REVIEW

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Aqueous solubility and true solutions

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To the naked eye there is no sharp boundary between true solutions, where solute molecules are fully dispersed in the solvent, and colloidal solutions, where the solute molecules form very small (diameter < 50 nm) water-soluble aggregates, and smaller aggregates (diameter < 5 nm) are not easily detected by light scattering. In some cases small aggregates can have higher affinity than the individual test molecules for some specific receptors, but most activity studies are based on interactions of individual test molecules with some specific receptor and, thus, it is more likely that aggregate formation will result in decreased apparent activity during high throughput screening (HTS) and false negative results. Furthermore, aggregate formation will influence the physicochemical properties of drugs, such as their aqueous solubility, chemical stability and partitioning. Formation of drug/cyclodextrin inclusion complexes can be used to mimic non-specific drug-receptor interactions. Studies in aqueous cyclodextrin solutions have shown that practically insoluble drugs (solubility < 0.05 mg/ml) form small molecular aggregates and formation of such aggregates increases with decreasing drug solubility. Novel methods for solubility determinations, which can distinguish between individual solute molecules and small molecular aggregates, can possible improve the efficacy of HTS for new drug candidates.

1. Introduction

Aqueous solubility of a biologically active compound is one of the key physicochemical determinant of its "drugability". Lipinski's rule of five is partly based on the ability of an active compound to interact with water molecules (Lipinski 2000). Aqueous solubility is one of three characteristics determining drug classification according to the Biopharmaceutics Classification System (BCS), the other two being dose and permeability (Amidon et al. 1995), and aqueous solubility influences the formulation approach used to convert biologically active compound into a usable drug. Lipinski has pointed out that minimum acceptable solubility of a drug with medium potency (dose 1 mg/kg) and medium intestinal permeability is 52 μg/ml but as high as 2.1 mg/ml for a low potency drug (dose 10 mg/kg) with low permeability (Lipinski 2000). In general, it is assumed that the aqueous solubility of an orally administered drug needs to be greater than 0.1 mg/ml to avoid dissolution limited absorption (Hörter and Dressman 2001). Oral bioavailability of poorly-soluble drugs is also susceptible to food effects, pH changes, gastrointestinal metabolism and efflux transporters. For aqueous eye drop solution the drug dose has to be soluble in 35 μl, in 100 μl for nasal administration and preferably in no more than few ml for injectable bolus administration of aqueous drug solution. Thus, determination of aqueous solubility is an integrated part of drug discovery and development.

When excess of solid drug particles are suspended in an aqueous solution, drug molecules are removed from the particle surfaces until equilibrium is reached between drug molecules leaving the surfaces and those returning to the surfaces. At this equilibrium the aqueous solution is saturated with the drug and the concentration of dissolved drug is referred to as its *equilibrium solubility* or simply as its *solubility*. *Intrinsic solubility* is the equilibrium solubility of an ionizable drug at pH where the drug is fully unionized. Solubility of a given drug in an aqueous solution of a fixed composition, pH and temperature should be a constant value. The *solution* formed, sometimes referred to as *true solution* (Gupta 2006), appears clear and is regarded as a homogeneous mixture of drug molecules (the solute) and water molecules (the solvent). *Colloids* consist of finely divided particles dispersed in solvent, such as water. In most cases colloids scatter light giving them turbid or opalescence appearance. If the particle size is comparable or larger than the wavelength of visible light (400–700 nm) then they scatter or absorb light independent of each other (appear milky) but particles smaller than 50 nm do not, in general, scatter visible light. To the naked eye there is no sharp division between true solutions, where solute molecules are fully dispersed in the solvent, and colloidal solutions, where the solute molecules form very small (diameter < 50 nm) water-soluble aggregates.

Many drugs possess amphiphilic properties and form micellar-like structures (aggregates) in aqueous solutions (Attwood and Florence 1983; Schreier et al. 2000; Serajuddin 2007; Fini et al. 1995) and carbohydrates like cyclodextrins are known to self-associate to form nanoparticles (Bonini et al. 2006; He et al. 2008; Jansook et al. 2010). The aggregate formation is concentration dependent and characterized by a critical aggregation concentration obtained by monitoring some physicochemical changes as function of solute concentration (Taboada et al. 1999; Taboada et al. 2004). Colloidal aggregates (30 to 400 nm in diameter) are known to cause promiscuous enzyme inhibition that leads to false-positive results during high throughput

screening (HTS) for biologically active compounds (Seidler et al. 2003). Then there are polar drugs that form dimers, trimers and higher order oligomers in aqueous solutions that are only couple of nm in diameter (Zhu and Streng 1996; Avdeef 2007). Formation of aggregates will influence the physicochemical properties of drugs, such as their aqueous solubility, chemical stability, partitioning and ability to form complexes with drug receptors.

2. Solubility determinations

Currently applied methods determine either thermodynamic solubility or kinetic solubility (Kerns and Di 2008). *Thermodynamic solubility* is characterized by addition of excess solid drug particles (e.g., drug crystals) directly to an aqueous solution and equilibration under constant agitation and solid drug particles are present during the whole equilibration period. The apparent thermodynamic solubility of a given drug varies with the solid form (different crystal polymorphs, amorphous forms, hydrates and purity) and, thus, it can vary between synthetic batches of the same drug. The most common method for determination of thermodynamic solubility is the equilibration *shake-flask method* (Higuchi et al. 1953). It is a bit time consuming but gives reproducible values. Modified shake-flask methods include the miniaturized shake-flask method (Glomme et al. 2005) and the heating method where drug precipitation is induced after formation of supersaturated solution (Loftsson and Hreinsdóttir 2006). *Kinetic solubility* is characterized by stepwise addition of solution of the compound to be tested in an organic solvent (e.g., DMSO) to an aqueous solution until drug precipitation is observed (turbidimetry or nephometry) or the concentration of dissolved drug becomes constant (UV spectrophotometry) (Kerns and Di 2008). Methods that determine kinetic solubility are relatively rapid and require only small amount (couple of mg) of the test compound and, thus, suitable for HTS of aqueous solubility during drug discovery. The small amount of organic solvent present in the aqueous test solution, as well as precipitation of amorphous or metastabile crystal forms, can lead to overestimation of the solubility. Thus, kinetic solubility is frequently greater than thermodynamic solubility. Presently *in silico* methods for solubility prediction are not sufficiently accurate to replace experimental determinations (Delaney 2005; Hopfinger et al. 2009). Even the well-known Henderson-Hasselbalch relationship only gives rough estimations of the pH-dependent solubility (Bergström et al. 2004; Avdeef 2007; Serajuddin 2007).

3. Does clear solution mean that a drug is fully dissolved?

While water belongs to the group VIA hydrides in the Periodic Table (i.e., H_2S, H_2Se and H_2Te), it is distinct from other members of the class based on melting and boiling point, as well as other physicochemical properties such as density, dielectric constant and surface tension (Chaplin 2006; Loftsson and Brewster 2008). These differences are attributed to the ability of water molecules to form intermolecular hydrogen bonds creating a strongly cohesive system. This cohesive property of water explains why hydrophobic compounds tend to aggregate in aqueous solutions. The basic theory behind aggregation of hydrophobic solute molecules in aqueous solutions (i.e., hydrophobic interaction) is well known and can be summarized as follows (Tanford 1980). In aqueous solutions hydrophobic solute molecules are not able to form strong bonds (hydrogen bonds, ion-dipole interactions) with surrounding water molecules and thus each solute molecule is surrounded by a flickering cage structure of cohered water molecules. This increase in water structuring results in a decrease in entropy ($\Delta S < 0$). The process of cage formation requires that some hydrogen bonds are broken ($\Delta H > 0$) while new ones are being formed ($\Delta H < 0$) resulting in overall positive or negative enthalpy, values that can be close to zero. Consequently, the process of dissolving hydrophobic molecules in water is entropy driven rather than enthalpy driven. If the hydrophobic solute molecules aggregate to form dimers, trimers and higher order oligomers fewer but larger cages are formed resulting in a decrease in the organization of water molecules (i.e., net increase in ΔS value) and more favorable free energy (i.e., net decrease in ΔG value). Thus, formation of small aggregates containing only couple hydrophobic solute molecules lowers the energy of the aqueous system. In drug discovery biologically active compounds are designed to interact with specific receptors and trigger specific pharmacological response. Such target selectivity and receptor affinity favors selection of lipophilic and poorly water-soluble compounds. Thus, HTS has produced compounds that are becoming less water soluble and more lipophilic, or in other words molecules that possess increased tendency to aggregate into dimers, trimers and higher order oligomers (Lipinski 2000).

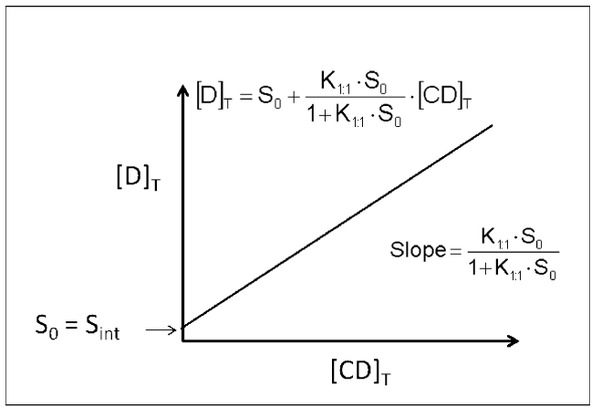
Turbidity of an aqueous aggregate solution depends on both the diameter of the aggregates and their concentration. Aqueous polymeric dispersions with particle diameter of 1000 nm appear milky white but become bluish semitransparent as the diameter is decreased to 50 nm with increasing transparency as the diameter is further decreased and become fully transparent at particle diameter 10 nm (Müller and Poth 2006). Microemulsions containing droplets that are about 50 nm in diameter are transparent and appear clear to the naked eye (Bowman et al. 2006). An aqueous 10% (w/w) amorphous silica (SiO_2) solution with particle size 7 to 8 nm (Ludox® SM, DuPont) appears to be a clear solution (Schoeman et al. 1994). Small aggregates of hydrophobic solute molecules are, in general, not detected during solubility determinations and trace concentrations of very small aggregates (diameter < 5 nm) are frequently not easily detected in aqueous solutions by light scattering. The aggregates dissociate in organic solvents and cannot be detected by liquid chromatography. Consequently drug does not have to be fully dissociated to generate an analytically clear solution and the experimentally determined solubility represents not only the concentration of monomers (D) but also that of dimers (D_2), trimers (D_3) and other water-soluble drug oligomers (D_n):

$$\text{Molar solubility} = [D] + 2 \cdot [D_2] + 3 \cdot [D_3] + \ldots + n \cdot [D_n] \quad (1)$$

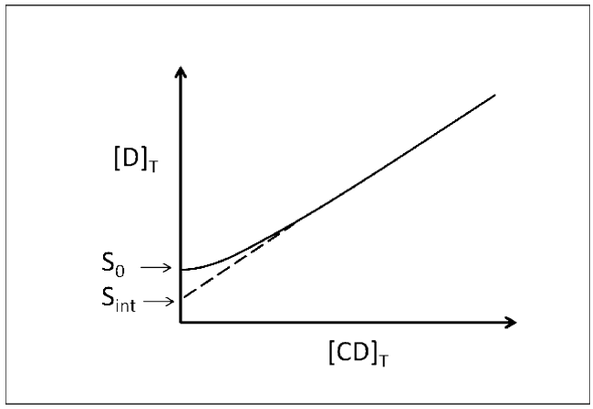
4. Nano-sized drug aggregates and receptor interactions

Even though trace amounts of small drug oligomers are not easily detected in aqueous solutions effects of their presence will be felt during HTS and formulation development. Although dimers and trimers of test molecules can have higher affinity than the monomer for some specific receptors (Nabiev et al. 1998) most activity studies are based on interactions (i.e., substrate-ligand binding) of individual test molecules with some specific receptor and, thus, it is more likely that formation of dimers and other oligomers will result in decreased activity during HTS.

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and somewhat lipophilic central cavity. Cyclodextrins are known to form inclusion complexes with various drugs by taking up some lipophilic moiety of a drug molecule



(A)



(B)

Fig. 1: Linear phase-solubility diagrams, with formation of 1:1 drug/cyclodextrin complex; (A) under ideal conditions where the drug solubility in pure complexation medium (S_0) is equal to the intercept (S_{int})(Higuchi and Connors 1965) and (B) under non-ideal conditions where $S_0 \neq S_{int}$. (Loftsson et al. 2005)

into the cavity. Formation of such drug/cyclodextrin inclusion complexes can be used to mimic non-specific drug-receptor interactions. Most common type of inclusion complexes in dilute aqueous solutions are 1:1 drug/cyclodextrin (D·CD) complexes where one drug (D) molecule forms a complex with one cyclodextrin (CD) molecule (Loftsson and Brewster 1996; Brewster and Loftsson 2007):

$$D + CD \overset{K_{1:1}}{\rightleftarrows} D \cdot CD \tag{2}$$

Cyclodextrin complexes with 1:1 drug:cyclodextrin stoichiometry give linear phase-solubility diagrams in aqueous cyclodextrin solutions:

$$[D]_T = S_0 + \frac{K_{1:1} \cdot S_0}{1 + K_{1:1} \cdot S_0} \cdot [CD]_T \tag{3}$$

Where $[D]_T$ is the total concentration of dissolved drug, S_0 is the solubility of the drug when no cyclodextrin is present, $K_{1:1}$ is the stability of the complex and $[CD]_T$ is the total cyclodextrin concentration (Higuchi and Connors 1965). When one drug molecule forms a complex with one cyclodextrin molecule the intercept (S_{int}) of the phase solubility diagram should be equal to the solubility (S_0) of the drug in the complexation media (Fig. 1). This is indeed the case when the drug solubility is greater than about 0.1 mg/ml. However, sharp deviation is observed when the aqueous solubility is less than about 0.05 mg/ml (Fig. 2) (Loftsson et al. 2005, 2007). These observations suggest that

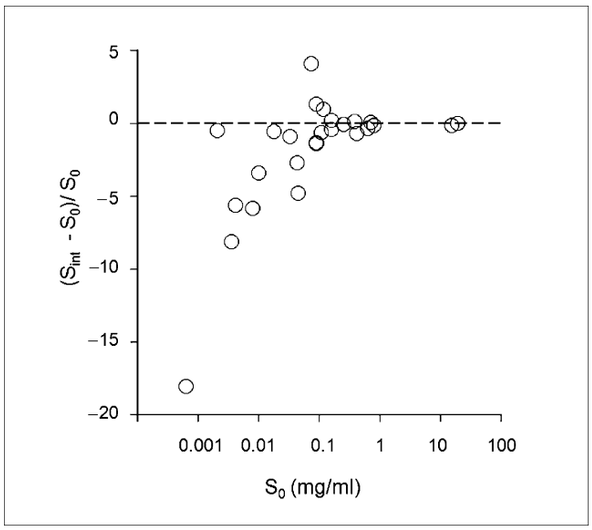


Fig. 2: Plot of the experimentally determined solubility (S_0) versus the relative molar deviation of the intercept (S_{int}) obtained from phase-solubility studies (see Fig. 1) at ambient temperature. (Loftsson et al. 2005, Loftsson et al. 2007) Theoretically S_0 should be equal to S_{int} and all the points should follow the dashed line. The figure contains data from phase-solubility studies of 26 different drugs (mean molecular weight 348.5 Da, range 178.3 to1202.6 Da; mean melting point 182 °C, range 19 to 293 °C) in pure water or dilute aqueous buffer solution

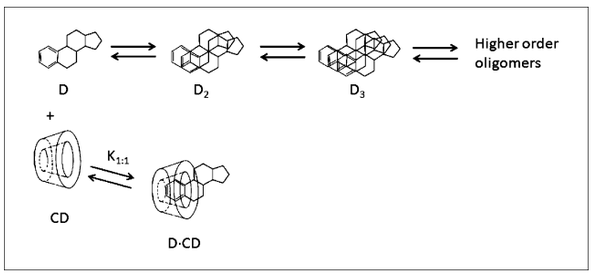


Fig. 3: Formation of drug/cyclodextrin complex in presence of drug dimers (D_2), trimers (D_3) and higher order aggregates that have negligible affinity for the cyclodextrin cavity

for practically insoluble drugs (solubility < 0.05 mg/ml) linear phase-solubility diagrams (Fig. 1) are shifted to right away from the Y-axis, and the further away the lower S_0 is. Such a shift is observed if the dissolved drug molecules in saturated solution are mainly in the form of aggregates that have lower affinity for cyclodextrins than the individual dissolved drug molecules (Fig. 3). Majority of new drug candidates under development are extremely water-insoluble with third of the compounds possessing water-solubility of less than 10 μg/ml and solubility less than 1 μg/ml is not uncommon (Li et al. 2009).

5. Conclusions

Currently applied methods for solubility determinations do not distinguish between true solutions, where individual solute molecules are fully dispersed in the aqueous media, and solutions of aggregated solute molecules forming water-soluble dimers, trimers and small oligomers. Formations of such aggregates can however influence both biological and physiochemical characterization of compounds during screening for new drug candidates. Novel methods that determine true drug solubility could possible improve the efficacy of HTS for new drug candidates.

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