Flux Analysis of Non-steady States in Dissolution-Membrane Permeation Studies of Poorly Soluble Drugs

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PURPOSE

The Dissolution-Membrane Permeability assay is an *In-Vitro* test in which a dissolution Donor Chamber is separated by a semi-permeable artificial membrane or a biological membrane from an absorption Acceptor Chamber. Dissolution-Membrane Permeability assays are widely utilized during pharmaceutical development to predict *in vivo* gastrointestinal uptake of poorly soluble drugs.(1)

Many solubilization techniques for poorly soluble compounds lead to complex solution conditions such as supersaturation occurring within the dissolution test solution. When a supersaturation phenomenon occurs, the concentration of the compound in the solution increases above its equilibrium solubility, then decreases to around the saturation concentration over time. In a Dissolution-Membrane Permeability test, the flux can also change over time with the change in the concentration in the dissolution chamber. In this study, we aimed to calculate the flux in the non-steady-state and compare the obtained *in vitro* data with *in vivo* data.

As a first step, we plotted the membrane permeation rate versus time in a simple system that does not cause supersaturation (Test 1). Next, we verified how the membrane permeation rate changes when supersaturation phenomena of drug compounds occurs in the dissolution vessels (Test 2). In addition, the membrane permeation rate was compared with *in vivo* data.

METHODS

Test 1 Comparing Flux Behavior in Formulations with Different Dissolution Rates.

Three Carbamazepine formulations were tested using the small volume Dissolution-Membrane Permeability Test (μFLUX, Pion, Fig. 1). The test solution used in the Donor Chamber was 15 mL FaSSIF and 15 mL Acceptor Sink Buffer (ASB, Pion) was used in the Acceptor Chamber. The Donor Chamber and Acceptor Chamber were separated by a hydrophobic PVDF membrane.

Test 2 Monitoring Flux Behavior in Supersaturated Conditions.

In this study, two formulations A and B, containing poorly soluble API (MW 700, BCS class 2, pKa 2.9) were evaluated. Both formulations used an API content of 100mg. A membrane permeation system (MacroFLUX, Pion, Fig. 2) was used in conjunction with the USP Apparatus2 dissolution tester (Model2500, Distek).
The test solution in the donor (dissolution vessels) was 750 mL SGF at the start of the assay, and 30 minutes after, was converted to 938 mL FaSSIF by adding concentrated SIF solution.12 mL of ASB was used in the Acceptor Chamber.
The membrane separating the dissolution vessel and the acceptor chamber was a 3.69cm^2 hydrophobic PVDF membrane, and the assay was carried out at three conditions of rotational speed of 25, 50, and 75 RPM.
The concentration of API in the dissolution vessels and acceptor chambers was monitored by in-situ UV Probes.

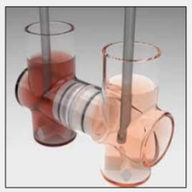

Fig.1 μFLUX system. The Donor Chamber and Acceptor Chamber are separated by a membrane.

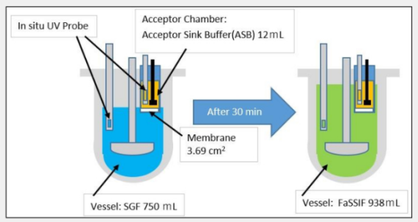

Fig.2 MacroFLUX assay. After 30 minutes, SIF solution was added.

RESULTS

Test 1 Comparing Flux Behavior in Formulations with Different Dissolution Rates.

Fig. 3 shows the concentration in the Donor Chamber, and Fig. 4 shows the concentration in the Acceptor Chamber. For all formulations, doses to the Donor Chamber were 4mg, and all tests were performed at n=4. In Fig.3, Formulation1 was the slowest to dissolve and Formulation 3 was the fastest. The flux (J) was calculated according to Equation (1), using the acceptor concentration (c) change over time, acceptor volume (V), and membrane area (A). (2)

$$J = \frac{dm}{A\,dt} = \frac{V}{A} \cdot \frac{dc}{dt} \qquad (1)$$

The flux J(t) at each timepoint is shown in Fig. 5.
For Formulation 3 with a high dissolution rate, the donor concentration reached a constant value after about 40 min and the flow rate reached a steady state after 50 min. Formulation 2 was completely dissolved after 150 minutes, and the flux also became a constant value. The flux at steady-state was 1.6×10^{-1} μg/cm^2/min for Formulation 2 and 3.
Formulation 1 continued to dissolve throughout the Assay, and the flux did not reach steady-state.

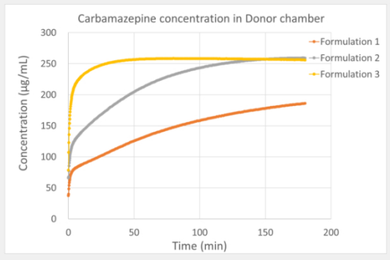

Fig.3 Carbamazepine concentration in Donor Chamber

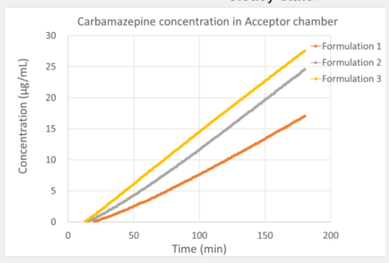

Fig.4 Carbamazepine concentration in Acceptor Chamber

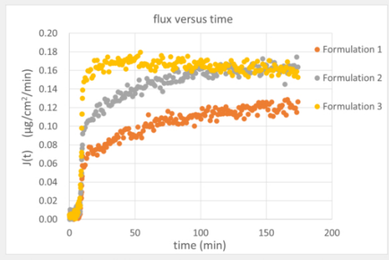

Fig.5 Real-time FLUX of Carbamazepine formulation

Test 2 Monitoring Flux Behavior in Supersaturated Conditions.

The compound was a BCS Class II weak base (pKa: 2.9). Theoretically, the API is more than 95% ionized in acidic SGF and exists in neutral free base form in FaSSIF at pH 6.5.

In the donor vessels, API dissolved up to 22 μg/mL in SGF, then decreased to the saturation concentration (<10μg/mL) after media conversion to FaSSIF, for both Formulations A and B (Fig. 6). The higher the rotation speed, the higher the degree of supersaturation following media conversion from SGF to FaSSIF. In the case of 75 RPM, the concentration decreased to the saturated concentration in about 50 minutes. In the case of 25 RPM, the supersaturation level was not as high and the concentration slowly decreased to the saturation concentration over about 120 minutes (Fig. 7). All tests were performed at n=4.

In the acceptor vessels, the rate of appearance of drug is highest soon after media conversion from SGF to FaSSIF and is greater at the faster paddle rotation speeds (Fig. 8). Formulation A displays higher concentrations than Formulation B.

The maximum membrane permeation rates were obtained within 10 minutes after the change of the test medium from SGF to FaSSIF for both formulations at all RPM settings (Fig. 9). At 75 RPM, the mean flux for 10 minutes after the test medium conversion was ~0.9 μg/cm^2/min for Formulation A, and 0.75 μg/cm^2/min for Formulation B. Maximum flux was 20% higher for Formulation A than B. After 10 minutes, the flux gradually decreased in both Formulations A and B, dropping to 0.1 μg/cm^2/min^2 two hours after the start of the test.
At low RPM, a lower flux was obtained. This is probably due to the low concentration on the donor side and the weak stirring force near the membrane, resulting in a thick unstirred water layer.

Results from human *in vivo* tests showed that Formulation A had higher values than Formulation B in both C_{max} and *AUC*. This suggests that the large flux after media conversion of the test solution in the membrane permeation test correlates with the *in vivo* absorption behavior of the drug.

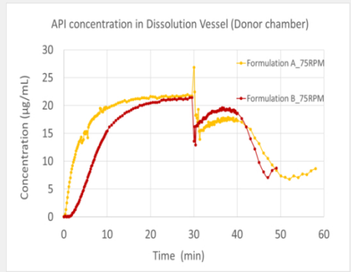

Fig.6 API concentration in Donor Chamber (75 RPM)

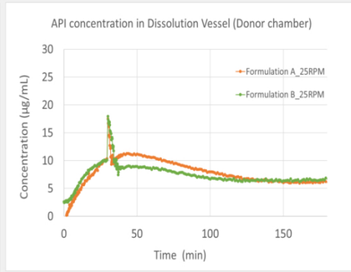

Fig.7 API concentration in Donor Chamber (25 RPM)

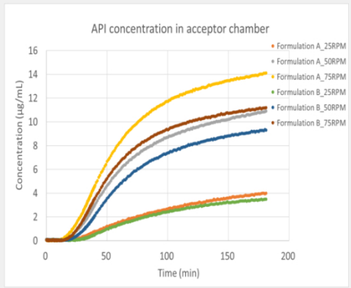

Fig.8 API concentration in Acceptor Chamber

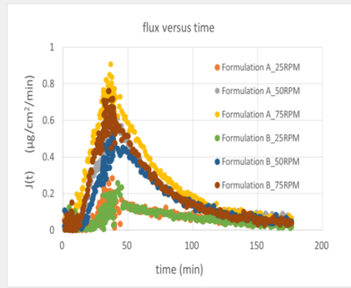

Fig.9 Real-time FLUX of each formulation and RPM settings

CONCLUSION

In Test 1, which observed changes in FLUX over time in detail, we succeeded in visually and quantitatively understanding the correlation between dissolution rate and FLUX.

In Test 2, *in vitro* flux and *in vivo* results for supersaturating formulations have shown a good relationship. Observing change of FLUX over time will provide more information about the absorption behavior of drugs in the human body.

Future Perspectives:
In this study, by plotting J(t), we were able to confirm changes in the apparent membrane permeability coefficient during the test.
In Test 1, the apparent membrane permeability coefficients P_e were 6×10^{-3} cm^{-1}min^{-1} for all formulations throughout the test period, and the membrane permeation rate was a constant value during the assay.
On the other hand, in Test 2 the apparent membrane permeability coefficient P_e showed a maximum of 8×10^{-2} cm^{-1}min^{-1} during the donor vessel supersaturation duration, and then decreased as the donor-side concentration decreased. The thickness of the unstirred water layer may influence the change in the apparent membrane permeability coefficient. We would like to carry out further verification by increasing the approach, such as measuring the particle size distribution of undissolved particles in the test solution.

As a future topic, we will verify the changes in flux and apparent membrane permeability coefficient by evaluating other compounds in a supersaturated system.

The goal is to make more accurate predictions of *in vivo* absorption based on commonly used dissolution testing methods.

REFERENCE

(1) Enikő Borbas, Szabina Kadár, Konstantin Tsinman, Oksana Tsinman, Dora Csicsa, Krisztina Takacs-Nova, Gergely Völgyi, Balint Sinko, Hajnalka Pataki. Prediction of Bioequivalence and Food Effect Using Flux and Solubility-Based Methods. Mol. Pharmaceutics 2019, (16), 4121−4130

(2) Konstantin Tsinman, Oksana Tsinman , Ram Lingamaneni , Saijie Zhu, Bernd Riebesehl, Arnaud Grandeury, Michael Juhnke, Bernard Van Eerdenbrugh. Ranking Itraconazole Formulations Based on the Flux through Artificial Lipophilic Membrane. Pharm. Res. 2018 (35) Article Number 161