Effects of drug-rich phase properties formed by liquid-liquid phase separation on the drug membrane permeability analyzed using μFLUX*

Keisuke Useda, Graduate School of Pharmaceutical Sciences, Chiba University

*μFLUX has been provided by PHYSIO MCKINA

Research Backgrounds and Objectives

Supersaturated formulations, including amorphous solid dispersion formulations, are widely used to improve the drug dissolution properties. Drug supersaturation in the gastrointestinal tract increases the amount of drug absorbed by passive diffusion, contributing to the improved gastrointestinal absorption of poorly water-soluble drugs. On the other hand, the drug supersaturation level in the bulk water phase has a thermodynamic limit, beyond which the drug cannot molecularly dissolve in the bulk water phase, and the phase separates from the aqueous phase as a drug-rich phase. This is called liquid-liquid phase separation (LLPS) or glass-liquid phase separation (GLPS), and the concentration at which LLPS and GLPS occur reflects the amorphous solubility of the drug. The drug-rich phase formed by LLPS, unlike the drug dissolved in bulk water, cannot permeate the membrane via passive diffusion. Therefore, the passive diffusion of the drug is thought to plateau at its amorphous solubility with no further enhancements possible. However, oral administration studies on supersaturated formulations in animals have shown that forming a drug-rich phase further improves drug absorption. This suggests that the drug-rich phase contributes to absorption improvement by mechanisms other than supersaturation formation in the bulk water phase. The mechanism of improved drug absorption through the formation of a drug-rich phase has been the subject of much debate. One of the debate topics is the Reservoir Effects of the drug-rich phase. When the dissolved drug is absorbed through the membrane in the presence of a drug-rich phase, the drug is supplied from the drug-rich phase, and the drug concentration in the bulk water phase is maintained at amorphous solubility, contributing to improved absorption. In addition, diffusion of drug-rich phase droplets in an unstirred water layer (UWL) has also been reported to contribute to enhanced absorption. A UWL refers to the poorly fluid microenvironment in the vicinity of the gastrointestinal epithelial cells. Diffusion in the UWL is the rate-limiting step for drug absorption, especially for hydrophobic drugs, which are absorbed with extreme rapidity in the gastrointestinal membrane. Diffusion of the drug-rich phase in the UWL is thought to increase the drug concentration in the UWL and contribute to improved absorption.

On the other hand, the effect of drug-rich phase properties on the ability to improve drug absorption still needs to be clarified and has yet to be sufficiently evaluated. Thus, to elucidate the impact of drug supersaturation level and drug-rich phase properties on gastrointestinal absorption, this study evaluated the drug absorption from supersaturated formulations containing drug-rich phases formed with different particle properties using Pion's μFlux.

Experiment

Cilnidipine (CND) was used as a poorly water-soluble model drug and AS-HF and AS-LF grades of hydroxypropyl methylcellulose acetate

succinate (HPMC-AS) as pharmaceutical excipients. 3 mg/mL each of AS-LF and AS-HF was dissolved in the buffer to prepare the HPMC-AS solution, respectively. CND stock solution, in which CND was dissolved in methanol, was added to each HPMC-AS solution to obtain a CND-supersaturated solution.

Results and Discussion

Table 1 shows the CND amorphous solubility in each HPMC-AS solution. The CND amorphous solubility was approximately 1.1 μg/mL in the HPMC-AS-free solution and was comparable in the AS-LF solution. Meanwhile, the CND amorphous solubility in the AS-HF solution was 0.56 μg/mL, about half that of the solution without HPMC-AS. Previous reports have shown that the distribution of polymers such as HPMC-AS in the drug-rich phase reduces the chemical potential of the drug in the drug-rich phase, which in turn reduces the drug concentration, or amorphous solubility, in the bulk water phase at equilibrium. These results suggest that AS-HF was partitioned from the aqueous phase into the CND-rich phase, decreasing the CND amorphous solubility.

Figure 1. shows the appearance of each CND-supersaturated solution prepared at a CND dose concentration of 1000 μg/mL. A whitish turbid appearance was observed in all solutions, with particle formation in each CND-supersaturated solution. The CND-supersaturated solution prepared in AS-HF solution exhibited a bluish color, suggesting the formation of smaller-sized particles. Particle size distribution measurements using dynamic light scattering showed that μm-order particles were formed in the HPMC-AS-free solution, while nanoparticles of about 450 nm were formed in the AS-LF solution and about 90 nm in the AS-HF solution. Polarized light microscopy of the particles formed in each CND-supersaturated solution showed the formation of amorphous droplets that did not exhibit crystallinity. The CND amorphous –solubility is not more than 1.1 μg/mL in each solution, and the prepared CND dose concentration of 1000 μg/mL is sufficiently higher than the CND amorphous solubility. These results indicated that the CND-rich phase was formed via LLPS in each CND-supersaturated solution prepared at a CND dose concentration of 1000 μg/mL.

Table 1. Amorphous solubilities of CND in non-polymer solution, AS-LF solution (3 mg/mL), and AS HF solution (3 mg/mL) at 37°C (n = 3, mean ± S.D.).

	Non-polymer solution	AS-LF solution (3 mg/mL)	AS-HF solution (3 mg/mL)
Amorphous solubility of CND (μg/mL)	1.08 ± 0.03	1.06 ± 0.01	0.56 ± 0.01

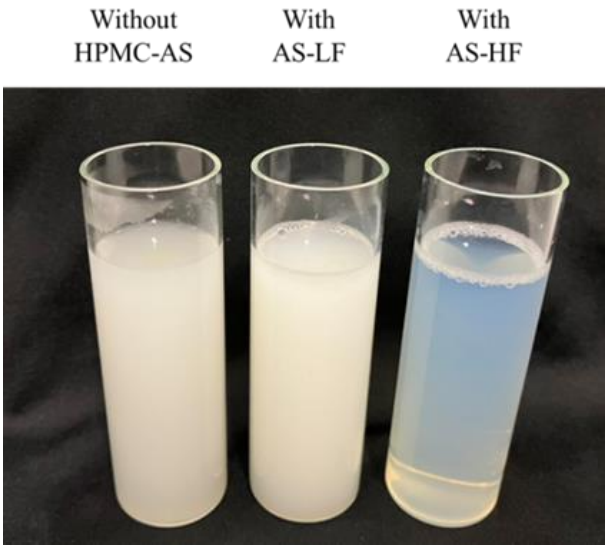

Figure 1. Appearance of CND-supersaturated solution without HPMC-AS, with AS-LF (3 mg/mL), and with AS-HF (3 mg/mL)

CND permeability tests were performed using μFlux to evaluate the membrane permeability of CND from each CND-supersaturated solution.

Figure 2 shows the CND concentration-time profiles on the acceptor side after adding each sample with a CND dose concentration of 1000 μg/mL to the donor side. A linear CND permeation was observed in all the samples after the initial lag time. Compared to the HPMC-AS-free solution, the sample containing HPMC-AS showed a CND permeation improvement, and a remarkable increase in the amount of CND permeation was observed for the AS-HF solution.

In order to evaluate the CND concentration-dependent change in membrane permeation rate, CND-supersaturated solutions were prepared at various CND dose concentrations, CND permeation tests were performed using μFlux, and the CND membrane permeation rate (flux) was calculated. The flux of CND obtained from each test plotted against the CND dose concentration is shown in Figure 3.

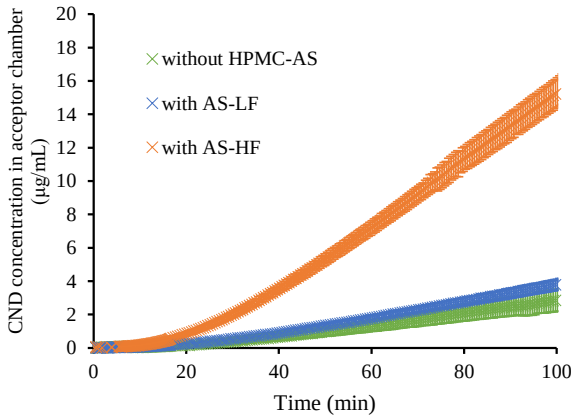

Figure 2. CND concentration in acceptor solution after administration of CND-supersaturated solution without HPMC-AS, with AS-LF (3 mg/mL), and with AS-HF (3 mg/mL) (n = 3, mean ± S.D.).

In the low CND dose concentration range (1.0 to 5.0 μg/mL), the flux increased linearly with CND dose concentration in all solutions. CND exhibited a similar flux in the HPMC-AS-free and AS-LF solutions, while the flux was lower in the AS-HF solution. In the range of CND dose concentrations above 5.0 μg/mL, the CND dose concentration-dependent rate of increase in the flux in HPMC-AS-free solutions was significantly reduced. Similarly, in the AS-LF solution, the flux increase rate with increasing CND dose concentration was decreased in the high CND concentration range. However, the flux increase rate was still greater than in the HPMC-AS-free solution. As a result, the flux from the AS-LF solution became higher than that from the HPMC-AS-free solution in the concentration range above the CND dose concentration of 5.0 μg/mL. Furthermore, in the AS- HF solution, the flux increase rate in the concentration range of CND dose concentration above 5.0 μg/mL was significantly greater than in

the HPMC-AS-free and AS-LF solutions. As a result, the amount of CND permeated from the AS-HF solution was significantly higher in the high CND concentration range.

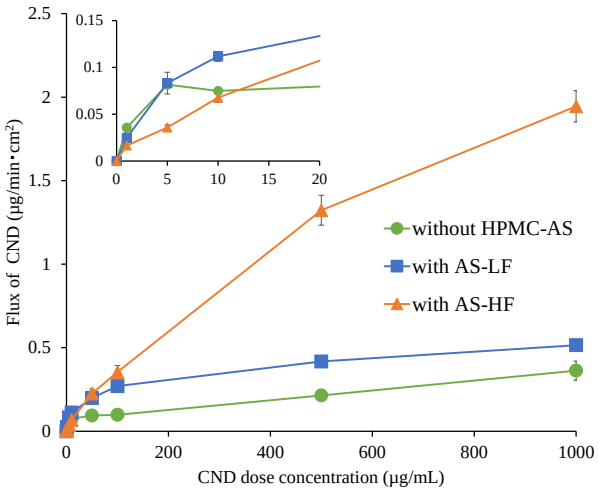

Figure 3. Mass frow rate of CND across PAMPA membrane with each CND-supersaturated solution prepared with various CND dose concentrations at 37 ℃ (n = 3, mean ± S.D.). Enlarged profiles at the lower CND dose concentration range are represented in the figure

In a parallel artificial membrane permeability assay (PAMPA), the flux of a drug is known to vary depending on the diffusibility in the UWL present in the donor and acceptor solutions in the lipid membrane and partitioning properties to the lipid membrane. CND is highly hydrophobic and has a high affinity for PAMPA membranes, so the rate of CND translocation near the membrane is exceptionally high. From this, the diffusibility of CND in the UWL is assumed to be the rate-limiting step for CND absorption. Figure 4 shows a schematic of the CND concentration in the UWL in the donor solution based on the results obtained from μFlux. As mentioned above, for hydrophobic drugs such as CND, the drug membrane migration rate is sufficiently faster than the diffusion rate of CND in the UWL. This creates a concentration gradient of CND in the UWL and reduces the CND concentration in the vicinity of the membrane compared to the concentration of CND dissolved in the bulk water. When the drug-rich phase is dispersed in the solution on the donor side, the drug-rich phase diffuses in the UWL, reducing the concentration gradient in the UWL and increasing the flux of the drug in the PAMPA. On the contrary, the diffusibility of the colloidal drug-rich phase is lower than that of the drug in a dissolved state. In environments where the drug dose concentration is near amorphous solubility, i.e., where the amount of drug-rich phase is not so high relative to the dissolved drug concentration, membrane permeability in the PAMPA would depend more on the amount of dissolved drug. Similarly, in this study, at CND dose concentrations of 1.0 to 5.0 μg/mL, the flux value of CND from the AS-HF solution was about half those from the HPMC-AS-free and AS-LF solutions. In the AS-HF solution, the CND amorphous solubility is shown to be about half that of the other two samples (Table 1). These results indicate a decrease in dissolved CND concentration in the vicinity of the PAMPA membrane, resulting in a reduction in CND flux within the AS-HF solution (on the left side in Figure 4). In the CND dose concentration range above the CND amorphous solubility, the CND concentration dissolved in the bulk water reaches a plateau at the CND amorphous solubility, even if the CND dose concentration is increased. On the other hand, as long as CND crystallization does not occur, the amount of the CND-rich phase increases with increasing CND dose concentration. The increase in the amount of the CND-rich phase is thought to increase the contribution of the CND-rich phase to membrane permeation. The μFlux

test results show that the increased rate of CND flux in the concentration range above 5.0 μg/mL of CND dose concentration is particularly large in the AS-HF solution. As a result, in the range of high CND dose concentrations, the order of flux values was AS-HF solution > AS- LF solution > HPMC-AS-free solution. In general, the smaller the particle diameter, the greater the diffusibility of the colloidal particles. The size of the CND-rich phase formed in each CND-supersaturated solution was maintained in the nano-scale size under the coexistence of HPMC-AS. Especially in the AS-HF solution, the CND-rich phase has been recognized to form on tens of nanometers. Based on the size of the CND-rich phase in each solution, the diffusibility of the CND-rich phase in the UWL is expected to increase in the order of HPMC-AS-free solution < AS-LF solution < AS-HF solution. In comparison to the HPMC-AS-free solution, the reason for the increase in flux in the AS-LF solution in the high CND dose concentration range is thought to be that the nanosized CND-rich phase in the AS-LF solution diffused effectively in the UWL and increased the CND concentration in the vicinity of the PAMPA membrane (on the right side in Figure 4). In addition, the effect of increased flux due to diffusion of the CND-rich phase in the UWL is particularly strong in the AS-HF solution, where a CND-rich phase of several tens of nm is formed. This may counteract the effect of reduced membrane permeation due to the decrease in CND amorphous solubility, resulting in significant improvement of the flux from the AS-HF solution in the high CND concentration range.

Conclusion

Drug permeation studies using μFlux showed that the size of the drug-rich phase formed by LLPS strongly affects the membrane permeability of the drug. This study indicates that utilizing more highly supersaturated formulations capable of forming nanosized drug-rich phases improves drug absorption beyond the absorption improvement performance expected from the achievable supersaturation level of the drug.

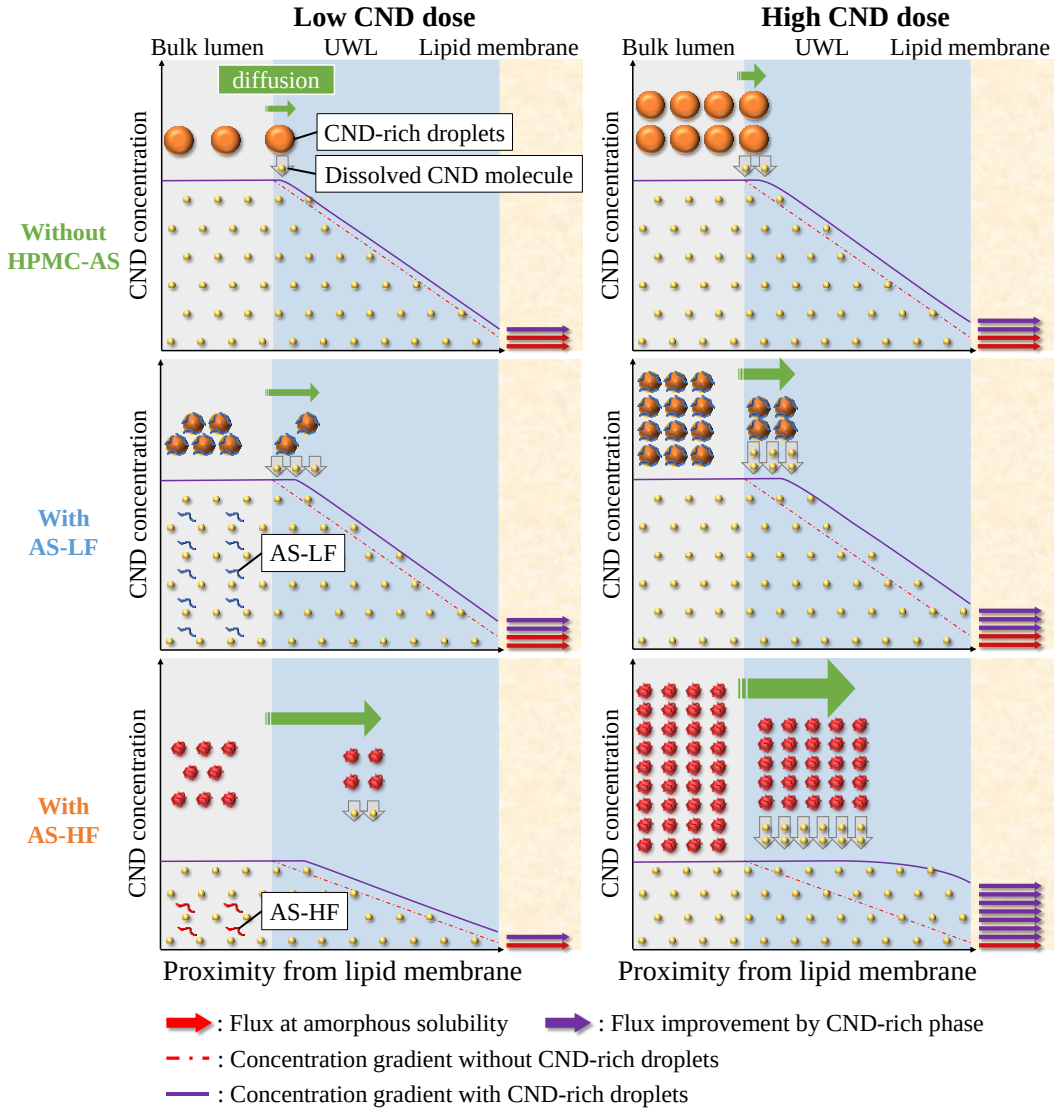

Figure 4. Speculated mechanism of flux improvement of CND by the formation of CND-rich phase in the bulk water phase.

PHYSIO MCKINA Co., Ltd. www.physiomckina.co.jp
Koshigaya Yayoi BLDG. 2F, Yayoi-cho 1-4, Koshigaya-SHI, SAITAMA, 343-0816, JAPAN
TEL : +81-50-3536-1817 FAX : +81-48-964-9930 E-mail : contact@physiomckina.co.jp