CHAPTER I

INTRODUCTION

It is generally believed that in order for a solid drug to be absorbed to any appreciable extent across the gastrointestinal membrane, it is preferable to have the drug go into solution in the gastrointestinal fluid prior to the absorption process. The rate determining step in the diffusion-controlled absorption process for orally administered of low aqueous solubility drugs is usually controlled by the dissolution rate of drugs in the biological fluid of the gastrointestinal tract (1-3).

The solution process of solid drugs in water can be divided into two steps. The first step occurred when the solid drugs is placed in water. The surface of solid particles are attached by the water molecule and break down the crystal lattice into a single molecule. This process consumes an input of energy and the energy that is uptaken in this step is called 'lattices energy'.

Therefore, the strength of the bond between the solute molecules in solid state obviously has effect on the solubility of the drug, the stronger bonds between them, the greater energy for separation. The second step is the hydration process, the isolated particles become enclosed by the water molecule. The energy which releases

in this step is named "hydration energy". Hence, the attractive interaction between solute and solvent molecule must be also considered. The solid drugs tend to be soluble in the solvent that exhibit similar attractive force. This observation is often stated very simply as "like dissolve like". Nonpolar substance is soluble in non-polar solvent while polar or ionic compounds dissolved in polar solvent. The physical and chemical nature of the solute such as structure, molecular size and shape of solute will affect the degree of interaction between both solute-solute molecules and solute-solvent molecules (1, 4).

There are many methods that can be used to enhance the dissolution of drug (1, 5). Since dissolution rate is directly proportional to surface area, reduction of particle size is one of the traditional methods. However, it is probable that, for some hydrophobic drugs, a reduction in particle size also reduce the effective surface area because of the resulting in agglomeration and aggregration of the fine particles (2, 5, 6). Lin and his co-workers (1980) (2) exhibited that the dissolution rate of glutetimide microsized (smaller than 5 u in size) was lower than the 100/120 mesh and 20/40 mesh particles.

Reduction of hydrophobicity is one the other methods that is used to improve solubilities of poorly soluble drugs and for this purpose, many surfactants are used. Surfactants can enhance water solubility of the

drug by increasing wettability, decreasing hydrophobicity and lowerring interfacial interaction between water and hydrophobic part of the molecule (2, 6, 7). In 1976, Chiou and his collaborators (7) reported that, after crystallized the poorly water-soluble drugs such as sulfathiazole, prednisolone and chloramphenicol in aqueous solution of non-ionic surfactant as polysorbate 80, the increasing in the dissolving rate of these drugs are produced. And also, in 1968, Elworthy and Lipcomb (8) showed the greatly increased in the dissolution of griseofulvin in water when it mixed with some non-ionic surfactants such as cetomacrogol and other hexadeconol derivatives. Furthermore, In 1989, Shah and coworkers (9) showed that the poorly water-soluble drugs such as griseofulvin, carbamazepine, clofibrate exhibited greatly improved solubility in the medium which consisted of surfactant, such as sodium lauryl sulfate, sodium cholate or sodium deoxycholate in aqueous solution.

Besides these two techniques, the molecular dispersion of a solid solute in amorphous form of solid solvent, glass dispersion is one of the techniques that can be used to enhance the dissolution rate. In 1969, Chiou and Reigelman (10) expressed that the glass solution prepared by melting griseofulvin with citric acid exhibited a very rapid dissolution rate of griseofulvin. Similarly, Allen (11) as well as Glanem (12) and their collaborators had shown that sugar glass dispersions lead

to heightening of the dissolution rate of corticosteroid and sulfamethoxazole. The elevation of dissolution rate resulted from the absence of lattices energy in the amorphous state (5, 6, 13).

Extensively, It is known that, besides sugar, urea and citric acid, bile acid is one of the compounds that have been able to form glass, a transparency and brittle solid, readily upon cooling from the liquid state. Resulting from lacking of lattices energy in the amorphous state, the glass dispersion of slightly water-soluble compound in amorphous form of weak acidic compounds as bile acids, may show greater dissolution rate in alkali medium than the crystal mixture. However, the investigation by Pathipvanich in 1984 (18) manifested that some of the bile acid derivative, as cholic acid chenodeoxycholic acid and also deoxycholic acid, showed the opposite result.

Cholic Acid

Bile acid is one of the physiological surfactants that play important roles in the digestion and absorption of dietary and fat-soluble substance such as vitamin K, B-carotene, when it presents in the salt form (14-17, 19). Beside their normal physiological function, bile salt can facilitate dissolution and absorption of poorly water-soluble drugs. For example, in 1969, Stoll and his

coworkers (20) cholic acid can enhanced the blepharoptonic activity of orally administrated reserpined in mice. In 1979, Miyazaki and his co-workers (21) showed that bile salt was able to improve dissolution behavior of indomethacin and phenylbutazone. In 1989, Shah (9) reported the effect of bile salts and other detergents on promoting the dissolution rate of water insoluble drugs such as griseofulvin, carbamazepine, cortisone acetate and clofibrate.

The structure of most common physiological bile acids is steroid structure with 24 carbon atoms possessing an acidic side chain and one or more hydroxyl groups which are α-and/or β-oriented in the 3, 6, 7, or 12 position as in Figure 1 and Table 1. As in this figure, the junction of A and B ring of 5B-cholane nucleus is kinked in the cis configuration with respected to ring B and hydrogen atom at 5 position is β-oriented (14-17, 22-24) . The report of Fini and Roda (15) in 1985 expressed that the solubility of bile acids were depended on a number, position, and orientation of hydroxyl groups. These hydroxyl groups affected solubility both by forming hydrogen bonds with water and by reducing the hydrophobic area of the steroid nucleus. Hence, the water-solubility of trihydroxyl bile acids such as cholic acid are greater than dihydroxyl bile acids such as deoxycholic acid.

Cholic acid, one of the three major groups of human bile acids, has three hydroxyl groups at 3, 7 and 12 position all of which are in α-orientation as in Figure 2. The perfective structure of cholic acid in Figure 2 illustrates that the three hydroxyl groups (OH) lining on the same side and below the equator of molecule exhibit planar polarity at lower plane of the molecule, while the methylene groups (CH_3) laying on another side and most of the steroid skeleton demostrate hydrophobic character (14, 16). Beside these three hydroxyl groups, existing at the end of the aliphatic chain, the terminal carboxyl group (COOH) also shows a strong hydrophillic character.

Like the other acid compounds, this carboxylic group can dissociated and turn into anionic form, when it dissolve in the medium with the pH above its pK_a. This dissociation will confer to consideration of the water solubility of the bile salt (15, 16, 25).

The dissociation constant of cholic acid has been determined by many investigators. In 1957, Ekwall and his co-wokers (26) used potentimetric titration to find out the pK_a of cholic acid and showed the 4.98 as the obtained value. In 1986, Cabral, D.J. and his collaborators (27) used C^{13} NMR spectra to determine this value and the value 4.6 was obtained for the cholic acids in the concentration below CMC (critical micelle concentration). Thus, the water solubility of cholic acid is limited when dissolved in acidic medium and

increased when placed in the medium with pH value above 5.

According to the study of Kunio Miki and his co-workers in 1990, to form the crystal structure, more than two molecules of cholic acid are linked to each other by strong hydrogen bonds (28, 29). All of these powerful bonds will be destroyed, when cholic acid is melted. After the quickly cooled step, It can be prospected that there are creation of some new intermolecular interactions in the homogeneous melted mixture of drug and cholic acid. The expected interactions are, of course, applied to cholic acid-drug, drug-drug, as well as cholic acid-cholic acid interactions. These interactions could lead to change in dissolution rate of the drug. Therefore, the solid-state interaction determining methods are necessary for this experiment.

The Experimental Methods for The Study of Solid-State Interaction

There are many techniques that are available to contribute information regarding the nature of interaction that may occur in solid state. These methods are:

1. Method Involving Heating : Thermal analytical system (TAS), differential scanning calorimetry (DSC), thermogravity (TG) and differential thermal

analysis (DTA) are known as the most common method to detect physicochemical interaction of two or more component system. The happening of the solid-state interaction affects on the change in thermal energy consumption of the solid system. In 1969, Guillory and his co-workers (30) used DTA technique to construct phase diagrams of deoxycholic acid-menadione and caffeine-phenobarbital for determination of their complexes. However, these methods can not be applied to the samples that decompose after melting and cannot indicate the type of interaction and the functional groups that are involved in the interaction.

2. X-ray Diffraction Methods : This method is one of the available methods widely used to study the structure of crystal (31). The x-ray diffraction pattern provided by the diffraction of x-ray radiation from the crystal plane can be used to determine the molecular arrangement in the crystal structure. But, without crystal plane in amorphous solid, the molecular arrangement in the glass mixture is unable to be elucidated by this valuable technique.

3. Spectroscopic Method : This method consist of many techniques that concern with absorption or emission of electronic radiation. The absorbed light which is consumed by the sample molecule can transform the status of the ordinary molecule. For example, UV light is able to rouse up the molecule in ground state to excited state,

infrared radiation can only alter the vibrational and rotational movement of the molecule and also the radio frequency in the range of 60-550 MHz can produce the change in magnetic properties of certain atomic nuclei, notably that of hydrogen and C^{13} isotope of carbon. In particular, only specific frequency are absorbed and others are virtually unaffected. For this reason, the spectra which obtained from these techniques are frequently used to define the molecular structure.

IR spectroscopic technique is one of the utilitarian means that can provide more information about the interaction in solid-state. Recently, Isumoto kono (1990) (31) used IR spectroscopy and other techniques to study the physical and chemical changes in solid-state of the amorphous mixture of flufenamic acid and magnesium aluminium trisilicate. This appropriated method will be described in detail under topic of infrared spectroscopic study.

4. Solubility Technique: The solubilizing character is one of the physicochemical properties that can be affected by the interaction between solute molecules. Hence, the alternation in the solid-solid interaction can be shown by the change in solubilizing character. In 1977, Allen and his co-workers (12) demonstrated that, because of the lacking in lattice energy in amorphous state, the dissolution of

corticosteroid was increased, when it was molecularly dispersed in sugar glass matrix. Thus, many investigators used this appropriated method to determine the change in the solid phase interaction. Mahammad Hassan and his collaborators (1990) (33) used dissolution testing method along with other techniques to determine inclusion complex between famotidine and B-cyclodextrin. This avialable technique will be discussed further under dissolution testing method.

In many instances, combination of two or more methods is required to contribute a more complete feature for the solid-state interaction. For our purpose, we choose the dissolution method and infrared spectroscopic method to determine the interaction in the mixture of cholic acid and the drugs. Dissolution testing method is a simple and inexpensive method. By means of comparing the releasing rate of drug from the mixture with the rate from the single compound, the irregular change in the dissolution rate of the mixture can be pondered to the occurence of the solid-state interaction in the mixture of drug and cholic acid. However, it is believed that this method can furnish only the existence of the interaction. To provide more information about this interaction, the infrared spectroscopic technique is an essential tools.

Dissolution for Non-Matrix System

The dissolution of a solid in aqueous medium may be regarded as being composed of two consecutive stages. The first stage is an interfacial reaction that results in the liberation of solute molecules from the solid phase and the saturated solution at the outmost layer of the solid surface. In the second stage, the dissolved molecules are transported through a thin stagnant layer of the liquid medium which exists at the solid-liquid interface to the bulk medium by diffusion mechanism, as in Figure 4.

Like any reaction that involves in consecutive stage, the overall rate of mass transport will be determined by the rate of the slowest stage. In the absence of a chemical reaction between solute and solvent, then, the slowest process is usually the diffusion of the liberated molecules across the static boundary, a stagnant layer of liquid at the solid-liquid interface. Under these conditions, the mathematical equation that was expressed by Noyes-Whithney and Nernst is used to define this phenomena. The equation may be written as follow (18):

$$\frac{dc}{dt} = \frac{DA}{Vh}(Cs-C) \qquad \text{Eq. 1}$$

where, Cs = The concentration of solute that required to saturate the solvent at the experimental temperature,

C = The concentration of solute in the bulk solution at time t,

$\frac{dc}{dt}$ = The dissolution rate,

D = The diffusion coefficient of the solute through the stagnant layer,

A = The surface areas of the undissolved solid in contact with the solvent,

V = The volume of dissolution medium,

and h = The thickness of the boundary layer.

This equation appear to be applied to the cases that (18, 34):

1. The escaping rate of solute molecule from the solid surface is much faster than the transporting rate in the unstirred layer. Therefore, the dissolution rate is controlled by the diffusion of the solute molecule through this stagnant liquid layer.

2. In the stagnant layer, there is a linear concentration gradient and this assumption as in the Figure 4 implies that:

2.1 The diffusion coefficient (D) is independented on the concentration but assume to be constant under the same solvent and stirring speed.

2.2 The concentration at the solid surface is equal to the saturation concentration (Cs)

2.3 A steady state exist in this layer.

According to these criteria, if the solute is removed from the dissolution medium by some processes at a much faster rate than it passed into the bulk medium, then the term (Cs-C) in Eq. 1 may be proximate to Cs. Alternatively, if the volume of the dissolution medium is so large that C is not allowed to exceed 10 % of the value of Cs then the same approximation may be made. In either of these circumstances dissolution is said to occurred under " sink condition " and Eq.1 may be simplified to:

$$\frac{dc}{dt} = \frac{DACs}{Vh} \qquad \text{Eq. 2}$$

This equqtion showed that, under condition of constant surface area, the character of dissolution of the single drug will follow zero order pattern. When the single drug is mixed with the other compound, such as mixed with cholic acid, the character of its dissolution pattern may be modified. The alternative characters are discussed in the next section.

Dissolution of Two Non-Interacting Components

Acceding to the Noyes-Whitney law, when a uniform, intimate, non-disintegrating mixture of two compounds, A

and B, is exposed to a solvent, both phase should initially dissolve at rate proportional to their solubilities and diffusion coefficient. After a short period of time, if the boundaries of compound A and B are receded by the medium at the same rate, both of these components will be coexisted at the solid-liquid interface, as case 1 in Figure 5. But, the compounds usually possess different solubilities and diffusion coefficients. Therefore, the solid boundary of the more soluble component will exhibit faster regression rate than the boundary of the less soluble compound. Hence, after the time zero, only the less soluble compound will exist at the solid surface as case 2 in Figure 5. The mathematical equation for this case was presented by W.I Higuchi and co-workers as followed (35):

$$\frac{N_A}{N_B} > \frac{D_A C_A}{D_B C_B} \qquad \text{Eq. 3}$$

where, N_A, N_B = The fraction of component A and B in the mixture,

D_A, D_B = The respective diffusion coefficients of component A and B,

and C_A, C_B = The solubilities of component A and B.

In this case, the solid-boundary regression of compound B is greater than the regression of component A.

Thus, only the component A exists at the surface of the tablet after the time zero and component B must be passed through this layer before they reach the stagnant layer. The dissolution rates of both compound A and B are calculated form the mathematical equation as followed:

$$V_A \frac{dC_A}{dt} = \frac{A\ D_A\ C_A}{h} \qquad \text{Eq. 4}$$

and

$$V_B \frac{dC_B}{dt} = \frac{W_A}{W_B} \frac{dC_A}{dC_B} \qquad \text{Eq. 5}$$

where, W_A = The amount of component A per unit volume of tablet,

W_B = The amount of component B per unit volume of tablet,

From Eq. 4, dissolution rate of the slow receding component, the component A, is depended on its diffusion coefficient and its solubility in the dissolution medium. Thus, the dissolution profile of this component will show zero order pattern. On the other hand, component B must diffuse through the surface component layer, hence the dissolution rate of component B, will be controlled by the receding rate of the surface component. Therefore, the character of the dissolution profile of component B will be changed from the zero-order pattern into matrix release pattern.

Dissolution of Matrix System

The releasing mechanism of the inner component can be driven by diffusion of the inner component through the solid matrix or outer component. The kinetic of this releasing pattern is affected by interaction between the two components. (11, 34, 35):

From the basic knowlegde from Noyes-Whitney's and Frick's law, T. Higuchi used them to predict the dissolution rate of the drug and other components dispersing in the inert matrix under sink condition and expressed it in the mathematical term as followed (35):

$$\frac{Q}{A} = [D\epsilon C (2W - Cs)t/\tau]^{1/2} \qquad \text{Eq. 12}$$

Where, Q = The cumulative amount of drug release,

D = The saturated coefficient of the drug in the innert matrix,

W = The amount of drug per unit volume of matrix,

ϵ = The porosity of tablet,

τ = The tortuosity.

From this equation, the dissolution profile of matrix release system will be linear when respect to square root time.

Commonly, it makes sense to think of the best regression model as the one that gives the best description for the relationship between independent and dependent variable for a set of data. To provide the best one, the statistic analysis is used as an essential device. As a general principle, the coefficient of determination, r^2, is one of the standard measurement of how well a set of data can be defined by the regression model. This quantity naturally varies between 0 and 1. However, the r^2 value always increase as more variable are added to the regression equation. Therefore, r^2 by itself is not a very good criteria for the best regression model. Frequently, this statistic value will be used in combination with other reasonable criteria to determine the best regression model.

Among the other statistic means for selecting the appropriated model, the optimal one is known as residual means square or residual variance, s^2. This statistic value can directly measure how accurately the regresssion equation predicts the dependent variable. Consequently, the smallest deviation of data from the regression line is obtained, when the residual means square is minimized. In this manner, when doing a linear regression, the criteria are to find the model that maximized r^2 and minimized s^2 value.

To define the effect of cholic acid on the dissolution, the comparison of the dissolution rate from

the best fitting model of the pure compound with the rate from the appropriated model of its respective physical and glass mixture is necessary.

Infrared Spectroscopic Study

Generally regarded as one of the method that provides unambiguous characterization of various molecular modification, IR spectroscopic method is used to deliver more information about cholic acid-drug interaction. To get more information about this interaction, a comparative study of the vibrational frequency of various functional groups in the three compounds, cholic acid, drugs and its mixture, is necessary. By the comparison, the noticably different character of individual band in the spectrum of the mixture reflects the modification in these particular groups.

Commonly, all of the atoms and chemical bonds in organic molecules vibrate with their specific frequencies. These motions are comprised of spring and ball system with constant movement. When these molecules are irradiated by infrared beam whose frequencies are changed continuously. Only the certain frequencies corresponding to that require to consume in the vibration of particular groups of atoms will be absorbed. The apropriate frequencies for the molecular vibrating system are not only depended on the

nature of the particular bond themselves, but are also affected by the entire molecules and its environment.

The IR absorption frequency of the sample will be displayed in the form of the spectrum which recorded the intensity of transmitted light versus wave number or wavelength. The position of the absorption band can be specified in unit of wavelength (micrometer, μm) or wavenumber (reciprocal centimeter, cm^{-1}). In our discussion, the position of the absorption band will be expressed in term of wavenumber, only.

From the basic knowledge, the character and position of the typical peak can be widely used to identify the organic substance. Therefore, the alternation in character of the absorption peaks of some particular groups in infrared spectrum may be reflected to the structural change or the occurence of the interaction between these attentive groups. On this ground, to get more information about the interaction in the two mixtures, physical and glass mixture, the understanding about the factor that affect on the position of the characteristic peak will be an essential means for this study. The interesting factors that we would like to concern with are included vibrational coupling, bond order, electronic effect, angle strain as well as association effect. These effects will be explained in detail as followed (38-44):

1 Vibrational Coupling : The two bonds existing in the adjoining part of the molecule and vibrating with approximately same frequencies are able to interact and give a mixed vibration in which both groups take part. This interaction is known as " vibrational coupling ".

The good example for this factor is the vibrational coupling in the sulfonyl functional group which has two co-center S=O group. These two identical groups might be vibrated with the same frequency, but in IR spectrum of many sulfonamide compounds, it shows two absorption bands at about 1350 and 1150 cm^{-1}. for asymmetrical and symmetrical vibration which result from in-phase and out-of-phase combination of these vibrations.

2 Bond Order : Like other spring and ball system, the tightening bond requires more vibration energy than the loose one. Hence, the vibrational energy is expected to be higher, when the bond strength is increased. In this manner, it can be predicted that the stretching of C=C and C=O bond will existed at higher frequency than C-C and C-O bond.

3 Electronic Effect : Regulating the physical and chemical properties of the substance, the distribution of electron in the molecule has been also devoted to influence on the appearance of the absorption peak in the IR spectrum. The change in the electron distribution by substitute group can affect the strength of the bonds

adjacent to the bond whose frequency are measured. By means of interference drawn from the chemical or physical behavior of molecule containing those groups, it was able to be classified the electronic effect into two groups. These three groups are consisted of inductive effect, resonance effect and field effect. The more details about them are as followed:

i) Inductive Effect : Inductive effect is the ability of an atom in molecule to attract electron to itself. This attractive ability can be measured by the difference in electronegativity value of the adjoining atom in the same molecule. This effect influence on the strength of the bond neighboring to the bond whose frequencies are measured and operated to modify the vibrating frequency by changing bond order. For example, an electron donating group as amino group in amide compound can lead the adjacent C=O bond weaken and lower the stretching vibration of C=O bond by 10-20 cm^{-1} while, an electron withdrawing group as chlorine in acylchloride will oppose this trend and show the opposite shift.

ii) Resonance Effect : Resonance effect accomplish from the distribution of electron in the molecule to contribute the more stable form whose total energy is minimum. By means of rehybridization of double bond and single bond which transfer of π - electron character from the multiple bond to the interventing single bond. Hence, unlike the inductive effect, the influence of resonance on the shift of the particular peak

dose not depended upon distance. In the infrared spectrum interpretation, both inductive and resonance effect are always pondered together. The difficulty of consideration in isolation of them is that in some molecules, inductive effect is more important than resonance effect, while the reverse is true in other. For instance, in phenyl ester, non-bonding electron on acyl oxygen molecule are partly drawn into the ring and the resonance effect may cause the shift of C=O stretching peak to lower frequency, but the powerful inductive effect of the oxygen atom bring the C=O streching band to the higher frequency position.

4. Bond Strain : Like the effect of bond order, the strain in atomic bond is also affected on the absorption frequencies of the particular group. Such in ketone compounds, the highest C=O stretching arise in the strained cyclobutanone. The reason is that the C-C(=O)-C bond angle is reduced below the normal 120^{o} leading to increase s character in C=O bond, the C=O bond is strengthened and so the stretching energy of C=O bond is increased. If the bond angle is push outward above 120^{o}, the opposite effect operates.

5 Association Effect : In general sense, the interaction between the molecules in the condensed phase will produce the change in the vibrational frequency. There are many types of interaction between the associated molecules. The typical cases of the

association effect are including: electron deficient bond in some alkaloid compound, lewis acid-base interaction in lewis acid-base complex and hydrogen bonding interaction in many associated compounds. Only the hydrogen bonding interaction will be discussed in this section.

Hydrogen bonding is by far the most common associative effect. This chemical bond is an especially strong kind of dipole-dipole attractive which a hydrogen atom serves as a bridge between two electronegative atoms, holding one by a covalent bond and the other by purely electrostatic force. The most common electronegative atom involving in the hydrogen bond are F, O, N.

Prominently noticed in IR spectra, hydrogen bonding manifests itself in very broad O-H and N-H stretching band at frequencies considerably lower than normal. For example, in the range from 3300 to 2500 cm^{-1}, we can observe a large extending and intense peak of hydrogen bonded N-H stretching in the spectra of azole compounds.

All of these factors influence on the location of the molecular vibrational peak in the IR spectrum. And it is difficult to isolate one effect from each other. Thus, to study the cholic acid-drug interaction in their respective mixture, both of the IR spectroscopic modification and the dissolution result must be pondered together.