Real Time Monitoring of Dissolution, Supersaturation and Precipitation Processes in Dynamically Changing Biorelevant Media

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INTRODUCTION

The timeframe of supersaturation and potential precipitation events may have profound effects on bioavailability. The aim of this study was to develop a practical, easily standardized and predictive *in vitro* dissolution method to monitor powder/formulation dissolution and concomitant precipitation processes in biorelevant media dynamically being changed from simulated gastric to simulated intestinal fluid. The importance of such API-sparing, two-stage, *in vitro* microdissolution tests to study drug-drug interaction in the case of pH-modifying agents was reported recently[1].

MATERIALS AND METHODS

The drug solution concentration versus time was evaluated in 1-20 mL of media using the µDISS Profiler™ (Pion Inc.) with the add-on integrated 8-channel µDISPENSER™, which can be programmed to change media during a dissolution run, e.g., by delivering a specially formulated concentrate that transforms SGF into FaSSIF.

Figure 1. µDISS Profiler™ instrument integrated with µDISPENSER™ unit.

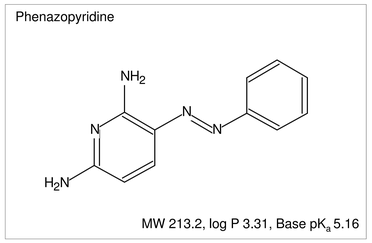

Figure 2. Physicochemical properties of Phenazopyridine.

Free acid A and its sodium A.Na and calcium A.Ca salts were received from an analytical service client company. Phenazopyridine hydrochloride (PZP) (Figure 2) was purchased from Sigma-Aldrich.

Conversion from X volume of SGF medium (pH 1.6) to 1.125*X volume of pH 6.5 buffer ($FaSSIF_{blank}$) and 1.53X volume of FaSSIF was performed on-the-fly by adding specially formulated concentrate solutions to SGF medium.

Reference Channel

When performing *in situ* UV-Vis measurement in dynamically changing media it is important to realize that molar absorptivity of the ionizable molecules like Phenazopyridine can change significantly depending on the ionization state of such compounds (Figure 3). Additionally, complex media components can alter the spectroscopic properties of the API.

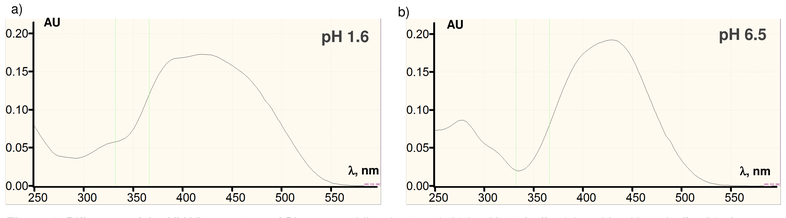

Figure 3. Difference of the UV-Vis spectrum of Phenazopyridine (15.8 µg/mL) in pH 1.6 buffer (a) and in pH 6.5 buffer (b) shown on the same absorbance units (AU) scale.

µDISS Profiler Command Software Rev. 4.5 implements a *Reference Channel* that monitors a solution of API of known concentration and applies a correction factor when apparent (measured) concentration deviates from the known one due to any changes in molar absorptivity of the API. This eliminates the need to collect multiple standards for different media.

RESULTS AND DISCUSSION

Two-Stage Conversion Experiment for Different Salts

Free acid A and its sodium and calcium salts were studied in parallel using a dual-stage media conversion assay, see Figure 4.

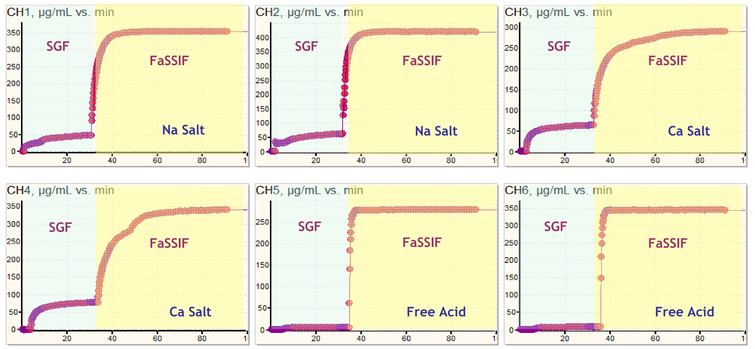

Figure 4. Concentration (µg/mL) versus time (min) profiles for Na (CH1&CH2) and Ca (CH3&CH4) salts of a free acid A (CH5&CH6) in two-stage micro-dissolution experiments where SGF medium (pH 1.6) was dynamically converted into FaSSIF.

Sodium and calcium salts of acid A stayed supersaturated within the first 30 min of the dissolution assay revealing kinetic solubility of 52±1 µg/mL and 68±5 µg/mL respectively while the free acid reached its solubility limit at 7.1±0.9 µg/mL (Table 1). After conversion of the media into FaSSIF, solubility limit for A.Na salt was reached in 20 – 30 min (277±23 µg/mL) and for calcium salt within 10 – 20 min (222±6 µg/mL).

It is interesting to note that free acid A was dissolving much faster than its salts and within several minutes after converting the SGF into FaSSIF the solubility was reaching 212±13 µg/mL. The faster dissolution of free acid was consistent with estimated particle sizes for all three forms[2].

Table 1. Solubility and effective radii estimated from the dissolution curves[2] of a free acid and its salts.

	SGF (pH 1.6)				FaSSIF			
	Solubility, µg/mL	STD	Effective Radius, µm	STD	Solubility, µg/mL	STD	Effective Radius, µm	STD
Sodium Salt	52.1	1.2	42.0	0.2	277	23	14.2	1.3
Calcium Salt	67.7	5.0	17.2	0.8	222	6	10.5	0.6
Free Acid	7.1	0.9	15.5	1.1	212	13	3.9	1.7

Dissolution and Precipitation of Phenazopyridine with Dynamic Media Change

The effect of pH change and lecithin/surfactant on dissolution and precipitation of PZP was studied using the multi-stage approach, Figure 5. The assay was started in pH 1.6 buffer at which the drug is quite soluble. After about 30 min an additional (continued)

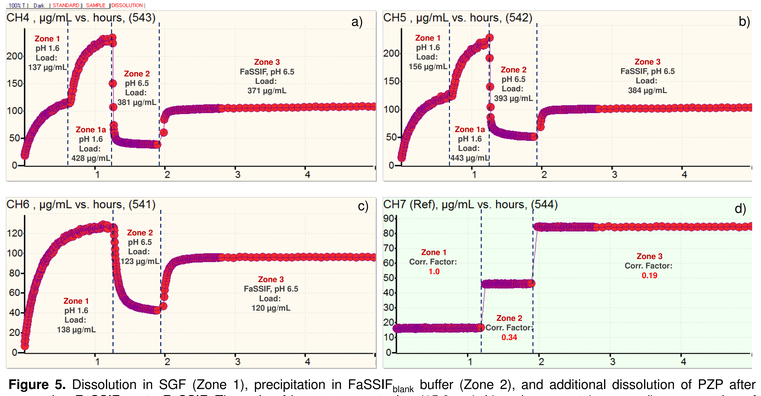

Figure 5. Dissolution in SGF (Zone 1), precipitation in $FaSSIF_{blank}$ buffer (Zone 2), and additional dissolution of PZP after converting $FaSSIF_{blank}$ to FaSSIF. The ratio of known concentration (15.8 µg/mL) and apparent (measured) concentration of PZP in the Reference Channel (d) was used to calculate concentration correction factors for Zones 2 and 3.

amount of PZP was added to channels 4 and 5 (Zone 1a in Figure 5, a - b) while channel 6 remained unchanged (Figure 5, c). Conversion of SGF medium (pH 1.6) to pH 6.5 after (Zone 2) by adding a 2 mL of phosphate buffer concentrate to 16 mL of SGF medium caused quick precipitation of PZP with no indication of supersaturation. When 0.45 mL of lecithin/taurocholic acid mixture was added (Zone 3), the apparent solubility of PZP increased ~2 times as seen in Figure 5, a – c. Figure 5, d demonstrates the application of the Reference Channel correction. Without corrections the concentration in Zones 2 and 3 would have been overestimated by 1.0/0.34 = 2.94 times and 1.0/0.19 = 5.26 times respectively.

Phenazopyridine µFLUX Measurement

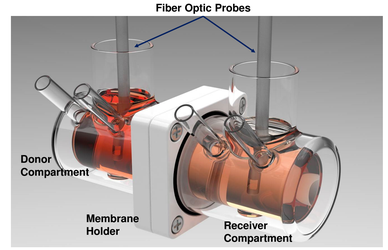

Figure 6. A fragment of the µFLUX™ apparatus (Pion Inc.) showing a pair of donor and receiver chambers. FO probes attached to the µDISS Profiler (Figure 1) monitor concentrations in the donor (left) and receiver (right) compartments. The chambers were separated by a Double-Sink™ PAMPA membrane (0.78 cm^2) mounted in the Membrane Holder.

In parallel with the dissolution experiments, a µFLUX assay was set up. In this assay API concentration was monitored in a pair of donor and receiver compartments separated by a filter-supported, GIT-optimized, artificial membrane[3] (Figure 6). The donor compartment was initially filled with 10 mL of the SGF while the receiver compartment contained 13 mL of Acceptor Sink Buffer at pH 7.4 (ASB-7.4, Pion Inc.). The media and the amount of PZP in the donor compartment was changed similarly to the procedure described previously.

Figure 7 demonstrates parallel concentration monitoring of PZP in the donor a) and the receiver b) compartments. It can be noted that in accordance with the pH-partitioning hypothesis there was no permeation (zero flux) while PZP was dissolving in SGF while the maximum flux in FaSSIF was 1.9 µg·min^{-1}·cm^{-2}.

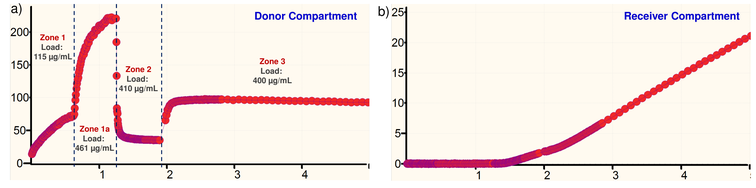

Figure 7. Dissolution in SGF, precipitation in $FaSSIF_{blank}$ buffer, and additional dissolution of PZP after converting $FaSSIF_{blank}$ to FaSSIF (a) are similar to Figure 5 a – b. The concentration profile of PZP in the receiver compartment (b) was used to calculate flux values.

CONCLUSIONS

This novel *in situ* approach to monitoring *in vitro* powder and formulation dissolution/precipitation effects can facilitate the fundamental understanding of processes that may be relevant *in vivo* and thus provide a rational basis for formulation design and development.

Dissolution profiling with fiber optic UV probes combined with integrated software controlling a dispensing unit provide means for streamlining *in situ* monitoring of kinetic processes (e.g., supersaturation), which is not achievable by other sampling and off-line analysis procedures.

REFERENCES

1. N.R. Mathias, *et al.* Assessing the Risk of pH-Dependent Absorption for New Molecular Entities: A Novel *In Vitro* Dissolution Test, Physicochemical Analysis, and Risk Assessment Strategy. *Mol. Pharmaceutics.* dx.doi.org/10.1021/mp400426f.
2. A. Avdeef, K. Tsinman, O. Tsinman, N. Sun, and D. Voloboy. Miniaturization of Powder Dissolution Measurement and Estimation of Particle Size. *Chem. Biodivers.* **2009**, *6* (11), 1796–1811.
3. K. Tsinman, and O. Tsinman. Dissolution-Permeability Apparatus with Integrated *In Situ* Concentration Monitoring of both Donor and Receiver Compartments. Poster presented at AAPS Annual Meeting and Exposition. November 10 -14, 2013, San Antonio, TX.