Supplementary Information

Measurement of encapsulation efficiency and *in vitro* release of colistin from liposomes by pressure ultrafiltration

Pressure ultrafiltration was used to separate free colistin solution from liposomes to enable determination of encapsulation efficiency[1] and drug release from liposomes as previously reported[2]. A 10 mL Amicon 8010 stirred pressure ultrafiltration cell fitted with a 25 mm regenerated cellulose YM10 ultrafiltration membrane (10,000 kDa nominal molecular weight cut-off) (Millipore Corp., Bedford, MA) was used to separate encapsulated colistin from free colistin. The ultrafiltration membranes were stored in 10% ethanol and were rinsed and soaked in Milli Q water prior to use. Potential adsorption of colistin to the YM10 ultrafiltration membrane was addressed by filtration of a solution of colistin sulfate through the membrane, and quantitation of the colistin concentration in fractions of the ultrafiltrate. The volume of solution that was required to pass through the membrane such that the concentration of drug in the ultrafiltrate was within 95% of that inside the cell was determined and used as the minimum filtrate volume for encapsulation measurements.

Encapsulation and release measurements involved placing 10 mL of liposome suspension in the ultrafiltration cell and applying pressure (nitrogen gas <400 kPa). Approximately 1 mL of ultrafiltrate was collected in two separate 500 μL fractions. The first fraction of ultrafiltrate was used to quantify the concentration of liposomes in the ultrafiltrate. Dynamic light scattering was used to measure the extent of light back scattered by the ultrafiltrate, using the raw count rate (kcps), as an indicator of the presence of colloidal particles[2]. A calibration curve was established by diluting the liposome preparation. A count rate of less than 500 kcps (derived count rate) indicated that <0.005% of the liposomes had passed the membrane. Consequently a count rate of <500 kcps was adopted as the criteria for effective absence of liposomes from the filtrate. Samples with scattering greater than 500 kcps were

rejected and new membranes employed. The second 500 µL fraction was retained for determination of drug concentration in the ultrafiltrate by HPLC.

For determination of *in vitro* release of colistin from liposomes, 10 mL of the liposome suspension was diluted into 40 mL phosphate buffered saline (PBS) and stored in a 50 mL polypropylene tube in a shaking water bath at 37°C for 72 h. This dilution factor was sufficient to disturb the extra-liposomal concentration to enable discrimination of release from the liposomes, without compromising analytical sensitivity. It should be appreciated that the real dilution factor that might occur in vivo likely differs from this and will depend on many variables including the disease state. At 0, 1, 2, 4, 8, 24, 48 and 72 h, a 5 mL sample was removed and passed though the pressure ultrafiltration cell. The filtrate was collected as two 500 µL fractions as described above. The concentration of drug in the ultrafiltrate and the total concentration of drug in the unseparated dispersion at each time point was determined by HPLC. For the aqueous ultrafiltrate samples (phospholipid free), 200 µL of methanol was added to 200 µL of ultrafiltrate and vortex mixed followed by centrifugation at 10,000 rpm. For liposome formulation samples (containing phospholipid), 200 µL of acetonitrile was added to 200 µL of formulation and vortex mixed. After centrifugation for 10 min at 11,200 rpm, the supernatant was diluted sufficiently with methanol to bring the concentration within the linear range. Encapsulation efficiency was calculated according to the equation below. It should be noted that the 'free' or non-liposome associated colistin was not removed prior to *in vitro* release studies.

$$\%encapsulated = \frac{drug\ in\ \ formulation - drug\ in\ \ filtrate}{drug\ in\ \ formulation}$$

References

1. Boyd BJ 2003. Characterisation of drug release from cubosomes using the pressure ultrafiltration method. Int J Pharm 260(2):239-247.
2. Wallace SJ, Li J, Nation RL, Boyd BJ 2012. Drug release from nanomedicines: selection of appropriate encapsulation and release methodology. Drug Deliv and Transl Res In press:DOI 10.1007/s13346-13012-10064-13344.