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General Commentary

Trends in the Assessment of Drug Supersaturation and Precipitation *In Vitro* Using Lipid-Based Delivery Systems

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The generation of drug supersaturation close to the absorptive site is an important mechanism of how several formulation technologies enhance oral absorption and bioavailability. Lipid-based formulations belong to the supersaturating drug delivery systems although this is not the only mechanism of how drug absorption is promoted *in vivo*. Different methods to determine drug supersaturation and precipitation from lipid-based formulations are described in the literature. Experimental *in vitro* setups vary according to their complexity and proximity to the *in vivo* conditions and, therefore, some tests are used for early formulation screening, while others better qualify for a later stage of development. The present commentary discusses this rapidly evolving field of *in vitro* testing with a special focus on the advancements in analytical techniques and new approaches of mechanistic modeling. The importance of considering a drug absorption sink is particularly emphasized. This commentary should help formulators in the pharmaceutical industry as well as in academia to make informed decisions on how to conduct *in vitro* tests for lipid-based delivery systems and to decide on the implications of experimental results.

Introduction

Poor water solubility is a main hurdle regarding the selection and development of new drug candidates in the pharmaceutical industry, and a key strategy to cope with solvation-limited drugs[1] is the use of lipid-based formulations (LBFs).[2-6] LBFs can promote drug absorption via different mechanisms, including drug solubilization in the gastrointestinal (GI) lumen and increase in drug permeation across the intestinal membrane.[3,7] A key mechanism is the promotion of drug supersaturation, which occurs after LBF dispersion in the aqueous environment and when formulation components are digested by intestinal lipases.[8-10] LBFs include different types of formulations,[11,12] most of which are supersaturating drug delivery systems (SDDS). SDDS is an umbrella term that also includes, for example, nanoparticulate formulations and amorphous solid dispersions.[13,14]

The degree of supersaturation is typically expressed as (super-) saturation ratio, *SR*, which is the ratio of the concentration of solubilized drug, C_{sol}, and the equilibrium solubility, C^*:

$$SR = \frac{C_{sol}}{C^*} \tag{1}$$

SR values larger than 1 generally indicate thermodynamically unstable solutions, for which precipitation will occur at least on a long time scale. Whether drug precipitation takes place on a shorter time scale, such as during GI transit, is determined by the kinetics of nucleation and growth. Supersaturation is a direct measure of the chemical potential difference in solution and in the bulk of the crystal phase, $\Delta\mu$.[15]

$$\Delta\mu = kT \ln(SR_a) \tag{2}$$

where k is the Boltzmann constant and T the temperature. Equation 2 includes the term SR_a, which corresponds to the supersaturation ratio calculated with chemical activities instead of concentrations. Equation 1 therefore provides an apparent value of drug supersaturation, which is expected to be a reasonable approximation for SR_a in most cases. However, some care is needed with very high supersaturation close to a liquid–liquid phase separation (LLPS).[16] Such spontaneous “oiling out” of hydrophobic drug from aqueous media is due to the strong driving force toward demixing. Recent drug diffusion studies across membranes demonstrated that the transport flux increased linearly with increasing drug concentration,

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but abruptly changed or leveled off beyond the LLPS.[17] The range around the LLPS showed clear changes in the thermodynamic activity, which would not be predicted from the apparent *SR* based on nominal concentrations as obtained using Equation 1.

Different types of LBFs have different potential to generate drug supersaturation.[18] The more hydrophilic LBFs typically promote drug supersaturation by the phase changes occurring during dispersion in aqueous media.[10] Formulations containing digestible lipids can additionally be hydrolyzed by intestinal lipases, which may trigger apparent drug supersaturation by reducing the solubilization capacity of lipidic excipients. This risk of drug precipitation is even more pronounced for basic compounds, for which the pH shift between stomach and intestine can be a general cause of excessive drug supersaturation.[8,18] All these mechanisms contribute differently to the overall supersaturation depending on the type of LBF. A special kind of self-emulsifying drug delivery system (SEDDS) was given the prefix "supersaturable" or S-SEDDS.[19-21] This approach targets a thermodynamically stable formulation that results in high drug supersaturation during formulation dispersion and digestion. To circumvent drug precipitation, such formulations contain polymers that help in maintaining the supersaturated state during GI transit.[22,23] It is important to differentiate this formulation approach from supersaturated self-nanoemulsifying drug delivery systems (super-SNEDDS).[22-24] The latter formulations are thermodynamically unstable because the drug is already supersaturated in the lipid mixtures. Such a formulation was shown to increase the oral bioavailability of the poorly soluble drug simvastatin in dogs.[23]

Several *in vitro* methods have been developed to simulate formulation dispersion and digestion under biorelevant conditions. However, the prediction of *in vivo* performance is still rather poor leading only occasionally to a rank-order level of correlation. For many years, it was assumed that *in vitro* drug precipitation of poorly soluble drugs would likely translate to an impaired oral absorption *in vivo* because of the slow redissolution of the precipitate in the GI tract. However, the drug may precipitate in a high-energy form like an amorphous state and thus redissolve quite rapidly. In light of several reports pointing to amorphous drug precipitates from LBFs,[22,24-26] one has to reconsider the dogmatic paradigm that *in vitro* precipitation is always an indicator for erratic formulation performance. Researchers in industry and academia are thus encouraged to investigate the solid state of a drug precipitate as part of *in vitro* formulation assessment.

Current trends in *in vitro* testing include solid state analysis of a drug precipitate and the use of novel analytical tools for real-time analysis of drug precipitation.[27] Finally, another important current discussion concerns the relevance of *in vitro* supersaturation and precipitation data given that in the intestinal lumen a drug is continuously removed via absorption, which thereby reduces the risk of excessive supersaturation followed by precipitation. Mathematical modeling may help here to gain a better understanding of factors that are difficult to study with an *in vitro* experiment.

The current trends in supersaturation and precipitation testing of LBFs will be discussed in the present article. A summary of findings of the "Lipid Formulation Classification System" (LFCS) research consortium[28] will provide an overview of current *in vitro* assays for LBF testing. A particular objective of this commentary is to facilitate the interpretation of *in vitro* findings to support researchers in elucidating adequate LBFs for oral delivery of biopharmaceutically challenging drugs.

Overview of In Vitro *Drug Release Tests for LBFs*

The main goal of *in vitro* LBF testing is to evaluate the capacity of a formulation to maintain the drug in a dissolved state during formulation dispersion and digestion, which is different from drug release testing using standard oral formulations. *In vitro* tests for LBFs simulate the process of formulation dispersion and digestion under gastric and intestinal conditions. However, there exist different levels of complexity for such assessment, which has not only historical reasons but also makes sense from a development viewpoint. Simple formulation dilution in water or biorelevant media[29,30] offers the advantage of miniaturization and high-throughput testing. Such dilution experiments have become a standard test for most research groups.[10,31-36] Experiments are conducted under mild agitation at either room temperature or 37°C, and different dilution ratios can be applied.[10,32-36] A high formulation dilution level (e.g., 1:200 w/w) is meaningful to simulate the final dilution in human GI fluids and helps in identifying early formulation candidates. Alternatively, a low dilution level (<1:5 w/w) may be of interest regarding the potential of drug precipitation in a bicontinuous formulation system, which is transiently formed during the dilution process.[37] Other specific dilution ratios can be considered during preclinical screening of LBFs for toxicological or pharmacokinetic studies.

Dilution tests provide information about the self-emulsification behavior of LBFs and can flag systems exhibiting drug precipitation. These data should be considered together with excipient properties, such as tolerability, influence on formulation stability, and biological effects on drug transporters or presystemic drug clearance. Such procedure of how to develop a LBF has been suggested by different authors.[7,38] Small-scale dilution screening is generally recommended as initial preselection of formulation candidates. In a second step, it is recommended to evaluate the formulations on a larger scale including dispersion testing in compendial dissolution equipment.

Dispersion tests in a paddle dissolution apparatus provide kinetic analysis of drug release using the final dosage form (e.g., liquid-filled soft or hard capsules). A comparison of such tests with corresponding *in vivo* results can be inferred from Griffin et al.[36] To obtain closer proximity to the *in vivo* situation, it is recommended to study the behavior of lipid-based systems under digestive conditions. Formulation lipolysis is in most cases affecting both phase behavior and solubilization of coadministered drug. Pharmaceutical application of lipolysis testing has already been reported in the late 1980s, but pioneer work at the University of Copenhagen (Denmark) and Monash University (Australia) was the basis for modern lipolysis assays.[39-43] Other research groups have further contributed to the development of *in vitro* lipolysis, and the various experimental conditions have been compared in review articles.[44-51]

It is certainly desirable that *in vitro* tests are comparable from one laboratory to another. For this reason, the LFCS research consortium was created with the main objective to evaluate and harmonize LBF testing protocols. Most work has been dedicated to *in vitro* lipolysis testing where experimental factors such as sampling, drug loading, and bile salt concentrations were evaluated using a series of different LBFs.[52-54] There was a special interest in determining *SR* as a measure of the driving force and hence the probability of intraluminal drug precipitation. Drug solubility was determined at intervals in the aqueous digestion phase (AP_{digest}) and *SR* was estimated as follows[53]:

$$SR = \frac{C_{sol}\left(AP_{digest}\right)}{C^{*}\left(AP_{digest}\right)} \quad (3)$$

where C_{sol} is the solubilized drug in AP_{digest} and C^{*} the corresponding equilibrium solubility. The authors further defined a maximum supersaturation value, SR^{M}, which is the ratio of the