^{sol(n)}\right) - max\left(0, \Delta G_{fus}\right)\right]/(RT\ ln(10)) \quad 3.5$$

where x_j^{sol} is the mole fraction of solid dissolved in the targeted solvent, μ_j^{pure} is the chemical potential of pure compound j, μ_j^{L} is the chemical potential of pure compound j at infinite dilution in the solvent, S and ΔG_{fus} is the Gibbs free energy of fusion. The ΔG_{fus} of solute at a particular temperature, T was estimated by reference solubility method. In this work, the solubility data of ascorbic acid at a particular temperature is used to determine ΔG_{fus}.

3.5 Ascorbic Acid Crystal Characterization

3.5.1 Sample Preparation

Ascorbic acid crystal was prepared by dissolving ascorbic acid in 25 mL solvent until the saturation point was achieved. Then the ascorbic acid were shaken for continuous 8 hours at 303 K. The solution is left overnight to let the supernatant equilibrate. An aliquot of supernatant from the solutions was filtered using 0.45 μm syringe filter prior to the drying process in vacuum oven at 323 K. The samples are left to dry and the mass were weighed until a constant mass weight was achieved.

3.5.2 Microscopic Analysis

The optical microscope used was a Nikon Diaphot 300 (Nikon, equipped with DinoXScope camera. Four times magnification was used at appropriate planar. Ascorbic acid sample is carefully transferred onto a clear glass slide. The plane is adjusted for clear resolution and image is captured at a point where the image is able to describe the habit of the crystal.

3.5.3 Scanning Electron Microscopic (SEM)

The morphology of crystals was examined with a scanning electron microscope (Philips 30 XL, Netherlands) operating at 20 kV (Qiang et al., 2007). The samples were mounted on a glass slide with double-sided adhesive tape and coated with gold under vacuum in an argon atmosphere prior to observation. Prior to scanning process, the sample must be in appropriate size, stable in vacuum and electrically conductive. It was then mounted on specimen holder with flat surface aluminum pegs. The sample was sprayed with conducting material. The specimen holder were loaded into the SEM chamber and the scanning were conducted from low to high resolution, depending on the area of interest. Spot size is controlled via the condenser lenses. A higher lens result in a smaller spot. Scan time were increased to decrease the noise level.

3.5.4 Powder X-Ray Diffraction (PXRD)

The dried sample crystals were grinded and positioned onto the silicon plate at the sample holder. The crystal form crystallized from each condition was determined by PXRD with D8 ADVANCED – BRUKER axS model. A small quantity of crystal was

mounted on a flat holder. The samples were scanned from 5.0° to 60.0° of 2θ range using 1-second step and 0.025-step size (Srinivasan and Devi, 2010).

3.5.5 Single X-Ray Diffraction (SCXRD)

Colorless rectangular crystal (0.37 × 0.20 × 0.20 mm^3) was used for data collection. Diffraction data were collected by ω-scan technique on a Bruker SMART APEX II CCD diffractometer (Bruker, Massachusetts, USA) equipped with CuKα radiation (λ = 1.5418 Å) (Chieng, Rades and Aaltonen, 2011, Srinivasan and Devi, 2010). The unit cell parameters were determined by the least-squares methods using 1292 reflections in the 2θ range 5.5° to 55.6°. The data were corrected for Lorentz-polarization and absorption effects. The structure was solved by direct methods using the SHELXS program and refined by a full-matrix least-squares calculation on F2 using SHELXL. All H atoms were placed at calculated positions and treated using a riding model, fixing the C-H distances at 0.96 Å and U iso (H) = 1.2U eq (C)], the N-H distance at 0.86 Å and U iso (H) = 1.2U eq (N)]. The final Fourier maps showed no peaks of chemical significance.

3.5.6 Diffraction Scanning Calorimeter (DSC)

Thermal activity of L-ascorbic acid was studied using TA Instruments Differential Scanning Calorimetric (DSC, New Castle, USA). It was conducted under a nitrogen atmosphere at a flow rate 25 cm^3/min. Approximately 3.5 mg of ascorbic acid was heated from room temperature 303 K to 773 K at a rate of 10 °K/min (Adam, 2012; Juhász et al., 2012). ΔH_{melt} was determined by calculating the area under the curve from when the time the sample started to melt (T_{melti}) until all samples have completely melted (T_{meltf}). The area under curve represented the combination of the heat of fusion and the underlying change in heat capacity.

3.5.7 Thermal Gravimetric Analysis (TGA)

TGA was used to measure the changes in the weight of specimen in a controlled nitrogen atmosphere. The analysis was conducted with a Thermo-gravimetric Analyser (TGA) Q500 supplied by TA Instruments (New Castle, USA). Sample with a weight range of 4-6 mg was placed in a platinum pan and heated from 303 K to 573 K at a constant heating rate of 10 K/ min (Adam, 2012; Juhász et al., 2012).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

Type of solvents were reported to give impacts on solubility and habit formation of the crystals. It is therefore, important to perform solvent screening and characterize the crystals prior to the crystallization processes. This chapter presents the investigation on the effects of temperature and polarity of solvents on the solubility and dissolution of ascorbic acid. The polar solvents chosen were water, methanol, ethanol and 2-propanol. The methods of generating solubility data are described in Section 4.2. The experimental solubility data were compared with previous work and enthalpy of dissolution were discussed. In Section 4.3, COSMO-RS was used as tool to predict solubility of ascorbic acid and the values were compared with analytical. Based on the data obtained, the strength of intermolecular forces (IMF) is also discussed. From the IMF obtained from COSMO-RS simulation will be discussed in relation of crystal habit. This work continues in section 4.4 with the characterization of ascorbic acid crystal grown from different solvents. The characterization was conducted to study the habit and morphology of ascorbic acid crystal (microscopic analysis and SEM), crystallography characteristic of ascorbic acid (PXRD and SCXRD) and also thermochemistry analysis (TGA and DSC). The experimental results were compared with those of previous works whenever possible.

4.2 Gravimetric analysis

The molar solubility of ascorbic acid in water, methanol, ethanol, and propan-2-ol was measured from 303 to 323 K is shown in Figure 4.1 using the method described in Section 3.3. The figure shows that ascorbic acid is highly soluble in water, followed

by methanol, ethanol and 2-propanol. Ascorbic acid shows highest solubility in high polarity solvent as compared to other solvents with lower polarity. Generally, polar molecule will be soluble in polar solvent. It is in agreement with the work done by Shalmashi with average percentage difference of 7.1%. The standard deviation of 3 repetition is represented using error bar. Solubility at 323 K have slightly higher standard deviation as compared to other sample which may due to evaporation of highly volatile solvents like methanol, ethanol and 2-propanol during weighing of the warm samples.

The solubility of ascorbic acid increases with the increase of temperature in all solvents used in this study. This finding is consistent with the solubility of other pharmaceutical compounds reported in the literature (Chen et al., 2013; Zhang et al., 2014). The increase of solubility with the increase in temperature is due to the increase in molecules' kinetic energy at higher temperatures that leads to more effective movement or interactions between solvents and solute molecules (Zhang et al., 2007; Zhang et al., 2014). This findings is expected since the solubility behavior for the selected solvents typically depends on the solvation or the ability of solvents to interact with solute molecules (Tang et al., 2015).

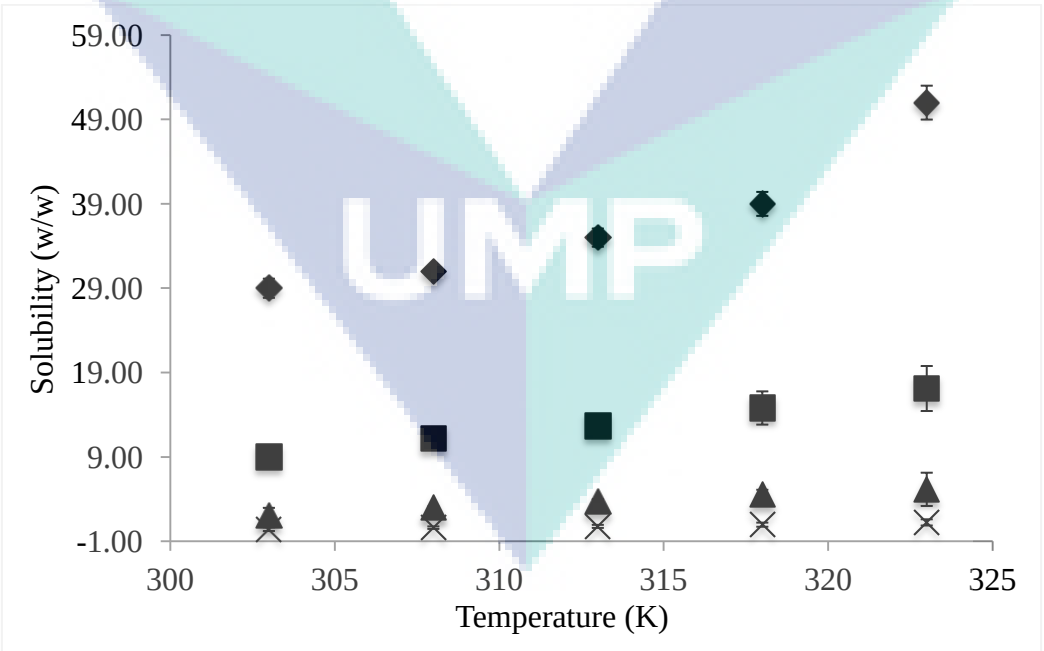

Figure 4.1: Experimental solubility data of ascorbic acid 10^3x in water (◆), methanol (■), ethanol (▲) and 2-propanol (✕). Error bars represent standard deviation from three measurements).

Figure 4.2 shows a comparison between the solubility data obtained by experiment and data from Shalmashi and Eliassi (2008). The R-squared (R^2) value is 0.98, signifying the reliability of the experimental solubility data as compared to literature. As temperature is increased, the solubility of ascorbic acid in water will increase by 0.001 (w/w) for every unit of temperature (K).

The solubility of ascorbic acid in all four solvents at 298 K were previously reported by (Shalmashi and Eliassi, 2008). Figure 4.2 shows linear graph to compare solubility of ascorbic acid obtained experimentally to work done by Shalmashi and Eliassi. In average, the experimental values only deviate less than 10% than those reported in their work. This indicates that gravimetric methods employed to measure the solubility in this work are reliable.

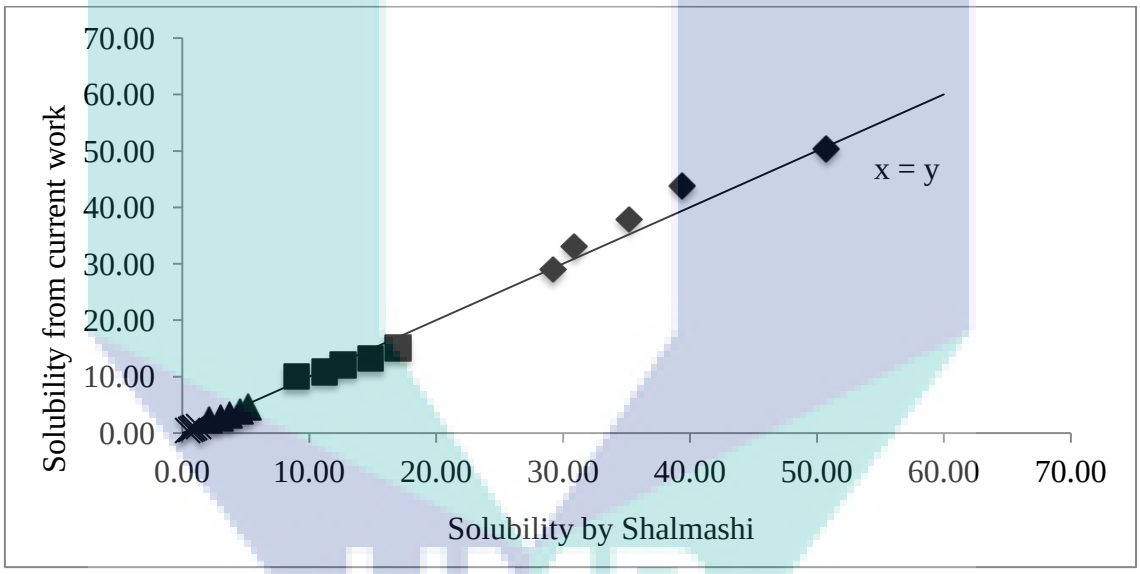

Figure 4.2: Experimental and published data for solubility of ascorbic acid in water(◆), methanol(■), ethanol(▲) and 2-propanol(✕). Solid lines are the solubility from liturature by Shalmashi (2008) with respect to each solvents.

The solubility data of ascorbic acid in four polar solvent are tabulated in Appendix: Table A.1 were used to construct a linearized van't Hoff plots as presented in Figure 4.3. The linearized van't Hoff plots is used to provide thermodynamics data such as enthalpy and entropy of dissolution (ΔH_{diss} and ΔS_{diss}) and Gibbs free energy change (ΔG_{diss}). The value of ΔH_{diss}, in kJ/mol calculated from the slope of the graphs indicates that the amount of heat absorbed or released during dissolution and the diffusion of ascorbic acid in each solvent used. The entropy of dissolution, ΔS_{diss}, in kJ/mol.K obtained from the y-intercept describing the spontaneity of dissolution

process (Chen et al., 2013; Zhang et al., 2014). The relation between ΔH_{diss} and ΔS_{diss} with the changes of Gibbs free energy, ΔG_{diss}, in different solvents is shown by Gibbs-Helmholtz equation as follows (Fang et al., 2015; Garcia-Delgado et al., 1992) as shows in Equation 4.2.

$$\Delta G_{diss} = \Delta H_{diss} - T\Delta S_{diss} \qquad 4.2$$

The R-squared (R^2) values of the van't Hoff plots are between 0.97 and 0.99, and thus signify the reliability of the experimental solubility data. Table 4.1 is tabulated to show the values of ΔG_{diss}, ΔH_{diss} and ΔS_{diss}, calculated within the range of 303 K to 328 K. From the table, it is found that the ΔG_{diss} , ΔH_{diss} and ΔS_{diss} are all positive is all four discussed solvents. All values are positive, which indicates that the dissolution processes were endothermic, entropy driven and not spontaneous. The highest values of ΔH_{diss} and ΔG_{diss} were obtained during the dissolution of ascorbic acid in 2-propanol, which indicates low solubility or poor solute−solvent interactions. ΔH_{diss} and ΔG_{diss} were the lowest ones in water, which indicate high solubility. The increase of ΔH_{diss} with the decrease in solvent polarity shows that ascorbic acid dissolution absorbed more energy as the polarity of the solvent decrease. This suggests that in order for ascorbic acid to dissolve in less polar solvent, more energy will be required to overcome its solute-solute intermolecular forces. ΔG is at its lowest in water, which suggest that the ascorbic acid crystal formed from water is the most stable as compared to the other solven

Table 4.1: Calculated ΔH_{diss}, ΔS_{diss} and ΔG_{diss} from Van 't Hoff linear plot

Solvents	ΔG_{diss} at 303 K kJ/mol	ΔH_{diss} kJ/mol	ΔS_{diss} kJ/mol
Water	12.82	21.84	0.04
Methanol	13.48	25.25	0.04
Ethanol	20.75	36.06	0.06
2-propanol	21.18	40.48	0.07

The positive values of ΔS_{diss} indicate that when the ascorbic acid undergoes dissolution in solvent, the spontaneity increases as the molecules gain more freedom. The values of ΔS increase from water < methanol < ethanol < 2-propanol. The values

increase because longer chain of compound rotates and vibrates in more ways than a shorter one.

Positive values of ΔH_{diss} indicate that dissolution of ascorbic acid is endothermic and it require more energy to break the bonds within the solute and solvent than the energy released when new bonds are formed. The ΔH_{diss} of ascorbic acid from water deviates by 4% from work done by Dallos and colleagues (Dallos, Hajós-Szikszay and Liszi, 1998). ΔH_{diss} increase as the polarity of the solvents decrease from water < methanol < ethanol < 2-propanol.

Positive values of ΔG_{diss} indicates that the dissolution of ascorbic acid is non-spontaneous at all temperatures. Based on kinetic molecular theory, as the temperature gets higher, the molecule will gain kinetic energy, therefore, more effective collision and interaction could occur between solvent and solute molecules (Silberberg, 2015).

Solubility of ascorbic acid is also influenced by the strength of the intermolecular forces that occur during the dissolution process. It has been reported in many literature that the intermolecular interaction between solute and solvent molecules through hydrogen bonding demonstrates a significant role in solubility (Chen et al., 2013; Mealey et al., 2014; Tang et al., 2015). When ascorbic acid dissolves in a solvent, the attractive forces in solute and solvent must be overcome. The dissolving ascorbic acid must be able to break up the aggregation of molecules in the solvent, that is, the hydrogen bonds between molecules or the London dispersion forces between molecules in a solvent, and the molecules of the solvent must have sufficient attraction for the solute particles to remove them one by one from their neighbors in the undissolved solute.

During dissolution process, ascorbic acid as a one of the cyclic polar molecule is able to achieve equilibrium easier with the smaller solvent molecules like water as compared to bigger solvent molecules because it is easier for the hydroxyl groups in ascorbic acid to establish hydrogen bonds with smaller size solvents, leading to better solubility (Shalmashi and Eliassi, 2008). The solubility of ascorbic acid in water shows the highest value than those in the other solvents. Thus, water may be the best solvent to dissolve ascorbic acid but it is not the easiest to be separated by evaporation method as it has strong polar-polar and hydrogen bond interaction between solvent and solute.

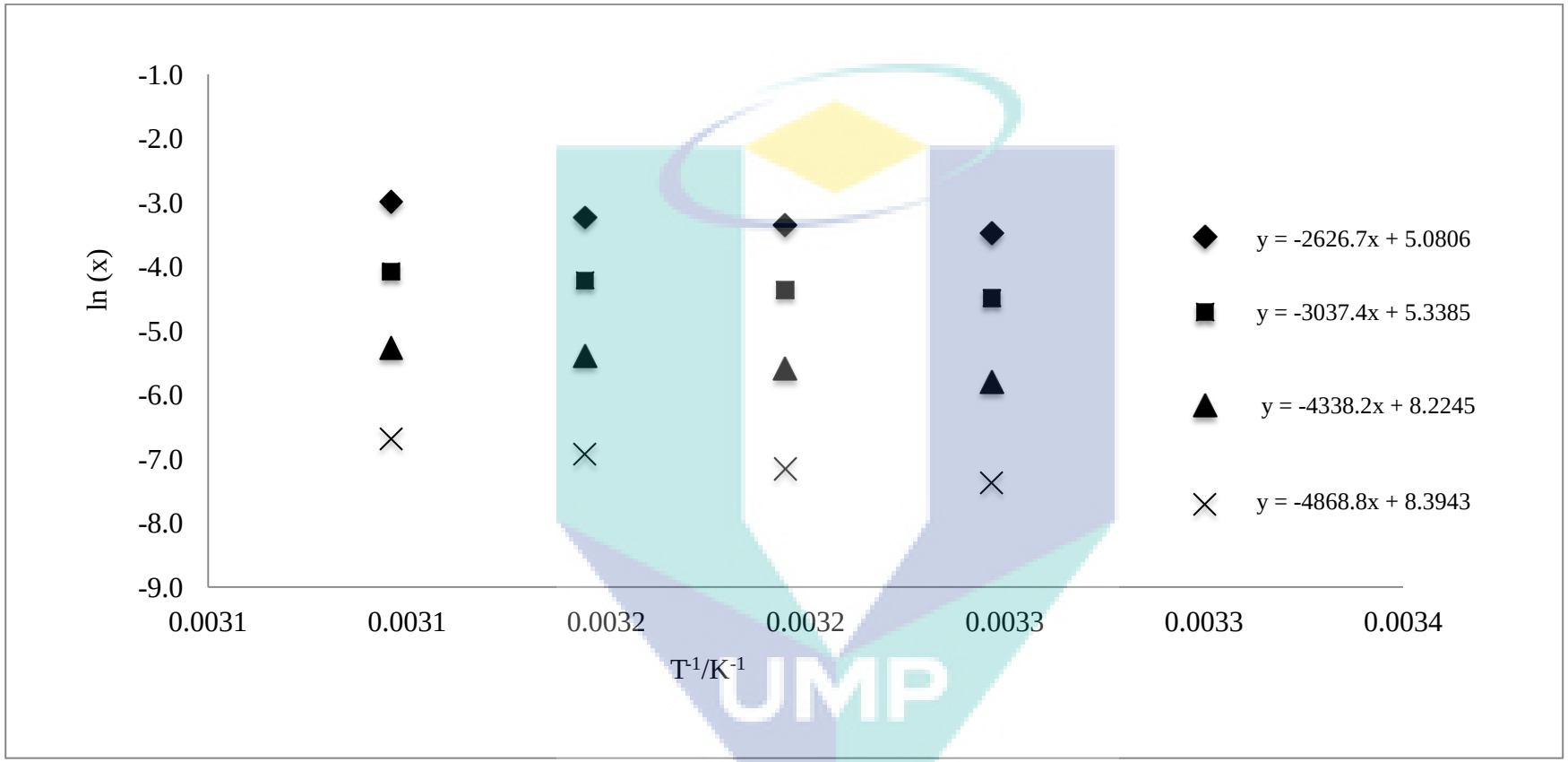

Figure 4.3: van't Hoff plot of ascorbic acid in water (◆), methanol (■), ethanol (▲) and 2-propanol (✕) against inverse temperature (K) ranging from 303 K to 323 K

4.3 Investigation on Ascorbic Acid Solubility and Intermolecular Interaction by COSMO-RS

Simple and fast determination of solubility is essential in pharmaceutical industry. Standard COSMO-RS function is able to predict the solubility of one compound based on its enthalpy of fusion (ΔH_{melt}) and melting temperature (T_m). However, ascorbic acid, which has four hydroxyl groups (-OH) and it does not obtain proper melting point because the compound decomposes before it could reach its melting temperature (Jingyan et al., 2013). Thus, ΔG was derived from the solubility experimental data has been used to reiterate the solubility of ascorbic and producing intermolecular interaction energy for evaluation. This is the first study that investigates the solubility of ascorbic acid in polar solvent using COSMO-RS.

4.3.1 Basic of COSMO-RS Simulation Process

COSMO-RS is based on compound local polarization charge densities, σ. The first step of the COSMO implementation was the development of a cavity-construction structure. Figure 4.4 depicts the molecular structure of ascorbic acid with its surface polarity already mapped out. Sigma profile of ascorbic acid is colour coded by the polarization charge density σ. Red areas denote strongly negative parts of the molecular surface and hence strongly positive values of σ. Deep blue marks denote strongly positive surface regions (strongly negative σ) and green denotes nonpolar surface.

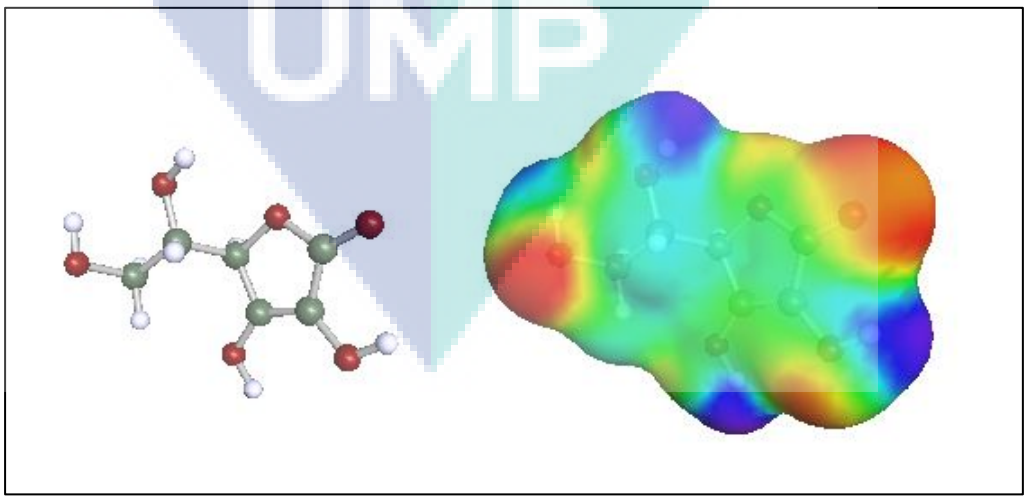

Figure 4.4 L-ascorbic acid (a) molecular structure; and (b) COSMO sigma profile image.

Figure 4.5 shows σ–profiles of solvents chosen in this scope of work. Water is used as the base of comparison to study other solvents' σ-profile. σ-region beyond ±1 e/Å^2 is considered strongly polar and can potentially form hydrogen bond (Klamt, 2005). σ-profile of all applied solvents range between 2 e/Å^2 to – 2 e/Å^2. In water, two peaks were detected at strongly positive and negative ends of the graph, indicating that water is a stable organic molecule that is able to accept and/or donate partial charge to form hydrogen bond. Methanol, ethanol and 2- propanol have about the same value as water in positive σ-range, however, in negative σ-range, the value is 50% less, describing that the solvents have less positive polar hydrogen as compared to water. This shows that the other solvents have half of the water capacity to accept partial charge to form hydrogen bond. The polarities of solvents are reduced from water, methanol, ethanol, and 2- propanol. The acceptance capacity is in line with the decrease in polarity value of the solvents.

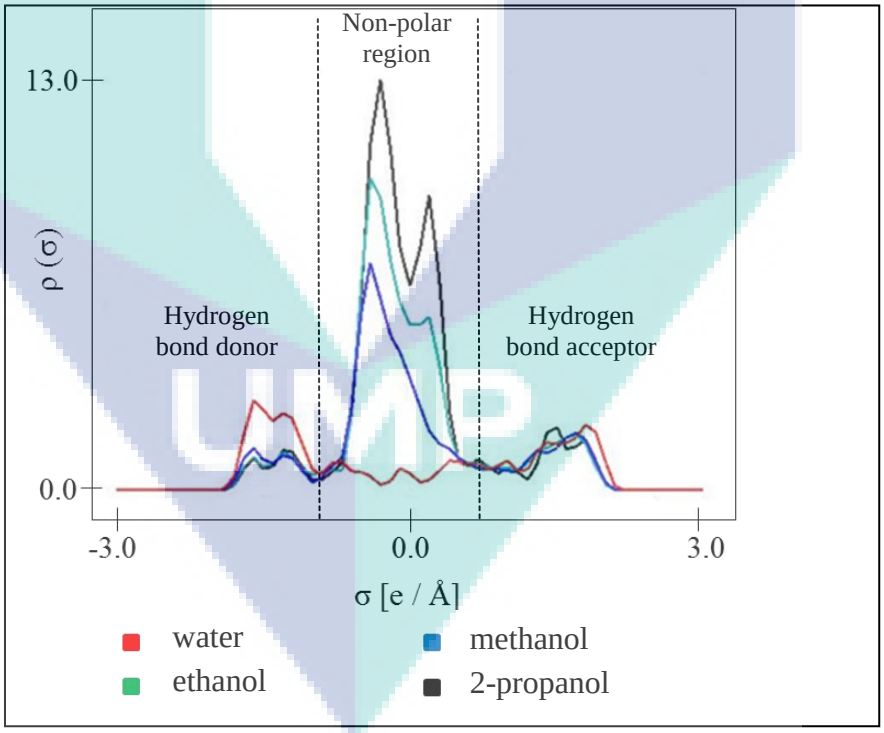

Figure 4.5 σ-profiles for water, methanol, ethanol and 2-propanol.

4.3.2 COSMO-RS Solubility Prediction

Figure 4.6 shows the solubility of ascorbic acid in four discussed solvents obtained by COSMO-RS simulation. The result shows that ascorbic acid is highly soluble in water, then followed by methanol, ethanol and 2-propanol. The solubility of ascorbic acid is the highest in the most polar solvent, which is water. The trend is mimicking the solubility data from gravimetric analysis. This shows temperature dependency in solubility of ascorbic acid. The solubility increases with the increase of the temperature due to the increase in molecules' kinetic energy at higher temperatures that leads to more effective collision between solvents with the solute molecules.

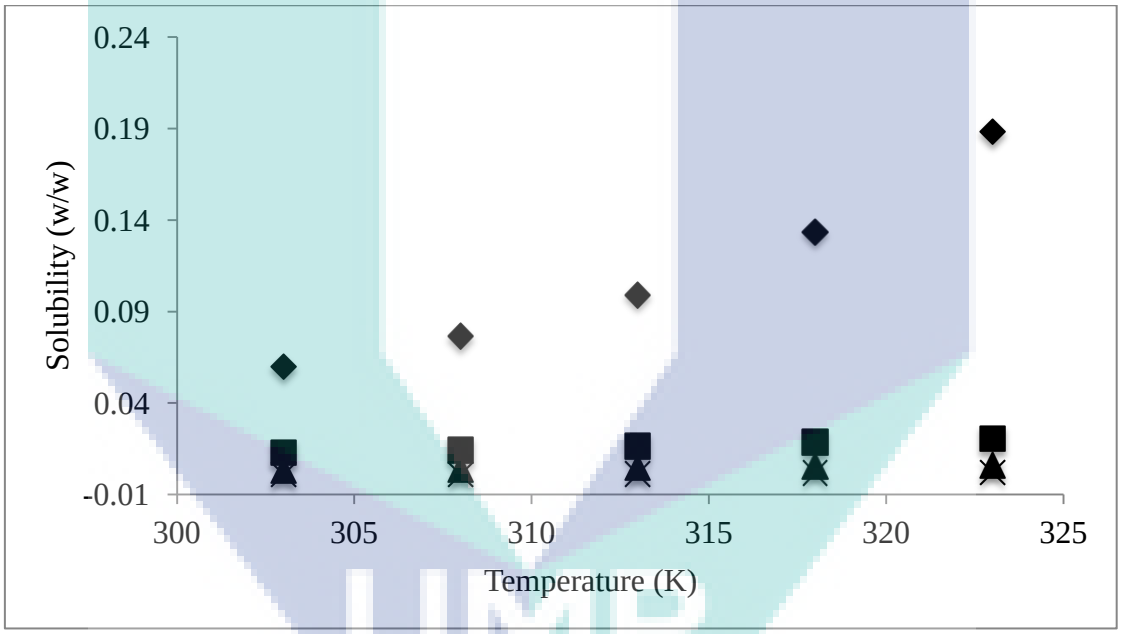

Figure 4.6: Solubility data obtain from COSMO-RS simulation for ascorbic acid in water(◆), methanol(■), ethanol(▲) and 2-propanol(✕)

Figure 4.7 show the comparison between solubility data obtained from the COSMO-RS calculation and experimental data using gravimetric method. The solubility pattern of ascorbic acid in water, methanol, ethanol and 2-propanol is in agreement with the experimental data. However, the solubility of ascorbic acid in water has bigger deviation when it is compared to experimental data even though it shows similar pattern. The sequence of solubility of ascorbic acid, as predicted by COSMO-RS, goes from the lowest to highest 2-propanol < ethanol < methanol < water. Data

show significant differences about 30 - 50%. This has been reported several times by other researchers that it is mainly due to the hydroxyl groups in ascorbic acid that had contributed to its high polarity, thus affecting its accuracy in COSMO calculation (Klamt et al., 2013, Klamt et al., 2012).

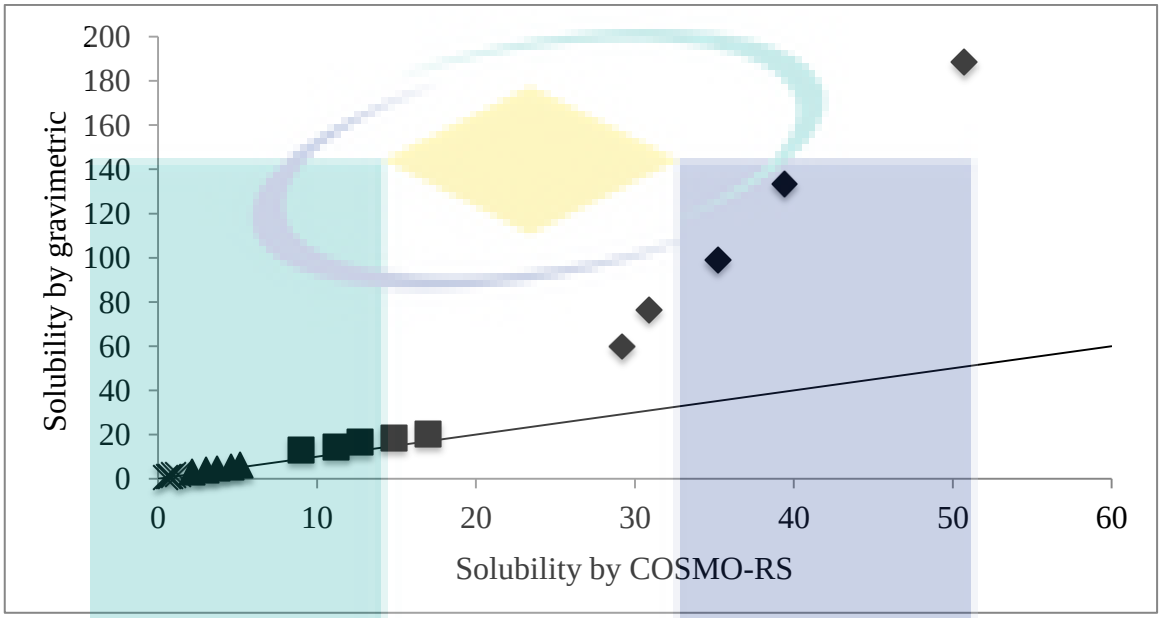

Figure 4.7: Comparison between ascorbic acid solubility data of current work and solubility data obtained by COSMO-RS in water (◆), methanol (■), ethanol (▲) and 2-propanol (✕). Solid lines represents linear line x = y.

Figure 4.8 shows the saturated mole fraction solubility of ascorbic acid against inverse temperature (K) in four different polar solvents derived from COSMO-RS simulation calculated within the range of 303 to 328 K. It shows highest solubility in water as compared to other solvents with lower polarity.

The R-squared (R^2) values of the van't Hoff plots are between 0.97 and 0.99, and thus signify the reliability of the data. The relationship between van't Hoff plot and ΔG_{diss} , ΔH_{diss} and ΔS_{diss} were discussed in Section 4.2. It is found that the ΔG_{diss} , ΔH_{diss} and ΔS_{diss} are all positive is all four discussed solvents. The positive values of ΔS indicate that when the ascorbic acid undergoes dissolution in solvent, the spontaneity increases as the molecules gain more freedom. Base on the simulation results, the values of ΔS was the highest in water with value of 0.13 kJ/mol and the lowest in methanol with the value of 0.26 kJ/mol. Then the ΔS increase from methanol, ethanol and 2-propanol.

This plot also gives positive values of ΔH_{diss} indicating that dissolution of ascorbic acid is endothermic in all solvents used. It absorb energy to break the bonds within the solute and solvent. The ΔH_{diss} of ascorbic acid from water is found to be the highest in these four solvents with value of 45.87 kJ/mol, contradicting with data obtained from gravimetric analysis. The lowest ΔH_{diss} is from methanol, ethanol and followed by 2-propanol. The reason of this difference is may due limitation of predicted approach that it does not take into account the additional strength of hydrogen bond interaction of the ascorbic acid and highly polar water molecules.

Table 4.2: Calculated ΔH, ΔS and ΔG from Van 't Hoff linear plot

Solvents	**ΔG at 303 K kJ/mol**	**ΔH kJ/mol**	**ΔS kJ/mol**
Water	6.78	45.87	0.13
Ethanol	10.94	18.82	0.03
Methanol	14.52	26.64	0.04
2-propanol	18.18	40.00	0.07

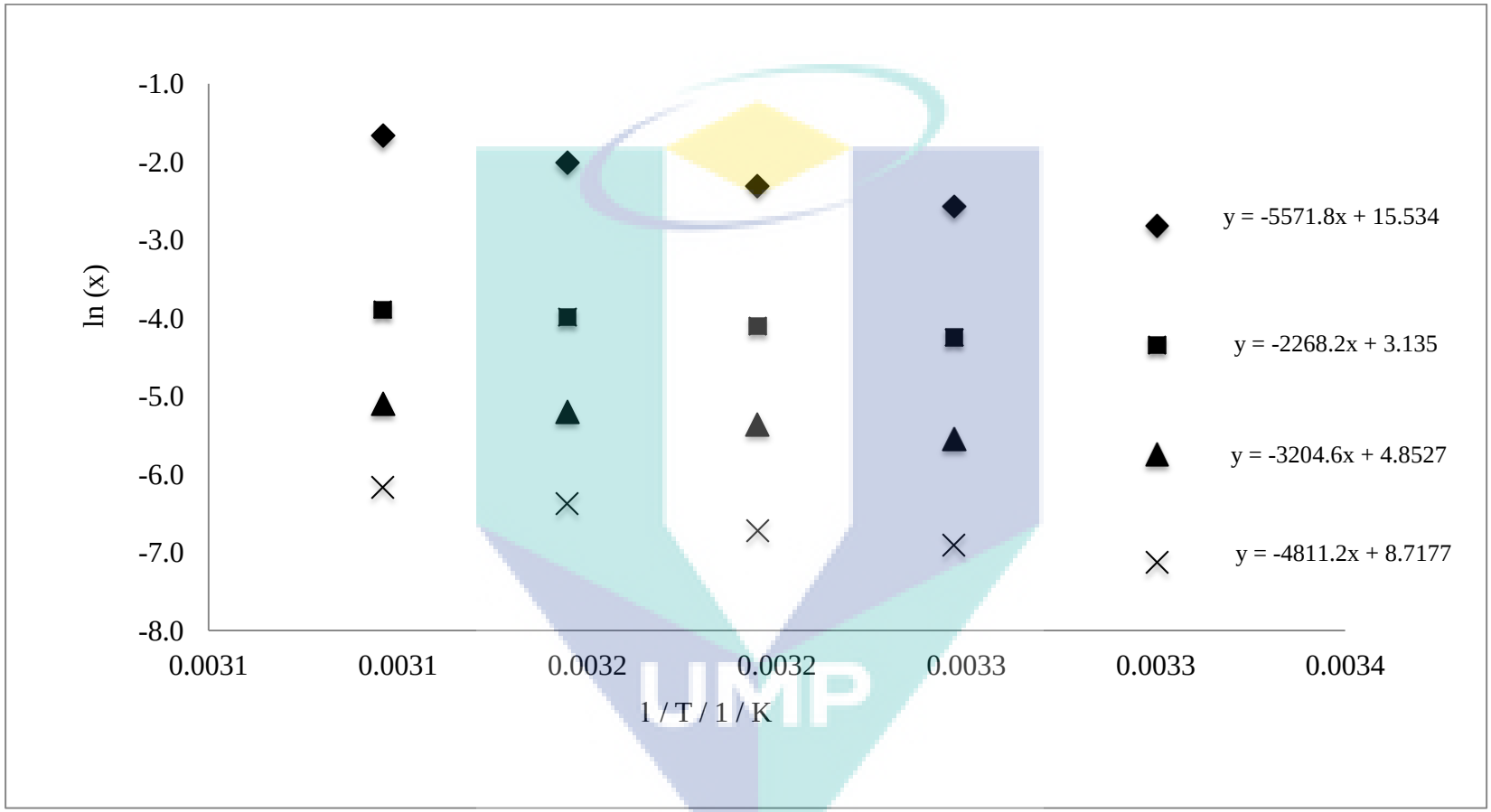

Figure 4.8: van't Hoff plot of ascorbic acid in water(◆), methanol(■), ethanol(▲) and 2-propanol(✕) against inverse temperature (1/K) ranging from 303 K to 323 K

4.3.3 Intermolecular Forces Interaction between Solute and Solvent

Intermolecular interaction always plays some role in determining the physical properties of a substance. Besides giving prediction to solubility data, COSMO-RS could also extend the data and obtain the excess information on intermolecular forces that are involved in ascorbic acid solubility. The intermolecular forces are van der Waals, hydrogen bond and electrostatic force. These interactions were quantified from the solubility calculation based on the local surface polarization charge densities in virtual conductor environment (Klamt, 1995). Electrostatic energy (E_{misfit}) represents the specific interaction energy between two surface segments. (E_{HB}) calculation is computed based on two molecule surfaces of opposite polarity that are in contact, while van der Waals interaction (E_{vdW}) are based on the number of atom in a molecule.

Figure 4.9 to Figure 4.12 shows molecular interaction energy, namely, hydrogen bond, van der Waals interaction and electrostatic energy in water, methanol, ethanol, and 2-propanol. Negative values indicate that the solvents are prone to accept partial electron and form hydrogen bond from ascorbic acid. The more negative values show the solvents are prone to accept more partial electron from ascorbic acid. On the other hand, positive values indicate that the solvents are prone to donate partial electron to the solute. Positive values indicate that the solvents are prone to donate partial electron to the solute. The figures illustrate that water has the highest hydrogen bond energy, representing stronger hydrogen bond acceptor (HBA) site as compared to other solvents. This is highly favorable because it prepares stronger binding site for ascorbic acid's hydroxyl's group to form hydrogen bonds and improve solubility. As the HBA in solvents decreases, the solubility of ascorbic acid in respective solvents decreases.

Ascorbic acid solubility is higher in solvent with high HBA and lower in those with less HBA available in solvent. In contrast, van der Waals energy was very low in water due to the size of water being very small compared to other solvents and the absence of non-polar carbon atom. However, with the increasing of carbon atom in other solvent molecules, van der Waals interaction can be seen to have increased consistently with the increase number of carbon atom. This observation is consistent with general rule that the bigger the molecule is, the stronger the van der Waals interaction will be because it provides more surface area, therefore increasing the molecules' interaction (Silberberg, 2015).

As seen in Figure 4.9 to Figure 4.12, small variations in the energies values are observed as the temperature increases. These indicate that the temperature only has a small effect on the molecular interaction energies. Tang et al. (2014) who studied the solubility of androstenedione in lower alcohols also reported that the microscopic interactions between molecules, which was modeled using COSMO-RS, did not show any significant changes as the temperature increases.

The changes of dispersion forces or van der Waals energy (H-vdW) of ascorbic acid molecules in the solvents studied are very small, except for water. The small variations in the H-vdW energies, which are between -12.72 kcal/mol and -12.32 kcal/mol are probably due to small changes of number of atoms in the solvents studied. The H-vdW energy for ascorbic acid in water is smaller than the other solvents, which is -10.14 kcal/mol. This is particularly due to the size of water molecule that is smaller than the size of other solvents under investigation. Tang et al. (2014) also found that the changes of H-vdW energies are very small for molecules that have similar size or number of atoms.

It is also observed from the four figures that the changes of electrostatic energies and the hydrogen bonding energies are quite apparent, which are from 5.296 kcal/mol to 2.973 kcal/mol and from -4.821 kcal/mol to -0.085 kcal/mol, respectively. The changes of H-HB energies are more significant than the H-MF energy. This finding may suggest that the hydrogen bonding interactions between ascorbic acid and solution are more responsible for the differences in the ascorbic acid solubility behaviour. The significant changes of H-HB energies are expected due to different hydrogen donor and acceptor propensity of ascorbic acid and solvents under investigation. A solvent molecule that has a stronger ability to donate or accept hydrogen bonding than the solute molecule may establish hydrogen-bonding network with the solute molecule, resulting in selective nucleation (Davey and Garside, 2002). Several literatures also reported that the stronger hydrogen bonding energies between solute and solvent molecules, the higher the H-HB energies are gained in the solutions, and thus, increasing the solubility values (Alevizou and Voutsas, 2014; Tang et al., 2014).

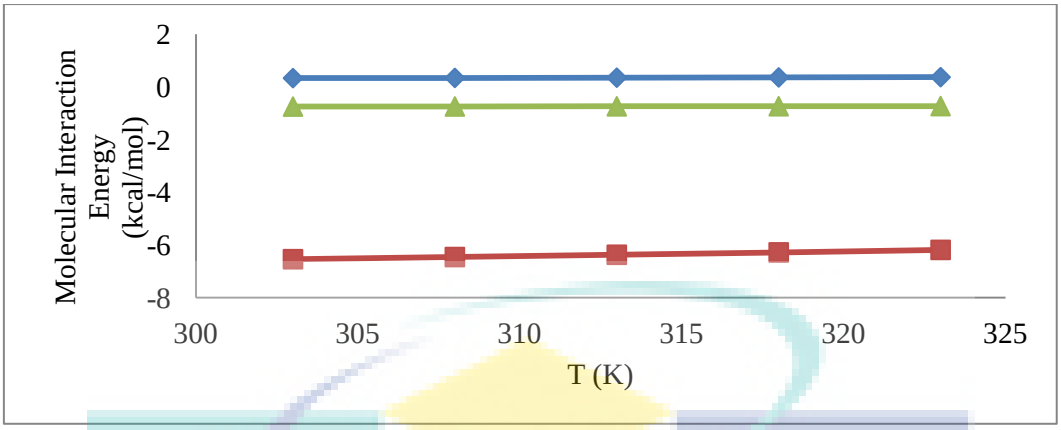

Figure 4.9: Molecular interaction, E_{misfit} (◆), E_{HB} (■), E_{vDW} (▲) at different temperatures between ascorbic acid with water

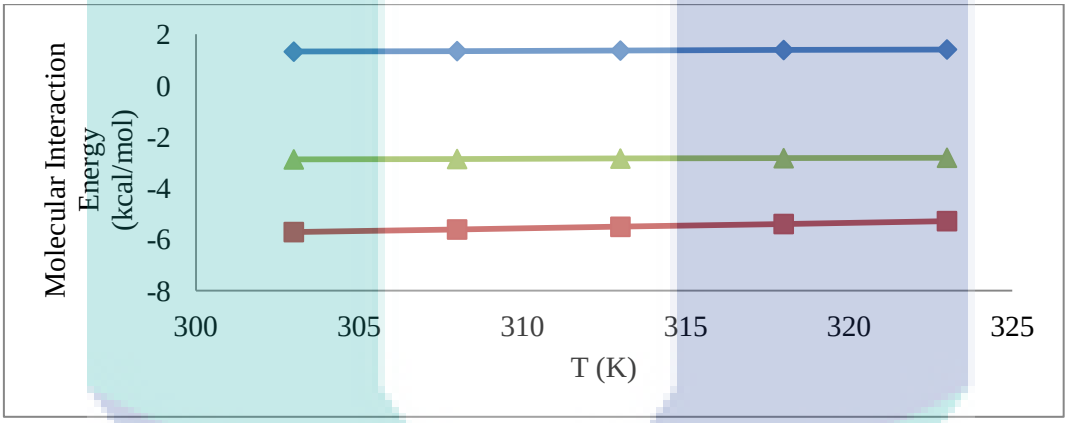

Figure 4.10: Molecular interaction, E_{misfit} (◆), E_{HB} (■), E_{vDW} (▲) at different temperatures between ascorbic acid with methanol

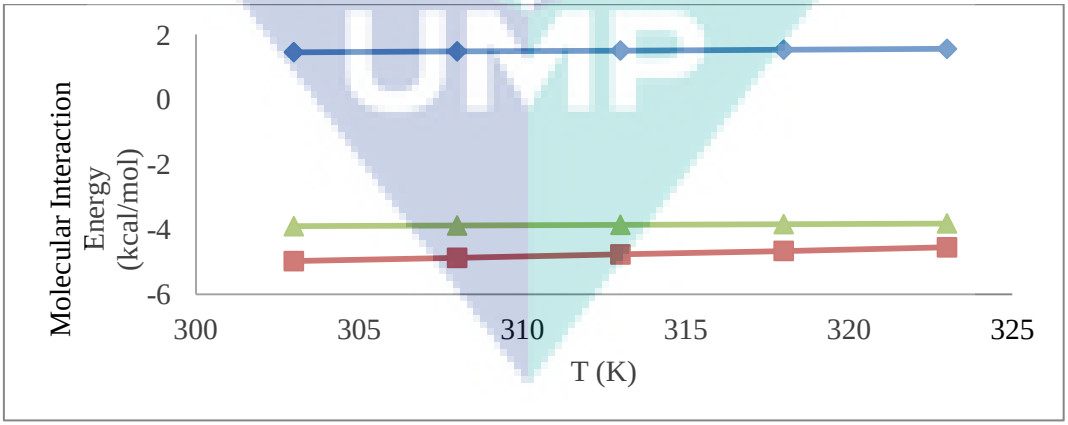

Figure 4.11: Molecular interaction, E_{misfit} (◆), E_{HB} (■), E_{vDW} (▲) at different temperatures between ascorbic acid with ethanol

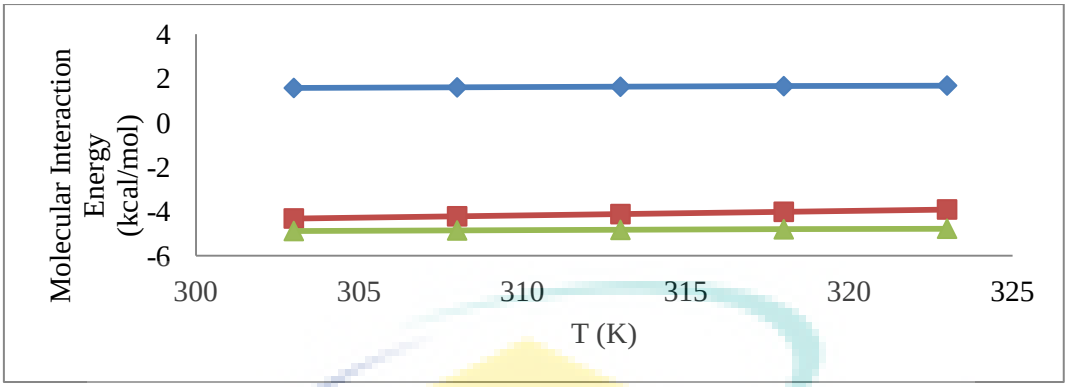

Figure 4.12: Molecular interaction, E_{misfit} (◆), E_{HB} (■), E_{vDW} (▲) at different temperatures between ascorbic acid with 2-propanol

In summary, ascorbic acid solubility in different solvents was calculated using COSMO-RS at temperatures ranging from 303 to 323 K. The solubility of ascorbic acid calculated using COSMO-RS mostly follows the same trend as the experimental values. It is also observed that the solubility increases with the increase of temperature. The capability of the model for the quantification of different types of interaction energies, namely, misfit, hydrogen bonding and van der Waals was utilized to identify the dominant interactions that determined the solubility behavior. The microscopic interactions energies predicted by COSMO-RS follows the solubility trend.

4.4 Characterization of Ascorbic Acid Obtained from Various Solvents

Ascorbic acid crystal grown in water, methanol, ethanol and 2-propanol showed differences in its habits. To understand the factors that influence the changes, the crystal was characterized using microscopic analysis to study its morphology. Crystal identity was determined using Powder X-Ray Diffraction analysis (PXRD) and Single Crystal X-Ray Diffraction Analysis (SCXRD). Thermal Gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) revealed each crystal thermodynamic properties. All these will be discussed with relation to intermolecular forces especially hydrogen bonding interaction. The ascorbic acid crystal used for this analysis was grown as outlined in section 3.5.

4.4.1 Microscopic Analysis

Figure 4.13 shows the figure of ascorbic acid crystal obtained from (a) water; (b) methanol; (c) ethanol; and (d) 2-propanol. The crystal formation was observed

under microscope at 4.0x resolution for crystal from water and 10x resolution for crystal from methanol, ethanol and 2-propanol. From the images, the habit of ascorbic crystal crystal that is obtained from water of ascorbic acid is in form of a prism. This is in confirmation with the work conducted by other researchers (Hvoslef, 1968a; Srinivasan and Devi, 2010).

Ascorbic acid that was crystallized from other solvents was observed to produce a tree-like formation which is known as dendrites. Figure 4.14 shows a dendritic growth of ascorbic acid crystal from ethanol. Dendritic growth commonly happened during evaporation process of high enthalpy crystallization (Mullin, 2001a). Individually, the crystal formed from other solvents showed a needle like habit. It is observed that width of the crystal was getting smaller as the polarity of the solvent reduced.

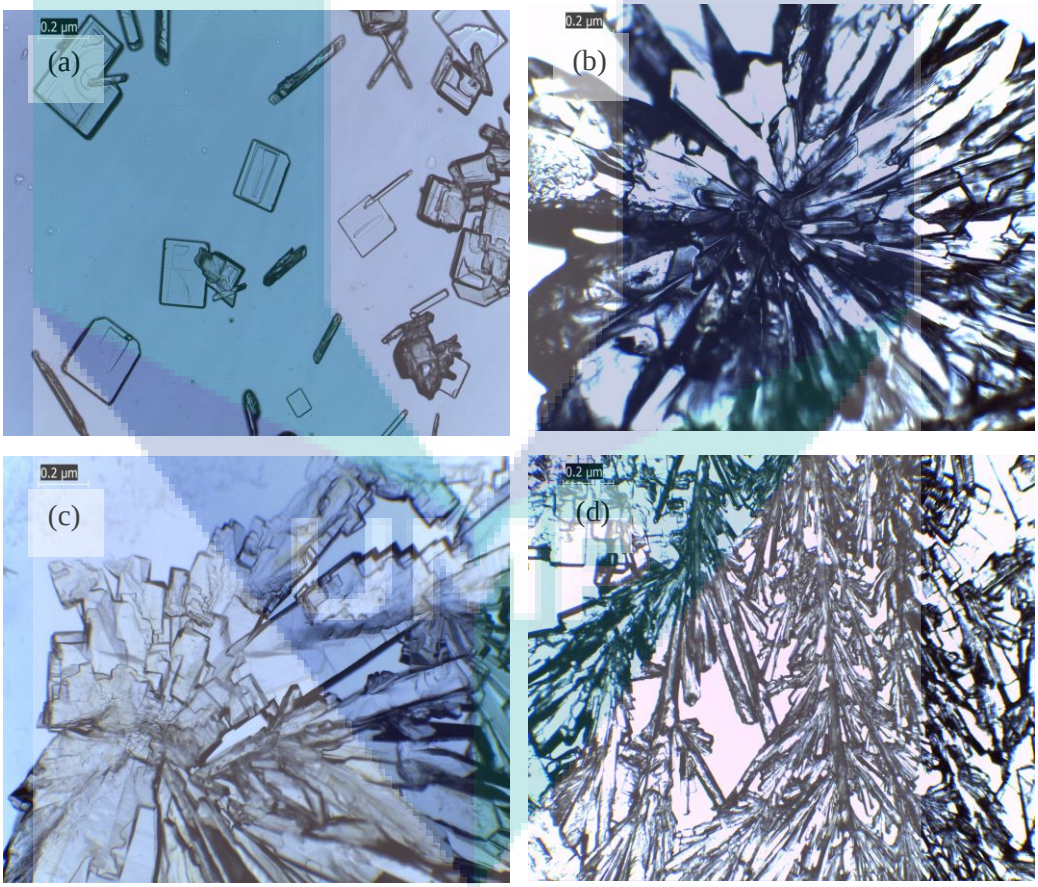

Figure 4.13: L-ascorbic acid crystal obtained from (a) water; (b) methanol; (c) ethanol; and (d) 2-propanol.

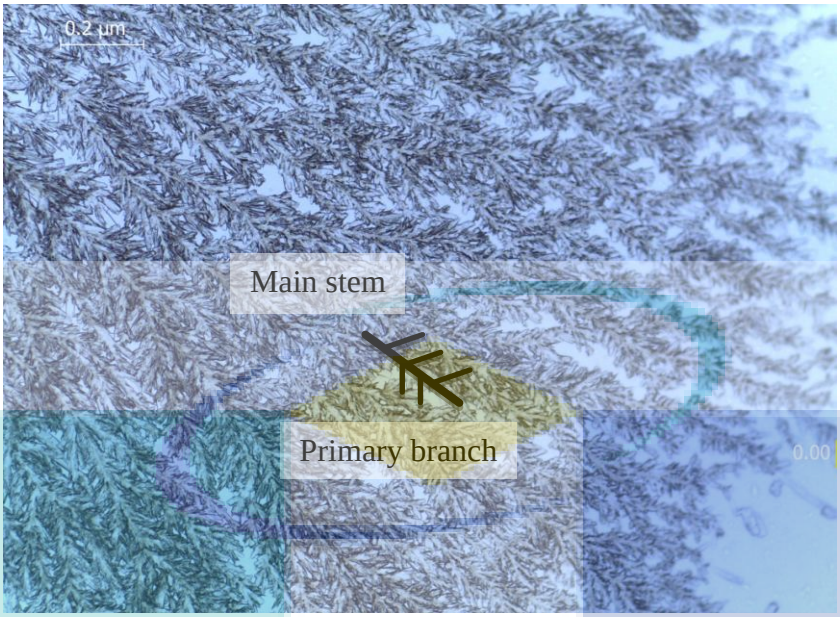

Figure 4.14: Dendritic growth of ascorbic acid crystal

The average size of crystals that formed from water is 0.0641 µm × 0.0562 µm. It has length to width ratio of 1:0.9, which is common characteristic of a prism habit crystal. The size of crystals that are obtained from methanol, ethanol and 2-propanol only been compared based on their width due to the dendritic growth of the crystal. The width of the crystal from higher polar solvent to lower polar solvent decrease from 0.0452 µm in methanol, 0.0221 µm in ethanol and the smallest was 0.0144 µm in 2-propanol.

Crystals grow from water has the tendency to grow into two axis, which makes the crystal prismatic, almost cubic-shaped. Bodor reported that the (010) face is more polar compared to (100) face (Bodor, 1995). Chen and Wang found that ascorbic acid has greater hydrogen bond propensities along *y* axis and *z* axis as compared to *x* axis (Chen and Wang, 2000). Figure 4.15 represent the image of ascorbic crystal from figure 4.13 and compare it with common miller indices faces side by side. Crystals grow along the *y* axis and *z* axis which have higher hydrogen bond density forming a prism, almost cubic shape. However, the growth of less polar *x* axis or (100) face is less than the other faces. In case of high cooling rate, (100) crystal face may disappear and the ascorbic acid crystal will appear to be platy.

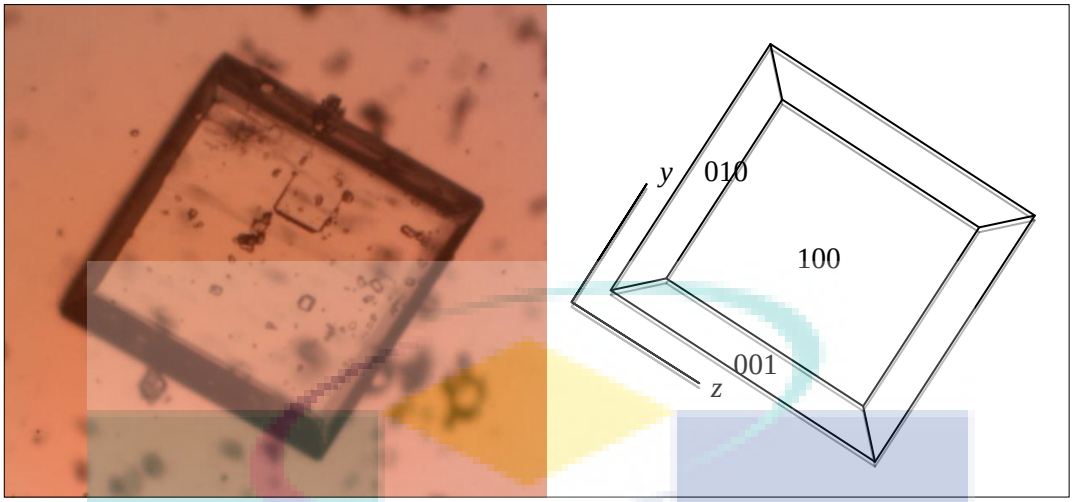

Figure 4.15: Comparison of experimental ascorbic acid crystal from water to miller indices representation of prism crystal

In methanol, ethanol and 2-propanol, there is only one site that may form hydrogen bond with ascorbic acid. Figure 4.16 shows an example of prism grown crystal from ethanol as solvent. It results to more elongated prism along *y* axis, and stunted growth on *z* axis. It suggests that *y* axis has higher hydrogen density as compared to other two axis. In different polarity of alcohol solvents, the size of crystal along *z* axis varies. The higher the polarity of the solvents, the longer *z* axis is observed.

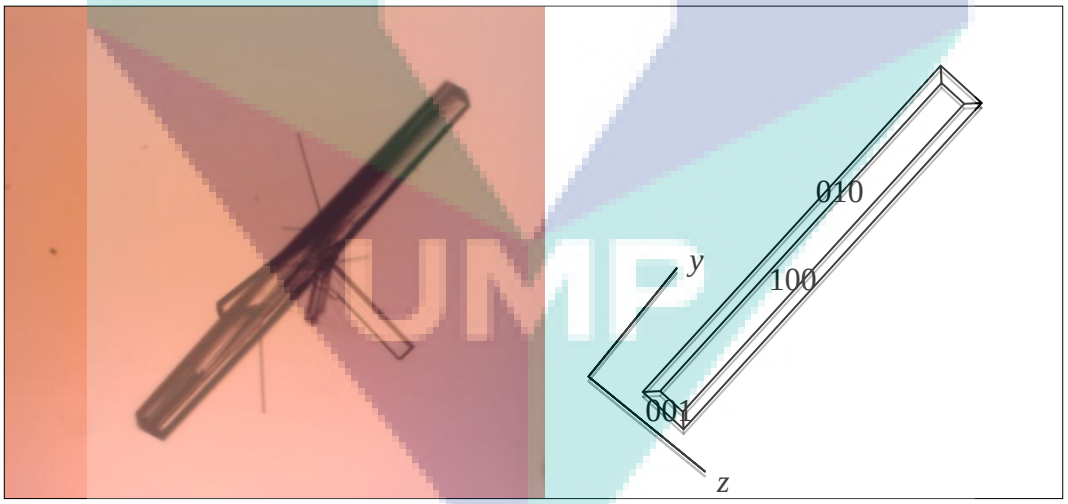

Figure 4.16: Comparison of experimental ascorbic acid crystal from ethanol to miller indices representation of prism crystal

Hydrogen bonding formed between ascorbic acid and solvents may also influence the formation of crystal habit. Solvent with more HBA site provides more potential for ascorbic acid to form stronger hydrogen bonding. Solute molecule must displace hydrogen bonded solvent molecule to grow a crystal face. The stronger the

hydrogen bond form, the slower the displacement process, thus slowing the growth rate of the crystal face. During crystallization, fast growing faces normally disappear; leaving the crystal bounded by the slowest growing faces. Growth will be slower with stronger hydrogen bond formed, hence influence the crystal morphology.

Uesaka and Kobayashi (2002) reported that higher humidity forms thicker needle-like crystal. Nevertheless, excess humidity will, eventually, have been absorbed between the crystals and transformed into an amorphous sticky-like material.

From the microscopic analysis, it shows that the crystal size gets smaller with the decrease of solvent polarity. The size is found to be smaller from water > methanol > ethanol and > 2-propanol.

4.4.2 Scanning Electron Microscope (SEM)

Figure 4.17 shows images from SEM for ascorbic acid crystallized from water, methanol, ethanol and 2-propanol. From the SEM images, crystal from water formed a cubic crystal. For ascorbic acid from methanol, ethanol and 2-propanol, the habit of the crystals become smaller and thinner in accordance with the results from microscopic analysis. The growth of ascorbic acid crystal is elongated to one direction, forming more needle-like crystal (acicular habit). And the width seems to vary depending on the strength of the solvents polarity. Ascorbic acid crystal habit is elongated and becomes thinner from growth in highly polar solvent like water as compared to less polar solvents like methanol and ethanol respectively. The less polar solvents produce thinner and more elongated ascorbic acid crystal.

As discussed in Section 4.2, solute-solvent interaction is due to the intermolecular forces strength. In this study, the hydrogen bond seems to be the most prominent intermolecular forces that plays a vital role in formation of ascorbic acid crystals. The stronger the hydrogen bond appears to be in the solvent, the stronger polarity of the solvent. The stronger polar solvents help the solutes to rearrange themselves on a specific polar face better. The growth rate of the crystal varies with different polar faces. Preferential growth of crystal faces changes (i.e. define) the habit of the crystal (Stoica, 2003).

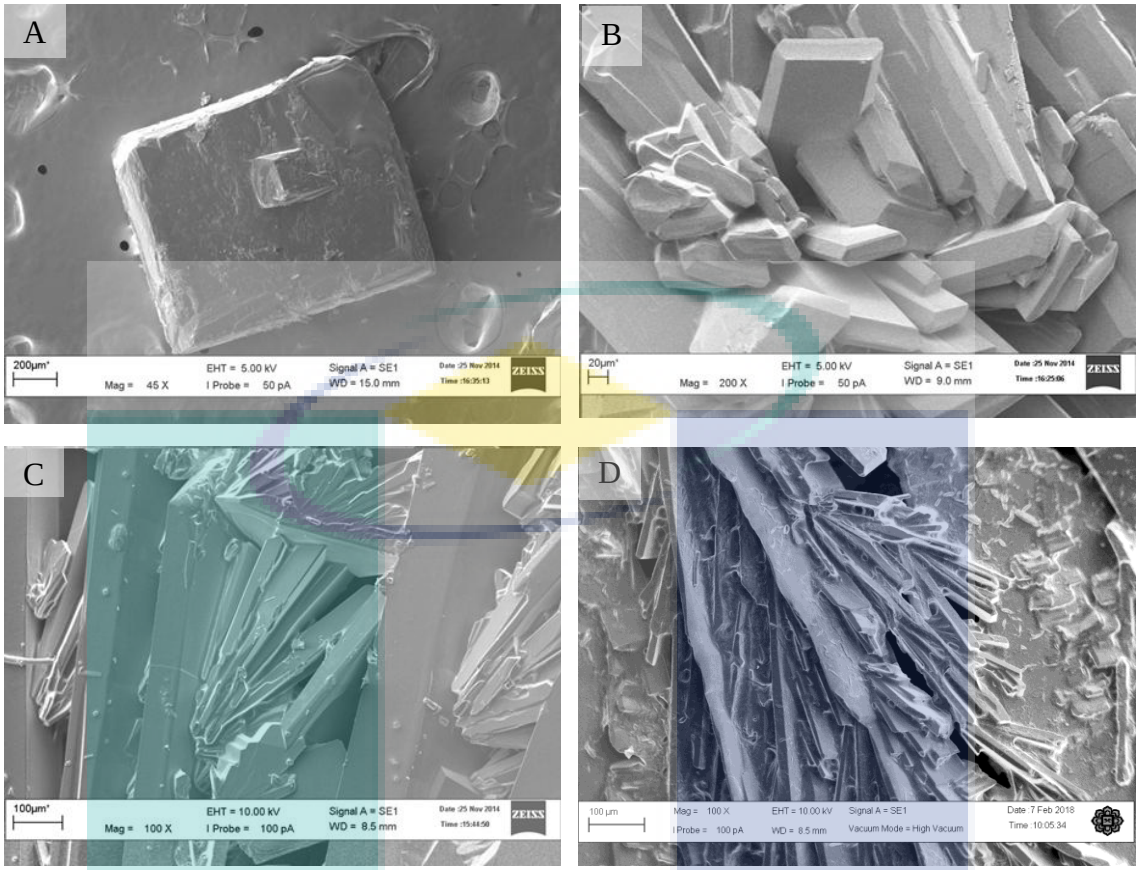

Figure 4.17: Scanning microscope image of ascorbic acid crystallized from solvents (a) water, (b) methanol, (c) ethanol, (d) 2-propanol

4.4.3 Powder X-ray Diffraction Analysis (PXRD)

Figure 4.18 shows the PXRD pattern on ascorbic acid obtained from CCDC standard library for LASCA 12 (Milanesio et al, 1997), compared to PXRD pattern of ascorbic acid crystallized from water, methanol, ethanol, and 2-propanol. From the data obtained, all ascorbic acid crystals tested are in monoclinic crystal system, $P2_1$ space group despite their differences in crystal habit and habit as discussed in previous section. Standard PXRD pattern from CCDC is used as a benchmark for analysis of crystals from different solvents. In general, ascorbic acid that is obtained from water, methanol, ethanol, and 2-propanol have the same crystal profile with varying intensity. The relative intensities were not a major issue because intensity varies by the differences in crystal habit, habit or morphology and the size of crystals, which resulted in the preferred orientation of crystals in the PXRD sample holder (Dash and Suryanarayanan, 1991). However, in recent studies, there is possibility to relate the PXRD relative intensity to crystal morphology (Inoue and Hirasawa, 2013)

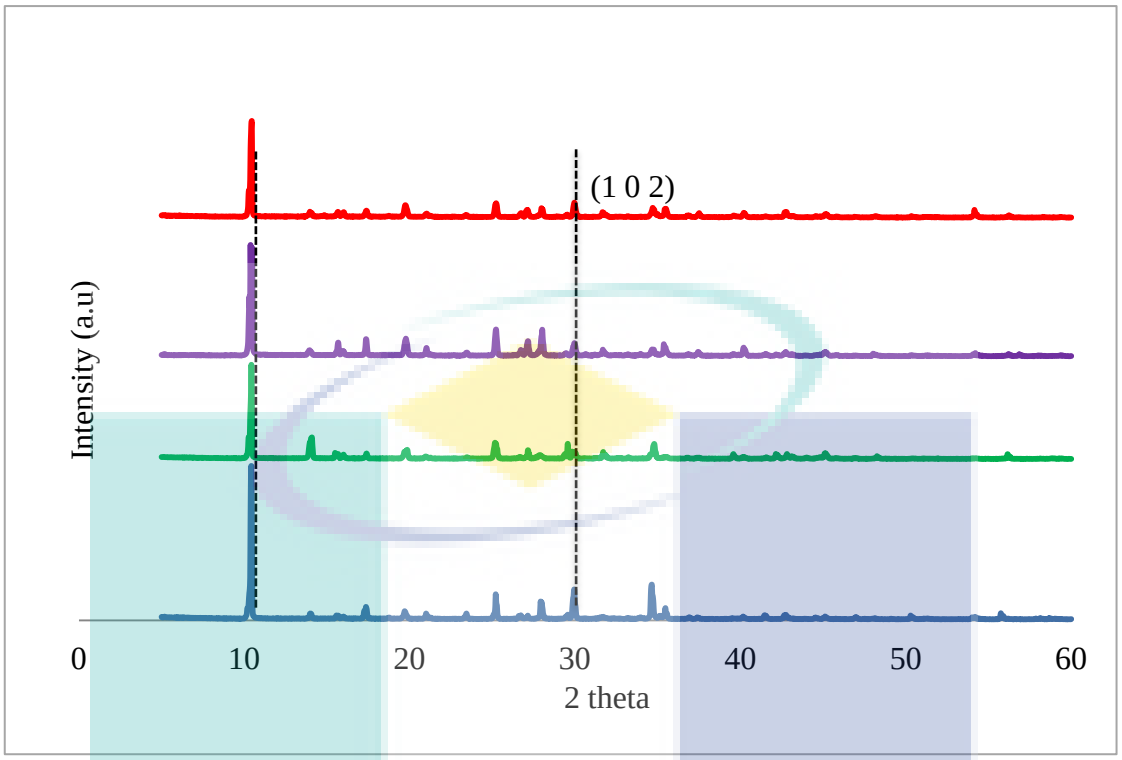

Figure 4.18: XRPD pattern from ascorbic acid crystallized in [A] water [B] methanol [C] ethanol [D] 2-propanol

The data is analyzed at four main peaks based on the crystal library. The points are at 10.466 °, 21.051 °, 28.059 °, and 30.029 °. Crystal from all solvents shows similar peaks at the respectives angles. However, there is a slight displacement at angle 28.059 ° for crystal from methanol, ethanol and 2-propanol. This observation may be due to impurities and imperfection in crystal form. On the other hand, at angle 26.726 °, there is only a tiny peak in standard crystal, crystal from water and from methanol. The peak, however, grows noticibly high from the crystal obtained from ethanol and 2- propanol. It may suggest that the ascorbic acid crystal from ethanol and 2- propanol may have developed crystal face in that direction.

From PXRD data, it is confirmed that ascorbic acid from different solvents share the same crystal phase even though the resulting shape and habit are different. The intensity of the PXRD peak may give some hint to the direction of crystal growth as well as the resulting shape. Therefore, modification of structure and habit of ascorbic acid crystal is possible by manipulating the solvent's polarity strength.

4.4.4 Single Crystal XRD (SCXRD)

Table 4.3 shows the values of lattice parameter from the work of Hvoslef, Devi and current work. The current work only includes crystal from water, methanol and ethanol as the crystal from 2- propanol is insufficient to form the test. From the single crystal data obtained, the values for axial length and angles for crystals from different solvents in this work is in agreement with the data from literatures. Ascorbic acid crystal is from monoclinic system with α and γ values of 90 ° and β not equal to 90 °. Current work records that it has 2 symmetry faces with the range of β axial angle between 99 °32' to 102 °18'.

It can be concluded that ascorbic acid crystals, despite exhibiting different habits while treated in different solvents, the molecular arrangement in unit cell is still the same. Thus, no new polymorph is formed or detected from this study, only varieties of habit of ascorbic acid crystals.

Table 4.3: The axial length and angles of single crystal of ascorbic acid from literature, ascorbic acid crystal from water, methanol and ethanol.

Crystal unit cell parameter	**Hvoslef, 1968a**	**Jones, Davey and Cox, 2005**	**Current work**		
			water	**methanol**	**ethanol**
a (Å)	16.95	17.29	17.30	17.31	17.30
B (Å)	6.32	6.35	6.35	6.35	6.35
C (Å)	6.38	6.41	6.41	6.41	6.41
α (°)	N/A	N/A	90.00	90.00	90.00
β (°)	102.30	102.11	102.18	100.32	99.32
γ (°)	N/A	N/A	90.00	90.00	90.00

4.4.5 DSC and TG Analysis

Figure 4.19 shows a comparison of DSC analysis conducted on pure ascorbic acid crystal, crystals from water, methanol, ethanol, and 2- propanol. The DSC curve of ascorbic acid in Figure 4.19 has a single endothermic peak, which matches the characteristic of nonpolymeric compound. The melting temperature of the first endothermic peak of ascorbic acid as shown in Figure 4.9 varied from 188.9 ℃ to 185.6 ℃. This observation suggests that the crystal formed from water, methanol,

ethanol, and 2- propanol is having almost similar quality because the melting temperature is near to its standard melting temperature, which is 191 ℃. The bump after the sudden drop indicates that the crystal melts with decomposition. The melting temperature and enthalpy of melting varies from 281.10 ℃ to 90.23 ℃ and from 229 kJ/mol to 91 kJ/mol, respectively.

In general, crystal obtained from other solvents besides water, shows lower melting temperature. This may be due to non-perfect crystal that tends to melt first, followed by larger, more stable crystal (Plato, 1969). It may also be suggested that the crystal from ethanol and 2-propanol is not properly formed due broader melting peaks. Broader melting peaks are the results of various size distribution of imperfect crystal. If peaks are broad, the onset temperature cannot be precisely determined and it loses its physical meaning. The peak temperature is then used to define the melting temperature

Up until the melting temperature, the crystal does not show any addition endothermic or exothermic peaks, which shows that ascorbic acid retains its phase until the melting process started. The bump (indicated in the circle) after the melting point region indicated that ascorbic acid decomposed after the melting process. This is a normal observation for organic compound.

The melting temperature and melting enthalpy for crystal from each solvents is summarized in Table 4.5. The differences in enthalpies of ascorbic acid in different solvent is due to different packing of the molecules. The melting temperature and enthalpy is found highest in ascorbic acid from water as it has better molecule arrangement and stronger attachment as compared to ascorbic acid from methanol, ethanol and 2-propanol as discussed in Section 4.3.3 and Section 4.4. When ascorbic acid has less pack arrangement, the melting temperature and enthalpy is found to be lower.

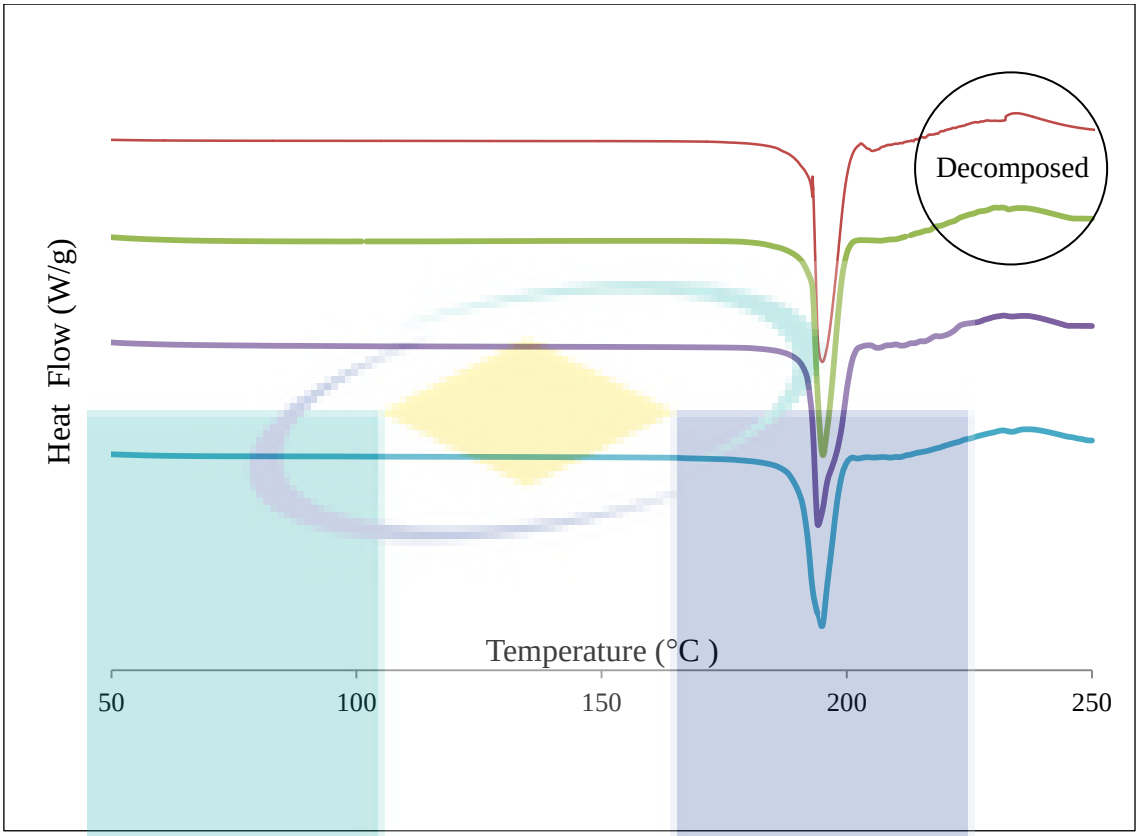

Figure 4.19: DSC analysis of ascorbic acid, crystallized acid from water, methanol, ethanol, and 2-propanol.

The thermal degradation of the ascorbic acid crystal from four solvents were studied using thermogravimetric techniques at temperature range of 20 ℃ to 500 ℃. Figure 4.20 to Figure 4.23 show TGA curves of ascorbic acid crystal obtained from water, methanol, ethanol and 2-propanol respectively. The derivative TG curves were presented to provide the percentage weight loss at temperature of 200 ℃ and 400 ℃. The ascorbic acid crystal TGA trace had several distinct weight loss transitions. In the lower temperature range between 20 to 200 ℃, there was a small weight loss that indicated moisture or low boiling point solvents evolving out of the matrix. From the figures, only crystal from water show very small weight loss during this stage. Crystal growth from alcohols shows more than 10% weight loss at temperature less than 200 ℃. This indicates that there is still traces of solvent in the crystal. This may due to dendritic growth that hinder the release of trapped solvent. It is also may due to the structure of ascorbic acid itself which consist of four hydroxyl groups. During this step, ascorbic acid tend to loose water and carbon dioxide molecules (Jingyan et al., 2013).

The major weight loss transition occurred between 200 ℃ to 400 ℃. This is when the ascorbic acid was further decomposed. The final was small weight loss transition from 400 ℃ to 500 ℃.

The curves in the four figures shows a significant weight change between 165 ℃ to 245 ℃, The change in weight is due to the decomposition of ascorbic acid crystals during the melting process. It may cause the decomposition of the substrate material or volatilization of residual solvents (Craig and Galwey, 2007). Neto and Pires reported that smaller molecular size and higher polarity solvent molecules such as water is easier to achieve equilibrium compared to ethanol because of the hydroxyl groups that exist in ascorbic acid can establish stronger hydrogen bonds, resulting in higher ΔH_f and melting temperature (Neto and Pires, 2009).

The prism habit of ascorbic acid is more stable than the needle-form by having higher melting point and enthalpy of fusion.

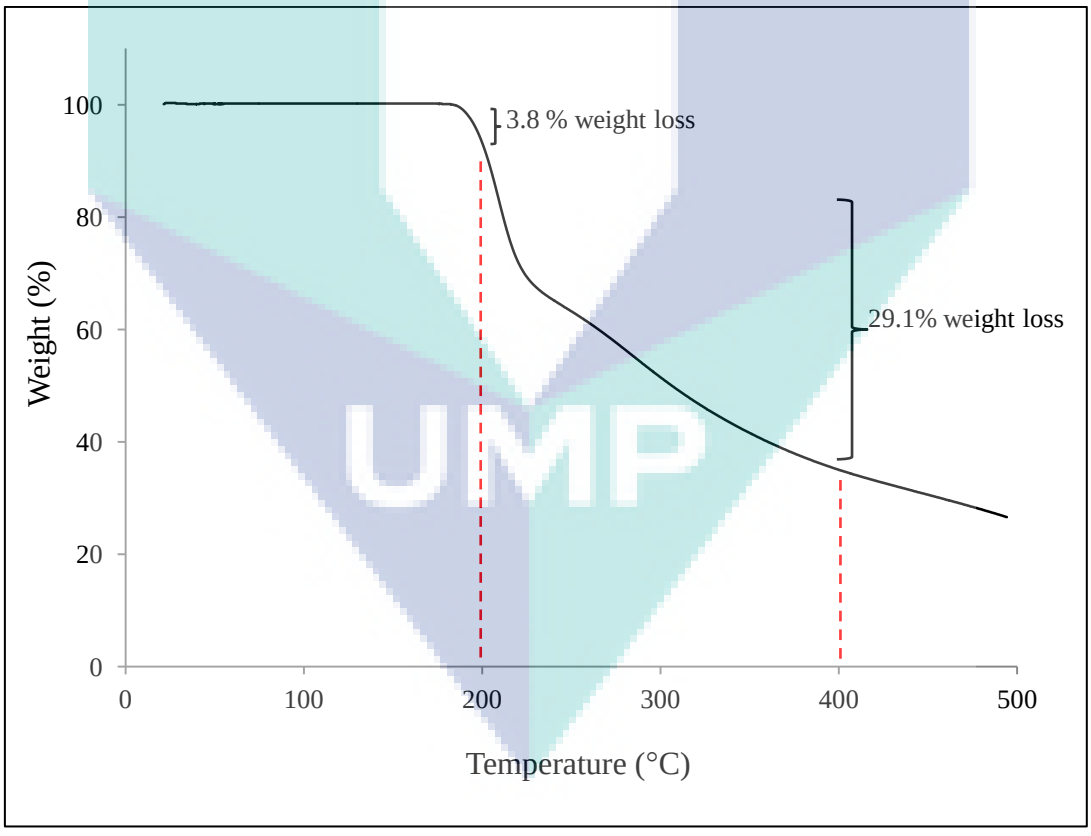

Figure 4.20: TGA curves of ascorbic acid crystal obtained from water and its derivatives

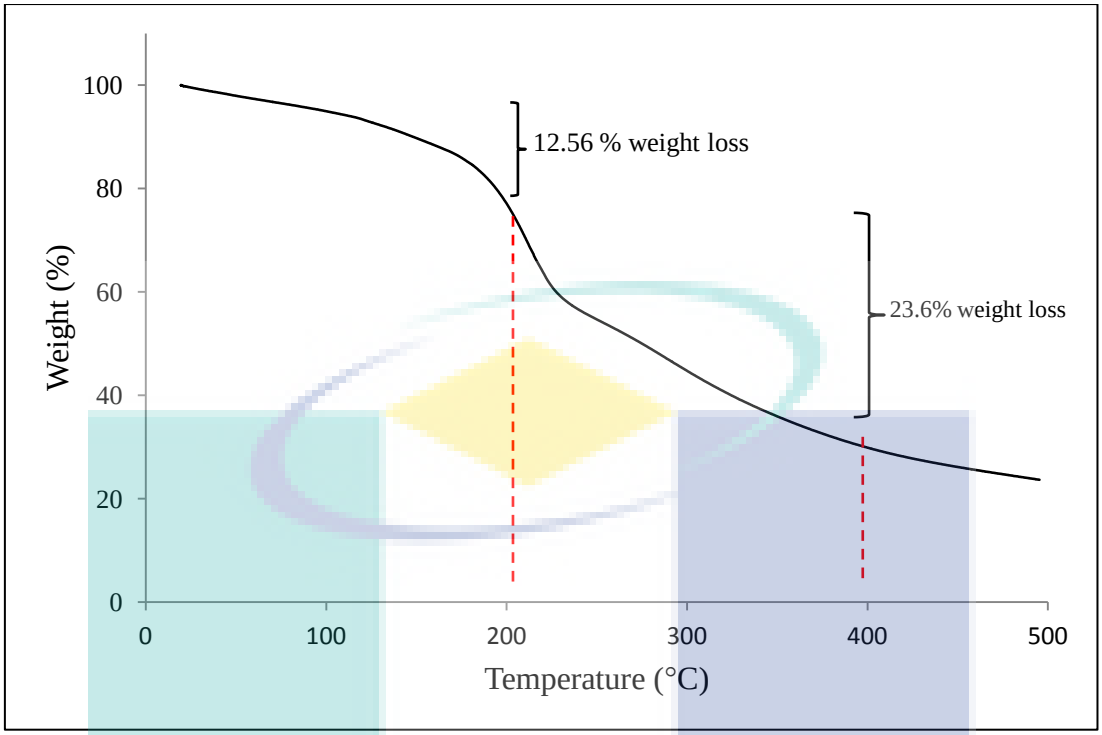

Figure 4.21: TGA curves of ascorbic acid crystal obtained from methanol and its derivatives

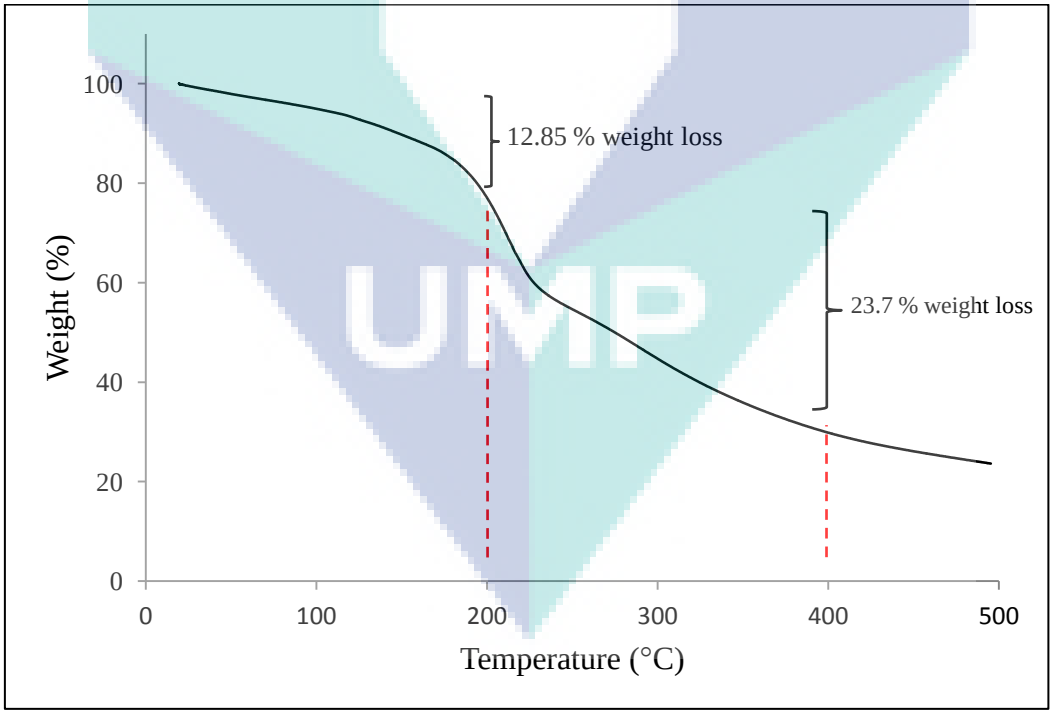

Figure 4.22: TGA curves of ascorbic acid crystal obtained from ethanol and its derivatives

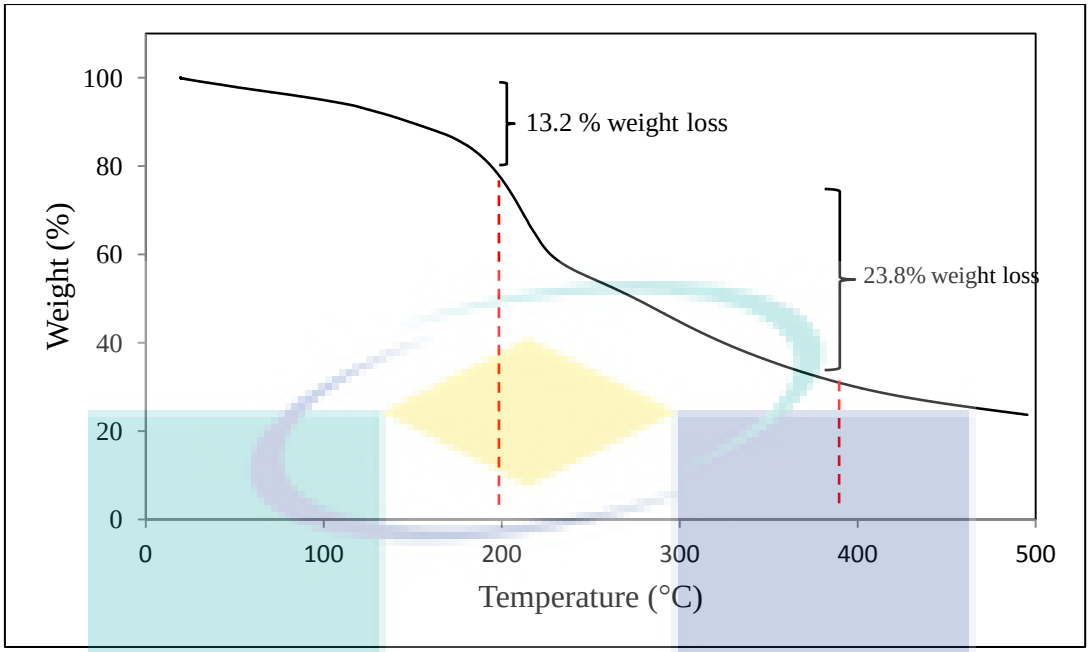

Figure 4.23: TGA curves of ascorbic acid crystal obtained from 2-propanol and its derivatives

Table 4.4 shows the summary of melting temperature and values of enthalpy of melting of ascorbic acid in various solvents. The melting temperature recorded for ascorbic acid crystal from water, methanol, ethanol, and 2-propanol is slightly lower than what had been recorded in the literature. This shows that even though ascorbic acid crystals have different habits by crystallizing it from different solvents, the thermal characteristic is almost the same. Meanwhile, the differences in melting enthalpies of ascorbic acid in different solvents are due to different packing of the molecules. Therefore, despite having various crystal habits, the packing of molecules is identical, parallel with data from a SCXRD, and thus, no event of polymorph is observed.

Table 4.4: Comparison of the T_{melt} and ΔH_{melt} of ascorbic acid obtained from water, methanol, ethanol and 2-propanol with previous studies.

	Jingyan (2013)	**Water**	**Methanol**	**Ethanol**	**2- propanol**
Melting Point (℃)	194.7	188.9	186.6	185.4	183.9
ΔH_{melt} (kJ/mol)	281.1	282.6	280.2	275.2	270.5

4.5 Summary

This work demonstrated that solvents polarity play an important role in the solubility and habits formation of ascorbic acid crystals. It was found that the solubility values increased with temperatures for all of the solvents tested in this work. Ascorbic acid demonstrates high solubility values in highly polar solvents. The solubility increase from 2-propanol, ethanol, methanol and water. Water, which is a polar protic solvent, is the best solvent for the dissolution of ascorbic acid as its shows the highest solubility value with lowest H_{diss} and G_{diss} with value 21.84 kJ/mol and 12.82 kJ/mol respectively compared to the other 3 solvents. The crystallization of ascorbic acid using different solvents with using evaporation method produced crystals with different habits. The results from crystallography data (PXRD and SCXRD) shown that the crystals obtained from all the solvents are just exhibiting different habit such as prism and needle like but they are not a polymorph. The thermal analysis (DSC and TGA) results showed that no solvent entrapment in the ascorbic acid crystals and the crystals were completely decompose during melting. The slight difference in T_{melt} may due to intermolecular interaction between solute and solvent during crystallization process. The results from optical analysis (microscopy analysis and SEM) showed that variety different habits, which were prism, needle-like and cubic shaped crystals, were produced depending on the polarity of the solvents used and the strength of intermolecular interaction.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The polar solvents used in the crystallization natural process play a significant role in the solubility and formation of different shape and habits of ascorbic acid crystals. The solvents used are polar solvents which are water, methanol, ethanol, and 2- propanol. The solubility data and crystal habits obtained are in agreement with the literature. It could be concluded that the solubility of ascorbic acid decreases by reducing the solvents' polarity. The solubility of ascorbic acid is highest in water with 335 g/L and it reduces from water > methanol > ethanol > 2- propanol. The Gibbs free energy change of dissolution of ascorbic acid in solvent highly influence the solubility data. Water is the best solvent for the dissolution of ascorbic acid as its shows the highest solubility value with lowest H_{diss} and G_{diss} with value 21.84 kJ/mol and 12.82 kJ/mol respectively.

The ascorbic acid solubility in different solvents calculated using COSMO-RS follows exactly the same trend as the experimental values. The solubility values increase when high polarity solvent is used. Solubility also increases with the increase of temperature. Analysis of the interaction energies, electrostatic energy (E_{misfit}), hydrogen bonding (E_{HB}) and van der Waals (E_{vdW}) revealed the important role of hydrogen bonding in solubility behavior. Different hydrogen bonding propensities in the solvents have contributed to different solubility values is also observed from the intermolecular forces estimation from COSMO-RS. Although the absolute solubility of ascorbic acid cannot be predicted precisely, COSMO-RS can quantitatively determine a reasonable and comparable solubility in different solvents.

The crystal formed is analyzed using microscopic analysis, SEM, PXRD, single XRD, TG, and DSC analysis. From these analysis, the crystal is showing a systematic change in habit, where the habit is cubic when formed in water and changed to more elongated into needle-like or a circular as the polarity is reduced. By reducing the polarity, it is also understood from DSC results that the crystal formed from weaker polarity, despite having smaller and longer habit, it still shares almost the same thermal properties. The ΔH_{melt} collected was in between 282.6 kJ/mol to 270.5 kJ/mol across the four solvents. The melting points recorded was in between 188.9 ℃ to 183.9 ℃ by crystals formed from water, methanol, ethanol and 2-propanol. The effect of solvent on ascorbic acid is due to the different in intermolecular interaction during crystal formation. Single crystal XRD (SCXRD) has confirmed that no polymorphism of ascorbic acid as the ability to form multiple habits is due to different intermolecular interaction while the molecular arrangement in a unit cell is still the same.

Further studies on ascorbic acid crystal could be conducted in order to specifically modify its size, habit and flow ability so that it is more feasible for direct tableting. Once the relationship is established, the crystallization process could be mass manufacture produce appropriate crystal to meet the purpose.

5.2 Recommendations

This study is far from finished since there are still a lot of areas that could be discovered for improvement. Suggestion and recommendations to improve this study of ascorbic acid includes the improvement in study of solubility of ascorbic acid. Different types of solvents like non-polar solvents should be used to study the interaction between ascorbic acid and non-polar solvents. This is also to confirm the affinity of ascorbic acid towards hydrogen bonding.

The solubility study shows that the habit of crystals is highly influenced by the type of solvents used. Therefore, the morphological simulation using molecular dynamics should be performed to investigate the types of intermolecular interactions that affect the growth of crystal faces and final habits of crystals in different solvents.

To study the solubility of ascorbic acid in different pH. The dissociation of weak acid is really dependent in the concentration of hydronium ion. Thus, by varying the concentration of hydronium ion, there will be the changes and the effects on it. By studying it in different concentrations, this study can be extended using Nyvlt equation to predict its nucleation order.

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APPENDIX A

Table A.1: Deviation, *D* of experimental ascorbic acid solubility, *s* in water, methanol, ethanol and 2-propanol at various temperatures, (*T)* against data by Shalmashi and Eliassi (2008)

Water			
T(K)	$S_{experimental}$	$S_{Literature}$	D[a]
303	0.0292	0.029	1%
308	0.0309	0.033	7%
313	0.0350	0.038	8%
318	0.0394	0.044	10%
323	0.0507	0.050	1%
Methanol			
	$S_{experimental}$	$S_{Literature}$	D[a]
303	0.0090	0.010	10%
308	0.0112	0.011	4%
313	0.0127	0.012	5%
318	0.0148	0.013	12%
323	0.0170	0.015	12%
Ethanol			
	$S_{experimental}$	$S_{Literature}$	D[a]
303	0.0021	0.002	8%
308	0.0030	0.003	11%
313	0.0037	0.003	16%
318	0.0046	0.004	18%
323	0.0052	0.005	13%
2-propanol			
	$S_{experimental}$	$S_{Literature}$	D[a]
303	0.0005	0.001	12%
308	0.0006	0.001	0%
313	0.0008	0.001	0%
318	0.0010	0.001	2%
323	0.0013	0.001	1%

[a]$D = \left|S_{Experimental} - S_{Literature}/S_{Literature}\right| \times 100$

Table A.2: Saturated mole fraction solubility x of ascorbic acid in different organic solvents at experimental pressure (0.1 MPa) and temperatures T from (303 to 323) K

Solvent	T (K)	x_{exp}	x_{COSMO}	Standard Deviation (%)
Water	303	0.0252	0.0599	2.45%
	308	0.0287	0.0764	3.38%
	313	0.0350	0.0991	4.53%
	318	0.0394	0.1335	6.65%
	323	0.0521	0.1885	9.64%
Ethanol	303	0.0101	0.0130	0.21%
	308	0.0113	0.0143	0.21%
	313	0.0135	0.0165	0.21%
	318	0.0149	0.0185	0.25%
	323	0.0165	0.0204	0.27%
Methanol	303	0.0021	0.0032	0.08%
	308	0.0033	0.0039	0.04%
	313	0.0037	0.0047	0.07%
	318	0.0046	0.0055	0.06%
	323	0.0054	0.0061	0.05%
2-propanol	303	0.0005	0.0008	0.02%
	308	0.0006	0.0010	0.03%
	313	0.0008	0.0012	0.03%
	318	0.0010	0.0017	0.05%
	323	0.0012	0.0021	0.06%

Table A.2 : Peak List of XRPD analysis

Sample name	Phase name	Formula	Space group
Water	Ascorbic Acid	$C_6H_8O_6$	$P2_1$ 4

2-theta (deg)	d (ang.)	Height (cps)	Int. I (cps ¥deg)	FWHM (deg)	Size	Phase name
10.466(6)	8.446(5)	457(21)	105.3(18)	0.181(5)	460(12)	Vitamin C, (2,0,0)
15.764(10)	5.617(4)	84(9)	20.0(7)	0.198(9)	422(20)	Vitamin C, (2,0,-1)
16.095(5)	5.5023(18)	29(5)	7.0(6)	0.20(3)	429(58)	Vitamin C, (1,0,1)
17.480(7)	5.069(2)	248(16)	63.8(12)	0.235(5)	357(8)	Vitamin C, (2,1,0)
19.805(7)	4.4790(16)	292(17)	92.1(15)	0.275(6)	306(6)	Vitamin C, (1,1,-1)
21.051(13)	4.217(3)	63(8)	24.5(10)	0.307(16)	275(14)	Vitamin C, (2,1,-1)
23.511(6)	3.7807(10)	48(7)	14.2(9)	0.25(2)	341(28)	Vitamin C, (3,1,-1)
25.292(6)	3.5185(8)	650(26)	132(2)	0.169(5)	502(15)	Vitamin C, (4,1,0)
26.726(12)	3.3328(14)	114(11)	31.9(12)	0.212(13)	402(25)	Vitamin C, (4,1,-1)
27.163(4)	3.2802(5)	165(13)	20.9(11)	0.102(7)	837(62)	Vitamin C, (5,0,-1)
28.059(8)	3.1774(8)	454(21)	132(2)	0.241(6)	355(8)	Vitamin C, (1,0,-2)
30.029(7)	2.9733(7)	599(24)	147(2)	0.186(6)	461(15)	Vitamin C, (1,0,2)
31.924(8)	2.8011(7)	176(13)	36.6(12)	0.141(11)	612(47)	Vitamin C, (6,0,-1)
34.718(17)	2.5817(12)	130(11)	32.5(14)	0.214(12)	405(23)	Vitamin C, (5,0,-2)
35.506(8)	2.5262(5)	59(8)	39(2)	0.55(4)	159(11)	Vitamin C, (4,2,0)
37.518(17)	2.3952(11)	39(6)	12.7(8)	0.22(2)	400(45)	Vitamin C, (5,1,-2)
39.54(3)	2.2774(14)	36(6)	11.1(8)	0.29(2)	309(25)	Vitamin C, (7,1,-1)
40.25(2)	2.2386(12)	46(7)	16.8(10)	0.33(2)	269(20)	Vitamin C, (1,2,-2)
41.550(19)	2.1716(9)	38(6)	9.5(6)	0.235(18)	378(29)	Vitamin C, (1,2,2)
42.25(5)	2.138(2)	20(4)	4.1(5)	0.19(4)	460(102)	Vitamin C, (2,0,-3)
42.778(11)	2.1121(5)	134(12)	22(3)	0.155(14)	574(53)	Vitamin C, (3,0,-3)
45.201(12)	2.0044(5)	62(8)	21.2(9)	0.232(16)	388(28)	Vitamin C, (8,1,0)
45.88(2)	1.9761(9)	21(5)	3.1(5)	0.14(2)	645(111)	Vitamin C, (3,2,2)
47.91(5)	1.8970(18)	13(4)	6.0(7)	0.43(5)	209(24)	Vitamin C, (6,2,-2)

LIST OF PUBLICATIONS

JOURNAL PAPERS

Hassan, S., Adam, F., Abu Bakar, M. R., & Abdul Mudalip, S. K. (2018). Evaluation of solvents' effect on solubility, intermolecular interaction energies and habit of ascorbic acid crystals. *Journal of Saudi Chemical Society*. https://doi.org/10.1016/j.jscs.2018.07.002

Hassan, S., Adam, F. & Abu Bakar, M. R. (2018). Thermal characteristic evaluation of different ascorbic acid crystal habits. *Journal of Chemical Engineering and Industrial Biotechnology*. https://doi.org/10.15282/JCEIB-V3-08.28/3/2018/3.3

ABSTRACT

Hassan, S., Adam, F. & Abu Bakar, M. R. Polymorph Detection On-set Nucleation in Ascorbic Acid Molecular Solution by H^+ Concentration at the 21st International Workshop on Industrial Crystallization (BIWIC 2014), September 10 – 12, 2014.

POSTER PRESENTATION

Hassan, S., Adam, F. & Abu Bakar, M. R. Solubility prediction of L-ascorbic acid in solvents using non-empirical method. Selected to present a poster at the International Conference on Industrial Pharmacy 2014 held at Swiss Garden Resort, Kuantan on 17th August 2014.

Hassan, S., Adam, F. & Abu Bakar, M. R. The effect of solvent on L-ascorbic acid crystal habit. Selected to present a poster at the Conference on Nano- and Bioresource Technology 2015 held at UKM Bangi on 29th March 2015.