SOLUBILIZATION OF POORLY WATER-SOLUBLE DRUGS: THEORY AND APPLICATIONS

by

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SIGNED: ____________________________  Yan He
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DEDICATION

To my parents, husband, brother, sister-in-law, and son
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ABSTRACT

This dissertation is based on the theory and applications of the most commonly used solubilization techniques: pH adjustment, cosolvency, micellization, complexation, and the combinations of pH adjustment with one of the other techniques.

Chapter 1 provides an overview for the methods which are available to formulate a poorly water-soluble drug based on its administration route.

Chapter 2 applies these commonly used techniques to solubilize two structurally related anticancer drugs. The efficiency of each technique is compared for both drugs side by side. It is observed that each technique is more efficient on the drug which has less polarity. However, the achievable final drug concentration in a formulation depends not only on the efficiency of the applied technique, but also on the drug’s water solubility.

Chapter 3 emphasizes the overall effectiveness of each technique on drugs which have different physicochemical properties. Solubilization profiles for the above techniques are generated for twelve compounds, eight of which are ionizable and studied under both unionized and ionized conditions. This chapter illustrates that the efficiency of the cosolvency, micellization, and complexation on both unionized and ionized drug species can be predicted from their polarities. Thus, the solubility of an ionizable drug can be estimated by using a given solubilizing excipient at any pH to meet the dose requirement.
Chapter 4 studies the effect of cosolvent on complex stability. A series of alcohols were used to illustrate the effect of cosolvent size and polarity on the solubilization of a compound. It is proposed that a ternary drug-ligand-cosolvent complex is formed in these combined systems.

This dissertation provides guidance for the selection of a solubilization technique for a compound based on the physicochemical properties and the dose requirement.
CHAPTER 1. INTRODUCTION

Many compounds that are identified to have high activity during early screening have low aqueous solubility (Gibbon and Sewing 2005). These compounds are mainly selected by high-throughput and receptor-based in vitro screening techniques. In the screening process, a certain degree of lipophilicity is often required for a drug to cross the cell membrane to reach the receptor site and a lipophilic group is often needed for the drug to have an affinity with the receptor (Yokogawa et al. 1990; Hageluken et al. 1994; Lipinski 2000; Lipinski et al., 2001). Unfortunately, compounds with high lipophilicity usually have low water solubility (Yalkowsky 1999).

A number of methods are available to improve the solubility of a poorly water-soluble compound. Some of them improve oral bioavailability by improving dissolution rate and apparent solubility without altering equilibrium solubility in water. Such techniques include particle size reduction, crystal form modification, solid dispersion, solid solution, and hot melt extrusion (Leuner and Dressman 2000; Garad 2004). These techniques are useful for solid dosage forms that are not applicable to solutions.

While solid dosage forms comprise the vast majority of the oral formulation market, liquid oral formulations provide higher bioavailability and can be used by patients who cannot swallow tablets or capsules (Strickley 2004). Furthermore, there is a great demand for liquid formulation for intravenous (IV) administration, which is often preferred for immediate bioavailability, easily adjustable and immediately stoppable
administration rate, and the absence of gastrointestinal irritation and vomiting (Jonkman-de Vries et al., 1996).

A soluble prodrug can be prepared when a compound has a functional group and the human body has a mechanism to cleave the prodrug into the active entity. This approach is usually costly and time consuming because a prodrug is considered as a new chemical entity (NCE) and the demonstration of the efficacy and safety of NCEs is required by the FDA (Kipp 2004). In recent years, attention has been placed on microparticulate entrapment systems for the purpose of controlled drug release and targeted drug delivery (Florence and Attwood 1998; Sinha et al., 2004). Such systems include microemulsion, liposome, and nanosuspension. They are colloidal systems and are not thermodynamically stable. They are usually expensive to prepare, unstable, and hard to sterilize.

The most commonly used solubilization techniques are the conventional pH adjustment, cosolvency, micellization, complexation, and pH adjustment combined with one of the other techniques. The efficiency, simplicity, and the stability of the true solutions are the main reasons that these techniques are well accepted. This dissertation is based on the applicability of these techniques.

Two structurally related anticancer drugs that have very different physicochemical properties and aqueous solubilities are studied with these techniques in Chapter 2. The efficiency of each technique is compared for both drugs side by side.

The emphasis of Chapter 3 is on the overall effectiveness of each technique on drugs which have different physicochemical properties. Solubilization profiles of all
techniques are generated for twelve compounds, eight of which are ionizable and studied under both unionized and ionized conditions. The solubility of each drug is presented as both the normalized value to its intrinsic solubility and the final concentration in mg/mL. The descriptors for each technique are generated from the experimental data. These descriptors are evaluated and correlated with respect to compound polarity. This chapter illustrates that the efficiency of the cosolvency and micellization can be predicted from drug polarity and that of the complexation can also be estimated. This chapter gives a guide on how to choose a technique to meet the dose requirement based on its efficiency and water solubility of the compound.

The combination of cosolvency and complexation is studied in Chapter 4 to understand the effect of cosolvent on complex stability. Some researchers have reported that cosolvents decrease drug solubility in the complex (Loftsson et al., 1993; Ono et al., 2001), while others reported an increase (Zung 1991; Savolainen et al., 1998; Loftsson et al., 1998; Loftsson et al., 2001; Faucci 2001). A series of alcohols with increasing molecular size and reducing polarity were used to illustrate the effect of cosolvent size and polarity on the solubilization of a compound. The solubilization curve in a solution contains a fixed amount of ligand, decreases to a minimum and then increases with increase cosolvent composition. It is proposed that a ternary drug-ligand-cosolvent complex is formed in these combined systems and descriptors are generated from the experimental data.

The theory of each technique is extensively investigated in each chapter. The high efficiency of the combinations of pH adjustment with either cosolvency,
micellization, or complexation is emphasized and illustrated by all studied ionizable compounds. This dissertation provides guidance for the selection of a solubilization technique for a compound based on the physicochemical properties and the dose requirement.
CHAPTER 2. SOLUBILIZATION OF TWO STRUCTURALLY RELATED ANTICANCER DRUGS: XK-469 AND PPA

1. Introduction

A large number of poorly water-soluble compounds are synthesized as the result of high-throughput combinatorial chemistry and the efficient receptor-based in vitro screening for new chemical entities. As reported by Gibbon and Sewing (2005), the percentage of compounds with predicted low aqueous solubility (≤ 5 µg/mL) has been increasing since the 60’s, especially for those compounds which have confirmed activity. Low water solubility is often an obstacle for the further development of a compound. In order to overcome this problem, a solubilization technique is usually needed. The most commonly used techniques are pH adjustment, cosolvent, micellization, complexation, and pH adjustment combined with one of these methods. The required magnitude of solubilization is dependent on the desired dose and the intrinsic solubility of a drug. The efficiency of a technique is largely determined by the physicochemical properties of the compound. In this chapter two structurally related compounds, XK-469 and PPA (Figure 2.1), are used as the model drugs to illustrate the effect of each commonly used solubilization technique.

XK-469 is a new synthetic antitumor agent with selective cytotoxicity for several murine solid tumors, including colorectal, mammary adenocarcinoma, ovarian cancer, and leukemia cell lines (Corbett et al., 1998; LoRusso et al., 1998; Ding et al., 2002).
PPA is a widely used herbicidal compound and has been explored by the National Cancer Institute for antitumor activity. Both drugs have the same ionizable site and similar pKa values.

The objective of this study is to provide guidance for solubilization on compounds with very different physicochemical properties and aqueous solubilities.

![XK-469 and PPA structures](image)

Figure 2.1. XK-469 and PPA structures

### 2. Materials and Methods

#### 2.1. Materials

XK469 {2-[4-[(7-chloro-2-quinoxalinyl)oxy] phenoxy- propanoic acid, NSC# 697887, CAS# 157435-10-4} was provided by the National Cancer Institute (Bethesda, MD). PPA (2-phenoxy- propanoic acid, NSC# 404102, CAS# 940-31-8) was purchased from Sigma (St. Louis, MO). HPβCD (hydroxypropyl-β-cyclodextrin, Cavasol®W7 HP Pharm) with an average molecular weight of 1390 and an average molar substitution of 0.6-0.9 hydroxy propyl group per anhydrous glucose unit was obtained from Wacker Biochem Corporation (Adrian, MI). SBEβCD (sulfobutyl ether-β-cyclodextrin, Captisol®) with an average molecular weight of 2160 and an average degree of
substitution of 7 was a gift from CyDex, Inc. (Lenexa, KS). ACN (acetonitrile), EtOH (ethanol), PG (propylene glycol), and Tween 80 (polysorbate 80), HCl standard solutions, NaOH standard solutions, and all other chemical were of reagent or HPLC grade and purchased from Aldrich (Milwaukee, WI).

All chemicals were used as received without further purification and the water was double-deionized.

2.2. Melting point (MP) measurement

The melting points of XK-469 and PPA were measured by DSC (differential scanning calorimetry, TA Instruments 910, model 910001-901). The heating rate was set to a 5°C/minute ramp.

2.3. Solvent preparation

Buffer systems: Buffer of pH 1.0 was prepared by adding 0.1M NaCl into the 0.1N HCl standard solution. Buffers in the pH range of 1.3-5.0 were prepared by mixing 0.1M monosodium citrate (C₆H₇NaO₇) with 0.1N HCl standard solution in different ratios. Buffers in the pH range of 5.0-8.0 were prepared by mixing 0.075M-0.15M monosodium phosphate (H₂NaO₄P) and disodium phosphate (HNa₂O₄P). Buffers in the pH range of 8.5 to 9.5 were prepared by 0.1M to 0.45M of trisodium citrate (C₆H₅Na₃O₇). Buffers in the pH range of 12.0 to 12.6 were prepared by 0.1M to 0.45M of trisodium phosphate (Na₃O₄P). The ionic strength of the buffers in the pH range of 1.0-8.0 were controlled at 0.2M by the addition of the appropriate amount of NaCl. Buffers with higher ionic strength in the pH range of 8.5-12.6 are needed to solubilize PPA.
Systems with solubilization agents: Cosolvent systems were made with 0 to 20% of EtOH or PG in buffer (v/v). Surfactant systems were made with 0 to 20% of Tween 80 in buffer (v/v). Complexant systems were made with 0 to 20% of HPβCD or SBEβCD in buffer (w/v). In order to study both compounds in the above systems with the same final pH of 1.0 ± 0.1 and 4.55± 0.1, buffers with initial pH of 1.0 and 4.55 were used for XK 469, while buffers with initial pH of 1.0 and 8.5 (0.42 M trisodium citrate) were used for PPA.

2.4. Solubility measurements

The solubilities of XK-469 and PPA were determined by the phase solubility analysis method of Higuchi and Connors (1965). Excess drug was added into duplicate 4 mL screw-capped vials which contained about 2 mL of the testing solvent. The vials were rotated end-over-end at a constant speed of 8 rpm on a Labquake® rotator (Barnstead International, Model # 415110, Dubuque, Iowa) at room temperature (25 ± 2°C) for 3 days, as a preliminary study indicated that 3 days are adequate to reach equilibrium. For sample vials with the target pH of 4.55, their pH was checked every day and buffers with the particular agent concentration were used to adjust the pH back to 4.55. Final pH was recorded at the end of the rotation.

The presence of suspended solid in the equilibrated samples was determined by the presence of a Tyndall beam produced by a laser pointer. The samples were filtered through 0.45 μm Acrodisc® syringe filters (Pall Gelman Laboratory, Ann Arbor, MI) and then diluted to the proper concentrations by 50% ACN in water before they were
analyzed by HPLC. All samples were prepared in duplicate and the solubility data were recorded as the average of the duplicates.

2.5. HPLC assay

HPLC (High Performance Liquid Chromatography) assays for both compounds were developed. An Agilent 1100 HPLC system was used (1100 autosampler, 1100 quaternary pump with degasser, 1100 thermostatted column compartment, and 1100 diode array detector) (Agilent, Palo Alto, CA). A Discovery® HS C18 column maintained at 26 °C was used as the stationary phase (150 x 4.6 mm, 3 µm, catalog#: 569252-U, Column#: 53309-06, bonded phase lot#: 4437, Silica lot#: 000710BO, Supelco, Milwaukee, WI). Acetonitrile and water with 0.1% TFA (trifloroacetic acid) at the ratio of 55 to 45 was used as the mobile phase for XK-469 and the same combination at the ratio of 35 to 65 was used for PPA. The flow rate was controlled at 1.0 mL/min. For XK-469, the effluent was monitored at 245 nm for 10 minutes and the drug retention time was 6.1±0.1 minute. For PPA, the effluent was monitored at 220 nm for 10 minutes and the drug retention time was 5.9±0.1 minute. The injection volume was 10 µl for both drugs. Both HPLC assays were validated for linearity and precision. For XK-469, the AUC (Area Under Curve) was linear with concentrations in the 0.03 to 338 µg/mL range with an R² at least 0.999. The relative intraday and interday standard deviations were 0.82% and 2.31%, respectively. For PPA, the AUC was linear with concentration range in the 0.4 to 242 µg/mL range with an R² at least 0.999. The relative intraday and
interday standard deviations were 0.47% and 1.91%, respectively. None of the solubilization agents interfered with either of the drug peaks.

3. Results and discussion

3.1. pH-solubility profile

Figure 2.2 exhibits the experimental pH solubility data (diamonds) of XK-469 and PPA, and the calculated lines, which are based on the Henderson-Hasselbach equation and the experimentally determined intrinsic solubilities, $S_u$, and $pK_a$ values:

$$S_w = S_u + S_i$$

$$= S_u \cdot (1 + 10^{(pH - pK_a)})$$

where $S_w = \text{total water solubility}$

$S_i = \text{ionized drug concentration}$

$S_u = \text{unionized drug solubility}$

XK-469 has an intrinsic solubility of 0.000274 mg/mL, while PPA has an intrinsic solubility of 2.82 mg/mL. It is known that the intrinsic solubility of a drug is determined by its polarity and crystallinity (Yalkowsky 1999). The polarity of a drug is commonly evaluated by the logarithm of its octanol-water partition coefficient (logP), while the crystallinity of a drug is usually evaluated by its melting point (MP). The calculated logP, ClogP (Biobyte® 4.0), for XK-469 and PPA are 3.85 and 1.88, respectively, and the measured MP for XK-469 and PPA are 184.5 °C and 116.0 °C, respectively. Evidently, the addition of chloro-quinoxalinyloxy to the phenoxy-propanoic acid not only
drastically reduced its polarity (with about a two unit increase in ClopP), but also improved the drug crystallinity to give it an increased melting point. Both effects contribute to an about 10,000-fold reduced intrinsic solubility of XK-469 compared to that of PPA. The physicochemical properties of XK-469 and PPA are listed in Table 2.1.

As shown in Figure 2.2, the experimental solubility of XK-469 follows the Henderson-Hasselbach equation quite well. Solubility is expected to keep increasing with increasing pH until it reaches the solubility product ($K_{sp}$) of the ionized XK-469 and the counter-ion from the buffer. The pH-solubility profile suggests that the solubility of PPA is approaching its solubility product ($K_{sp}$) of the ionized PPA and the counter-ion ($Na^+$) from the buffer when the pH is greater than 4.8.

![Figure 2.2. pH solubility profiles of XK-469 and PPA](image.png)
Table 2.1. Intrinsic solubility and physicochemical properties of XK-469 and PPA

<table>
<thead>
<tr>
<th></th>
<th>XK-469</th>
<th>PPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance:</td>
<td>white powder</td>
<td>off-white powder</td>
</tr>
<tr>
<td>MP (°C):</td>
<td>184.5</td>
<td>116</td>
</tr>
<tr>
<td>ClogP:</td>
<td>3.85</td>
<td>1.88</td>
</tr>
<tr>
<td>$S_u$ (mg/mL):</td>
<td>0.000274</td>
<td>2.82</td>
</tr>
<tr>
<td>pKa:</td>
<td>2.7</td>
<td>2.9</td>
</tr>
</tbody>
</table>

3.2. Cosolvency

Both XK-469 and PPA were studied by cosolvency at pH 1.0 and pH 4.55. In both cases, the solubilization curves in Figure 2.3 exhibit an exponential increase in drug solubility with respect to the cosolvent concentration. These curves can be described by the log-linear model, which was proposed by Yalkowsky et al. (1972), and Yalkowsky and Rubino (1985):

$$S_{tot} = S_w \cdot 10^{\sigma_w \cdot C_{cos}}$$

(2)

where $\sigma_w =$ solubilization power of cosolvent for drug

$C_{cos} =$ cosolvent concentration in percent

The solubilization power, $\sigma_w$, is the initial slope of logarithm solubility vs. cosolvent concentration. When the solubility is studied under the unionized condition, the $S_w$ and $\sigma_w$ can be expressed as $S_u$ and $\sigma_u$, respectively. The values of $\sigma_u$ for XK-469 and PPA by using EtOH and PG as the cosolvent are obtained from the solubilization curves at pH 1.0 (i.e. the bottom curves of Figure 2.3) and are listed in Table 2.2.
Applying cosolvency to an ionizable drug under the ionized condition can be a more efficient means to solubilize the drug. Li and coworkers quantitatively described the combined effect of the cosolvency and pH adjustment by breaking down the total dissolved drug into the dissolved unionized drug and ionized drug with the introduction of the concept of the $\sigma_i$ (Li et al., 1998, 1999a,b):

$$S_{tot} = S_w \cdot 10^{\sigma_{app} \cdot C_{cos}}$$

$$= S_u \cdot 10^{\sigma_u \cdot C_{cos}} + S_i \cdot 10^{\sigma_i \cdot C_{cos}}$$

(3)

where

- $S_w$ = drug water solubility
- $S_i$ = the water solubility of the ionized drug at a specific pH
- $\sigma_{app}$ = drug apparent cosolvent solubilization power
- $\sigma_i$ = ionized drug cosolvent solubilization power

Clearly, the value of $\sigma_i$ can be calculated from Equation 3 after obtaining the value of $\sigma_u$ from the unionized condition, and the values of $S_u$ and $S_i$ from the pH-solubility profile. In other words, the value of $\sigma_i$ is obtained by subtracting the solubilization curve under the unionized condition from the solubilization curve under the ionized condition. Although the value of $\sigma_{app}$ from a solubilization curve is dependent on the tested pH, the values of $\sigma_u$ and $\sigma_i$ are independent of the tested pH as they are the properties of the unionized and ionized drug species.

The values of $\sigma_i$ for XK-469 and PPA using EtOH and PG as the cosolvent were calculated from the difference between the top curves and the bottom curves of Figure 2.3 and are listed in Table 2.2. With the $S_u$, $\sigma_u$, and $\sigma_i$ values, and a $S_i$ value calculated
from $S_n$ and $pK_a$ by the Henderson-Hasselbach equation (Equation 1), the total solubility of an ionizable drug by a given cosolvent at any pH can be estimated using Equation 3.

It was found that the $\sigma_n$ for a given cosolvent is proportional to the drug’s polarity (Millard et al., 2002). Since XK-469 is less polar than PPA, its $\sigma_n$ value is greater than that of PPA, when either EtOH or PG was used. The $\sigma_n$ values of both XK-469 and PPA are in agreement with the dataset of Millard et al., as shown in Figure 2.4.

EtOH is more efficient in solubilizing both XK-469 and PPA because it is less polar than PG. It appears that PG has a similar or slightly greater polarity than the charged PPA, since it slightly desolubilizes the latter with the evidence of a negative $\sigma_i$ value.
Figure 2.3. Solubilization of XK-469 and PPA by cosolvency
Figure 2.4. The dependence of the solubilization power $\sigma_i$ of EtOH on drug polarity

Table 2.2. Solubilization powers of XK-469 and PPA using EtOH or PG as the cosolvent

<table>
<thead>
<tr>
<th></th>
<th>XK-469</th>
<th>PPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sigma_{\text{app}}$ (%$^{-1}$, pH 1.0)</td>
<td>0.043</td>
<td>0.016</td>
</tr>
<tr>
<td>$\sigma_{\text{app}}$ (%$^{-1}$, pH 4.55)</td>
<td>0.018</td>
<td>0.004</td>
</tr>
<tr>
<td>$\sigma_i$ (%$^{-1}$)</td>
<td>0.043</td>
<td>0.016</td>
</tr>
<tr>
<td>$\sigma_i$ (%$^{-1}$)</td>
<td>0.017</td>
<td>0.003</td>
</tr>
<tr>
<td>PG:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sigma_{\text{app}}$ (%$^{-1}$, pH 1.0)</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>$\sigma_{\text{app}}$ (%$^{-1}$, pH 4.55)</td>
<td>0.017</td>
<td>-0.0001</td>
</tr>
<tr>
<td>$\sigma_i$ (%$^{-1}$)</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>$\sigma_i$ (%$^{-1}$)</td>
<td>0.017</td>
<td>-0.0005</td>
</tr>
</tbody>
</table>
3.3. **Micellization**

This technique was studied at pHs 1.0 and 4.55 with Tween 80, the most extensively used nonionic surfactant in pharmaceuticals (Powell et al., 1998). The solubilization curve by micellization is usually linear after the surfactant critical micelle concentration, **CMC**. Many surfactants, including Tween 80, have very small **CMC** values and the effect of the **CMC** is usually not observed in the solubilization curves.

Conventionally, there are two descriptors to evaluate a surfactant. The solubilization capacity, \( \kappa \), is the slope of the solubilization curve. The unit of \( \kappa \) depends on the units of the drug solubility and surfactant concentration. Using this descriptor, the solubilization curve can be expressed by the following equation (Yalkowsky 1999):

\[
S_{\text{tot}} = S_w + \kappa \cdot (C_{\text{surf}} - C_{\text{CMC}}) = S_w + \kappa \cdot C_{\text{mic}} \approx S_w + \kappa \cdot C_{\text{surf}}
\]

where

- \( \kappa \) = surfactant solubilization capacity
- \( C_{\text{surf}} \) = surfactant concentration in percent (v/v)
- \( C_{\text{CMC}} \) = critical micellar concentration in percent (v/v)
- \( C_{\text{mic}} \) = micellar surfactant concentration in percent (v/v)

The micellar partition coefficient, \( K^m \), is the unitless ratio of drug concentration in the micelle phase to its concentration in water. This descriptor assumes that all of the drug in excess of the aqueous solubility is in the micelle phase:

\[
K^m = \frac{S_M}{S_w}
\]

In order to obtain a meaningful value of \( K^m \), the drug solubility in the water and in the micelle phase have to be in the same units. Because the micelles are distributed in the
aqueous phase, the drug solubility in the micelle phase can not be measured directly and has to be calculated by the following:

\[
S_M = \frac{A_{\text{mic}}}{V_{\text{mic}}}
\]  

(6)

where \(A_{\text{mic}}\) = the amount of drug in the micelle phase

\(V_{\text{mic}}\) = the volume of the micelle phase

The value of \(A_{\text{mic}}\) is equal to the drug concentration difference with and without the surfactant multiplied by the total volume of the solution. The value of \(V_{\text{mic}}\) is the volume of surfactant in the total volume of the solution. Drug solubility is most frequently expressed in mg/mL and surfactant concentration is often reported as percentage. Using these two units, the amount of drug in the micelle phase of one milliliter solution is \((S_{\text{tot}} - S_w)\) mg and the total volume of surfactant in 1 mL solution is \(C_{\text{surf}}/100\). Replacing \(A_{\text{mic}}\) and \(V_{\text{mic}}\) by \((S_{\text{tot}} - S_w)\) and \(C_{\text{surf}}/100\), respectively, the following equation is obtained for \(S_M\):

\[
S_M = \frac{100 \cdot (S_{\text{tot}} - S_w)}{C_{\text{surf}}}
\]  

(7)

Note, the value of \(\frac{(S_{\text{tot}} - S_w)}{C_{\text{surf}}}\) is the slope of the solubilization curve, \(k\). Thus, the above equation can be simplified to:

\[
S_M = 100 \cdot k
\]  

(8)

Inserting \(S_M\) into Equation 5, a relationship of \(K^m\) and \(k\) is found:
Rearrangement of Equation 9 results in Equation 10:

\[ \kappa = \frac{K_m \cdot S_w}{100} = 0.01 \cdot K^m \cdot S_w \]  

where \( \kappa \) is expressed as the product of \( K^m \) and \( S_w \) with a numerical value which is determined by the units of drug solubility and surfactant concentration.

If the CMC is small enough to be ignored, the drug solubility can be expressed in terms of the micellar partition coefficient by inserting Equation 10 into Equation 4:

\[ S_{tot} = S_w + 0.01 \cdot K^m \cdot S_w \cdot C_{surf} \]  

The \( K^m \) values of unionized XK-469 and PPA using Tween 80 were calculated from the solubilization curves in the bottom part of Figure 2.5 and are listed in Table 2.3. Since the definitions of the micellar partition coefficient and the octanol-water partition coefficient are similar, it is natural to correlate these two parameters (Collete and Koo 1975; Tomida et al., 1978; Alvarez-Nunez and Yalkowsky 2000). Figure 2.6 shows that the \( K^m \) values of both drugs are in excellent agreement with the trend of the data set used by Alvarez-Nunez and Yalkowsky.

The top part of Figure 2.5 shows that Tween 80 solubilizes more ionized drug than unionized drug. These solubilization curves are described by Equations 4 and 11.
with the water solubility at the tested pH and the apparent $k$ or $K^m$ values. The effect of the combination of pH adjustment and micellization can also be quantitatively evaluated by breaking down the total drug solubility into the unionized and ionized drug with the descriptors for both species (Li et al., 1999a,b; Jinno et al., 2000):

$$S_{tot} = S_u + \kappa_u \cdot C_{surf} + S_i + \kappa_i \cdot C_{surf}$$  

(12)

$$S_{tot} = S_u + 0.01 \cdot K^m \cdot S_u \cdot C_{surf} + S_i + 0.01 \cdot K^m \cdot S_i \cdot C_{surf}$$  

(13)

The descriptors for the ionized species of both drugs were obtained from the solubilization curves in the top part of Figure 2.5 and are listed in Table 2.3. Knowing the values of $K^m_u$ and $K^m_i$, the solubility of a drug by a given surfactant can be estimated at any pH.

The amphiphilic characteristics of these two drugs make micellization an efficient technique. The slopes ($k$) of the PPA solubilization curves by Tween 80 are much greater than those of XK-469, which indicates that Tween 80 solubilizes more PPA. However, the micellar partition coefficients of XK-469 are greater than those of PPA. The reason for this is that the slope is the product of the drug water solubility and the micellar partition coefficient. For a drug that has a high water solubility, such as PPA, this product can be large even if its micellar partition coefficient is not. The water solubility of unionized PPA is about 10,000-fold greater than that of XK-469. Although the micellar partition coefficient of PPA by Tween 80 is about 270-fold smaller than that of XK-469, its solubilization capacity by Tween 80 is still much greater.
Solubilization capacity measures how much drug can be solubilized by a surfactant, while micellar partition coefficient evaluates the efficiency of the micelles to extract drug from the water phase. Once the drug water solubility is known, the two descriptors can be calculated from each other. As $K_m$ is directly related to a drug’s polarity, the charged PPA is too polar to have a measurable $K_m$ value, which means that the charged PPA does not partition into the micelles and the combination of pH adjustment and micellization does not have a synergistic solubilizing effect.

Figure 2.5. Solubilization of XK-469 and PPA by micellization
Figure 2.6. The dependence of the micellar partition coefficient of Tween 80 on drug polarity

Table 2.3. Solubilization descriptors of XK-469 and PPA using Tween 80

<table>
<thead>
<tr>
<th></th>
<th>XK-469</th>
<th>PPA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$K_{app}$ at pH 1.0 (mg/mL vs. %)</strong></td>
<td>0.026</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>$K_{app}$ at pH 4.55 (mg/mL vs. %)</strong></td>
<td>0.098</td>
<td>0.94</td>
</tr>
<tr>
<td><strong>$K_u^m$</strong></td>
<td>9489</td>
<td>35</td>
</tr>
<tr>
<td><strong>$K_i^m$</strong></td>
<td>171</td>
<td>$\approx$0</td>
</tr>
</tbody>
</table>

3.4. Complexation

XK-469 and PPA were solubilized by two FDA approved complexation agents, HPβCD and SBEβCD. The experimental data are plotted in Figure 2.7. These agents solubilize a drug by the formation of non-covalent inclusion complexes. In most cases,
only the 1:1 complex is formed. The binding constant of a 1:1 complex can be expressed in the following equation:

\[
K_{1:1} = \frac{SL}{S_w \cdot L}
\]  

(14)

where \(SL\) = complex concentration

\(L\) = free ligand concentration at equilibrium

This equation can be rearranged to calculate the concentration of the complex. However, free ligand concentration is hard to measure. Because the ligand in the solution exists as either free ligand or complex, \(L\) can be replaced by the total ligand concentration, \(L_{tot}\), subtracting the complex concentration, \(SL\). After a rearrangement, the following equation is obtained:

\[
SL = \frac{K_{1:1} \cdot S_w}{1 + K_{1:1} \cdot S_w} \cdot L_{tot}
\]  

(15)

The measured total drug concentration is the summation of the free drug concentration and the concentration of the complex, i.e.

\[
S_{tot} = S_w + SL
\]

\[
= S_w + \frac{K_{1:1} \cdot S_w}{1 + K_{1:1} \cdot S_w} \cdot L_{tot}
\]  

(16)

Since \(K_{1:1} \cdot S_w \ll 1\) for most poorly water-soluble drugs, Equation 16 can be approximated by:

\[
S_{tot} \approx S_w + K_{1:1} \cdot S_w \cdot L_{tot}
\]  

(17)
This type of solubilization curve is linear with a slope of $K_{1:1} \cdot S_w$. This slope is referred to as the solubilization capacity, $\tau$, which is analogous to $k$ in the micellization technique. The solubilization curve expressed in $\tau$ is as the following:

$$S_{tot} = S_w + \tau \cdot L_{tot}$$  \hspace{1cm} (18)

Sometimes a higher order of complex can form and the solubilization curve could be concave up, as in the case of the solubilization of XK-469 by HPβCD at pH 1.0 (Figure 2.7). When this happens, the measured total drug solubility is a summation of the free drug and all of the complexes, while the free ligand concentration is equal to the total ligand concentration subtracting the sum of the concentrations of all complexes. The solubilization curve of XK-469 by HPβCD at pH 1.0 fits in a 2-order polynomial, which indicates that both 1:1 and 1:2 complexes are formed. Using both 1:1 and 1:2 binding constants and adding all of the dissolved drug forms together, the total drug solubility can be expressed by the following approximate equation:

$$S_{tot} = S_w + SL + SL_2$$
$$\approx S_w + K_{1:1} \cdot S_w \cdot L_{tot} + K_{1:2} \cdot S_w \cdot L_{tot}^2$$  \hspace{1cm} (19)

The synergistic effect on solubilization of an ionizable compound by the combination of pH adjustment and complexation is exhibited in the solubilization curves of XK-469 by HPβCD and SBEβCD, and the solubilization curve of PPA by HPβCD (Figure 2.7). These three solubilization curves can be described by Equations 17 and 18 with $S_w$ at the tested pH and the apparent $K_{1:1}^{'}$ or $\tau$. 
As in the other two techniques, the total drug solubility by a combination of pH adjustment and complexation can be quantitatively evaluated by breaking down the total dissolved drug to the dissolved unionized drug (free unionized drug and unionized drug complexes) and the dissolved ionized drug (free ionized drug and the ionized drug complexes) (Tinwalla et al., 1993; Johnson et al., 1994; Okimoto et al., 1996; Li et al., 1998). In the simplest and the most common case, which only has 1:1 complexes formed for both unionized and ionized drugs, the solubilization curve is linear and the total drug solubility can be expressed by the following equation:

\[ S_{tot} = S_u + K_u^{1:1} \cdot S_u \cdot C_L + S_i + K_i^{1:1} \cdot S_i \cdot C_L \]  \hspace{1cm} (20)

The binding constants for both unionized and ionized drug species from Equations 17, 19 and 20, and the apparent slopes (solubilization capacities) from Equation 18 were calculated and are listed in Table 2.4. Again, the drug solubility at any given pH can be estimated with the values of \( K_u \) and \( K_i \).

The relationship between the solubilization capacity and the binding constant in complexation is analogous to the solubilization capacity and the micellar partition coefficient in micellization. For the same reason, the solubilization capacities of cyclodextrins are greater for PPA, even though the binding constants are much greater for XK-469.

The binding constants in Table 2.4 indicate that SBE\( \beta \)CD has a higher affinity than HP\( \beta \)CD with both unionized drugs. However, this is not true for the charged XK-469 and PPA. This turnaround can be explained by the fact that the negative charge on the sulphate groups of SBE\( \beta \)CD can repel the negative charged compound (Zia et al.,
Furthermore, the polarity of the drug influences its affinity for a complexation ligand. The affinity of the charged PPA for both complexing agents are negligible. Although binding constant was observed to be strongly related to the polarity of the compound in a homologous series, the relationship is often different from one series to another (Matsui and Mochida 1979; Uekama et al., 1980; and Uekama 1981). This difference might lie on the fact that homologous series have the similar structural properties which allow them to fit similarly in the ligand cavity, while polarity is the driving force for a compound to be squeezed out of water.

The solubilization curve of PPA by SBEβCD at pH 4.55 has a slight negative slope. This might due to the fact that having SBEβCD in the solution reduces the water concentration, i.e., in a 20% SBEβCD solution, the water concentration is reduced to about 80%. Because the affinity of PPA with SBEβCD is very small, the increased amount of drug as complex can not overcome the loss of drug as the result of the reduced amount of water.
Figure 2.7. Solubilization of XK-469 and PPA by complexation
Table 2.4. Solubilization parameters of XK-469 and PPA using HPβCD and SBEβCD

<table>
<thead>
<tr>
<th></th>
<th>XK-469</th>
<th>PPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPβCD:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\tau_{app}$ at pH 1.0 (mg/mL vs.%)</td>
<td>N/A</td>
<td>0.87</td>
</tr>
<tr>
<td>$\tau_{app}$ at pH 4.55 (mg/mL vs.%)</td>
<td>0.11</td>
<td>0.73</td>
</tr>
<tr>
<td>$K_u^{1:1}$ (M$^{-1}$)</td>
<td>1886</td>
<td>157</td>
</tr>
<tr>
<td>$K_u^{1:2}$ (M$^{-1}$)</td>
<td>7.2</td>
<td>N/A</td>
</tr>
<tr>
<td>$K_i^{1:1}$ (M$^{-1}$)</td>
<td>2078</td>
<td>≈0</td>
</tr>
<tr>
<td>SBEβCD:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\tau_{app}$ at pH 1.0 (mg/mL vs.%)</td>
<td>0.007</td>
<td>0.62</td>
</tr>
<tr>
<td>$\tau_{app}$ at pH 4.55 (mg/mL vs.%)</td>
<td>0.098</td>
<td>-0.09</td>
</tr>
<tr>
<td>$K_u^{1:1}$ (M$^{-1}$)</td>
<td>5543</td>
<td>245</td>
</tr>
<tr>
<td>$K_i^{1:1}$ (M$^{-1}$)</td>
<td>1082</td>
<td>≈0</td>
</tr>
</tbody>
</table>

3.5. Selection of a technique

The solubility data of XK-469 and PPA in 20% of each excipient at pH 1.0 and 4.55 are listed in Table 2.5. In order to compare the efficiency of each technique, the ratios of the observed solubility to the intrinsic solubility of the drug are also listed. All of the applied techniques are much less efficient on PPA than they are on XK-469. This is because all of these techniques are more or less related to a compound’s polarity and XK-469 is much less polar than PPA.

Cosolvency increases drug solubility exponentially. It is generally not very efficient at relatively low concentration. At a concentration of 20%, both EtOH and PG
are much less efficient than other excipients. Under the unionized condition, 20% Tween 80 is the best technique for both drugs. However, at pH 4.55, which is about 2 units above the pKa, 20% HPβCD can dissolve about four times more XK-469 than 20% Tween 80 does. If the buffer is adjusted to an even higher pH, a greater effect of HPβCD is expected for the solubilization of XK-469.

Table 2.5. Solubilities of XK-469 and PPA and the ratios of total solubility to the intrinsic solubility

<table>
<thead>
<tr>
<th>pH 1.0</th>
<th>Solubility (mg/mL)</th>
<th>Ratio (S/S₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>XK-469</td>
<td>PPA</td>
</tr>
<tr>
<td>Water (S₀):</td>
<td>0.000274</td>
<td>2.82</td>
</tr>
<tr>
<td>20% EtOH:</td>
<td>0.00198</td>
<td>5.89</td>
</tr>
<tr>
<td>20% PG:</td>
<td>0.00109</td>
<td>4.47</td>
</tr>
<tr>
<td>20% Tween 80:</td>
<td>0.520</td>
<td>22.6</td>
</tr>
<tr>
<td>20% HPβCD:</td>
<td>0.153</td>
<td>20.2</td>
</tr>
<tr>
<td>20% SBEβCD:</td>
<td>0.153</td>
<td>15.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH 4.55</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water:</td>
<td>0.0195</td>
<td>80.8</td>
<td>71</td>
<td>29</td>
</tr>
<tr>
<td>20% EtOH:</td>
<td>0.0441</td>
<td>95.4</td>
<td>161</td>
<td>34</td>
</tr>
<tr>
<td>20% PG:</td>
<td>0.0425</td>
<td>80.6</td>
<td>155</td>
<td>29</td>
</tr>
<tr>
<td>20% Tween 80:</td>
<td>1.20</td>
<td>99.6</td>
<td>4378</td>
<td>35</td>
</tr>
<tr>
<td>20% HPβCD:</td>
<td>5.85</td>
<td>95.4</td>
<td>21355</td>
<td>34</td>
</tr>
<tr>
<td>20% SBEβCD:</td>
<td>2.09</td>
<td>79.0</td>
<td>7628</td>
<td>28</td>
</tr>
</tbody>
</table>

4. Conclusions

The efficiency of a solubilization technique is directly related to the physicochemical properties of a compound. The effectiveness of cosolvency and micellization is dependent on drug polarity. The less polar a drug is, the more effectively
it is solubilized. This is also true in complexation for those compounds that have similar fit into the ligand cavity.

Of the two drugs, XK-469 is much less polar than PPA. Even though it has an intrinsic solubility about 10,000-fold lower than that of PPA, its solubility is much more altered by the common techniques.

Both of the drugs are weak acids. An ionizable compound can be simply formulated by pH adjustment. If pH adjustment itself is not enough to solubilize a compound to a desired concentration, a combination of pH adjustment with cosolvency, micellization, or complexation might be applied. The synergistic effect of pH adjustment and these techniques can lead to a very efficient drug solubilization.
CHAPTER 3. SOLUBILIZATION TECHNIQUE SELECTION

1. Introduction

Two structurally related anticancer drugs were studied in Chapter 2 by the most commonly used solubilization techniques: pH adjustment, cosolvency, micellization, complexation, and pH adjustment combined with one of the other techniques. So far, twelve drugs have been thoroughly studied by these techniques in this laboratory, which are AMPB, BPU, carbendazim, cyclosporin A, estrone, flavopiridol, fluasterone, griseofulvin, naproxen, PG 300995, PPA, and XK-469 (Ran et al., 2001; Kan 2003; Zhao et al., 1999; Ni et al., 2002; He et al., 2005; Ran et al., 2005; Peterson 2001; Li et al., 1998; Li et al., 1999; El-Sayed et al., 2000; Jain et al., 2001). Since they were studied under similar conditions, these drugs form an ideal dataset to evaluate the overall effectiveness of each technique on drugs which have different physicochemical properties. The aim of this chapter is to further understand each solubilization technique and to be able to estimate its efficiency in solubilization of a compound based on its dose requirement, water solubility, structure, and the physicochemical properties.
2. Background

2.1. pH adjustment

Adjustment of pH only applies to drugs which have an ionizable group. After a drug is ionized, it is more hydrophilic. Consequently, the solubility of a weak acid is higher when it is in an aqueous medium which has the pH greater than its pKa. The total solubility of an acidic drug is described by the Henderson-Hasselbach equation:

$$S_w = S_u + S_i$$
$$= S_u \cdot (1 + 10^{(pH - pK_a)})$$

where 
$$S_w = \text{total water solubility}$$
$$S_i = \text{ionized drug concentration}$$
$$S_u = \text{unionized drug solubility}$$

The bigger the difference between the pH and pKa, the more drug dissociates, which leads to a greater solubility.

Similarly, a basic drug dissociates in an aqueous medium where the pH is lower than its pKa. The solubility of a basic drug at a given pH can be calculated with the flipping of the pH and pKa in the exponential term of Equation 1, i.e.:

$$S_w = S_u \cdot (1 + 10^{(pK_a - pH)})$$

Other approaches may have to be explored when a drug does not have an ionizable site or its total solubility is not high enough in the acceptable pH range.
2.2. Cosolvency

Cosolvency is the use of a water miscible organic compound to increase drug solubility in an aqueous medium. A cosolvent reduces the overall polarity of the aqueous medium as it reduces water hydrogen bond density (Yalkowsky 1999). The solubility of a poorly water-soluble drug usually exhibits an exponential dependence on the cosolvent concentration. This relationship is described by the following equation (Yalkowsky et al., 1972; Yalkowsky and Rubino 1985):

\[ S_{tot} = S_w \cdot 10^{\sigma_w \cdot C_{cos}} \]  

(3)

where \( \sigma_w \) = solubilization power of cosolvent for drug

\( C_{cos} \) = cosolvent concentration

The solubilization power, \( \sigma_w \), is the initial slope of a log solubility vs. cosolvent concentration plot. With the values of \( S_w \) and \( \sigma_w \), the drug solubility in an aqueous solution at a given cosolvent concentration, or the concentration of cosolvent required to obtain a given drug concentration, can be calculated. This approach is based on the relative polarity difference of the drug and the cosolvent. For any specific drug, the least polar cosolvent usually has the best solubilization power. Also, a given cosolvent usually provides the highest \( \sigma_w \) value for the most nonpolar drug.

2.3. Micellization

Surfactant increases the solubility of a solute by incorporation of the solute molecules into micelles. A general linear solubilization curve is observed for this
technique. The drug solubility is constant at all surfactant concentrations below the critical micellar concentration, \textit{CMC}, and increases linearly with surfactant concentration above the \textit{CMC}. Most surfactants in the pharmaceutical industry have very small \textit{CMC} values. Therefore, the \textit{CMC} effect is usually not observed in the solubilization curves and the micellar surfactant concentration can be approximated by the total surfactant concentration. The solubilization curve can be described by the following equation (Yalkowsky 1999):

\[ S_{tot} = S_w + \kappa \cdot (C_{surf} - CMC) \]
\[ = S_w + \kappa \cdot C_{mic} \]
\[ \approx S_w + \kappa \cdot C_{surf} \]

where \( \kappa \) = surfactant solubilization capacity
\( C_{surf} \) = surfactant concentration
\( CMC \) = critical micellar concentration
\( C_{mic} \) = micellar surfactant concentration

The descriptor, \( \kappa \), is the surfactant solubilization capacity, which is the slope of the solubilization curve. It measures the ability of a surfactant to solubilize a drug. This technique is also commonly evaluated by \( K^m \), the micellar partition coefficient, which measures the ability of micelles to extract drug from water. In another words, \( K^m \) measures the affinity of the solute to the micelles. While the unit of \( \kappa \) is dependent on the units of the drug solubility and the surfactant concentration, \( K^m \) is a unitless number.
as it is defined to be the ratio of the drug concentration in the micelle phase to its concentration in the aqueous phase:

\[ K^m = \frac{S_M}{S_w} \]  

(5)

In order to obtain a meaningful value of \( K^m \), the drug solubility in the aqueous and micelle phase have to be in the same units. The following relationship of \( K^m \) and \( \kappa \) was derived in Chapter 2 for drug solubility in units of mg/mL and the surfactant concentration as percentage:

\[ \kappa = 0.01 \cdot K^m \cdot S_w \]  

(6)

Note, the numerical factor in the above equation is determined by the units of drug solubility and surfactant concentration. When the CMC is small enough to be ignored, the drug solubility can be expressed in terms of the micellar partition coefficient by inserting Equation 6 into Equation 4:

\[ S_{tot} = S_w + 0.01 \cdot K^m \cdot S_w \cdot C_{surf} \]  

(7)

2.4. Complexation

The most widely applied complexation technique in pharmaceutics is inclusion complexation, which is based on the noncovalent interaction of the nonpolar region of drug with the nonpolar region of the complexation ligand cavity. This inclusion thermodynamically increases the solution stability as it minimizes contact of both nonpolar regions with water. The solubilization curve of a drug by a complexation agent is usually linear. The slope of the solubilization curve is again called the solubilization
capacity, denoted as $\tau$. The total drug solubility with the respect of the ligand concentration, $C_L$, can be expressed as:

$$S_{\text{tot}} = S_w + \tau \cdot C_L$$  \hspace{1cm} (8)$$

Again, the slope of the solubilization curve, $\tau$, evaluates the ability of a ligand to solubilize a drug. Alternatively, the drug-ligand binding constant, $K$, can be used to evaluate the affinity between the drug and the ligand because the complex is in equilibrium with both the free ligand and the free drug in the aqueous medium. In the solution of a poorly water-soluble drug, most ligand remains as the free ligand. Thus, the free ligand concentration can be approximated as the ligand concentration (Li et al., 1998). In most cases, only a 1:1 complex is formed, the solubilization curve can be expressed by the following equation:

$$S_{\text{tot}} = S_w + K^{1:1} \cdot S_w \cdot C_{L,\text{free}}$$
$$\approx S_w + K^{1:1} \cdot S_w \cdot C_L$$  \hspace{1cm} (9)$$

Comparing Equations 8 and 9, the value of $K^{1:1}$ can be calculated from the solubilization curve slope divided by the drug water solubility:

$$K^{1:1} = \frac{\tau}{S_w}$$  \hspace{1cm} (10)$$

Note that a unit conversion factor is needed in this equation if the drug concentration and the ligand concentration are not expressed with the same unit in the solubilization curve.
For thermodynamic convenience, it is traditional to calculate a binding constant with drug and ligand in molar concentrations. Therefore, $K^{1:1}$ usually has units of M$^{-1}$.

Sometimes a higher order complex can form and the solubilization curve could appear as concave up. When this happens, the measured total drug solubility is a summation of the concentrations of free drug and all of the complexes. If the solubilization curve happens to fit in a 2-order polynomial, it indicates that both 1:1 and 1:2 complexes are formed. The solubilization curve can be expressed by the following equation with $K^{1:1}$ and $K^{1:2}$:

$$S_{tot} = S_w + DL + DL_2$$

$$\approx S_w + K^{1:1} \cdot S_w \cdot L_{tot} + K^{1:1} \cdot K^{1:2} \cdot S_w \cdot L_{tot}^2$$

where $DL = $ concentration of the 1:1 complex

$DL_2 = $ concentration of the 1:2 complex

2.5. Combined pH adjustment with cosolvency, micellization, or complexation

Applying multiple techniques can be advantageous for drugs that can not be optimally solubilized by a single technique. It also enables the use of a smaller amount of any single excipient to minimize side effects (He et al., 2003). The most efficient and the least costly combinations utilize pH adjustment and one of the other three methods. Combinations among cosolvency, micellization, and complexation are not usually very efficient for the solubilization of a drug due to the potential of the competitions between the drug and the various solubilizing components.
Equations 3-11 are applied to cosolvency, micellization, or complexation with or without pH adjustment. When the solubilization is studied in a medium where the drug is not dissociated, i.e. the solution pH is lower than the pKa of an acidic drug or higher than the pKa of a basic drug, $S_w$ in these equations can be replaced by $S_u$ and the obtained descriptors are for the unionized drug species. On the other hand, when the solution pH is higher than the pKa of an acidic drug or lower than the pKa of a basic drug, $S_w$ in these equations is the total dissolved drug, which is $S_u + S_i$, and the descriptors are called apparent descriptors because their values depend on the solution pH.

In the following section, these techniques are quantitatively characterized by descriptors for unionized and ionized drug species. Since the values of these descriptors are independent of solution pH, the drug solubility at any pH and excipient concentration can be estimated.

2.5.a. Combination of pH adjustment and cosolvency

When a cosolvent is used to solubilize an ionizable drug, the apparent total solubility is the summation of the concentrations of the unionized and ionized drug that is solubilized (Li et al., 1998; Li et al., 1999a,b):

$$S_{tot} = S_u \cdot 10^{\sigma_u \cdot C_{cos}} + S_i \cdot 10^{\sigma_i \cdot C_{cos}}$$ (12)

where $\sigma_u =$ unionized drug cosolvent solubilization power

$\sigma_i =$ ionized drug cosolvent solubilization power
The polarity of any drug is increased when it is ionized. Consequently, the solubilization power of a cosolvent for an ionized drug species, \( \sigma_i \), is invariably less than that on a unionized drug species, \( \sigma_u \). However, this combination can still be beneficial in solubilization of a compound which has a positive \( \sigma_i \), because the cosolvent increases the solubility of both the unionized and ionized drug species. In fact, as the concentration of \( S_i \) can be much greater than \( S_u \), cosolvent can solubilize the ionized drug species much more than it does for the unionized drug species.

2.5.b. Combination of pH adjustment and micellization

The solubilization curve with the descriptors for both unionized and ionized species can be described by (Li et al., 1999a,b; Jinno et al., 2000):

\[
S_{tot} = S_u + \kappa_u \cdot C_{surf} + S_i + \kappa_i \cdot C_{surf} \quad (13)
\]

\[
S_{tot} = S_u + K_{i}^{m} \cdot S_u \cdot C_{surf} + S_i + K_{i}^{m} \cdot S_i \cdot C_{surf} \quad (14)
\]

Micelles in aqueous medium dissolve ionized drug in the same manner as they dissolve unionized drug. The solubilization capacity of the ionized drug species, \( \kappa_i \), is dependent on \( S_i \), which is dependent on solution pH. On the other hand, the micellar partition coefficient for the ionized species, \( K_{i}^{m} \), is an intrinsic descriptor for the ionized drug species as it is pH independent. The value of \( K_{i}^{m} \) will likely be smaller than that of the unionized species, \( K_{u}^{m} \). However, because \( S_i \) can be much greater than \( S_u \), the total solubilized ionized species can be much greater than that of the unionized species. In any
case, drug solubility at any pH and the surfactant concentration can be estimated using

\( K_u^m \) and \( K_i^m \).

### 2.5.c. Combination of pH adjustment and complexation

Like a micelle, a complexation ligand can increase the concentrations of both unionized and ionized drugs as described by the following equations (Tinwalla et al., 1993; Johnson et al., 1994; Okimoto et al., 1996; Li et al., 1998):

\[
S_{tot} = S_u + \tau_u \cdot C_L + S_i + \tau_i \cdot C_L \tag{15}
\]

\[
S_{tot} = S_u + K_u^{1:1} \cdot S_u \cdot C_L + S_i + K_i^{1:1} \cdot S_i \cdot C_L \tag{16}
\]

As in the micellization technique, while the solubilization capacity of the ionized drug species, \( \tau \), is dependent on solution pH, the binding constant of the ionized drug with the ligand, \( K_i^{1:1} \), is independent of pH and the total solubilized ionized species can be much greater than that of the unionized drug species. A drug solubility can be estimated at any pH after obtaining \( K_u^{1:1} \) and \( K_i^{1:1} \).
3. Materials and methods

3.1. Materials

The following twelve drugs were used in this study: flavopiridol, AMPB, XK-469, and BPU (NCI, Rockville, MD); carbendazim and PG 300995 (Procter & Gamble Company, Cincinnati, OH); fluasterone (Aeson Therapeutics Inc., Tucson, AZ); cyclosporin A (Institute of Microbiology, Fujian, China); griseofulvin, PPA, naproxen, and estrone (Sigma, Milwaukee, WI).

The following five solubilizing agents were used. EtOH (ethanol), PG (propylene glycol), and Tween 80 (polysorbate 80) (Sigma, Milwaukee, WI); HPβCD (hydroxypropyl-β-cyclodextrin, Trappsol®) (Cyclodextrin Technologies Development Inc., Gainesville, FL); SBEβCD (sulfobutyl ether-β-cyclodextrin, Captisol®) (CyDex, Inc., Lenexa, KS). HPβCD has an average molecular weight of 1390 and an average degree of substitution of 4.4. SBEβCD has an average molecular weight of 2160 and an average degree of substitution of 7.

All other chemicals were of reagent or HPLC grade and purchased from Aldrich (Milwaukee, WI). All chemicals were used as received without further purification and the water was double-deionized.

3.2. Methods

The solubilities of the above mentioned drugs were measured in aqueous solutions, containing various concentrations of cosolvent, surfactant, or complexation
ligand. The pH was controlled for ionizable drugs. The experimental details are described in the original papers.

4. Results and discussion

4.1. Solubilization profiles

The solubilization profiles of the twelve drugs are plotted in Figures 3.1-3.20. The first four profiles are for the nonelectrolyte drugs. They were studied without pH adjustment and are sorted according to their logP. The rest of the profiles are for the ionizable drugs under both uncharged and charged conditions and also are sorted according to the logP of the unionized form. The X-axis gives the percent solubilizing agent, while the primary Y-axis describes solubility of the drug normalized to its intrinsic solubility. For conventionality, the secondary Y-axis shows the drug concentrations in mg/mL. The experimental data are plotted as symbols, while the trends of applied techniques are illustrated by lines. The solubilization curves by EtOH and PG are shown as dotted lines with the experimental data in open and filled circles, respectively. The solubilization curve with Tween 80 is a broken line with the experimental data in open triangles. The solubilization curves of HPβCD and SBEβCD are solid lines with the experimental data in open and filled rectangles, respectively. The liquid solubilizing agents are expressed in percent as volume to volume, while the solid solubilizing agents are given as percent in weight to volume. In order to facilitate comparison of the various solubilizing agents, the data are extrapolated to 40% of the excipient.
Griseofulvin

logP: 2.2  
MP (°C): 220  
S_u (mg/mL): 0.0086

C_{17}H_{17}ClO_6  
CAS# 126-07-8

Figure 3.1. Solubilization profile of griseofulvin. EtOH (○), PG (●), Tween 80 (△), HPβCD (□), and SBEβCD (■)
Cyclosporine A

logP: 3.4
MP (°C): 153
S_u (mg/mL): 0.0028

C_{62}H_{111}N_{11}O_{12}
CAS# 59865-13-3

Figure 3.2. Solubilization profile of cyclosporine A. EtOH (○), PG (●), Tween 80 (▲), HPβCD (□), and SBEβCD (■)
Estrone

logP: 3.7
MP (°C): 265
$S_u$ (mg/mL): 0.0006

Figure 3.3. Solubilization profile of estrone. EtOH (○), PG (●), Tween 80 (Δ), HPβCD (□), and SBEβCD (■)
Fluasterone

\[
\begin{align*}
\text{logP:} & \quad 5.0 \\
\text{MP (°C):} & \quad 206 \\
\text{S_u (mg/mL):} & \quad 0.00005
\end{align*}
\]

\[\text{C}_{19}\text{H}_{27}\text{FO}\]

\[\text{CAS}\# 112859-71-9\]

Figure 3.4. Solubilization profile of fluasterone. EtOH (○), PG (●), Tween 80 (Δ), HPβCD (□), and SBEβCD (■)
Carbendazim

logP: 1.5  \hspace{1cm} \text{C}_9\text{H}_9\text{N}_3\text{O}_2
MP (°C): 330  \hspace{1cm} \text{CAS#} 10605-21-7
pKa (basic): 4.5
S_u (mg/mL): 0.0064

\[\text{pH 7.0}\]

Figure 3.5. Solubilization profile of unionized carbendazim. EtOH (○), PG (●), Tween 80 (Δ), HPβCD (□), and SBEβCD (■)
Carbendazim

pKa (basic): 4.5  
$S_w$ (mg/mL): 1.33

![Chemical structure of Carbendazim](image)

Figure 3.6. Solubilization profile of carbendazim by combinations of pH adjustment with EtOH (○), PG (●), Tween 80 (△), HPβCD (□), and SBEβCD (■)
PPA

logP:  1.7  
MP (°C):  116  
pKa (acidic):  2.9  
$S_u$ (mg/mL):  2.82  

C$_{10}$H$_{10}$O$_3$  
CAS# 940-31-8  
NSC# 404102

Figure 3.7. Solubilization profile of unionized PPA. EtOH (○), PG (●), Tween 80 (△), HPβCD (□), and SBEβCD (■)
PPA

pKa (acidic): 2.9
$S_w$ (mg/mL): 80.82

Figure 3.8. Solubilization profile of PPA by combinations of pH adjustment with EtOH (○), PG (●), Tween 80 (△), HPβCD (□), and SBEβCD (■)
Flavopiridol

logP: 1.9  
MP (°C): 21 
\( pK_a \) (basic): 5.7  
\( S_u \) (mg/mL): 0.022

\[
\begin{align*}
\text{CAS}\# &\quad 146226-40-6 \\
\text{NSC}\# &\quad 649890
\end{align*}
\]

\[
\begin{align*}
\text{C}_{21}\text{H}_{20}\text{ClNO}_5
\end{align*}
\]

Figure 3.9. Solubilization profile of unionized flavopiridol. EtOH ( ), PG ( ), Tween 80 (Δ), HPβCD (□), and SBEβCD (■)
Flavopiridol

pKa (basic): 5.7
Sw (mg/mL): 0.60

![Flavopiridol Chemical Structure]

Figure 3.10. Solubilization profile of flavopiridol by combinations of pH adjustment with EtOH (○), PG (●), Tween 80 (Δ), HPβCD (□), and SBEβCD (■)
PG300995

\[
\text{log P: 2.2} \quad \text{C}_{10}\text{H}_{7}\text{N}_3\text{S} \\
\text{MP (°C): 273} \quad \text{CAS# 1204-64-4} \\
\text{pK_a (basic): 4.2} \\
\text{S_u (mg/mL): 0.036}
\]

Figure 3.11. Solubilization profile of unionized PG300995. EtOH (○), PG (■), Tween 80 (△), HPβCD (□), and SBEβCD (●)
PG300995

pKa (basic): 4.2
$S_w$ (mg/mL): 1.75

Figure 3.12. Solubilization profile of PG300995 by combinations of pH adjustment with EtOH (○), PG (●), Tween 80 (Δ), HPβCD (□), and SBEβCD (■)
Naproxen

logP: 3.0
MP (°C): 153
pKa (basic): 4.4
$S_u$ (mg/mL): 0.0091

$\text{C}_4\text{H}_7\text{O}_3$

CAS# 22204-53-1

Figure 3.13. Solubilization profile of unionized Naproxen. EtOH (○), PG (●), Tween 80 (△), HPβCD (□), and SBEβCD (■)
Naproxen

pKa (basic): 4.4
$S_w$ (mg/mL): 6.64

![Chemical structure of Naproxen](image)

Figure 3.14. Solubilization profile of naproxen by combinations of pH adjustment with EtOH (○), PG (●), Tween 80 (△), HPβCD (□), and SBEβCD (■).
AMPB

\[
\text{logP: 4.0} \quad \text{C}_{14}\text{H}_{12}\text{N}_{2}\text{S}
\]

\[
\text{MP (°C): 196} \quad \text{CAS# 178804-04-1}
\]

\[
\text{pKa (basic): 2.8} \quad \text{NSC# 674495}
\]

\[
\text{S_u (mg/mL): 0.0012}
\]

\[
\text{pH 8.0}
\]

---

Figure 3.15. Solubilization profile of unionized AMPB. EtOH (○), PG (●), Tween 80 (Δ), HPβCD (□), and SBEβCD (■).
AMPB

pKa (basic): 2.8
$S_w$ (mg/mL): 0.0439

Figure 3.16. Solubilization profile of AMPB by combinations of pH adjustment with EtOH (○), PG (●), Tween 80 (Δ), HPβCD (□), and SBEβCD (■).
XK-469

logP: 4.1  
MP (°C): 185  
pKa (acidic): 2.7  
S_u (mg/mL): 0.000274

C_{17}H_{13}ClN_{2}O_{4}  
CAS# 157435-10-4  
NSC# 697887

Figure 3.17. Solubilization profile of unionized XK-469. EtOH (○), PG (●), Tween 80 (△), HPβCD (□), and SBEβCD (■)
XK-469

\[
pK_a (\text{acidic}): \quad 2.7
\]
\[
S_w (\text{mg/mL}): \quad 0.0195
\]

Figure 3.18. Solubilization profile of XK-469 by combinations of pH adjustment with EtOH (○), PG (●), Tween 80 (△), HPβCD (□), and SBEβCD (■)
BPU

$\text{logP: } 4.3$
$\text{MP (°C): } 130$
$\text{pK}_a \text{ (basic): } 5.0$
$S_u \text{ (mg/mL): } 0.00003$

$\text{C}_{22}\text{H}_{24}\text{BrN}_5\text{O}_3$
$\text{CAS# } 134742-19-1$
$\text{NSC# } 639829$

Figure 3.19. Solubilization profile of unionized BPU. EthOH (♂), PG (●), Tween 80 (Δ), HPβCD (□), and SBEβCD (■)
BPU

pKa (basic): 5.0
$S_w$ (mg/mL): 0.21

Figure 3.20. Solubilization profile of BPU by combinations of pH adjustment with EtOH (∗), PG (●), Tween 80 (∆), HPβCD (□), and SBEβCD (■)
In these profiles, cosolvency (circles with dotted lines) produces the least solubilization at low concentration, but can be the most effective approach at high concentration as in the solubilization of the unionized PG300995 and flavopiridol. Furthermore, because EtOH is less polar than PG, it is more effective than PG in solubilizing all studied drugs. The concentration at which cosolvency becomes the most effective depends largely upon the drug molecule. For example, EtOH solubilizes flavopiridol better than any other agent above 33%, while ETOH and PG are the least effective solubilizing agents for most drugs studied below 40%. However, cosolvents can still be efficient as they are usually accepted at relatively high concentrations in parenteral formulations. For example, the well accepted vehicle of 10% EtOH and 40% PG can be more efficient than the other methods. Furthermore, because cosolvents increase drug solubility exponentially, they can be especially useful in formulations for external use.

It is clear from Figures 3.1 to 3.20, that the solubilization capacities of the two complexation agents, HPβCD and SBEβCD (solid lines), rarely differ by more than a factor of 2 for nonelectrolytes and unionized weak electrolytes. However, the negatively charged SBEβCD is invariably more efficient than both the neutral HPβCD and Tween 80 (broken lines) to solubilize the following positively charged drugs: carbendazim, PG300995, flavopiridol, AMPB, and BPU. When a drug is negatively charged, such as the PPA anion, naproxen anion, and XK 469 anion, the efficiency of SBEβCD is less than that of HPβCD.
There appears to be no strong systematic difference between the ability of the two cyclodextrins and Tween 80 on a weight basis to solubilize all studied nonelectrolytes and unionized weak electrolytes. The differences of the solubilization capacities for micellization and complexation are less than 4-fold for all of the drugs except griseofulvin and cyclosporine A, both of which have Tween 80 solubilization capacities significantly larger than that of complexation. Not surprisingly, they are the only molecules without a ring that is able to fit into a 7 Å cyclodextrin cavity. Ran et al. (2001) postulated that the conformation of cyclosporine A in aqueous media would be closer to the illustration in Figure 3.21a than to the published arrangement for the crystal, as illustrated in Figure 3.21b. This would make cyclosporine A less suitable for incorporation into the cyclodextrin cavity. This hypothesis is supported by the fact that the calculated logarithm of the octanol-water partition coefficient, ClogP, from the structure in Figure 3.21b is 14.4 (BioByte Corp., Claremont, CA), whereas the measured logarithm of the octanol-water partition coefficient, MlogP, is only 2.9. The calculation of the ACD/Labs™ software might incorporate the consideration of the conformation information, which gives a value 3.4 (Advanced Chemistry Development, Inc., Toronto, ON, Canada).
Figure 3.21. Cyclosporine A schematic conformations: a. in aqueous media. b. in crystal.
4.2. **Descriptors** \((σ, κ, K^m, τ, \text{ and } K^{1:1})\)

In order to quantitatively evaluate all of the tested solubilizing agents, the descriptors, \(σ, κ, K^m, τ, \text{ and } K^{1:1}\) for both unionized and ionized drug species, were calculated from the experimental data and evaluated with respect to drug polarity as reflected by the logarithm of the octanol-water partition coefficient. Since ACD/Labs™ software provides calculated logP values for both charged and uncharged species, it is used in this chapter. The descriptors for all drugs, along with their logP values, are listed in Tables 3.1-3.3, and are used in the Figures 3.22-3.27. For practical convenience, all descriptors, except \(K^{1:1}\), are calculated using drug solubility in units of mg/mL and the solubilizing agent concentration in units of percentage.

4.2.a. **Cosolvency** \((σ)\)

The efficiency of a cosolvent for a drug is usually evaluated by its \(σ\) value. The experimental \(σ_u\) values of EtOH and PG for the twelve unionized drugs and the \(σ_i\) values for the eight ionized drug species along with their logP values are listed in Table 3.1a and b, respectively. The EtOH and PG data are plotted in Figures 3.22 and 3.23. The filled diamonds are for the unionized drugs and the open triangles are for the ionized drugs. As expected, the solubilization power of the cosolvent is inversely proportional to the drug polarity because a cosolvent increases drug solubility by reducing the overall polarity of the solution. The regressions of both figures were obtained and drawn as the solid lines:

\[
\text{EtOH: } σ = 0.0069 \cdot \log P + 0.0227
\] (17)
where $\sigma$ represents both unionized and ionized compounds.

The dotted lines in Figures 3.22 and 3.23 are the calculated values based on the results of Li and Yalkowsky (1994) and the results of Millard et al. (2002), which examined 103 compounds with EtOH concentrations of 0 to 50%, and 81 compounds with PG up to 100%, respectively. The average absolute errors (AAE) of the calculated and experimental data of the $\sigma$ values by using EtOH or PG as the cosolvent are 0.012 and 0.009, respectively. Thus, when EtOH or PG is used as the solubilizing agent, a $\sigma_u$ value for a nonelectrolyte and a $\sigma_i$ values for a charged compound can be reasonably estimated from the compound logP by using the reported regression or the regression generated from this study. This could provide a general idea of the efficiency of the cosolvency before effort is put into the lab work.
Table 3.1a. Solubilization power (\(\sigma_u\)) of EtOH, and PG

<table>
<thead>
<tr>
<th>Compound</th>
<th>logP (u)</th>
<th>(\sigma_u) (exp.)</th>
<th>(\sigma_u) (calc.)</th>
<th>(\sigma_u) (exp.)</th>
<th>(\sigma_u) (calc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbendazim</td>
<td>1.5</td>
<td>0.020</td>
<td>0.025</td>
<td>0.018</td>
<td>0.018</td>
</tr>
<tr>
<td>PPA</td>
<td>1.7</td>
<td>0.016</td>
<td>0.041</td>
<td>0.010</td>
<td>0.033</td>
</tr>
<tr>
<td>Flavopiridol</td>
<td>1.9</td>
<td>0.066</td>
<td>0.028</td>
<td>0.049</td>
<td>0.021</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>2.2</td>
<td>0.050</td>
<td>0.026</td>
<td>0.031</td>
<td>0.019</td>
</tr>
<tr>
<td>PG300995</td>
<td>2.2</td>
<td>0.037</td>
<td>0.030</td>
<td>0.026</td>
<td>0.023</td>
</tr>
<tr>
<td>Naproxen</td>
<td>3.0</td>
<td>0.031</td>
<td>0.036</td>
<td>0.027</td>
<td>0.029</td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>3.4</td>
<td>0.026</td>
<td>0.039</td>
<td>0.025</td>
<td>0.032</td>
</tr>
<tr>
<td>Estrone</td>
<td>3.7</td>
<td>0.057</td>
<td>0.042</td>
<td>0.041</td>
<td>0.034</td>
</tr>
<tr>
<td>AMPB</td>
<td>4.0</td>
<td>0.041</td>
<td>0.044</td>
<td>0.037</td>
<td>0.036</td>
</tr>
<tr>
<td>XK-469</td>
<td>4.1</td>
<td>0.043</td>
<td>0.047</td>
<td>0.030</td>
<td>0.039</td>
</tr>
<tr>
<td>BPU</td>
<td>4.3</td>
<td>0.083</td>
<td>0.053</td>
<td>0.059</td>
<td>0.045</td>
</tr>
<tr>
<td>Fluasterone</td>
<td>5.0</td>
<td>0.058</td>
<td>0.045</td>
<td>0.044</td>
<td>0.037</td>
</tr>
</tbody>
</table>

Table 3.1b. Solubilization power (\(\sigma_i\)) of EtOH, and PG

<table>
<thead>
<tr>
<th>Compound</th>
<th>logP (i)</th>
<th>(\sigma_i) (exp.)</th>
<th>(\sigma_i) (calc.)</th>
<th>(\sigma_i) (exp.)</th>
<th>(\sigma_i) (calc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA</td>
<td>-2.1</td>
<td>0.003</td>
<td>-0.004</td>
<td>0.000</td>
<td>-0.010</td>
</tr>
<tr>
<td>Flavopiridol</td>
<td>-1.2</td>
<td>0.051</td>
<td>0.003</td>
<td>0.044</td>
<td>-0.003</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>-1.0</td>
<td>0.008</td>
<td>0.005</td>
<td>0.006</td>
<td>-0.002</td>
</tr>
<tr>
<td>Naproxen</td>
<td>-0.8</td>
<td>0.008</td>
<td>0.007</td>
<td>0.005</td>
<td>0.000</td>
</tr>
<tr>
<td>PG300995</td>
<td>-0.3</td>
<td>0.013</td>
<td>0.011</td>
<td>0.013</td>
<td>0.004</td>
</tr>
<tr>
<td>XK-469</td>
<td>0.3</td>
<td>0.017</td>
<td>0.015</td>
<td>0.017</td>
<td>0.008</td>
</tr>
<tr>
<td>BPU</td>
<td>0.8</td>
<td>0.032</td>
<td>0.019</td>
<td>0.014</td>
<td>0.012</td>
</tr>
<tr>
<td>AMPB</td>
<td>1.5</td>
<td>0.034</td>
<td>0.024</td>
<td>0.025</td>
<td>0.017</td>
</tr>
</tbody>
</table>
Figure 3.22. EtOH $\sigma$ vs. drug logP

Figure 3.23. PG $\sigma$ vs. drug logP
4.2.b. Micellization and complexation ($\kappa$, $K^m$, $\tau$, and $K^{1:1}$)

The solubilization efficiency of a surfactant or a complexation ligand is evaluated by its solubilization capacity. As described in the background section, these capacities for surfactant and complexation are described by $\kappa$ and $\tau$. The $\kappa_u$, $\kappa_i$, $\tau_u$ and $\tau_i$ values for the studied drugs in Tween 80, HPβCD, and SBEβCD are listed in Table 3.2. The average values of the unionized drug species in Table 3.2a are 0.15, 0.13, and 0.12 for Tween 80, HPβCD, and SBEβCD, respectively. This indicates that they have similar overall abilities on a weight basis to solubilize these drugs. Furthermore, the nonionic solubilizing agents, Tween 80 and HPβCD, have similar overall solubilization abilities for all data in Table 3.2 with average values of 0.15 and 0.16, respectively. The lack of a simple relationship between logP and logarithms of $\kappa$ for Tween 80 or $\tau$ for the cyclodextrins is observed in Figures 3.24a, 3.25a, and 3.26a.
Table 3.2a. Solubilization capacity ($\kappa_u$) for Tween 80 and ($\tau_u$) for HPβCD, and SBEβCD

<table>
<thead>
<tr>
<th>Compound</th>
<th>logP$_u$</th>
<th>$\kappa_u$ (exp.)</th>
<th>HPβCD $\tau_u$ (exp.)</th>
<th>SBEβCD $\tau_u$ (exp.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbendazim</td>
<td>1.5</td>
<td>0.005</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>PPA</td>
<td>1.7</td>
<td>0.99</td>
<td>0.87</td>
<td>0.62</td>
</tr>
<tr>
<td>Flavopiridol</td>
<td>1.9</td>
<td>0.093</td>
<td>0.076</td>
<td>0.101</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>2.2</td>
<td>0.032</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>PG300995</td>
<td>2.2</td>
<td>0.034</td>
<td>0.019</td>
<td>0.033</td>
</tr>
<tr>
<td>Naproxen</td>
<td>3.0</td>
<td>0.332</td>
<td>0.368</td>
<td>0.385</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>3.4</td>
<td>0.109</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Estrone</td>
<td>3.7</td>
<td>0.029</td>
<td>0.125</td>
<td>0.098</td>
</tr>
<tr>
<td>AMPB</td>
<td>4.0</td>
<td>0.041</td>
<td>0.013</td>
<td>0.019</td>
</tr>
<tr>
<td>XK-469</td>
<td>4.1</td>
<td>0.026</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td>BPU</td>
<td>4.3</td>
<td>0.091</td>
<td>0.034</td>
<td>0.038</td>
</tr>
<tr>
<td>Fluasterone</td>
<td>5.0</td>
<td>0.016</td>
<td>0.058</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Table 3.2b. Solubilization capacity ($\kappa_i$) for Tween 80 and ($\tau_i$) for HPβCD, and SBEβCD

<table>
<thead>
<tr>
<th>Compound</th>
<th>logP$_i$</th>
<th>$\kappa_i$ (exp.)</th>
<th>HPβCD $\tau_i$ (exp.)</th>
<th>SBEβCD $\tau_i$ (exp.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA</td>
<td>-2.1</td>
<td>-0.050</td>
<td>-0.140</td>
<td>-0.710</td>
</tr>
<tr>
<td>Flavopiridol</td>
<td>-1.2</td>
<td>0.899</td>
<td>0.449</td>
<td>1.085</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>-1.0</td>
<td>0.000</td>
<td>0.035</td>
<td>0.088</td>
</tr>
<tr>
<td>Naproxen</td>
<td>-0.8</td>
<td>0.102</td>
<td>0.103</td>
<td>-0.070</td>
</tr>
<tr>
<td>PG300995</td>
<td>-0.3</td>
<td>0.033</td>
<td>0.144</td>
<td>0.411</td>
</tr>
<tr>
<td>XK-469</td>
<td>0.3</td>
<td>0.033</td>
<td>0.111</td>
<td>0.091</td>
</tr>
<tr>
<td>BPU</td>
<td>0.8</td>
<td>0.045</td>
<td>0.599</td>
<td>1.752</td>
</tr>
<tr>
<td>AMPB</td>
<td>1.5</td>
<td>0.072</td>
<td>0.233</td>
<td>0.422</td>
</tr>
</tbody>
</table>
Figure 3.24. Solubilization descriptors of Tween 80 vs. drug logP: a. Log $\kappa$; b. log $K^m$. An arbitrary value of 0.1 is assigned to the slight negative $\kappa$ or $K^m$ and represented as $\circ$. 
Figure 3.25. Solubilization descriptors of HPβCD vs. drug logP: a. Log \( \tau \), b. log \( K_{1:1} \). An arbitrary value of 0.1 is assigned to the slight negative \( \tau \) or \( K_{1:1} \) and represented as •.
Figure 3.26. Solubilization descriptors of SBEβCD vs. drug logP: a. Log $\tau$, b. log $K_{1:1}$. An arbitrary value of 0.1 is assigned to the slight negative $\tau$ or $K_{1:1}$ and represented as ○.
The micellar partition coefficients of the unionized and ionized drugs in Tween 80 \((K^m)\), and the drug ligand complex equilibrium constants of the unionized and ionized drugs in HP\(\beta\)CD and SBE\(\beta\)CD \((K_{1:1}^L)\) are listed in Table 3.3. For each drug, \(K^m\) and \(K_{1:1}^L\) were calculated from their corresponding \(\kappa\) and \(\tau\) values and their aqueous solubilities. The logarithms of \(K^m\) and \(K_{1:1}^L\) are plotted vs. drug logP in Figures 3.24b, 3.25b, and 3.26b. Both \(K^m\) and \(K_{1:1}^L\) trend to increase with drug logP along with the ionized compounds (open symbols) tending to be less well solubilized than their neutral counterparts (filled symbols). The regression of each figure was obtained and plotted as the solid line:

\[
\begin{align*}
\text{Tween 80: } \log K^m &= 0.6461 \cdot \log P + 1.2391 \\
\text{HP\(\beta\)CD: } \log K_{1:1}^L &= 0.7995 \cdot \log P + 0.6183 \\
\text{SBE\(\beta\)CD: } \log K_{1:1}^L &= 0.5163 \cdot \log P + 2.1172
\end{align*}
\]

where \(K^m\) and \(K_{1:1}^L\) are for both unionized and ionized compounds.

The dependence of \(K^m_u\) on compound logP was studied by Alvarez-Nunez and Yalkowsky (2000) for 43 compounds with Tween 80 as the solubilizing agent. The values of \(K^m_u\) and \(K_{1:1}^m\) calculated from their regression are listed in Table 3.3a and b and plotted in Figure 3.24b as the dotted line. Evidently, the \(K^m_u\) and \(K_{1:1}^m\) values can be reasonably estimated from the compound logP by using either the reported regression or
the regression generated from this study. The efficiency of Tween 80 can be predicted for both nonelectrolytes and electrolytes with pH adjustment before performing any lab work.

Table 3.3a. Micellar partition coefficient \( K_u^m \) of Tween 80, and binding constant \( K_u^{1:1} \), \( M^{-1} \) of HPβCD and SBEβCD

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \log P_u )</th>
<th>( K_u^m ) (exp.)</th>
<th>( K_u^m ) (calc.)</th>
<th>HPβCD</th>
<th>SBEβCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbendazim</td>
<td>1.5</td>
<td>81</td>
<td>29</td>
<td>47</td>
<td>74</td>
</tr>
<tr>
<td>PPA</td>
<td>1.7</td>
<td>35</td>
<td>2075</td>
<td>157</td>
<td>245</td>
</tr>
<tr>
<td>Flavopiridol</td>
<td>1.9</td>
<td>421</td>
<td>68</td>
<td>486</td>
<td>1035</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>2.2</td>
<td>370</td>
<td>42</td>
<td>29</td>
<td>87</td>
</tr>
<tr>
<td>PG300995</td>
<td>2.2</td>
<td>93</td>
<td>132</td>
<td>73</td>
<td>205</td>
</tr>
<tr>
<td>Naproxen</td>
<td>3.0</td>
<td>3682</td>
<td>675</td>
<td>7282</td>
<td>14402</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>3.4</td>
<td>394</td>
<td>1417</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Estrone</td>
<td>3.7</td>
<td>4995</td>
<td>2912</td>
<td>31861</td>
<td>39280</td>
</tr>
<tr>
<td>AMPB</td>
<td>4.0</td>
<td>3393</td>
<td>5181</td>
<td>1572</td>
<td>3469</td>
</tr>
<tr>
<td>XK-469</td>
<td>4.1</td>
<td>9489</td>
<td>10381</td>
<td>1886</td>
<td>5543</td>
</tr>
<tr>
<td>BPU</td>
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<td>301945</td>
<td>49997</td>
<td>159374</td>
<td>278474</td>
</tr>
<tr>
<td>Fluasterone</td>
<td>5.0</td>
<td>35559</td>
<td>6243</td>
<td>185305</td>
<td>223558</td>
</tr>
</tbody>
</table>

Table 3.3b. Micellar partition coefficient \( K_i^m \) of Tween 80, and binding constant \( K_i^{1:1} \), \( M^{-1} \) of HPβCD and SBEβCD

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \log P_i )</th>
<th>( K_i^m ) (exp.)</th>
<th>( K_i^m ) (calc.)</th>
<th>HPβCD (exp.)</th>
<th>HPβCD (exp.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA</td>
<td>-2.1</td>
<td>-0.1</td>
<td>0.01</td>
<td>-1</td>
<td>-2</td>
</tr>
<tr>
<td>Flavopiridol</td>
<td>-1.2</td>
<td>147</td>
<td>0.1</td>
<td>257</td>
<td>399</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>-1.0</td>
<td>-0.01</td>
<td>0.1</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Naproxen</td>
<td>-0.8</td>
<td>2</td>
<td>0.2</td>
<td>-2</td>
<td>-2</td>
</tr>
<tr>
<td>PG300995</td>
<td>-0.3</td>
<td>2</td>
<td>1</td>
<td>33</td>
<td>52</td>
</tr>
<tr>
<td>XK-469</td>
<td>0.3</td>
<td>169</td>
<td>2</td>
<td>660</td>
<td>1021</td>
</tr>
<tr>
<td>BPU</td>
<td>0.8</td>
<td>21</td>
<td>6</td>
<td>1160</td>
<td>1802</td>
</tr>
<tr>
<td>AMPB</td>
<td>1.5</td>
<td>164</td>
<td>26</td>
<td>1374</td>
<td>2135</td>
</tr>
</tbody>
</table>
There are large discrepancies for the binding constants of the cyclodextrins reported from literature. In this research, the regressions of Figures 3.25b, and 3.26b indicate that both \( K_n^{1:1} \) and \( K_i^{1:1} \) can be predicted from the logP of unionized and ionized drug species. As with cosolvency and micellization, the efficiency of HPβCD or SBEβCD with or without pH adjustment can be predicted based on the compound logP.

The values in Table 3.3a and b also indicate that Tween 80, HPβCD and SBEβCD have similar efficiencies when they are evaluated by \( K^m \) and \( K^{1:1} \). The logarithms of these values are plotted in Figure 3.27 vs. logP with their regressions. Note, \( K^{1:1} \) was calculated with the molar concentrations of drug solubility and excipient.

Figure 3.27. \( \log K^m \) and \( \log K^{1:1} \) for Tween 80, HPβCD, and SBEβCD vs. logP. Tween 80 (□), HPβCD (+), and SBEβCD (+).
5. Conclusions

Combinations of pH adjustment with another technique can be a powerful means of increasing the solubility of an ionizable drug. The solubilization profiles in Figures 3.1-3.20 illustrate the efficiency of EtOH, PG, Tween 80, HPβCD, or SBEβCD to solubilize the unionized and ionized drugs. Equations 12, 14, and 16 in the background section explain why the combination of pH adjustment with cosolvency, micellization, or complexation can be a very powerful solubilization technique for an ionizable drug.

Although the effect of a solubilizing agent on the ionized drug species is generally smaller than that on its unionized counterpart, i.e., values of \( \alpha_i \), \( K_{i}^{m} \), \( K_{i}^{1:1} \), are usually smaller than the values of \( \alpha_u \), \( K_{u}^{m} \), \( K_{u}^{1:1} \), the free ionized drug concentration can be much greater than that of the unionized species, i.e., \( S_i \) can be much greater than \( S_u \). This depends on the medium pH and on the drug pKa. Since the concentration of a drug species is a function of both the free drug in the medium and the effect of the solubilizing agent, the total concentration of the ionized drug can be much greater than that of the unionized species.

The cosolvent solubilization power, \( \alpha \), is related to drug polarity because the cosolvent decreases intermolecular interactions in the medium. Therefore, the polarity of the entire medium is uniformly reduced by the addition of cosolvent. The micellar partition coefficient, \( K^{m} \), is dependent on logP because it is analogous to the octanol-water partition coefficient. The drug-ligand binding constant, \( K^{1:1} \), is also dependent on
logP because it can be thought of as a ligand-water partition coefficient. However steric hindrance is another factor determining drug incorporation into the ligand cavities. The dependence of $\sigma_u$ and $K^m_u$ on drug logP was studied by other researchers and the reported regressions were used to calculate these values for these twelve drugs. The calculated values in Tables 3.1 and 3.4, and Figures 3.22, 3.23, and 3.24b and the regression analysis of this study indicate that reasonably accurate $\sigma$ and $K^m$ values can be estimated solely based on polarity of the unionized and ionized drug species for EtOH, PG or Tween 80 as the solubilizing agent. Furthermore, Figure 3.26b indicates that the value of $K^{1:1}$ can also be estimated from the logP of the uncharged and charged drug species.

Therefore, despite the fact that the solubilization capacities of micellization and complexation, $\kappa$ and $\tau$, cannot be predicted from structure because they are only partially dependent upon drug polarity, the solubilization power of cosolvency, $\sigma$, the micellar partition coefficient of micellization, $K^m$, and the binding constants of complexation, $K^{1:1}$, can be predicted. This study shows that not only $\sigma_u$, $K^m_u$, $K^{1:1}_u$ are strongly dependent on drug logP, the descriptors for the charged counterparts, $\sigma_i$, $K^m_i$, $K^{1:1}_i$ are also dependent on the logP of the ionized species (Figures 3.22, 3.23, 3.24b, 3.25b, and 3.26b). Surprisingly, the values of both ionized and unionized drugs for each of the five tested solubilizing agents fall on a single line. The results of regression analysis for these excipients were given in Equations 17-21. Thus, the efficiency of a solubilizing agent
can be predicted for charged and uncharged drugs by cosolvency, micellization, or complexation, and by combinations of these techniques with pH adjustment.
CHAPTER 4. SOLUBILIZATION OF FLUASTERONE IN COSOLVENT/CYCLODEXTRIN COMBINATIONS

1. Introduction

The solubility of a drug in aqueous media determines many aspects of its efficacy for delivery and absorption. Solubilization can be achieved by: pH control, cosolvency, complexation, micellization, or a combination effect of any of the above. Applying multiple techniques can be advantageous for drugs that cannot be optimally solubilized by a single technique. It also enables the use of a smaller amount of any single excipient. The combined use of cosolvency and complexation is a particularly interesting case. The effect of cosolvents and complexants when used individually are well understood. Cosolvents work by reducing the hydrogen bond density of water and consequently its ability to “squeeze out” nonpolar solutes from aqueous systems. In other words, they increase the solubility of nonpolar drugs by reducing the polarity of the aqueous mixture. Inclusion ligands, such as the cyclodextrins, increase drug solubility by reversibly incorporating the nonpolar portion of the drug into their nonpolar cavities. Some researchers have reported that cosolvents decrease drug solubility in the complex (Loftsson et al., 1993; Ono et al., 2001), while others reported an increase (Zung 1991; Savolainen et al., 1998; Loftsson et al., 1998; Loftsson et al., 2001; Faucci 2001). According to Connors (1997), at least five different explanatory hypotheses have been proposed for the effect of the solvent in drug-complexant systems. Some of these
hypotheses attribute this effect to changes of the hydrophobic driving force for formation of the drug complex. Some attribute it to changes in the solvophobic characteristics of the medium. And still others proposed a decrease in the stoichiometric equilibrium with the addition of organic cosolvent.

Li et al. (1999) reported that the solubility of fluasterone, a new chemoprevention agent and a type 2 diabetes drug (Figure 4.1), is first decreased and then increased as ethanol is added to a solution containing hydroxypropyl-β-cyclodextrin. This study is aimed at quantitatively characterizing the effect of cosolvent size and polarity on the solubility of fluasterone in the presence of complexation ligand. Methanol, ethanol, and n-propanol were chosen as the cosolvents because they are a series with increasing molecular size and reducing polarity.

![Fluasterone](image)

Figure 4.1. Fluasterone

2. Materials and Methods

2.1. Materials

Fluasterone (micronized) was provided by Aeson Therapeutics Inc., (Tucson, AZ). HPβCD (Hydroxypropyl-β-Cyclodextrin, Trappsol®) with an average molecular weight
of 1390 and an average degree of hydroxypropyl substitution at 4.4 was obtained from Cyclodextrin Technologies Development Inc., (Gainesville, FL). ACN (Acetonitrile), MeOH (Methanol), EtOH (Ethanol), EtOAc (Ethyl Acetate), and n-PrOH (n-Propanol) were of reagent or HPLC grade and purchased from Aldrich (Milwaukee, WI). All chemicals were used as received without further purification and the water was double-deionized.

2.2. Solubility determination

The solubility of fluasterone in water was measured via the phase-solubility method followed by extraction. Sample bottles containing excess Fluasterone in 100 mL water were rotated for 6 days at room temperature on an end-over-end mechanical rotator (Glas-Col Laboratory Rotator, Terre Haute, IN). The saturated fluasterone-water solutions were filtered with a 0.45 µm membrane, and then the filtrates were extracted 3 times with 10 ml of EtOAc. The organic phases were then combined and dried under nitrogen. The residue was redissolved into 2 mL of 75% ACN in water for HPLC analysis.

Similarly, the solubilities of fluasterone in aqueous systems containing cosolvent, HPβCD, or combined cosolvent-HPβCD were measured by the phase-solubility method. Solvent systems of 0 to 88% (v/v) cosolvent and/or 0 to 20% (w/v) HPβCD in water were prepared by adding an exact amount of HPβCD into the water or cosolvent/water mixture. Saturated fluasterone solutions were obtained by filtering excess fluasterone from 2 mL of above solvent systems after the samples vials were rotated for 6 days. The diluted
filtrates were analyzed by HPLC. All the samples were prepared and analyzed in duplicate.

2.3. **HPLC analysis of fluasterone**

The HPLC assay method reported by Li et al. (1999) with a Beckman Gold System (Fullerton, CA) was used: A Pinnacle 5 µm C8 amine column (150 cm X 4.6 mm, Restek, Bellefonte, PA) was used as the stationary phase with 75% ACN and 25% water as the mobile phase. The flow rate was controlled at 1.1 ml/min (125 Solvent Module) for 9 minutes. The sample injection volume was 100 µL (507 Autosampler) and the analytes were detected at 220 nm (168 Detector). The retention time of Fluasterone was 6.3 minutes. None of the solubilizing agents interfered with the assay.

3. **Background**

3.1. **Fluasterone in water or cosolvent solutions**

The solubility of fluasterone in water, i.e., the intrinsic solubility, was determined by HPLC to be 0.000155 mM (0.045 µg/mL).

The exponential dependence of the solubility of the unionized non-polar solutes on cosolvent concentration in a semi-aqueous solution is described in Equation 1, which can be written in log-linear form as Equation 2 (Yalkowsky et al., 1972; Yalkowsky and Rubino 1985):
\[ S_{\text{tot}} = S_u \cdot 10^{\sigma_u \cdot C_{\text{cos}}} \quad (1) \]

\[ \log S_{\text{tot}} = \log S_u + \sigma_u \cdot C_{\text{cos}} \quad (2) \]

where

- \( S_{\text{tot}} \) = total apparent drug solubility
- \( S_u \) = intrinsic drug solubility
- \( C_{\text{cos}} \) = cosolvent concentration
- \( \sigma_u \) = cosolvent solubilizing power for the solute

Figure 4.2 shows the solubility of fluasterone in the cosolvent solutions. The solubility data in all tested concentrations of MeOH, EtOH, and up to 40% n-PrOH conform to the predictions of Equations 1 and 2. As expected, the solubility of fluasterone increases least in MeOH (the most polar solvent) solution and most in n-PrOH (the least polar solvent) solution. The \( \sigma \) values are 0.19 M\(^{-1}\), 0.34 M\(^{-1}\), and 0.78 M\(^{-1}\) for MeOH, EtOH, and n-PrOH, respectively.
3.2. Fluasterone in complexant solution

The linear dependence of the solubility of a non-polar drug with HPβCD is described in Equation 3:

$$S_{tot} = S_u + K_b \cdot S_u \cdot L_{tot}$$  \hspace{1cm} (3)$$

where $K_b$ = the binding constant for the binary complex DL

$L_{tot}$ = total ligand concentration

Figure 4.3 shows the measured fluasterone solubility in solutions of 0 to 20% HPβCD. The fluasterone-HPβCD binary complex equilibrium constant calculated from the slope is $1.8 \times 10^5$ M$^{-1}$. Since the concentration of binary drug ligand complex is given by the
last term in Equation 3 \( (K_b \cdot S_u \cdot L_{tot}) \), there is a linear increase in total drug solubility with increasing ligand concentration.

![Graph showing the solubility of fluasterone in HPβCD solution](image)

Figure 4.3. Solubility of fluasterone in HPβCD solution

3.3. **Fluasterone in solutions of cosolvent and HPβCD**

The presence of ternary complexes of nonpolar drug with cosolvent in the β-cyclodextrin cavity has been reported by many researchers (Schuette et al., 1993; Milewski et al., 1998; Evans et al., 2000). On the other hand, some researchers (Ono et al., 2001; Zhao et al., 2002; Yang et al., 2003) have reported competition between guest drug molecule and competitor to complex with the cyclodextrin when the drug and the competitor have similar polarity and molecular size. When fluasterone dissolves in a
solvent system of either MeOH, EtOH, or n-PrOH with HPβCD, it is likely to form a ternary complex because each cosolvent is much smaller and are much more polar than fluasterone. Some of the fluasterone will dissolve in the medium as free drug, $S_u$, some will dissolve by inclusion in the ligand cavity as binary complex, $DL$, and some will dissolve by inclusion with the cosolvent in the ligand cavity as a ternary complex, $DLC$. The total solubility of fluasterone is the sum of the concentrations of all three of these drug species, i.e.,

$$S_{tot} = S + DL + DLC$$  \hspace{1cm} (4)

The concentrations of these species are described by Equations 5, 6, and 7

$$S = S_u \cdot 10^{\sigma \cdot C_{\cos}}$$  \hspace{1cm} (5)

$$DL = \left( K_b \cdot 10^{-\rho_b \cdot C_{\cos}} \right) S_u \cdot 10^{\sigma \cdot C_{\cos}} \cdot L_{tot}$$

$$DL = K_b \cdot S_u \cdot L_{tot} \cdot 10^{(\sigma - \rho_b) C_{\cos}}$$  \hspace{1cm} (6)

$$DLC = \left( K_t \cdot 10^{-\rho_t \cdot C_{\cos}} \right) S_u \cdot 10^{\sigma \cdot C_{\cos}} \cdot L_{tot} \cdot C_{\cos}$$

$$DLC = K_t \cdot S_u \cdot L_{tot} \cdot C_{\cos} \cdot 10^{(\sigma - \rho_t) C_{\cos}}$$  \hspace{1cm} (7)

where

- $K_t$ = constant for the ternary complex $DLC$
- $\rho_b$ = destabilizing power of the cosolvent for the binary complex $DL$, i.e., the effect of the cosolvent on $K_b$ as described by Li et al. (1999)
- $\rho_t$ = destabilizing power of the cosolvent for the ternary complex $DLC$, i.e., the effect of the cosolvent on $K_t$ as described by Li et al. (1999)
Note that the free drug concentration in Equation 5 is equal to the total drug concentration in Equation 1, when only cosolvent is added into the system. Equations 6 and 7 account for two effects of the cosolvent on complex formation. First, cosolvent addition favors complexation because it provides more solute in solution according to $10^{\sigma C_{\text{cos}}}$. Second, cosolvent addition disfavors complexation because it reduces the driving force for solute incorporation into the CD cavity by $10^{-\rho_b C_{\text{cos}}}$ or $10^{-\rho_t C_{\text{cos}}}$. Note that $\rho$ can be either positive or negative depending upon whether the cosolvent destabilizes or stabilizes the ternary complex.

In order to obtain the total drug concentration, Equations 5, 6, and 7 are inserted into Equation 4 to give Equation 8:

$$S_{\text{tot}} = S_u \cdot 10^{\sigma C_{\text{cos}}} + K_b \cdot S_u \cdot L_{\text{tot}} \cdot 10^{(\sigma-\rho_b)C_{\text{cos}}}$$

$$+ K_t \cdot S_u \cdot L_{\text{tot}} \cdot C_{\text{cos}} \cdot 10^{(\sigma-\rho_t)C_{\text{cos}}}$$  \hspace{1cm} (8)

Equation 8 expresses the total drug solubility as a function of both the ligand and cosolvent concentrations with 5 constants for each system: $\sigma$ is the cosolvent solubilization power. $K_b$ and $K_t$ are the formation constants for the binary and ternary complexes, respectively. $\rho_b$ and $\rho_t$ are the cosolvent destabilizing powers for the binary complex and ternary complexes, respectively.

Equation 8 can be rearranged to give Equation 9, which expresses the total solubility as a linear function of ligand concentration:

$$S_{\text{tot}} = S_u \cdot 10^{\sigma C_{\text{cos}}} + \{K_b \cdot S_u \cdot 10^{(\sigma-\rho_b)C_{\text{cos}}}$$

$$+ K_t \cdot S_u \cdot C_{\text{cos}} \cdot 10^{(\sigma-\rho_t)C_{\text{cos}}} \} \cdot L_{\text{cos}}$$  \hspace{1cm} (9)
4. Results and Discussion

The measured solubilities of fluasterone in aqueous systems of MeOH and HPβCD, EtOH and HPβCD, and n-PrOH and HPβCD are plotted as symbols in Figures 4.4 as a function of cosolvent concentration at several fixed HPβCD concentration (from 20% to 0%, w/v). The data strings are organized in order of reducing HPβCD concentration from the top to the bottom in each system. The symbols for the various HPβCD concentrations are listed in Table 4.1.
Figure 4.4. Dependence of fluasterone solubility on cosolvent in cosolvent-HPβCD systems  
A: MeOH and HPβCD;  B: EtOH and HPβCD;  C: n-PrOH and HPβCD
Table 4.1. The symbols and HPβCD concentrations of the data strings in Figure 4.4

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Concentration of HPβCD (%)</th>
<th>Concentration of HPβCD (%)</th>
<th>Concentration of HPβCD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>△</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>□</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>⋅</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>○</td>
<td></td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>×</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>+</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>-</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>◊</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The experimental data in Figure 4.4 were used to obtain the constants in Equation 8 via non-linear multiple regression. The values of the constants for each water-cosolvent-HPβCD-fluasterone system are listed into Table 4.2.

Table 4.2. Parameters of the solvent systems

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MeOH</th>
<th>EtOH</th>
<th>n-PrOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\sigma(M^{-1}))</td>
<td>0.19</td>
<td>0.34</td>
<td>0.78</td>
</tr>
<tr>
<td>(\rho_b(M^{-1}))</td>
<td>0.30</td>
<td>0.52</td>
<td>1.58</td>
</tr>
<tr>
<td>(\rho(M^{-1}))</td>
<td>0.21</td>
<td>0.34</td>
<td>0.79</td>
</tr>
<tr>
<td>(K_b(M^{-1}))</td>
<td>1.8E+05</td>
<td>1.8E+05</td>
<td>1.8E+05</td>
</tr>
<tr>
<td>(K_r(M^2))</td>
<td>4.3E+04</td>
<td>1.4E+04</td>
<td>9.1E+03</td>
</tr>
</tbody>
</table>
The solubility of fluasterone can be calculated by Equation 8 under any chosen cosolvent-ligand concentrations, once the constants are generated as in Table 4.2. The calculated solubilities are indicated as the solid lines in Figure 4.4. Clearly, they are in good agreement with the experimental data. The average absolute error (AAE) for the logarithms of the measured and calculated data are 0.05, 0.05, and 0.17 for the MeOH, EtOH, and n-PrOH systems, respectively.

Interestingly, the solubility of fluasterone decreases and then increases as the cosolvent concentration increases. This effect is quantitatively explained in Equation 8 by the changes in the concentration of each drug component. As cosolvent composition increases, the free drug, $S$, exponentially increases as indicated in Equation 5. However, the changes of the other two drug components could be in either direction. As indicated in Equation 6, the binary drug-ligand complex, $DL$, can exponentially increase, decrease, or it can remain steady with increasing cosolvent composition, depending on whether the term $(\sigma \rho_b)$ is greater than, less than, or equal to zero. Similarly, the change of the ternary drug-ligand-cosolvent complex, $DLC$, is described by Equation 7. This change can also go in either direction with increasing cosolvent composition, depending on whether the term $(\sigma \rho)$ is positive, negative, or equal to zero. The summation of the three drug components determines the shape of the total drug solubility curve. This model explains the observed reduced and then increased solubility of fluasterone in the three tested systems. It also explains why some researchers observed a decrease in total drug solubility with increasing cosolvent composition, while others observed an increase.
Comparing the three graphs in Figure 4.4, the most polar and smallest size cosolvent, MeOH, gives the least reduction in total drug solubility. The calculated parameter ($K_t$) in Table 4.2 indicates that MeOH has the greatest ability to form a drug-ligand-cosolvent complex. This could be attributed to its greater ability to fit into the spaces within the HPβCD cavities that are not occupied by drug. The increase of the ternary complex overcomes the loss of the binary complex to give an increase in total drug solubility.

The least polar and largest cosolvent, n-PrOH, produces the most pronounced reduction in solubility because it reduces the binary drug-ligand complex the most. The calculated parameter ($K_t$) in Table 4.2 indicates that n-PrOH has the least ability to form a ternary complex. The main contribution of the increased drug solubility is the free drug as a result of the high cosolvent solubilization power ($\sigma$) of n-PrOH.

The solubilities of fluasterone are shown as a function of HPβCD concentration in Figure 4.5, which contains the rearranged data of Figure 4.4. Each data string represents a fixed cosolvent concentration and is organized in each graph in order of reducing cosolvent concentration (from 88% to 0%, v/v) from the top to the bottom. The cosolvent concentrations and symbols are listed in Table 4.3. The linear dependence of fluasterone solubility on HPβCD in each cosolvent system is in good agreement with Equation 9. Note, that if the data sets in graphs D, E and F are vertically connected at 20% HPβCD, they correspond to the data strings on the top of graphs A, B, and C, respectively. The vertical data sets from right to left in Figure 4.5 correspond to horizontal data strings from top to bottom in Figure 4.4. The measured solubility data are provided in the appendix.
Figure 4.5. Dependence of fluasterone solubility on complexation ligand in cosolvent-HPβCD systems. D: MeOH and HPβCD; E: EtOH and HPβCD; F: n-PrOH and HPβCD
Table 4.3. The symbols and cosolvent concentration of the data strings in Figure 4.5

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Concentration of cosolvent (%, w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D (MeOH)</td>
</tr>
<tr>
<td>▲</td>
<td>88.6</td>
</tr>
<tr>
<td>◆</td>
<td>75.9</td>
</tr>
<tr>
<td>■</td>
<td>63.2</td>
</tr>
<tr>
<td>○</td>
<td>50.6</td>
</tr>
<tr>
<td>—</td>
<td>37.9</td>
</tr>
<tr>
<td>+</td>
<td>25.3</td>
</tr>
<tr>
<td>—</td>
<td>12.7</td>
</tr>
<tr>
<td>*</td>
<td>6.3</td>
</tr>
<tr>
<td>×</td>
<td>3.2</td>
</tr>
<tr>
<td>△</td>
<td>1.3</td>
</tr>
<tr>
<td>□</td>
<td>0.3</td>
</tr>
<tr>
<td>◊</td>
<td>0.0</td>
</tr>
</tbody>
</table>

5. Conclusions

Fluasterone solubility in combined cosolvent and complexant solutions is the summation of the free drug in the semi-aqueous media, the drug-ligand binary complex, and the drug-ligand-cosolvent ternary complex. The presence of cosolvent reduces the overall polarity of the aqueous media while it has a tendency to be included in a ternary complex based on its small molecular size. The simple Equation 8 can explain a decrease, increase, or decrease followed by increase in drug solubility with the addition of a cosolvent to a HPβCD and drug solution. It accounts for the effect of cosolvent size...
and polarity on the solubilization of the drug, the destabilization of drug-ligand complex and the formation of a ternary drug-ligand-cosolvent complex.
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