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Research paper

A polychromatic turbidity microplate assay to distinguish discovery stage drug molecules with beneficial precipitation properties

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ABSTRACT

A material sparing microplate screening assay was developed to evaluate and compare the precipitation of discovery stage drug molecules as a function of time, concentration and media composition. Polychromatic turbidity time course profiles were collected for cinnarizine, probucol, dipyridamole as well as BMS-932481, and compared with turbidity profiles of monodisperse particle size standards. Precipitation for select sample conditions were further characterized at several time points by size, morphology, amount and form via laser diffraction, microscopy, size based particle counting and X-ray diffraction respectively.

Wavelength dependent turbidity was found indicative of nanoprecipitate, while wavelength independent turbidity was consistent with larger microprecipitate formation. A transition from wavelength dependent to wavelength independent turbidity occurred for nanoparticle to microparticle growth, and a decrease in wavelength independent turbidity correlated with continued growth in size of microparticles. Other sudden changes in turbidity signal over time such as rapid fluctuation, a decrease in slope or a sharp inversion were correlated with very large or aggregated macro-precipitates exceeding 100 μm in diameter, a change in the rate of precipitate formation or an amorphous to crystalline form conversion respectively.

The assay provides an effective method to efficiently monitor and screen the precipitation fates of drug molecules, even during the early stages of discovery with limited amounts of available material. This capability highlights molecules with beneficial precipitation properties that are able to generate and maintain solubility enabling amorphous or nanoparticle precipitates.

1. Introduction

Many contemporary small molecule drug compounds demonstrate low aqueous solubility, which often hinders oral absorption (Augustijns et al., 2014; Di et al., 2012). Supersaturation, a state in which concentration exceeds equilibrium solubility, can drive increased oral drug exposures (Miller et al., 2012; Brouwers et al., 2009). The supersaturated state is however thermodynamically unstable and precipitation can negate the supersaturation advantage, especially at higher doses. Fortunately, precipitation is not always detrimental. For instance, rapidly redissolving nanoparticles (Sigfridsson et al., 2011) and higher energy, more soluble solids such as amorphates (Murandi et al., 2010) can lead to improved oral absorption. Understanding the consequences of precipitation during drug discovery can aid in the design, optimization and selection of better drug candidates (Leeson and St-Gallay, 2011) with beneficial precipitation properties. In contrast, less robust drug molecules are more likely to demonstrate solubility limited oral absorption which increases clinical formulation developability risks (Hann and Keseru, 2012).

Precipitation from supersaturated solutions is a complex phenomenon, with the onset time, rate of formation, particle size and polymorphic form dependent upon multiple interacting factors: the particular molecule, time-frame, concentration and media composition (Ozaki et al., 2012; Kostewicz et al., 2004). Following in vivo dosing, inter-subject differences in gastrointestinal fluid volumes, motility, pH and composition (Kararli,

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1995; Hatton et al., 2015; Abuhelwa et al., 2017) affect both the solubility and concentration of drug molecules. The interplay of these factors influence whether, when and how drug molecules precipitate in-vivo, and the inter-subject variability can lead to oral exposure variability (Amidon et al., 1995; Martinez and Amidon, 2002). Given this, precipitation assessments conducted across a range of conditions is more representative of the range of in-vivo precipitation fates.

The throughput of conventional particle size and polymorphic form analyses (i.e. laser diffraction particle sizing, single particle counting, X-ray diffraction and microscopy) is insufficient to track time dependent precipitate changes across multiple concentrations, media conditions and drug molecules. Other spectroscopic techniques such as fiber optic UV, Raman, infrared and focused beam reflectance measurements (FBRM) have also been used for in-situ precipitation analysis during dissolution experiments (Kuentz, 2014). These techniques are not however amenable to higher throughput conditions. Furthermore, all of the above techniques generally require more material than is available in the drug discovery environment.

Monochromatic turbidimetric analyses are effective means to monitor for precipitation over time (Kostewicz et al., 2004; Chandran et al., 2011). In a microplate format this technique is well suited for molecules in the discovery space, requiring little material and enabling parallel analyses across multiple conditions (Morrison et al., 2014). Combining the turbidity assessments with complimentary final supernatant concentration analyses (Morrison et al., 2014) can indicate whether higher energy precipitates such as amorphates with greater apparent solubility than the original crystalline form are present. While valuable, this information is however somewhat limited. A change in turbidity indicates an active precipitation event, but not whether it represents a change in the nature or abundance of the precipitate. Polychromatic turbidity utilizing multiple wavelengths of light provides the opportunity to distinguish precipitate size, since light scattering is wavelength dependent for submicron but not supramicron sized particles (Bohren and Huffman, 1983; Wang and Hallett, 1996). This particle size distinction is not possible with monochromatic turbidity since the signal is convoluted by both the size and concentration of the particles.

To explore the feasibility of this approach, polychromatic turbidity precipitation profiles for several poorly soluble model drug molecules were collected over multiple concentrations and in different simulated intestinal media using a microplate reading spectrophotometer. These turbidity profiles were compared with profiles of monodisperse particle size standards as well as particle size distributions of the drug molecule precipitates collected at several time points. Some samples also demonstrated unusual directional changes in turbidity signal such as a rapid fluctuation, a decrease in slope or a sharp inversion. Further precipitation characterization was undertaken for these samples using orthogonal analysis techniques in order to validate the turbidity pattern interpretations: polarized light and dark field microscopy, size based particle counting and X-ray diffraction.

It is important to note that this assay is not intended to provide in-vitro to in-vivo correlations, but rather rank drug molecules based upon physicochemical properties known to be advantageous to oral or parenteral delivery. Furthermore, this assay is not intended to replace development stage characterization techniques, although such techniques were used as validation tools in this work. Instead, the assay provides valuable insights into drug molecule precipitation behaviour within the more resource constrained discovery environment. The assay also reveals suitable conditions and time frames for more rigorous investigations of precipitation phenomenon. Additionally, although not explored in this work, the assay can be adapted to evaluate the performance of precipitation inhibiting excipients across multiple parameters including excipient type, amount and combinations.

2. Experimental

2.1. Materials and media preparation

Polystyrene sphere particle standards were obtained from Duke Scientific. Dipyridamole, cinnarizine and probucol were purchased from Sigma-Aldrich. BMS-932481 was synthesized, purified and characterized in house (Toyn et al., 2016). Spectroscopic grade dimethylsulfoxide (DMSO) was purchased from EMD and acetonitrile, methanol, and water were HPLC-grade and obtained from JT Baker or Sigma-Aldrich. Filtered water was obtained from a Barnstead Nanopure Diamond filtration system.

Polypropylene 96-well microplates were purchased from Corning. Flat-bottom 500 μL capacity Pyrex glass well inserts and round base support plates were purchased from Analytical Sales and Service. These glass insert wells provided good optical clarity, solvent resistance and cost effective disposability to prevent cross contamination. Phosphate buffer solutions were prepared with sodium monobasic phosphate and sodium dibasic phosphate from Sigma-Aldrich. The pH was measured with a Beckman Φ350 pH/Temp/mV Meter and adjusted as necessary with 1 M NaOH or 1 M HCl solutions from Sigma-Aldrich. Simulated intestinal fluid powder (SIF, original version) was purchased from Biorelevant.com and used to prepare FaSSIF according to the specified guidelines.

2.2. Analytical procedures

2.2.1. Polystyrene particle size standard sample preparation and turbidimetric analysis

Polystyrene sphere stock suspensions were vortexed for 20 s with a VWR Analog Vortex Mixer to suspend the particles. Aliquots were diluted with pH 6.5 phosphate buffer (30 mM) to a final concentration of 0.5 mg/mL. After 20 s of vortexing, 400 μL samples were transferred to Pyrex glass insert microplates. Turbidity was analyzed at 400 nm, 500 nm, 600 nm and 700 nm with a Tecan Infinite M200 instrument. Samples were resuspended immediately prior to analysis using the instrument's internal shaking capability (60 rpm for 20 s).

2.2.2. Model drug sample preparation and turbidimetric analysis

Model drug samples were prepared following a previous published procedure (Morrison et al., 2014). Master DMSO stock solutions (20 mg/mL for cinnarizine, 40 mg/mL for dipyridamole and probucol) were prepared and aliquots were serially diluted with DMSO. Serial dilution factors of 1.00, 1.33, 1.20, 1.25, 1.33 and 1.50-fold yielded secondary DMSO stock solution concentrations of 20, 15, 12.5, 10, 7.5 and 5 mg/mL for cinnarizine, and 40, 30, 25, 20, 15 and 10 mg/mL for both probucol and dipyridamole. After aqueous dilution, the final test concentrations were 1000, 750, 625, 500, 375 and 250 μg/mL for cinnarizine, and 2000, 1500, 1250, 1000, 750 and 500 μg/mL for both probucol and dipyridamole. A 60 mg/mL DMSO stock solution of BMS-932481 was prepared and portions were diluted to 40 mg/mL and 20 mg/mL with DMSO. Following aqueous dilution, the final test concentrations were 3000, 2000 and 1000 μg/mL. Twenty microliter aliquots of the DMSO solutions were transferred to Pyrex glass insert microplates. Samples were diluted 20-fold by adding 380 μL of either phosphate buffer or FaSSIF and mildly vortexed for 20 s at the lowest setting sufficient to achieve orbital mixing. Turbidity was monitored with a Tecan Infinite M200 instrument at 400 nm, 500 nm, 600 nm and 700 nm. The 400 nm data was excluded for dipyridamole and BMS-932481 due to electronic absorption at this