Thermodynamics of Highly Supersaturated Aqueous Solutions of Poorly Water-Soluble Drugs—Impact of a Second Drug on the Solution Phase Behavior and Implications for Combination Products

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ABSTRACT: There is increasing interest in formulating combination products that contain two or more drugs. Furthermore, it is also common for different drug products to be taken simultaneously. This raises the possibility of interactions between different drugs that may impact formulation performance. For poorly water-soluble compounds, the supersaturation behavior may be a critical factor in determining the extent of oral absorption. The goal of the current study was to evaluate the maximum achievable supersaturation for several poorly water-soluble compounds alone, and in combination. Model compounds included ritonavir, lopinavir, paclitaxel, felodipine, and diclofenac. The "amorphous solubility" for the pure drugs was determined using different techniques and the change in this solubility was then measured in the presence of differing amounts of a second drug. The results showed that "amorphous solubility" of each component in aqueous solution is substantially decreased by the second component, as long as the two drugs are miscible in the amorphous state. A simple thermodynamic model could be used to predict the changes in solubility as a function of composition. This information is of great value when developing co-amorphous or other supersaturating formulations and should contribute to a broader understanding of drug–drug physicochemical interactions in *in vitro* assays as well as in the gastrointestinal tract. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2583–2593, 2015

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INTRODUCTION

It has been estimated that up to 75% of emerging drug candidates have suboptimal aqueous solubility, leading to issues with biological testing and *in vivo* delivery.[1] Various approaches thus need to be taken in order to solubilize the drug to enable testing and product development. In the lab, these typically involve the use of an organic solvent in which the compound is soluble such as dimethyl sulfoxide, followed by a large dilution into an aqueous medium. In the context of *in vitro* assays where the drug is introduced to the test solution from an organic solvent, it has been observed that phase separation may occur, resulting in the formation a non-crystalline drug-rich colloidal phase. This phenomenon has been termed promiscuous aggregation and has received widespread attention since the formation of drug aggregates are thought to be responsible for many of the false results obtained during high-throughput enzymatic screening of compound activity.[2] Studies have also suggested that the formation of aggregates reduces the cytotoxicity of anticancer drugs.[3] These negative effects of aggregate formation have led to the development of screens for promiscuous aggregation behavior.

In contrast, it has been suggested that the formation of colloidal aggregates may be favorable for oral drug delivery. Frenkel et al.[4] observed that anti-HIV compounds that formed colloidal aggregates of 100 nm or less had a higher than expected oral bioavailability and suggested that the small aggregates improved uptake across the gastrointestinal tract. Friesen et al.[5] noted that high energy amorphous dosage forms form colloidal species upon dissolution and that these could improve the bioavailability of poorly water-soluble drugs. Clearly, the formation of colloidal drug aggregates is important in a number of different areas of drug discovery and delivery and therefore it is important to understand the physical chemistry of these systems.

Recently, it has been demonstrated that aggregate formation occurs in supersaturated solutions when the concentration of the compound in solution exceeds the solubility of its amorphous form.[6,7] At these concentrations, which are typically 5–50× the crystalline solubility,[8] the miscibility limit of the liquid form of the compound is exceeded and liquid–liquid phase separation (LLPS) occurs leading to the formation of a drug-rich phase of initially colloidal dimensions.[2,6] The term drug-rich phase is thus probably more appropriate than the less specific term "drug aggregates," and this terminology will be adopted henceforth. Because the stable form of most compounds of interest is crystalline at room temperature, the drug-rich phase is a water-saturated supercooled liquid[7] or a glass[9] and crystallization can subsequently occur. For some compounds, LLPS will never be observed as compounds will crystallize directly.[10] For other compounds, the duration of the non-crystalline drug-rich phase will be highly dependent on the compound[10,11] and the composition of the medium.[6,11] For low melting point compounds that are liquid at room temperature, LLPS can still occur, but crystallization will not occur. The "amorphous solubility" (and thus the concentration where the drug-rich phase will form) can be estimated from the crystal solubility and the free energy difference between the crystal and supercooled liquid.[8]

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Excellent agreement has been observed between the predicted "amorphous solubility" and the concentration at which the drug-rich phase forms for a number of compounds.[8] LLPS in aqueous media has been observed to occur upon dilution of a drug solubilized in an organic solvent,[7–9] as a result of a decrease in solubility due to a pH change,[12] following dissolution of amorphous solid dispersion formulations,[6] and by changing solubility due to temperature changes.[7]

The formation of a drug-rich phase has important implications on the thermodynamics of the solution. When the drug-rich phase forms, the free drug concentration in the bulk phase reaches a maximum.[13] Addition of more compound increases that amount of the drug-rich phase, but not the concentration in the bulk aqueous phase. Given that many chemical processes are driven by free drug concentration (more precisely the chemical potential of the solute which is a constant in an equilibrated two phase system) rather than the total concentration, the formation of the drug-rich phase can result in response versus concentration plots that are non-linear. For example, there is a maximum in the transport rate of a drug across a semipermeable membrane that coincides with the concentration where the drug-rich phase first forms.[13] In addition, the drug-rich phase can interact with additional species that are present in solution. Thus, it has been suggested that enzymes adsorb to the surface of the nanosized drug-rich phase, leading to their inactivation.[14] In addition, it has been found that environment sensitive fluorescence probes register a more hydrophobic environment in the presence of the drug-rich phase relative to in a single-phase aqueous solution, which indicates that the probes preferentially mix with the drug-rich phase.[7] This latter observation has considerable potential implications for drug evaluation and delivery if a similar mixing of a second drug species were to occur. For example, combination drug products such as Kaletra™, which contains the anti-HIV agents, ritonavir and lopinavir, as an amorphous solid dispersion, are increasingly being used to improve therapeutic efficacy as well as patient compliance. Furthermore, it is common that two or more different drugs are administered at the same time as separate dosage forms, resulting in both species being present in solution together following dissolution. In addition, for some types of *in vitro* drug screening such as metabolic assays, it is common to use a substrate molecule in combination with the new compound under evaluation. If one compound readily forms a drug-rich phase, and the critical concentration for LLPS is exceeded, then the second species might mix with the drug-rich phase, leading to a change in the free drug concentration of the second species, which may then impact its biological activity.

The goal of this study was to evaluate the formation of drug-rich phases in solutions containing more than one solute, and to evaluate the impact of a second solute on the thermodynamics of the solution, in particular in terms of the free drug concentration of each species. The hypothesis to be evaluated is that the free drug concentration of a given compound will be reduced in the presence of a second compound that readily undergoes LLPS to form a drug-rich phase. Ultracentrifugation was used to remove the drug-rich phase and free drug concentration was evaluated by HPLC analysis of the supernatant. A side-by-side diffusion cell was used to study the transport rate of solutes across a semi-permeable membrane and to evaluate the thermodynamic activity of solutes in solutions containing a drug-rich phase. Model systems included ritonavir and lopinavir, and ritonavir and paclitaxel. These systems were chosen because ritonavir is often dosed concomitantly with other agents because of its potent inhibition of cytochrome P450 CYP3A4, which leads to a bioavailability boost for the second compound if it is a substrate for this enzyme.[15] Ritonavir readily forms a drug-rich phase when a certain concentration is exceeded.[7]

MATERIALS AND METHODS

Materials

Ritonavir, lopinavir, felodipine, and paclitaxel were purchased from Attix Pharmaceuticals (Montreal, Canada). Diclofenac was purchased from Spectrum chemicals (New Brunswick, New Jersey). Hydroxy propyl methyl cellulose (HPMC) was a gift from Shin-Etsu Chemicals (Niigata, Japan). The structures of the compounds used are given in Figure 1. A dialysis membrane made of regenerated cellulose with a molecular weight cutoff size of 6000–8000 kDa, purchased from Spectrum Laboratories Inc., (Rancho Dominguez, California) was used in the diffusion cell. Paclitaxel appeared to be a non-stoichiometric hydrate and had around 3.6% moisture at ambient conditions. Stock solutions of the drugs were prepared in methanol. Felodipine was selected as a model compound to investigate the effect of a compound that forms a drug-rich phase at a very low concentration, whereas diclofenac (p*K*a 4.2)[16] was used as it is ionized at the pH used in these studies. The media used for the diffusion studies was 100 mM phosphate buffer, pH 6.8 to which 10 μg/mL of HPMC was added to prevent crystallization for the duration of the experiment.

Methods

Crystalline and Amorphous Solubility Determination

The equilibrium solubility of ritonavir, lopinavir, and paclitaxel was determined by adding an excess of the crystalline material to 15 mL of 100 mM phosphate buffer, pH 6.8, and placing the resultant suspensions in a shaker bath at 25°C for 2 days. The supernatant was separated from the solid material by ultracentrifugation and the concentration of the drug was determined using HPLC as described below. The concentration where LLPS occurred, resulting in the formation of a drug-rich phase, which is in close agreement with the "solubility" of the amorphous form of the compound,[7] was determined by three different methods.

1. Ultracentrifuge method: 150 μL of a 10 mg/mL methanolic solution of the drug was added to 15 mL of 100 mM phosphate buffer, pH 6.8 containing 10 μg/mL HPMC, at 25°C with stirring. HPMC (10 μg/mL) was added to avoid nucleation and crystallization of the drugs for the duration of the experiment. The solution was then centrifuged at 40,000 rpm (274,355×g) to pellet the drug-rich phase using an Optima L-100 XP ultracentrifuge (Beckman Coulter Inc., Brea, California) with a SW 41 Ti swinging bucket rotor attachment. Supernatant (1 mL) was then diluted with methanol to twice the volume and then analyzed by HPLC as described below. Ultracentrifuge experiments were also undertaken in the absence of polymer to see if there is any effect of polymer concentration on the concentration where the drug-rich phase formed. For ritonavir and lopinavir, no change in