SOLVENT AND TEMPERATURE EFFECT IN THE POLYMORPHISM STUDY OF 2,6-DIHYDROXYBENZOIC ACID CRYSTAL

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ABSTRACT

This research is about the solvent and temperature effect in the polymorphism study of 2,6-Dihydroxybenzoic Acid (2,6DHB) Crystal. Polymorphism is the ability of substance to crystallize into different crystal structures. Polymorphism is important, as difference in physical properties of polymorphs can impact on issues such as solubility, bioavailability, formulation, patent protection, regulatory compliance and downstream particle handling such as filtration, drying, milling and compaction. The physical properties of polymorphs can be affected by variation of solvent and temperature. Currently, the solvent selection for crystallization is often trial and error procedure (Gu et al., 2004) and polymorphs of 2,6-DHB from some solvent is still unknown. This study is aimed to provide guideline for rational choices of solvent with different properties for polymorph screening and as extension to Adam (2011) research. The selection of solvent in this study were based on the polarity of 2,6-DHB solute and solvents molecule. Solvent chosen were Benzene Formic Acid and Diethylamine. Meanwhile, the temperature that is going to be used are 20, 35 and 50 °C. The methodology for this study is gravimetric methods for the crystallization and for analysis methods, Van’t Hoff equation, XRD, DSC, TGA and optical microscope are going to be used. But due to time limitations only optical microscope analysis method is used. Different type of solvent at specific temperatures have a significant effect in polymorphism of 2,6-DHB crystal and its physical properties. Based on the study, the rank of 2,6-DHB solubility in different solvents are Diethylamine > Formic Acid > Ideal > Benzene. Low solute-solvent interactions and poor solubility was observed in Benzene. The polymorphic characterization analysis has concluded that a Form 1 (plate-like) crystal was observed in Formic Acid and Diethylamine and the crystals are transforming to Form 2 (needle-like) based on observation. Form 2 (needle-like) was observed in Benzene due to prolonged time to achieve supersaturation and limitations of analysis.
ABSTRAK

CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter comprises the background of study. A brief introduction about the study will be described. Later, the problem statement, research objectives and scope of study will be emphasized. This chapter also addressed the significance or rationale of the study.

1.2 Background Of Study

2,6-Dihydroxybenzoic Acid (2,6-DHB) is a white crystalline powder and has been evaluated as an active pharmaceutical ingredients (API). In pharmaceuticals, it is used in the treating of tumors and commonly employed as reagent in the synthesis of pharmaceutical materials especially antipyretic, analgesic and anti-rheumatism agents (Adam et al., 2011).

Majority of active pharmaceutical ingredients (APIs) including 2,6-DHB are produced in solid forms or crystal forms. They are frequently delivered to the patient in the solid state as part of an approved dosage form such as in the form of tablets or capsules. Solids dosage form provides a convenient, compact and generally stable format to store an API. 2,6-DHB can exhibit polymorphism in crystalline form.
1.2.1 What is Polymorphism?

Polymorphism is the ability of a solid material to exist in more than one form or crystal structure. It is estimated about 80-90% of organic compounds can exhibit polymorphism (Stahly, 2007). Different polymorphs of a compound are chemically identical which same molecular structure but exhibit different physical properties. Polymorphs are known to present significant differences in the physicochemical properties such melting point, density, morphology, solubility and colour. As a result, it may have an impact on the stability (physical and chemical), bioavailability and processability during manufacturing and/or in the final product (Heinz et al., 2009). The different crystalline forms showed by one substance may result from a variation in crystallization temperature or difference of solvent (Sangwal, 2009; Mullin, 2001; Tung, 2009).

1.2.2 Why Polymorphism Study is Important?

In term of pharmaceuticals, polymorphism study is important because majority of Active Pharmaceutical Ingredients (APIs) are produced in crystal forms and can exhibit polymorphism. Severe consequences of drug products might happen due to the inconsistencies in the solid phase produced during the manufacturing and storage of drug substance. The approximate shape of crystals or crystal morphology can affect downstream particle handling such as filtration, drying, milling and compaction (Chen and Trout, 2010). It is also an important attribute of powdered materials that affects the ease with which a pharmaceutical formulation can be pressed into a tablet (Mirza et al., 2009).

Llinas and Goodman (2008) said that more recently, the polymorphism study of drugs has been the subject of strong interest in the pharmaceutical industry. In particular, the variation in solubility between different polymorphs is important, as it can affect drug efficacy, bioavailability and more importantly safety of the final product. They also said that new polymorphs can still appear without notice because polymorphs can transform spontaneously from less stable to more stable forms, and the most stable polymorph will be the least soluble.
In this situation, polymorphism study is important to discover and characterize the most stable form, ideally while a drug candidate is still in the discovery phase, so that the form will be the one used for subsequent testing. Polymorphs test is regulatory requirement for new chemicals for Food and Drug Administration (FDA) to ensure that the final product is safe. It is also significant for intellectual property considerations because crystal forms with superior properties can be protected by patents (Chawla and Bansal, 2003).

1.3 Problem Statement

2,6-Dihydroxybenzoic Acid (2,6-DHB) can exist in three different crystalline forms which are Form I (metastable), Form II (stable) and monohydrate which shows different physical properties (Adam, 2011). The physical properties of polymorphs can be affected by variation of solvent and temperature. Currently, the solvent selection for crystallization is often trial and error procedure (Gu et al., 2004) and solubility data for some solvents are not available in the literature. In fact, polymorphs of 2,6-DHB from some solvent is still unknown. These problems have result to this proposed research.

1.4 Research Objectives

1.4.1 To establish the solubility function of 2,6-DHB acid polymorphs in a specific solvent type at different temperatures.
1.4.2 To identify and characterize the effect of different solvents towards polymorphic forms of 2,6-DHB.

1.5 Scope of Study

1.5.1 Selection of solvent is based on the polarity of solvent molecules and 2,6-DHB solute molecules. The solvents are Benzene, Formic Acid and Diethylamine.
1.5.2 Gravimetric method is used to study the solubility of 2,6-DHB at 20, 35 and 50°C.
1.5.3 Solubility is determined by using Van’t Hoff equation.
1.5.4 Polymorphic form of 2,6-DHB is going to be characterized by using optical microscope, XRD, DSC and TGA.

1.6 **Rationale/Significance of Study**

The type of solvent is the key factor in polymorphic selectivity and crystal morphology. It is important understand the polymorphism of 2,6-DHB by the effect of solvent type and temperature. Research conducted by Adam (2011) has proved that 2/3 of 2,6-DHB crystals from different solvent exhibit polymorph. Hence, this study is aimed to provide guideline for rational choices of solvent with different properties for polymorph screening and extension to Adam (2011) research. This research also will provide information about the polymorphs and may lead to further research.
CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter comprises of the literature from fundamental theory to previous study findings. It started with crystallization and polymorphism theory, and later followed by previous findings for solubility and polymorphic forms of 2,6-DHB.

2.2 Crystallization

Crystallization is a physical or chemical process or action which results in the formation of regularly-shaped, -sized, and -patterned solid forms known as crystals. It is concerned with the development from solution or melt of the crystalline state (Blagden et al., 2007). In pharmaceutical industry, crystallization is significant as a separation process for intermediates and often serves as the final step in the manufacture of active pharmaceutical ingredients (API).

2.3 Kinetics of Crystallization Process

Crystallisation is a process of molecular aggregation in a solution, leading to the formation of nuclei and, later, crystal growth and eventually creating a crystalline phase. Supersaturation, nucleation and crystal growth are the major physical phenomena associated with crystallization (Banga et al., 2004).
2.3.1 Supersaturation

Supersaturation is defined as the concentration of the solute in excess of saturated concentration under given conditions of temperature (Banga et al., 2004). The number of potential solid forms which are thermodynamically accessible depends on the level of supersaturation. This is because different solid forms have different solubility (Chen et al., 2011). Supersaturation is the driving force of the crystallization; hence the rate of nucleation and growth is driven by the existing supersaturation in the solution (Tung, 2009). It is composed of two zones; the metastable and unstable zones (see Figure 2.1 below). The two zones are defined so that the metastable region shows crystals growing without nucleating, whereas in the unstable region crystals appear after nucleation.

![Concentration of solute during crystallization](image)

**Figure 2.1** Concentration of solute during crystallization

Source: Bange et al. (2004)
2.3.2 Nucleation

Nucleation is a molecular assembly process, where a critical number of molecules are needed to achieve the phase change from solution into a crystal or solid (Blagden et al., 2007). The nuclei are stable only when they achieve a critical size and the critical size is governed by parameters such as supersaturation and temperature (Chen and Trout, 2010).

2.3.3 Crystal Growth

Crystal growth will take place after nucleation is achieved and it will lead to the growth of embryonic crystals into a larger size and shape. Crystal lattice of the molecular solids and the effects of solvent selection and additives on the process of crystal growth are three key drivers that related with regard to the shape of growing crystals (Blagden et al., 2007). The process of crystal growth to into visible size involves at least two stages: (1) formation of stable 3D nuclei and (2) development of stable 3D nuclei into crystals with well-developed faces (Sangwal, 2007).

2.4 Polymorphism

In pharmaceuticals, polymorphism is often described as the ability of a drug substance to exist as two or more crystalline phases that have different arrangement and/or conformation of the molecules in the crystal lattice (Yu et al., 2003).

According to International Conference on Harmonization (ICH) Guideline Q6A, polymorphism is defined to include polymorphs, solvate, and amorphous form as shown in Figure 2.2. Amorphous solids or known as solid that lacks of the long-range order of a crystal consist of disordered arrangements of molecules and do not hold a distinguishable crystal lattice. Crystal forms that contain either stoichiometric or nonstoichiometric amounts of solvent are called solvates. Solvates are commonly known as hydrates when the incorporated solvent is water.
Different polymorphs of a solid substance are chemically identical but will exhibit different physical properties and behaviour, such as different solubility and melting points. The different crystalline forms showed by one substance may result from a variation in crystallization temperature or difference of solvent (Mullin, 2001; Tung, 2009). Crystal polymorph sometimes undergo transformation without a change of external form, the result being an aggregate of very small crystals of the stable modification confined within boundaries of the original unstable form (Mullin, 2001).
2.5 Physical Properties of Polymorphs

As mentioned before, different polymorphs of a compound are chemically identical which same molecular structure but exhibit different physical properties.

2.5.1 Solubility

Solubility is defined as the equilibrium (maximum) amount of solute that can be dissolved in a specific solvent system (Tung, 2009). According to (Yu et al., 2003) polymorphs in pharmaceuticals may have different aqueous solubility and dissolution rates. When the differences are big between, the bioavailability maybe adjusted it will be difficult to formulate a bioequivalent drug product using a different polymorph. Aqueous solubility of a drug is usually determined using the equilibrium solubility method which involves suspending an excess amount of a solid medicine in a selected aqueous medium.

Solubility correlates to the strength of solvent-solute interactions which are influenced by solvent properties such as hydrogen bond acceptor or donor propensity, polarity/dipolarity, dipole moment, dielectric constant, viscosity, surface tension and cohesive energy (Gu et al., 2004).

The solubility reflects a strong solute-solute interactions exist in the solvents which solubility is lower then ideal solubility and strong solute solvent interaction when solubility is higher than ideal solubility (Adam, 2011). The solubility can be obtained by using Van’t Hoff Plot.

2.5.2 Enthalpy and Entropy of Dissolution

Dissolution in crystallization can be explained as the breakdown of the crystal lattice into individual ions, atoms or molecules and their transport into the solvent. $\Delta H_{diss}$ can be defined as a process of solute going into solution and it represents the energy required for the solute molecules to overcome the intermolecular forces in the solution.
(Adam, 2011). Meanwhile, Entropy of Dissolution $\Delta S_{diss}$ is the disorder of the system for dissolution process.

### 2.5.3 Stability of Polymorphs

In pharmaceuticals, the most stable polymorphic form of a medicine substance is often used in a formulation because it has the lowest potential for conversion from one polymorphic form to another (Yu et al., 2003). On the contrary, metastable (less stable form) or even amorphous might be chosen to enhance the bioavailability of medicine product. One polymorph may convert to another during process or storage, particularly when a metastable form. Gibbs free energy, thermodynamics activity and solubility provide the definitive measures of relative polymorphic stability under defined conditions of temperature and pressure.

### 2.6 Crystal Morphology

Crystal morphology is the appearance or approximate shape of crystals. It is also called crystal habit (Tung, 2009). Although crystal can be classified into seven general systems, the relative sizes of the face of particular crystal can differ considerably.

Crystals may grow rapidly, or be stunted, in one direction; thus an elongated growth of the prismatic habit gives a needle-shaped crystal (acicula r habit) and a stunted growth gives a flat plate-like crystal (tabular, platy or flaky habit). Ostwald’s Step Rule suggests that in general, the least stable polymorph will crystallize first instead of most stable polymorph (Threlfall, 2003).

Crystallization from solutions of a substance in different solvents generally results in change habit. The degree of supersaturation of a solution often exerts a significant influence on the crystal habit, and so can the state agitation of the system (Mullin, 2001).
2.7 2,6-Dihydroxybenzoic Acid Solute

2,6-DHB is categorized under carboxylic acid group which OH can act as a proton donor and C=O can act as the proton acceptor in forming hydrogen bond either between the solute itself or the solvent. 2,6-DHB Acid is a polar compound.

Figure 2.3 Molecular structure of 2,6-DHB

2.7.1 Solubility of 2,6-Dihydroxybenzoic Acid

Adam (2011) has conducted polymorphism study for 15 types of solvent and they found that low solubility for 2,6-DHB Acid in toluene and xylene isomers which are o, p, m-xylene solvents which indicate strong solvent-solvent interaction and weak solvent-solute interaction exist in the system. Cyclohexane and hexane was found to be low solubility or poor solute-solvent interaction to accelerate the dissolution process because of they have the highest value of heat of compared to other solvents studied in that research. The results in Adam et al. study found that the rank of solubility of 2,6-DHB in solvents are methanol > ethanol > IPA > EA > acetonitrile > acetone > IPE > diethylether > tert-butyl methyl ether > IDEAL > chloroform > toluene > o-xylene > m-xylene > p-xylene > hexane > cyclohexane. The ideal solubility of 2,6-DHB is obtained from Davey et al. (2001) study.
According to Grant and Haguchi (1990), at a specific temperature, the polymorphic form with higher activity will also have higher solubility and lower stability. This has been proved in Adam et al. study that polymorph of Form I (metastable) which is grown from toluene at 20°C, m-, p-, o-xylene at 35, and 50°C which have lower melting point from the DSC measurements and plate-like shape show the difference in activities and escaping tendencies. Davey et al. (2001) show that as the temperature increase, the solubility of 2,6-DHB in specific solvents also increase.

Figure 2.4 Ideal solubility data for 2,6-DHB

Source: Davey et al. (2001)
2.7.2 Polymorphic Forms of 2,6-Dihydroxybenzoic Acid

The first crystal structure of this material was found in 1994 by Gdaniac et al. who obtained crystals from chloroform solution. 2,6-DHB can exist in three types of polymorphic forms. Form I is metastable, Form II is stable and monohydrates (Chen and Trout, 2010; Adam et. al, 2011b).

Davey et al. (2001) found that 2,6-DHB Acid crystallize as Form I from toluene and exhibit plate-like crystals. In chloroform, 2,6-DHB Acid crystallize as Form II and exhibit needle-like crystals.

Chen and Trout (2010) found that crystallization of 2,6-DHB in single solvents such as ethanol, toluene, chloroform exhibit thin needle like crystal while in mixture of toluene and diethylether the aspect ratio of needle-like crystals is increased.

Adam (2011) in their study found that Form I and Form II crystal shape were obtained as platelike and needle like respectively. They also conclude that solvents crystallize the Form II (stable) at all temperatures except in o-xylene, p-xylene and m-xylene and toluene which crystallize metastable Form I.

2.8 Function of Solvents in Crystallization

Industrial Solvents Handbook defines solvent as a substance in which another substance is dissolved forming a solution. It is used to suspend or modify the physical properties of a material. Many organic liquids can act as solvents it is use as ingredients in the production of various products. Some solvents selectively favor the crystallization of a particular form or forms. In addition, by varying the solvent, it will affect crystal morphology, crystallization kinetics, formulation and drug stability (Tung, 2009).
2.8.1 Solvents Selection

An important consideration when choosing a solvent for crystallization is the solute to be crystallized should be readily soluble in the solvent and it should be easily deposited from the solution in the desired crystalline form after cooling, evaporation, etc. Solvents can be classified as being polar or non-polar; the earlier description given to liquids which have high dielectric constants (e.g. water, acids, alcohols, and the latter refers to liquid of low dielectric constant (e.g. aromatic hydrocarbons).

Based on the nature of intermolecular bonding interactions, solvents can be divided into three main classes which are 1) polar protic; 2) dipolar aprotic; 3) non-polar aprotic. In polar protic solvents, the solvent molecules interact by forming strong hydrogen bonds. In order to dissolve, the solute must break these bonds and replace them with bonds of similar strength. Dipolar aprotic solvents are characterized by high dielectric constants, the solvent molecules interact by dipole-dipole interactions. If the solute is also dipolar and aprotic, it can interact readily with the solvent molecules forming similar dipole-dipole interactions. In non-polar aprotic solvents (low dielectric constants), molecules interact by weak Van Der Waals forces (Mullin, 2001).

In this research, there are three types of solvents chosen which are Benzene, Formic Acid and Diethylamine. Below is the summary table for some of the properties of these solvents.
Table 2.1 The solvent properties considered in solvent selection for the polymorph screening

<table>
<thead>
<tr>
<th>Solvent Type</th>
<th>Molecular Structure</th>
<th>Boiling Point (°C)</th>
<th>Dipole Moment</th>
<th>Polar/Non-Polar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td><img src="image" alt="Benzene" /></td>
<td>101</td>
<td>0.00</td>
<td>Non-polar</td>
</tr>
<tr>
<td>Formic Acid</td>
<td><img src="image" alt="Formic Acid" /></td>
<td>80.1</td>
<td>1.41</td>
<td>Polar</td>
</tr>
<tr>
<td>Diethylamine</td>
<td><img src="image" alt="Diethylamine" /></td>
<td>55.5</td>
<td>0.92</td>
<td>Polar</td>
</tr>
</tbody>
</table>

Source: Gu et al. (2004)
CHAPTER 3

RESEARCH METHODOLOGY

3.1 Introduction

This chapter comprises of methodology for experimental work and analysis for this study. It emphasizes on material and properties needed for this study, experimental work and analysis method.

3.2 Materials and Properties

2,6-DHB (C₇H₆O₄) is a white crystalline powders with 98% purity. There are three types of solvents that are going to be used which are Formic Acid, Benzene and Diethylamine. All this materials were obtained from Sigma Aldrich.

3.3 Experimental Work

In this research, Gravimetric Method was used in order to obtain polymorphs from the crystallization of 2,6-DHB Acid with the three type of solvents at different temperatures.

The saturated solution is prepared by dissolving a certain amount of 2,6-DHB Acid powder into 5-25 mL solvent until excess solute is observed. After saturation is achieved, the solution is shaken using Thermomixer with speed at 400 rpm and the time is according to temperature shown below.
**Table 3.1** Shaking time for saturated solution at specific temperature

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
</tr>
</tbody>
</table>

**Figure 3.1** Thermomixer

The solution is left for about one hour after shaking at the specific temperature until the supernatant forms. The solution is then filtered using 0.45µm membrane filter and left in the vacuum oven for drying. Finally, the polymorphs are characterized using optical microscope, XRD, DSC and TGA.
3.4 Solubility and Solvation Study

The solubility can be calculated using the equation below (Adam, 2011).

\[
\text{solubility} = \frac{\text{mol solute}}{\text{mol solvent}}, \text{at saturation point} \quad (3.1)
\]

The Van’t Hoff Plot is plotted by using the equation as expressed below. The slope from the Van’t Hoff Plot is \( \frac{\Delta H_{\text{diss}}}{R} \) while the y-intercept is \( \Delta S_{\text{diss}} \).

\[
\ln(x) = \frac{\Delta H_{\text{diss}}}{RT} + \frac{\Delta S_{\text{diss}}}{T} \quad (3.2)
\]

3.5 Characterization of Polymorphs

In order to determine and characterize the polymorphs, these three analysis techniques are going to be used which are optical microscopy, XRD, DSC and TGA.
These analysis techniques are the most frequently used in polymorphs characterization (Chieng et al., 2011).

### 3.5.1 Optical Microscopy

Optical microscope is used for crystal habit imaging of polymorph formed (Adam, 2011) Microscopy characterizes polymorphs through the optical and morphology properties of crystal and it can reveal possible difference in crystal habit (Yu et al., 2003).

![Optical microscope](image)

**Figure 3.3** Optical microscope

### 3.5.2 X-Ray Diffraction

X-ray Diffraction (XRD) is used to directly detect crystal lattice properties. Changes in the diffraction pattern can imply the existence of a new polymorph (appearance or disappearance of polymorph-specific diffraction peaks), or a smaller change in the crystal lattice, such as the introduction of a slight disorder or change in lattice parameters (small decreases in peak intensity or minor peak shifts) (Chieng et al., 2011).
When a 2-D diffraction pattern is recorded, it shows concentric rings of scattering peaks corresponding to the various d-spacing in the crystalline lattice. The positions and the intensities of the peaks can be used for identifying the underlying structure (or phase) of a solid material. For example, the diffraction lines of diamond would be different from graphite even though they both are made of carbon atoms. This phase identification is important because the material properties are highly dependent on the structure.

3.5.3 Thermal Gravimetry Analysis and Differential Scanning Calorimetry

TGA and DSC is used to differentiate polymorphs on the basis of the phase transitions they undergo during heating and can be used to obtain additional information regarding these phase transitions including melting, desolvation, crystallization, and glass transition.

These thermal methodologies can also be used to determine the relative stability among polymorphs and to differentiate enantiotropic and monotropic systems. For an enantiotropic system, the relative stability of a pair of solid forms inverts at some transition temperature beneath the melting point, whereas in a monotropic system, a single form is always more stable beneath the melting point (Yu et al., 2003).

DSC determines polymorphism samples by monitoring the thermal events during heating while TGA measures any changes in the weight of specimen while varying temperature in a controlled nitrogen atmosphere (Adam, 2011).
CHAPTER 4

RESULT & DISCUSSION

4.1 Introduction

In this chapter, the solubility of 2,6-DHB in specific solvent will be discussed. The solubility function of 2,6-DHB is established and discussed using Van’t Hoff Plot. Besides, the characterization of 2,6-DHB solid crystals which crystallized from three different solvents are discussed using optical microscopy to check the crystal habit.

4.2 Solubility of 2,6-Dihydroxybenzoic Acid

In the experiment, a certain weight amount of 2,6-DHB solute was dissolved in 7 mL of each type of solvent. The solute is added into the solvent solution until excess solute is observed in the glass vial. At this point, once there is no any solute dissolve in the solvent, it signifies that the solution is achieving the saturation point. Saturation also indicates that the solute concentration in the solution is at its solubility limit (Tung, 2009). Hence, the solubility function is established by plotting the Van’t Hoff Plot as discussed in Section 3.4.
Table 4.1  Weight of 2,6-DHB dissolved in three different types of solvent

<table>
<thead>
<tr>
<th>Temperature(°C)</th>
<th>Mass of 2,6-DHB Dissolved (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benzene</td>
</tr>
<tr>
<td>20</td>
<td>0.0053</td>
</tr>
<tr>
<td>35</td>
<td>0.0089</td>
</tr>
<tr>
<td>50</td>
<td>0.0288</td>
</tr>
</tbody>
</table>

In Section 2.8.1 (Table 2.1), the molecular geometry of each solvent shows that benzene is nonpolar solvent meanwhile formic acid and diethylamine is polar. The solubility is calculated using equation 3.1 in Section 3.3. In Table 4.2, the solubility at saturation is recorded at 20°C, 35°C and 50°C and the solubility is calculated in mol/mol.

Table 4.2 Solubility data of 2,6-DHB in three different solvent(mol/mol)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Molsolute/Molsolvent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benzene</td>
</tr>
<tr>
<td>20</td>
<td>0.0004</td>
</tr>
<tr>
<td>35</td>
<td>0.0007</td>
</tr>
<tr>
<td>50</td>
<td>0.0024</td>
</tr>
</tbody>
</table>

Figure 4.1 shows the solubility trend of 2,6-DHB in different solvent at increase temperature. It was obvious that as the temperature increase, the solubility of 2,6-DHB also increase. Among all the solvent, Benzene has the lowest solubility and small solubility increase as a function of temperature.
Figure 4.1 Graph of solubility versus temperature for 2,6-DHB in three different solvent

As from the literature (Section 2.5.1), solubility correlates to the strength of solvent-solute interactions which are influenced by solvent properties such as hydrogen bond acceptor or donor propensity, polarity/dipolarity, dipole moment, dielectric constant, viscosity, surface tension and cohesive energy (Gu et. al, 2004). In order, to determine the strength of solvent-solute interactions, Van’t Hoff Plot is referred to compare the solubility to the ideal solubility. The Van’t Hoff equation is referred to Equation 3.2 in Section 3.4.