Simulation of Label-Free PK Evaluation of Nanoparticles in Complex Media

Purpose

It would be highly desirable if nanoparticle drug products could be measured in a complex biological matrix such as plasma, as this information could enable label-free nanoparticle pharmacokinetics (PK) evaluation. An experiment was performed to determine if Microfluidic Resistive Pulse Sensing (MRPS™) might be capable of achieving this.

Methods

90nm Nanobead NIST Traceable sizing standards (Polysciences, Warrington, PA) were used as surrogate nanoparticles (NP) for evaluation of particle concentration in mouse plasma* using the Spectradyne nCS1 MRPS instrument (Figure 1). The particle concentration of the stock was first determined by preparing dilutions in a suitable buffer solution and quantifying using the nCS1. Subsequently, mouse plasma was spiked with the particles to achieve a dilution series for examination with the nCS1 instrument. A background sample of mouse plasma alone was used to determine the background subtraction for subsequent samples containing spiked particles. The derived particle concentrations from the plasma samples were compared to the “true” values calculated from the dilution of the particle stock in buffer. For all measurements, 200-nm Nanobead NIST Traceable sizing standards (Polysciences, Warrington, PA) were also spiked in at a known concentration to serve as an in-measurement control for instrument function.

Results (continued)

The 90nm bead solution was prepared with a target concentration of 1E13 p/mL and measured after serial dilution by the nCS1 to have a stock concentration of 1.07E13 p/mL (Figure 2).

The 90nm particle stock was diluted in mouse plasma to the following concentrations: 0 (plasma only), 5E10, 1E11, 1E12, 1E13 p/mL. Further dilutions (with 1% Tween 20 in 1X PBS, filtered at 20nm) were made with each of these to 100-fold prior to measurement. Note 208nm controls also added.

Figure 3: Measurement of the 90 nm bead solution diluted in mouse plasma to the following concentrations: 0 (plasma only), 5E10, 1E11, 1E12, 1E13 p/mL. Further dilutions (with 1% Tween 20 in 1X PBS, filtered at 20nm to remove any measurable particulates) were made with each of these to 100-fold prior to measurement. Note 208nm controls also added.

Figure 4: Measurement of the 90nm bead solution diluted in mouse plasma to the following concentrations: 0 (plasma only), 5E10, 1E11, 1E12, 1E13 p/mL. Further dilutions (with 1% Tween 20 in 1X PBS, filtered at 20nm to remove any measurable particulates) were made with each of these to 1000-fold prior to measurement. Note 208nm controls also added. Overlaying the 100X and 1000X final dilutions clearly shows a simple 10X shift in concentration, as expected (Figure 5). This is an indication of the excellent linearity of the MRPS concentration measurements.

Finally, a plasma-only sample signal was subtracted from each measurement to remove the background signal from the plasma. Figure 6 shows the results in a tabular and graphical format, with concentration measured in the range 80-130nm, confirming the detection limit of ~1E11p/mL, at both the 100-fold (Figure 3) and 1000-fold (Figure 4) dilutions.

Conclusions

The detection limit for the simulated 90nm drug nanoparticles in plasma using MRPS was ~1E11p/mL. The next step will be to attempt duplication of these results using real drug NPs under the same conditions. Future efforts will also aim to reduce the matrix background without affecting NP concentration, in order to lower the concentration detection limit. MRPS is uniquely qualified for these measurements, because it measures each particle individually using an electrical signal. Optical techniques such as DLS or NTA cannot be used for this type of analysis, due to their sample-dependent Limit of Detection.

References


* All samples were collected in accordance with regulations and established guidelines for humane treatment of research animals and were reviewed and approved by an Institutional Animal Care and Use Committee