Novel Device and Method for *in situ* UV Dissolution and Precipitation Monitoring in 96-well Plate

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INTRODUCTION

It has been shown[1,2], that disk intrinsic dissolution rate (IDR) can be determined from powder dissolution experiments for BCS Class III and IV (low soluble) compounds and that particle size information could be extracted from powder dissolution experiments. This research investigates if such studies could be conducted in the 96-well microtitre plate format. A newly developed device was applied to asses the feasibility of performing *in situ* (real time) UV concentration monitoring experiments in volumes as low as 100 -200 µL.

MATERIALS AND METHODS

Seven compounds studied previously[1] by µDISS Profiler™ (*p*ION INC) were selected for the study. Two different solutions of these compounds were prepared using methanol (MeOH) and DMSO as solvents. In addition slurries of compounds in DI water were prepared. Table 1 below shows final concentrations in the MeOH and DMSO stock solutions and in the DI water slurries.

Table 1. Concentrations used in the experiment and resulting upper limits of the experiment.

		Methanol stock		DI water slury		DMSO stock	
Sample Name	**MW**	**Stock Conc, mM**	**Upper limit, µg/mL**	**Stock Conc, mM**	**Upper limit, µg/mL**	**Stock Conc, mM**	**Upper limit, µg/mL**
Carbamazepine	236.27	60.27	678	45.29	510	51.13	575
Furosemide	330.75	36.52	575	34.95	550	38.40	605
Griseofulvin	352.8	7.88	132	11.85	199	14.29	240
Hydrochlorothiazide	297.7	82.10	1164	77.80	1103	73.63	1044
Dipyridamole	504.64	18.67	449	38.17	917	20.09	483
Ketoprofen	254.3	88.79	1075	89.11	1079	88.24	1069
Naproxen	230.27	29.27	321	26.06	286	20.50	225

The standard buffer at pH 4.5 prepared according to USP Vol. 23 (28 mM acetic acid, 22 mM sodium acetate, NaOH) was used for dissolution and solubility studies.

MeOH stock solutions (10 µL) were added into columns 1 – 4 of the 96-well UV transparent plate (Greiner) with rows A – G designated to a particular compound according to the order in the Table 1. The plate was set open in the desiccator overnight for evaporation. Slurry and DMSO solutions were prepared before the beginning of the experiment and 10 µL aliquots were manually added to the columns 5 – 8 and 9 – 12 respectively.

The dissolution experiment was continued for 60 min. After that solutions were filtered and scanned by a UV plate reader (SpectraMax 190, Molecular Devices) for validation purposes.

In Situ Concentration Monitoring in 96-well Microtitre Plate

The new prototype instrument µDISS Scanner™ (*p*ION INC) allowing *in situ* concentration monitoring in 96-well microtitre plate format was used to evaluate if powder dissolution/solubility experiments could be done in 0.1-0.2 mL of buffer. A unique stirring mechanism employed by the instrument (see Figure 1) allows uninterrupted in-well stirring while not blocking UV light for real time concentration detection, eliminating the filtration step.

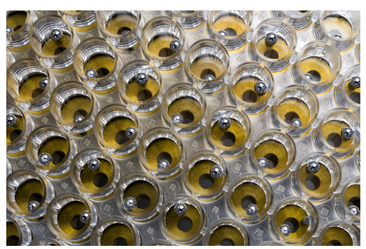

Figure 1. A 96-well UV transparent plate preloaded with *disposable* ferromagnetic balls is placed on top of the stirring cassettes. Hollow centers allow for un-interrupted light transmission. The driving mechanism sets all magnets in rotating motion inducing synchronous motion of the stirring balls inside the wells.

RESULTS AND DISCUSSION

Dissolution/Precipitation Profiles in 96-well Plate

Figure 2 shows the print screen of the results from multi time point dissolution/precipitation experiment performed *in situ* in 96-well plate format. The study clearly indicated feasibility of such assays.

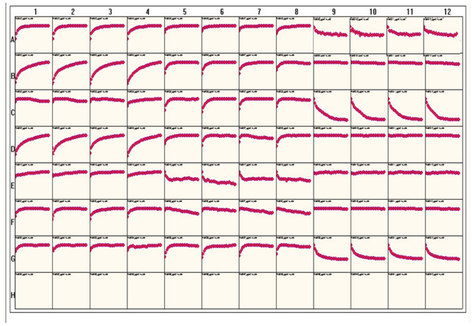

Fig. 2 Print screen of dissolution/ precipitation profiles in 96-well microtitre plate. Horizontal lines in columns 9-12, rows B, D, E and F indicate absence of precipitation in the presence of 5% DMSO in the background

Dissolution Profiles from Evaporated MeOH Stock and DI Slurry

In columns 1 – 4 (MeOH evaporation) tiny particles were visible in rows A (carbamazepine), B (furosemide), C (griseofulvin), D (hydrochlorothiazide) and G (naproxen). No particles could be seen in rows E (dipyridamole) and F (ketoprofen). In rows C and E it was evident that traces of compound were above the buffer liquid level indicating that MeOH solution was crawling up well walls during evaporation and amount of compounds available for dissolution study was unknown. Figure 3 (a) compares final concentrations measured *in situ* with concentrations measured after filtration in the columns 1 – 4.

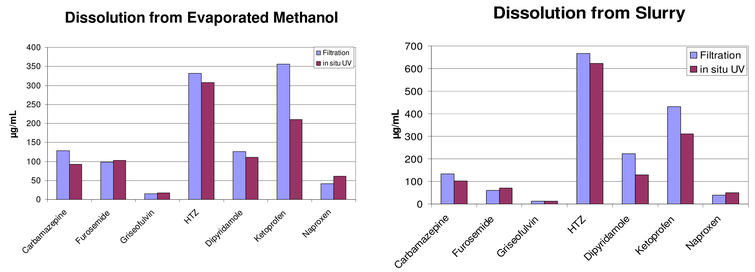

Fig. 3. Comparison final concentrations in solutions measured *in situ* by µDISS Scanner and externally after filtration and UV scanning in conventional 96-well UV plate reader.

In columns 5 – 8 (compounds introduced as slurry), the different degree of leftover solid was found in all wells. The heaviest seemed to be in rows B, E and F. Figure 3 (b) compares final concentrations measured *in situ* with ones measured in filtered solutions for these columns.

The form of the solid in wells A1 – A4 (carbamazapine) looked different from what was seen in wells A5 – A8 indirectly indicating possibility of different solid form (polymorph/hydrate/anhydrate) resulting after MeOH evaporating.

The magnified dissolution profile of hydrochlorothiazide (pH 4.5) introduced as DI water slurry is shown on Figure 4. Three replicates were averaged (blue symbols in Fig. 4) and the data were fitted (dashed line in Fig. 4) using approach[2] to extract particle size information from the dissolution profile. As evident from Fig. 4, the model described the experimental points very well and particle size analysis indicated effective particle radius to be 28 µm - good agreement with results reported in Ref. 2. The specific surface area estimation (0.84 $cm^2\ mg^{-1}$) is within 20% from one measured by Coulter method (1.05 $cm^2\ mg^{-1}$).

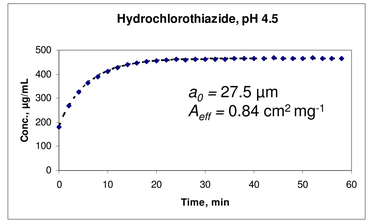

Fig. 4. Dissolution profile of hydrochlorothiazide from 96-well dissolution experiment (0.22 mg in 0.2 mL of USP pH 4.5 buffer, 25℃). Points represent averages over 3 replicates with errors (not shown) within 10 – 15%.

In situ Precipitation Monitoring

Monitoring concentration in real time allowed easily identifying compounds that precipitated and ones that stayed in the solution possibly in the super-saturated state. Precipitating compounds seemed to reach equilibrium within 60 min of monitoring, as could be seen from the examples on Fig. 5.

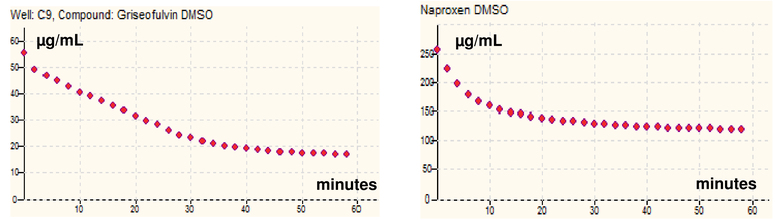

Fig. 5. Precipitation profile of griseofulvin (a) and neproxen (b) from 96-well *in situ* concentration monitoring experiment (10 µL of DMSO stock in 0.2 mL of USP pH 4.5 buffer, 25℃).

It is evident that presence of DMSO in the background increases solubility of studied compounds. It is suggested that for follow up studies no more than 1% DMSO in the background (e.g., 2 µL DMSO stock in 200 µL of buffer) be used.

CONCLUSIONS

To our knowledge, a non-invasive real-time monitoring high throughput microtitre plate based dissolution apparatus does not exist in the market today. Such instrument will make it possible to conduct critical dissolution studies much earlier in the drug development process and substantially reduce the associated cost per sample.

Cost and speed favor *in situ* UV/-VIS detection over HPLC-UV because it does not require external pumps, tubing, and filtration devices to dynamically monitor concentration thereby further reducing the cost and maintenance requirements for the assay.

The *in situ* concentration monitoring method in the 96-well microtitre plate format has benefits over current high throughput solubility methods (e.g., µSOL[3], *p*ION) by enabling dynamic non-invasive monitoring of concentration of a dissolving/precipitating compound, ensuring that equilibrium is reached (rather than relying on arbitrarily selecting a single time point for analysis).

The method uses 3 – 5 times less material for solubility determination and up to 10,000 less material comparing with traditional dissolution studies.

It eliminates the need for vacuum pumps, manifolds and filter plates, thereby further reducing costs and avoiding non-specific binding to the filters.

REFERENCES

(1) Tsinman, K; Avdeef, A; Tsinman, O; Voloboy, D. Powder Dissolution Method for Estimating Rotating Disk Intrinsic Dissolution Rates of Low Soluble Drugs. *Pharm. Res.* **2009**, 26 (9), 2093 - 2100.
(2) Avdeef, A; Tsinman, K; Tsinman, O; Sun, N; Voloboy, D. Miniaturization of Powder Dissolution Measurement and Estimation of Particle Size. *Chem. Biodiversity* **2009**, 6 (11), 1796 - 1811.
(3) Avdeef A. & Tsinman K. Measurement of Solubility-pH Profiles. US Patent 6,569,686 B2.