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

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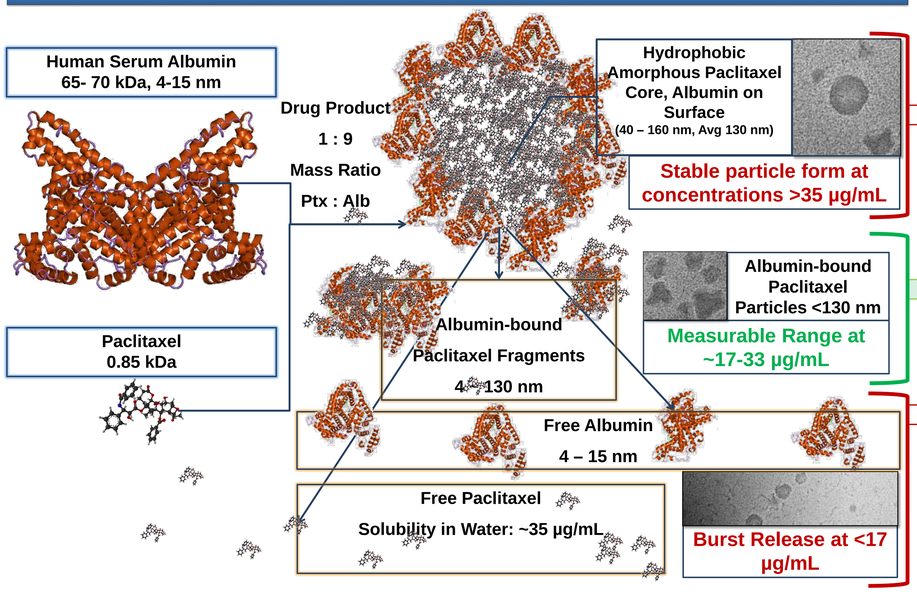
PURPOSE

This work evaluates potential *in vitro* dissolution methods for particulate albumin bound paclitaxel (Ab-Ptx), which undergoes rapid release at sink conditions. The evaluated methods, namely *in situ* UV-Vis analysis and particle sizing were then used to determine release profile for Ab-Ptx.

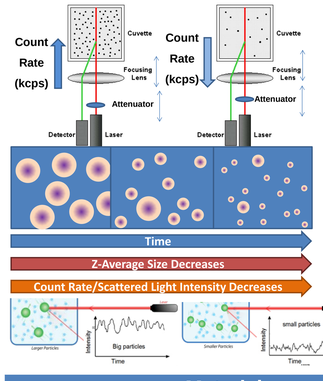
INTRODUCTION



Ab-Ptx is 9:1 parts Albumin:Paclitaxel, with stable particles around 130 nm when dispersed above maximum paclitaxel solubility. Concentrations lower than paclitaxel solubility result in rapid complete or partial breakdown of particles

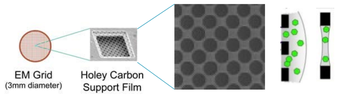
METHODS

DYNAMIC LIGHT SCATTERING

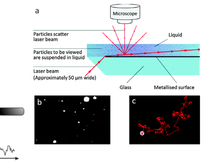


UV-VIS ANALYSIS

CRYO ELECTRON MICROSCOPY



NANOPARTICLE TRACKING

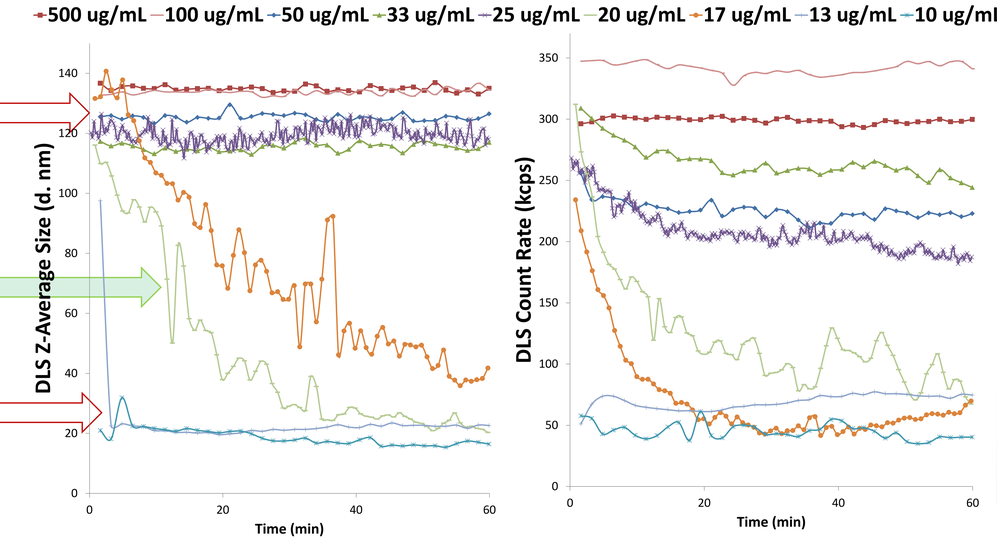


DLS count rate is proportional to concentration. Size should decrease as a particulate drug degrades over time. UV-Vis analysis with fiber optic probes can measure the drug concentration in real-time

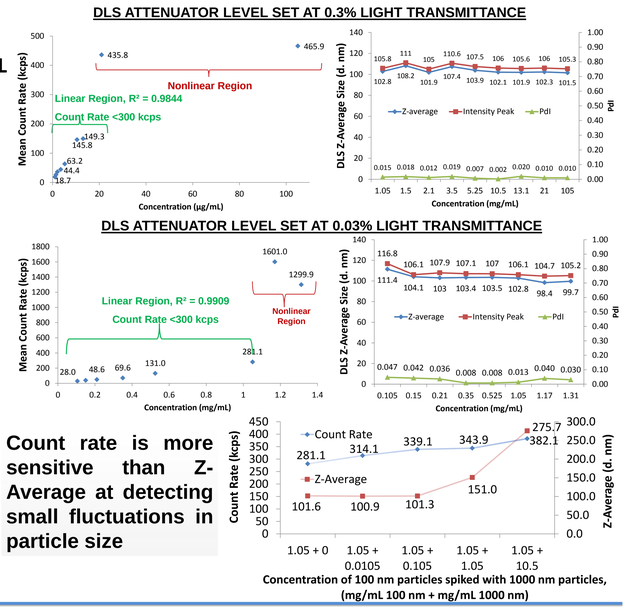
Materials	Instruments
Albumin-bound paclitaxel drug product	8 channel Mini-Bath™ (Pion) with magnetic stir bars
Human Serum Albumin, Fraction V, modified Cohn prepared, Calbiochem	Pion Rainbow Dynamic Dissolution Monitor, 8 fiber-optic probes, 10 mm PL
Paclitaxel, >99%, LC Laboratories	Malvern Zetasizer NanoZS; quartz cuvette (ZEN2112)
3000 and 4000 Series Nanosphere™ Size Standards	Malvern NS500 with EMCCD and 405 nm blue laser
NaCl, >99% pure, Sigma Aldrich	Orion Star™ A211 pH Benchtop Meter
Formvar and Quantifoil grids, uranyl acetate	Jeol 1400 TEM/STEM; Leica EM GP plunge freezer

RESULTS

DLS is capable of capturing gradual particle breakdown that occurs over the course of one hour for concentrations only near the estimated maximum paclitaxel solubility of 35 µg/mL. Z-Average size is less sensitive than raw signal count rate, which is proportional to total particle concentration. Findings confirm that particle stability is dependent on total paclitaxel concentration in solution

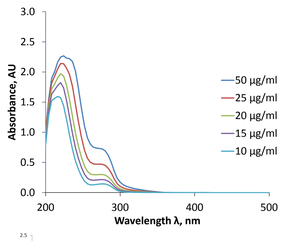


Validation of DLS count rate (kcps) as a function of concentration using 100 ± 3nm NIST-traceable size standards shows that at a given attenuation setting, count rate is linearly proportional to concentration

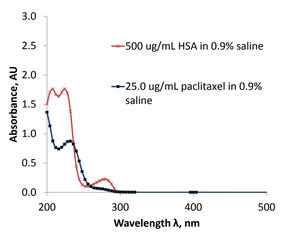


Count rate is more sensitive than Z-Average at detecting small fluctuations in particle size

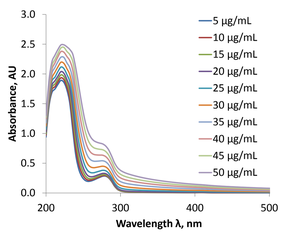
UV-Vis spectra of Ab-Ptx drug product in 0.9% saline, blanked for 0.9% saline



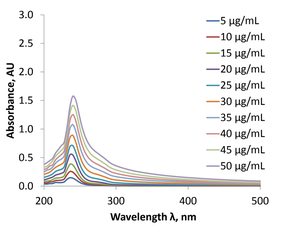
Superimposed UV-Vis spectra of Paclitaxel and HSA suspensions in 0.9% saline

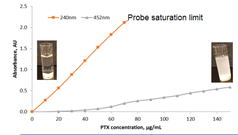


UV-Vis of 1:9 ratio of paclitaxel:HSA, in 0.9% saline, blanked for 0.9% saline



UV-Vis of 1:9 ratio of paclitaxel:HSA, blanked for both 0.9% saline and 4.5% HSA

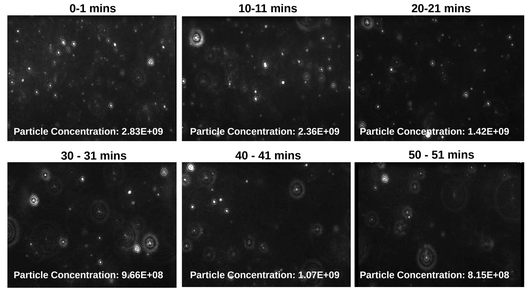




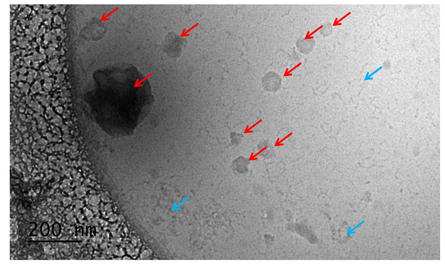
UV-Vis is unable to distinguish between dissolved and particulate paclitaxel in solution, only measuring total concentration

Nanoparticle tracking analysis shows a decreasing particle concentration of Ab-Ptx over time at 17-25 µg/mL

Nanoparticle Tracking of 1:250 diluted Ab-Ptx (20 µg/mL Paclitaxel) 1 Hr Time Course



Cryo-TEM shows that Ab-Ptx stock has irregular particles 40 - 200 nm (red arrows) and fragments (blue arrows)



CONCLUSIONS

- Although direct measurements of drug concentration are favorable for dissolution tests, particle sizing methods such as DLS may support dissolution findings for particulate drug formulations that undergo rapid release.
- A change in paclitaxel particle size and signal count rate with time may correspond to drug dissolution. At concentrations near maximum paclitaxel solubility, a gradual breakdown in size occurred over 1 hour, which was confirmed with other methods.
- UV-Vis analysis could not differentiate paclitaxel in particulate vs dissolved form

Acknowledgements

Imaging and particle size measurements were performed at the FDA White Oak Advanced Characterization Facility (ACF). DLS/NTA diagrams were adapted from materials available at Malvern.com.

Disclaimer

The views expressed in this poster do not necessarily reflect the official policies of the Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government. Alternative DLS-based dissolution methods for Ab-Ptx were originally outlined in FDA-2015-P-0732.

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