Continuous Monitoring of Nanoparticle Albumin-Bound Paclitaxel Dissolution Profiles Using Dynamic Light Scattering and In Situ UV/Vis Fiber-Optic Probes

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Purpose
To ensure the bioequivalence of a proposed nanoparticle albumin-bound paclitaxel (Nab-Ptx) generic, the FDA recommends that these products be qualitatively (Q1) and quantitatively (Q2) similar to the currently marketed reference product. In addition, the Agency recommends that these products have comparative physicochemical characteristics, including particle size, morphology, and in vitro release kinetics. Conventional in vitro dissolution tests, under sink conditions, can give rise to rapid release rates for nanosuspensions, making it difficult to assess differences in product manufacture or formulation. Nab-Ptx is a stable particle at or above a paclitaxel concentration of 35 μg/mL. Below this concentration it undergoes a rapid burst release. Non-conventional analysis techniques that can discern particulate and solubilized drug concentration in near-real time are, therefore, required. One approach is to obtain direct dissolution data from a rapid analysis methodology such as UV-Vis and/or supplement this with indirect data from changes in solution turbidity or particle sizing. Techniques such as dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) can provide an indirect measure of paclitaxel release rate in near real-time under a variety of exposure conditions.

Methods
To directly measure the release profile of Nab-Ptx in a range of both sink and non-sink conditions, a low volume, temperature controlled, 8 channel Mini-Bath™ (Pion) was equipped with magnetic stir bars and a Rainbow Dynamic Dissolution Monitor® (RDDM, Pion) with 8 fiber-optic probes and customizable path lengths. Basic Beer-Lambert-Law and Chemometric data analysis was performed. Dynamic light scattering (Malvern Zetasizer Nano ZS) was used to monitor the change in particle hydrodynamic size (Z-Average) and signal count rate (the number of photons scattered by particles in kilo-counts per second, kcps) over time under sink and non-sink conditions. In addition, nanoparticle tracking analysis (Malvern NS500 equipped with an EMCCD and a 405 nm blue laser) was used to confirm DLS results via higher resolution but slower analysis measurements of the change in particle size and concentration. Cryo-TEM imaging was performed using a Jeol 1400 TEM/STEM equipped with a grid plunge freezer to examine morphology of the drug product. Pharmaceutical grade human serum albumin (HSA), paclitaxel, and NaCl were acquired to be used as standards and dispersants. Nab-Ptx was used as supplied by diluting the lyophilized powder in sterile 0.9% saline solution.

Results
Real time monitoring of Nab-Ptx dissolution with fiber optic UV-Vis probes revealed that it was not possible to distinguish between paclitaxel particles and freely dissolved paclitaxel in solution as both forms absorbed at λmax (~240 nm). Despite the overlapping UV spectrum from HSA (λmax at 220 - 260 nm), paclitaxel UV absorbance was found to follow Beer-Lambert’s law up to a concentration of 70 ug/mL, at which point the probe became saturated. At or above the threshold solubility of Nab-Ptx, the presence of solid nanoparticles gave rise to an additional UV-Vis scattering effect, which was monitored at 452 nm in this study. As such, an indirect measurement of dissolution was developed by following the change in particle hydrodynamic size (Z-Average) and signal count rate (the number of photons scattered by particles in kilo-counts per second, kcps) over time under sink and non-sink conditions. Cryo-TEM imaging of Abraxane diluted at a ratio of 1:10 (500 μg/mL paclitaxel) showed the main size group as irregularly shaped roughly spherical particulates with a diameter ranging from 40-150 nm as well as smaller fragmented particulates < 20 nm in diameter.

Conclusion
UV-Vis analysis of Nab-Ptx identified the presence of HSA and paclitaxel, but could not differentiate between the fraction of free and particulate paclitaxel in solution. DLS measurements provided an indirect indication of paclitaxel release as a change in particle size with time that was dependent on the paclitaxel concentration. These findings confirm that particle sizing methods such as DLS are useful techniques to support dissolution characterization for nanoparticle drug formulations that undergo rapid release.