SOLUBILITY STUDY OF 2, 6-DIHYDROXYBENZOIC ACID IN XYLENE, METHANOL AND ACETONITRILE USING HPLC METHOD

RAJA NUR NADHIRAH BT RAJA OTHMAN

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Faculty of Chemical &Natural Resources Engineering

UNIVERSITI MALAYSIA PAHANG

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ABSTRACT

The solubility of 2, 6-dihydroxybenzoic acid in three different solvent types was determined at temperature 20ºC, 35 ºC, 50 ºC. The solubility is correlates to the strength of solvent solute interaction which is dependent on the solvent properties. The right solvent is select based on the ability to form the hydrogen bond acceptor or donor between 2, 6-DHB solute molecule. The objective in this experiment is to develop the solubility measurement technique in 2, 6-DHB in different solvent using HPLC method and to find the Van Hoff correlation as a function of solvent type. The solvent under study were methanol, o-xylene and acetonitrile. 0.05M KH_2PO_4 at ph 3.2 and methanol was used as the mobile phase.2, 6-DHB acid was detected at 254nm with an UV-DAD detector and the flow rate is 1.0ml/min. RP-C18 was used and performed at ambient temperature. The solubility of the solute in the solvent at specific temperature can be determined by comparing with the standard calibration curve created using HPLC. From the observation during the saturated solution preparation, methanol can make 2, 6-DHB more soluble followed by acetonitrile and o-xylene and from the Van Hoff plot shows that methanol and 2, 6-DHB has strong solute-solvent interaction.

ABSTRACT

Kebolehlarutan asid 2, 6-Dihydroxybenzoic dalam tiga jenis pelarut yang berlainan telah ditentukan pada suhu 20ºC, 35ºC, 50 ºC. Kebolehlarutan berhubung kait dengan kekuatan interaksi antara pelarut dan bahan yang dilarutkan dimana ianya bergantung pada sifat bahan pelarut seperti kekutuban,boleh menerima dan memberi hydrogen untuk menghasilkan ikatan hydrogen. Pelarut yang paling baik dipilih berdasarkan kemampuan untuk membentuk ikatan hidrogen dengan menerima atau menberi hydrogen kepada bahan larut. Objective dalam tesis ini adalah untuk membangunkan prosedur pengukuran kebolehlarutan tiga jenis bahan pelarut dalam melarutkan 2,6- DHB menggunakan HPLC dan untuk mengetahui tentang hubung kait Van Hoff dengan setiap jenis bahan pelarut yang diuji dalam tesis ini. Bahan pelarut yang diuji adalah methanol,acetonitirle and o-xylene. 0.05M KH_2PO_4 pada ph 3.2 dan methanol gred HPLC telah digunakan sebagai bahan yang membawa 2, 6-DHB dalam sampel kepada pengesan didalam HPLC.2,6-DHB dikesan pada gelombang 254nm menggunakan pengesan UV-DAD pada kadar alir 1.0ml/min.RP-C18 telah digunakan pada suhu persekitaran. Kebolehlarutan asid 2,6-Dihydroxybenzoic di dalam pelarut boleh ditentukan dengan membuat perbandingan terhadap klaribasi ukuran.Daripada pemerhatian ,2,6-DHB lebih larut dalam methanol kemudian acetonitrile dan paling kurang larut pada o-xylene.2,6-DHB dalam methanol mempunyai ikatan yang kuat berdasarkan plot Van Hoff.

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ºC - Degree Celsius

LIST OF ABBREVIATION

DHB - Dihydroxybenzoic acid

HPLC -High performance Liquid Chromatography

API -Active Pharmaceutical Ingredient

CHAPTER 1

INTRODUCTION

1.1 Research Background

2, 6-dihydroxybenzoic acid is one of the active pharmaceutical ingredients which is commonly use as a substance to produce pharmaceutical product such as antipyretic and analgesic drug. Many of the drug candidates are so hydrophobic that their effects in the organism, related to their bioavailability are dependent on the techniques used by the pharmaceutical industry to make them more soluble (Blagden et al.,2007).These technique include ,among others, manipulation of solid state structures(polymorph changes and amorphous forms)(Nordstrom et al, 2006).Other than is the salt formation, solubilization in cosolvent and miscellar solutions(Millard et al,2002).Pharmaceutical cocrystallization is a relatively new technology to improve solubilization (Basavoju et al,2008). In the pharmaceutical industry, most of the active pharmaceutical ingredients are in the form of solid produced through crystallization and so solubility is important because it can affects the drug efficacy, its future development and formulation efforts and also influences the pharmaco-kinetics, such as the release, transport and the degree of absorption in the organism(Mota,Carneiro,Queimada.2009).

2,6-DHB compound is under carboxylic acid group which OH can act as a proton donor and C=O acts as the proton acceptor in the forming the hydrogen bond not only between the solute itself but also the solvent. In this study, the types of solvent used might influenced the solubility because each solvent have different properties such as hydrogen bond acceptor/donor propensity and dipole moment as listed in table 1.Gu et al have classified the solvent properties as shown in table 1.

Table 1.1: The solvent properties considered in solvent selection

Solvent	Solvent class	Dipole moment
Methanol	Polar protic	1.70
Acetonitrile	Dipolar aprotic	3.92
o-xylene	Apolar protic	0.07

Dipolar aprotic solvents acts as H-bond acceptor, protic solvent able to acts as H-bond donor, apolar aprotic solvent can neither act as H-bond acceptor nor donor with 2,6-DHB solute in hydrogen bonding formation

1.2 PROBLEM STATEMENT

In drug development, poor solubility has been known as the cause of many failures and able to influences the drug efficiency and the degree of absorption in the organism. The development of solubility data is significant in order to select an appropriate solvent. Some thermodynamic models can be used to predict solubility, the availability of experimental data is still fundamental for an appropriate model development and evaluation (Elsevier et al., 2009). In this research, the solubility measurement of 2,6-dihyroxybenzoic acid was developed by using high performance liquid chromatography (HPLC).The solvent sunder the study have different properties and leading to different interactions with 2, 6-DHB.

1.3 RESEARCH OBJECTIVE

1. To develop the solubility measurement technique of 2,6-dihyroxybenzoic in different organic solvent using HPLC method
2. To find the Van Hoff correlation as a function of solvent type

1.4 SCOPE OF STUDY

To achieve the objective of this research, three main research fields was carried which are the 2,6-dihyroxybenzoic acid was dissolved in different organic solvent which are methanol, o-xylene and acetonitrile .The solubility of saturation solution was measured at three different temperature in order to identify the effective temperature using HPLC method and the Van Hoff plot was used to study the strength of solute –solvent interaction.

1.5 SIGNIFICANT OF STUDY

Most of the active pharmaceutical ingredients are in the form of solid produced through crystallization. As one of the active pharmaceutical ingredients, the solubility data of 2, 6-DHB in different solvent must be developed in order to choose the right solvent and the appropriate temperature to make the 2, 6-DHB more soluble.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

In this chapter is containing data from the previous research and facts that related to this study.

2.2 SOLUBILITY

2.2.1 DEFINITION OF SOLUBILITY

Solubility is defined as the maximum quantity of a substance that can be completely dissolved in a given amount of solvent. The solubility of a substance becomes especially important in the pharmaceutical field because it often represents a major factor that controls the bioavailability of a drug substance (Augustijns and Brewsier, 2007).

Solution are said to be saturated if the solvent has dissolved the maximal amount of solute permissible at a particular temperature (Augustijns and Brewsier ,2007).When solution of solids in liquid are considered, the solubility is given in terms of the weight dissolved in a given in terms of the weight dissolved in a given weight or volume of the solvent at a specified temperature (G.Thompson,2006). If a solid can dissolve in a liquid, it is said to be soluble in that liquid. As we add more solid to a liquid the solution becomes more concentrated. The greater the solubility of a substances the more concentrated it is possible to make the solution(G.Thompson,2006).When we add solute to solvent a point is reached where no more will dissolve, under the specified conditions,the solution is saturated.

The concentration of the solute in a saturated solution is the solubility of the solute in that solvent at that temperature (G.Thompson, 2006).Saturation of a solution is also defined as the point where the solution is in equilibrium with the undissolved solute. If less solute is added to the solvent than is required to give a saturated solution. This yields a supersaturated solution. Such solutions can often be prepared by utilizing the greater solubility possible at higher temperatures (G.Thompson, 2006).

2.2.2 SOLUBILITY DETERMINATION METHOD

The shake-flask method is based on the phase solubility technique that was developed 40 years ago and still the most reliable and widely used method for measurement today (Higuchi and Connors, 1965).The method can be divided into five step: sample preparation, equilibrium, separation of phases, analysis of the saturated solution and residual solid, and data analysis and interpretation (Yalkowky and Banerjee,1992).High performance liquid chromatography (HPLC) is the most commonly used analytical tool for the analysis of saturated solution. Its advantages over the ultraviolet method are that it can detect impurities and any instability (Augustijns and Brewsier ,2007).

2.2.3 FACTORS THAT EFFECTING THE SOLUBILITY

The solubility of one substance in another is determined by the balance of intermolecular forces between the solvent and solute and the entropy change that accompanies the salvation.Factors such as temperature and pressure will alter this balance, thus changing the solubility(I.Y. Nekrasov 1996).Solubility (metastable)also depends on the physical size of the crystal or droplet of solute or strictly speaking on the specific or molar surface area of the solute. For highly defective crystals, solubility may increase with the increasing degree of disorder (Yuen.C,2003). Both of these effects occur because of the dependence of solubility constant on the Gibbs energy of the crystal.

The solubility of a given solute in a given solvent typically depends on temperature. For most solids and liquids that dissolved in liquids, the rule is that solubility increases with increasing temperature (Campbell K.Mary, 2007). Heat is required to break the bonds holding the molecules in the solid together. At same time, heat is given off during the formation of new solute-solvent bonds (Ophardt E, 2003) .The other factors are the nature of the solvent and the solute. The more similar two compounds are, the more likely that one will be soluble in the other. Here the rule is “like dissolves like”. This is not an absolute rule, but it does apply in most cases. Like mean similar in terms of polarity. In other words, polar compounds dissolve in polar solvents, and nonpolar compounds dissolve in nonpolar solvents. A popular aphorism used for predicting solubility also uses the rule “like dissolves like” (Kenneth J.1994). This statement indicates that a solute will dissolve best in a solvent that has a similar chemical structure to itself. The overall salvation capacity of a solvent depends primarily on its polarity. The solubility is favored by entropy of mixing and depends on enthalpy of dissolution and the hydrophobic effect. Pressure has little effect on the solubility of liquid or solids.

2.3 2, 6-DIHYROXYBENZOIC ACID

2, 6-DHB is an aromatic carboxylic acid containing carboxyl group and two –OH group bonded directly to benzene ring and also a white and crystalline organic compound.2, 6 –DHB is a polar compound and one of the active pharmaceutical ingredient (Lake, 2002)

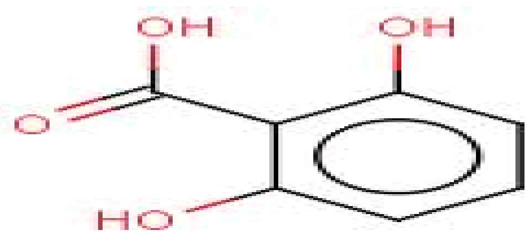

Figure 2.3: Molecular structure of 2,6-DHB

2.4 ACTIVE PHARMACEUTICAL INGREDIENT

2.4.1 DEFINITION

Any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that when used in the production of a drug becomes an active ingredient of the drug product (ICH Q7a, 1999). APIs can exist in a variety of distinct solid forms and each form displays unique physicochemical properties that can profoundly influence the bioavailability manufacturability purification, stability and other performance characteristics of the drug. (Byrn et al,1999)

2.5 ORGANIC SOLVENT

Solvent dissolved other chemicals into a solution in which the solvent chemically bonds the dissolved compound. Molecules in the solvent position themselves around the individual molecules from the dissolved substances, forming a compound that is more thermodynamically stable and, therefore, less reactive under standard conditions than the solvent and dissolved chemical on their own (Lowery et al, 1987).Organic solvent are widely used to dissolve and disperse fats, oils, waxes, pigment, varnishes, rubber and many other substances. Most organic solvents can be classified into chemical groups based on the hydrogen and carbon atoms and the presence of different functional groups (Public Health Guardian, 2002).Chemical groups that are commonly used are straight or branched chains of carbon and hydrogen, cyclic hydrocarbons, ester, aromatic hydrocarbon, alcohols, ketone (Public Health Guardian, 2002).

2.5.1 XYLENE

Xylene is a clear, colorless, sweet smelling solution of three aromatic hydrocarbon isomers produced from crude oil through a process called alkylation. Xylene ,also known as dimethylbenzene, is an organic molecules and apolar aprotic solvent which is can neither act as H-bond acceptor nor donor(Gu et al,2004).That exist in three forms, known as isomers: ortho-,meta- and para-xylene. The isomers refer to the positions of the methyl groups (composed of a carbon and two hydrogen atoms) on the benzene ring at the center of the xylene molecule. It is this structure that makes xylene a good solvent(Lowery and Richardson ,1987).A dipole moments is the strength of the magnetic field created by a molecule with an unbalanced distribution of electrons in its covalent bonding structure (Kohl.A, 2010).

Because of the xylene possesses a benzene ring structure, which requires the sharing of an electron between the six carbon atoms in the structure, it contains a large dipole(Kohl.A, 2010).As the molecule polarized, it is able to bond tightly with other polarized, it is able to bond tightly with other polarized molecules and act as a solvent (Kohl. A, 2010).Hydrogen bonding occurs when a chemical with a small dipole moment is attracted to the magnetic charge on another weakly polarized chemical (Kohl.A, 2010). With xylene, the effect of the benzene ring's electron sharing covalent bonding means other electrons within the molecule's bonds are attracted toward the ring structure, which causes weak polarization. This allows the molecule to exert hydrogen bonds and act as a solvent (Kohl.A,2010).The structure of xylene is such that it creates an unstable distribution of electrons and thus can be easily polarized by the presence of strongly, or evens some weakly, polarized molecules, and also affects polarization in other molecules (Kohl.A, 2010). The result of this polarization is that the two molecules can form bonds with one another via Van der Waal's force. The effect is that the polarizing chemical is surrounded and dissolution occurs (Kohl.A, 2010).

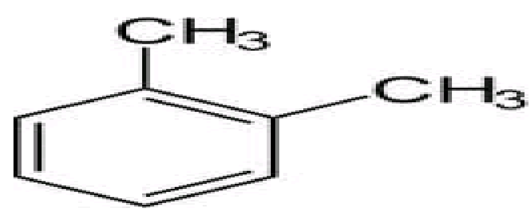

Figure 2.5.1: Molecular structure of xylene

2.5.2 METHANOL SOLVENT

Methanol is a chemical with formula CH30H.It is the simplest alcohol and is a light, volatile, colorless, flammable, liquid with a distinctive odor that is very similar to but slightly sweeter than ethanol(National Institute for Occupational Safety and Health,2008).Methanol is a polar protic solvent (Gu et al,2004). Protic refer to hydrogen atom attached to an electronegative atom.

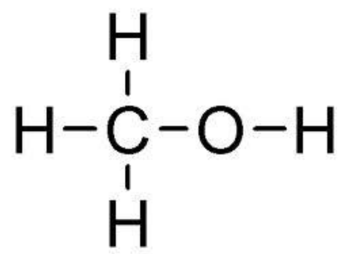

Figure 2.5.2: Molecular structure of methanol

2.5.3 ACETONITRILE SOLVENT

Acetonitrile is dipolar aprotic solvent (Gu et al,2004) .In hydrogen bonding dipolar aprotic solvent acts as H-bond acceptor. Its low viscosity and low chemical reactivity make it a popular choice for liquid chromatography. Industrially, it is used as a solvent for the manufacture of pharmaceutical and photographic film (Spanish Ministry of Health 2002)

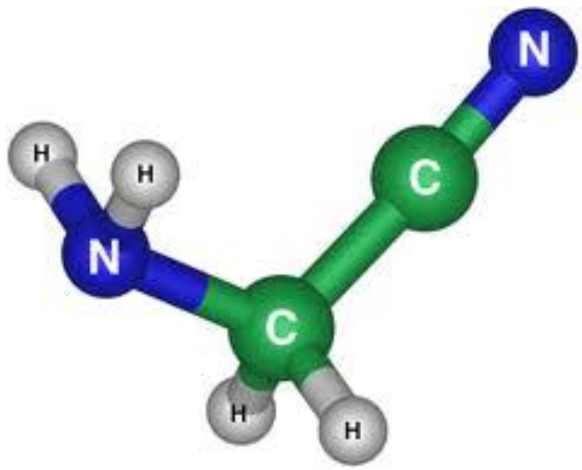

Figure2.4. 3: Molecular structure of acetonitrile

2.5.4 SOLVENT PROPERTIES

The solvent properties may be described by solvent proper parameters, including molecular description such as hydrogen bond donor or acceptor propensity descriptors and bulk properties (Gu et al,2004)The main factors which influence the properties of organic solvents are the number of carbon atoms present, the presence of only single bonds saturated molecules or double or triple bonds unsaturated molecules between adjacent carbon atoms and the presence of functional groups. The solvent properties of organic solvents tend to increase with fewer numbers of carbon atoms in the molecules. Unsaturated molecules tend to be more reactive than their saturated counterparts (Public Health Guardian, 2002).

CHAPTER 3

METHODOLOY OF RESEARCH

3.1 INTRODUCTION

In this chapter is about all the materials that used and the procedures that have been taken in order to obtain the solubility data. The materials are 2, 6-dihydroxybenzoic acid, methanol, acetonitrile , o-xylene and water. The mobile phase is a mixture of 0.05M Potassium dihydrogen phosphate (KH_2PO_4) (ph=3.2) and methanol (volume 3:2).

3.2 MATERIALS

In this experiment, 2, 6-dihydroxybenzoic acid is in the powder form and the purity is 98%.Methanol, acetonitrile and o-xylene are anhydrous. Methanol, 0.05M Potassium dihydrogen phosphate (KH_2PO_4) and formic acid were used to prepare the mobile phase and the methanol is a HPLC grade. All the materials are kindly provided by the Sigma Aldrich Company.

3.3 MOBILE PHASE PREPARATION

The mobile phase is a mixture of 0.05M Potassium dihydrogen phosphate (KH_2PO_4) (ph=3.2) and methanol (volume 3:2).To get 0.05M KH_2PO_4, 6.8 g of KH_2PO_4 was dissolved in 1 liter of water and the ph was adjusted using the ph meter to 3.2 by adding the formic acid.

3.4 STANDARD SOLUTION PREPARATION

2, 6-dihydroxybenzoic acid standard solution to create a calibration curve was prepared .Five different concentration was prepared by adding 0.2g, 0.4g, 0.8g, 1.6g and 3.2 g into 50 ml volumetric flask containing 30 ml of methanol and 20 ml of water.

3.5 SATURATED SOLUTION PREPARATION

Saturated solution was prepared by mixing an excess 2, 6-DHB into 5-15 ml of solvent which are methanol, acetonitrile and o-xylene on a hot plate at the desired temperature. Saturated solution means no more solute can be dissolved into the solvent.