# Enhancements and Limits in Drug Membrane Transport Using Supersaturated Solutions of Poorly Water Soluble Drugs

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**ABSTRACT:** Amorphous solid dispersions (ASDs) give rise to supersaturated solutions (solution concentration greater than equilibrium crystalline solubility). We have recently found that supersaturating dosage forms can exhibit the phenomenon of liquid–liquid phase separation (LLPS). Thus, the high supersaturation generated by dissolving ASDs can lead to a two-phase system wherein one phase is an initially nanodimensioned and drug-rich phase and the other is a drug-lean continuous aqueous phase. Herein, the membrane transport of supersaturated solutions, at concentrations above and below the LLPS concentration has been evaluated using a side-by-side diffusion cell. Measurements of solution concentration with time in the receiver cell yield the flux, which reflects the solute thermodynamic activity in the donor cell. As the nominal concentration of solute in the donor cell increases, a linear increase in flux was observed up to the concentration where LLPS occurred. Thereafter, the flux remained essentially constant. Both nifedipine and felodipine solutions exhibit such behavior as long as crystallization is absent. This suggests that there is an upper limit in passive membrane transport that is dictated by the LLPS concentration. These results have several important implications for drug delivery, especially for poorly soluble compounds requiring enabling formulation technologies. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2736–2748, 2014

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## INTRODUCTION

Low aqueous solubility of emerging drug candidates is a major hurdle in drug development. Many of the small molecules showing therapeutic activity against biological targets of interest have very low aqueous solubility either because they are lipophilic or have a high melting point, or because of the combined effect of these two factors. Therefore, there is a great deal of interest in formulating such compounds into dosage forms that can supersaturate.[1–3] It has been noted that these lipophilic small molecules, when present at elevated supersaturations in aqueous media, can form colloidal drug aggregates that can subsequently coalesce leading to an increase in size, and/or crystallize.[4–10]

Colloidal aggregates of small molecules are routinely observed in the high-throughput enzymatic screens employed during drug discovery and are formed when concentrated aqueous solutions of drugs are generated by the solvent shifting method.[7] In this method, a small aliquot of a concentrated solution of the drug, dissolved in an organic solution, is added to an aqueous solution to generate the desired concentration, often resulting in a highly supersaturated solution. A supersaturated solution is one where the concentration (or more rigorously, the chemical potential of the solute) exceeds that of a solution saturated with respect to the crystalline solid. These aggregates have attracted attention because they frequently lead to false positives in enzyme-based assays via nonspecific protein inhibition. Aggregation may also lead to false negatives as molecules in an aggregated state may be unable to interact with proteins. Compounds undergoing this phenomenon have been termed "promiscuous aggregators." Studies by Doak et al.,[11] Frenkel et al.,[4] along with several other research groups have demonstrated that an array of active pharmaceutical ingredients (APIs) form nanosized aggregates. The formation of colloidal aggregates is concentration dependent and occurs only above a critical concentration termed the critical aggregate concentration. The formation of colloidal species has also been reported to occur following dissolution of amorphous solid dispersions (ASDs). One of the earliest studies reported the formation of colloidal species after dissolving a dispersion of polyvinyl pyrrolidone (PVP) and β-carotene.[12] ASDs prepared with hydroxypropylmethyl cellulose acetate succinate (HPMCAS) are also reported to generate colloidal species.[13] Other systems where colloid formation has been noted include felodipine and hydroxypropylmethlyl cellulose (HPMC),[14] mangostin and PVP,[15] and lopinavir/ritonavir formulations with copovidone.[16] Recent studies have demonstrated that the formation of colloidal species upon dissolution of an amorphous dispersion only occurs when a critical concentration of dissolved drug has been achieved.[17] The formation of these colloidal species has been postulated to be beneficial for oral drug delivery.[13]

It has been suggested that the underlying phenomenon causing the formation of colloidal aggregates, either from the solvent

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switch method, or from dissolution of an ASD, is liquid–liquid phase separation (LLPS).[7,17] LLPS can also occur from other supersaturation generating systems for example during lipolysis of a self-microemulsifying drug delivery system[18] or as a result of pH changes.[19] Highly supersaturated solutions will undergo LLPS when a certain threshold concentration is exceeded whereby a homogeneous one-phase system separates into two liquid phases. LLPS is also referred to as oiling-out or liquid–liquid demixing.[20–27] LLPS has been widely observed in a variety of systems including polymer blends, proteins, metals, and is often prevalent, albeit undesired, during industrial crystallization.[24,27,28] Svärd et al.[29] reported LLPS for the water–vanillin system. Kiesow et al.[30] reported LLPS during the crystallization of 4-4′-dihydroxydiphenylsulfone and used modeling to predict its occurrence. In the context of aqueous solutions of hydrophobic drugs with melting points above the experimental temperature, LLPS is the separation of a supersaturated drug solution into two liquid phases, one of which is drug rich and hydrophobic, that is, colloidal aggregates that predominantly consist of the drug, whereas the other phase is water rich and contains only a low concentration of drug.[17] The two phases are in dynamic equilibrium, although both phases are thermodynamically metastable and the system is supersaturated.

In formulated products, additives such as polymers, surfactants, complexing agents, and lipids are routinely used to improve solubility and dissolution profiles *in vitro* in the hope that this will enhance systemic drug concentrations *in vivo*. Often, extremely high-concentration enhancements are reported based on approaches that involve assaying the solution phase. This approach of measuring solution concentration can be problematic when attempting to predict *in vivo* performance because it does not discriminate between the two mechanisms of achieving enhanced solution concentrations: supersaturation versus solubility enhancement. True supersaturation occurs when there is an increase in the chemical potential of the solute relative to a saturated solution in which the solute chemical potential is the same as that of the crystalline solid. For example, polymers at low concentrations do not typically enhance equilibrium solubility,[31] but enable supersaturated solutions to be generated and maintained after dissolving an amorphous formulation by inhibiting crystallization from supersaturated solutions. In contrast, micellar surfactant, pH adjustment, cyclodextrins, and cosolvents increase the equilibrium solubility of the crystalline solid (although supersaturation may be generated when the resultant system is diluted). As the crystalline solid is in equilibrium with the solution, it is clear that an increase in solution concentration can be achieved without an increase in the chemical potential of the solute. Because membrane transport is driven by the chemical potential gradient,[32] it is important to consider the thermodynamic properties of the solution rather than the total solution concentration. Thus, it is well established that membrane transport can be decreased by the presence of micellar surfactant,[33] cyclodextrins,[34] but is increased by supersaturated solutions as long as crystallization is prevented.[33,35–41] Although most of the studies on the relationship between membrane transport and supersaturation have been in the context of transdermal delivery, there is an increasing interest in exploiting supersaturated solutions to enhance oral absorption. Recent studies have demonstrated enhanced flux across intestinal membranes when perfused with supersaturated solutions.[40–45] However, the impact of the colloidal species generated in highly supersaturated solutions has not been fully evaluated to date. Diffusion data from Alonzo et al.[46] suggested that the flux reached a maximum when concentrations equal to or above the "amorphous solubility" were generated. Thus, at very high concentrations in the supersaturated regime, it appears that there may be a breakdown in the relationship between concentration and diffusion rate.

The goal of the current study was to demonstrate that small organic molecules dissolved in aqueous solution at concentrations at and above their amorphous solubility undergo LLPS, and that the LLPS concentration corresponds to the maximum achievable diffusive flux for a supersaturated solution. Felodipine and nifedipine were selected as model drugs to evaluate the impact of supersaturation and LLPS on membrane transport. The approach of Corrigan et al.[35] was utilized, whereby crystallization inhibitors were added to the donor solution to sustain supersaturation for sufficient time to enable diffusion measurements to be made; the polymer, hydroxypropyl methyl cellulose was used as the crystallization inhibitor. Phase diagrams were constructed to understand the concentrations at which liquid–liquid and liquid–solid phase transformations occurred.

## MATERIALS

Felodipine and nifedipine were purchased from Attix Pharmaceuticals (Toronto, Ontario, Canada) and Euroasia (Mumbai, India), respectively. HPMC Pharmacoat grade 606 was obtained from ShinEtsu (Shin-Etsu Chemical Company, Ltd., Tokyo, Japan). Dissolution media used in all experiments comprised 50 mM pH 6.8 phosphate buffer (ionic strength = 0.155 M) without or with predissolved polymer at a concentration of 100 μg/mL and 1 mg/mL for felodipine and nifedipine, respectively. Methyl alcohol was purchased from Pharmco Products, Inc., Brookfield, Connecticut. Molecular structures of the model compounds are shown in Figure 1. Regenerated cellulose membrane with a molecular weight cutoff (MWCO) of 6–8 K was obtained from Spectrum Laboratories, Inc. (Rancho Dominguez, California).

## METHODS

### Crystalline Solubility Measurements

Equilibrium crystalline solubility was determined using a modification of the shake flask method. An excess of crystalline felodipine and nifedipine was equilibrated in 20 mL scintillation vials with 50 mM pH 6.8 phosphate buffer in an agitating water bath (Dubnoff metallic shaking incubator; PGC Scientific, Palm Desert, California). Vials were wrapped in aluminum foil to protect the samples from light. Preliminary experiments indicated that equilibrium was reached by 48 h. Samples were agitated for 48 h at 20°C, 25°C, 30°C, 37°C, and 45°C before subjecting them to ultracentrifugation to separate excess solid from the supernatant (which is saturated with drug). An Optima L-100 XP ultracentrifuge equipped with Swinging-Bucket Rotor SW 41 Ti (Beckman Coulter, Inc., Brea, California) was used and samples were centrifuged at 274,356*g* for 15 min. The supernatant obtained was diluted with acetonitrile: 50 mM phosphate buffer (50:50) in a 1:1 ratio and 200 μL samples were injected into an Agilent 1100 high-performance liquid chromatography system (Agilent Technologies, Santa Clara, California). The chromatographic separation was performed with