Investigating the Particle Engineering of Colistin for Pulmonary Delivery

Teresa Jong, Jian Li, John D.F. Hale, David A.V. Morton, and Ian Larson

Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), VIC 3052, Australia

KEYWORDS: powder formulation, spray drying, inverse phase gas chromatography, colistin sulphate, antibiotic

Abstract: The aim was to develop an antibiotic dry powder inhaler formulation that delivers a high drug load to the lower respiratory tract. Particle size of the colistin sulphate (colistin) formulations was determined by laser diffraction. The spray dried formulations were made with a Büchi 190 spray drier, where varied concentrations of the amino acid L-leucine were added. An *in-vitro* method was used to determine the aerosolisation behaviour of each formulation from a Rotahaler and then analysed using a validated HPLC assay. Images of the formulations were taken with a scanning electron microscope. The surface energy of each formulation was determined through inverse gas chromatography. Spray drying colistin (SDC) increased the number of particles <6.5 μm from 23.6% to 83%. This contributed to a significantly (p<0.001) increased fine particle fraction (FPF) (from 15.3% to 38.8%). To further improve the FPF, the colistin formulation was co-spray dried with L-leucine at concentrations of 5, 10 and 20%, which resulted in FPF values of 44.9%, 35.7% and 49.6%, respectively, although these FPF values were not significantly (p>0.05) different from those of SDC. The spray dried colistin particles appeared highly corrunculated and formed cenospheres. Among the spray dried formulations there were no apparent difference in the appearance of the particles. There was a correlation between dispersion performance and total surface energy, with the original colistin obtaining a significantly (p<0.001) higher total surface energy, 307.6 mJ/m2 with a dispersion of 15.3% compared to SDC total surface energy, 161.5mJ/m2 with a dispersion of 38.8%. Therefore a lower total surface energy increases dispersion efficiency. This study has lead to the development of inhalable colistin dry powder formulation that delivers approximately 40% efficiency from a simple passive inhaler device. Particle engineering, such as altering particle size, surface morphology and surface energy, can play a significant role in the extent of drug aerosolised to the lower respiratory tract.

Introduction:

Antibiotics used for the treatment of lung infections can be delivered by the intravenous, oral and other routes, but inhalation may provide a better targeted route for lung infections. Colistin, a cyclic polypeptide linked to a fatty acyl tail, is an old antibiotic with potential nephro- and neuro-toxicity when traditionally administered intravenously. However, with its high efficacy, it has received a new lease of life (1), notably if dose can be minimized by direct delivery to the lung. Dry powder inhalers have the ability to deliver high loads of antibiotics directly to the site of infection in the lungs (2). To increase the efficiency of dry powder inhalers, properties such as particle size, particle morphology and surface energy can be altered (3,4).

Spray drying may increase the dispersion of inhaled drugs (4), and for example co-spray drying the drug with leucine has lead to reduced powder density and an increase in dispersibility (4). However, there is yet to be a fully satisfactory explanation of how spray drying increases this dispersibility. There have been claims that the addition of leucine in spray dried formulations alters the surface energy of the particles allowing the formulation to better aerosolise (4). In previous studies, it was found that aerosol performance could be related to surface energy due to its influence on inter-particulate forces (5), where two condensed systems interact at the interface it creates thermodynamic work of adhesion, that is dependent on the individual surface energy of the two components (6). The surface energy can determine the relative strength of adhesion and agglomeration and thus the extent of dispersion. Also, there have been claims that spray drying with leucine alters the drug particle morphology, where the particle is no longer spherical and exhibits a wrinkled dimple effect (7), thereby changing the contact geometry and aerodynamics of the particles to increase dispersibility. Delivery and deposition in the lower region of the lungs is dependent on the powder's dispersibility which is controlled by the interparticle forces (3). Therefore, altering the surface morphology and surface energy of drug particles should affect the interparticle and interfacial forces, therefore altering the amount of drug to be delivered (3).

The aim of this research was to investigate whether the increase in dispersion is due to the change in surface energy or the changes in morphology of the drug particles.

Materials and Methods:

Spray dried powders containing colistin sulphate (Zhejiang Shenghua Biok Biology Co., Ltd. China), together with varying percentages of L-leucine, (analytical grade, from Sigma-Aldrich, USA) were obtained using a Buchi Mini-Spray Drier 190 (Buchi AG, Switzerland). L-leucine was added to assess its potential to improve aerosolisation behaviour. Aqueous solutions were prepared containing a total of approximately 2% w/v solids loading where this solute comprised colistin sulphate, or colistin sulphate:L-leucine in ratios 95:5, 90:10 or 80:20 w/w. These were spray dried at 2 mL/min liquid flow rate, with inlet temperature adjusted to maintain an outlet temperature of approximately 60°C and with an atomiser air flow rate of 600 L/hr, and aspirator set to maximum. Particles were collected and analysed for various physico-chemical properties. The particle size distributions were determined by laser diffraction as suspensions in ethanol (Malvern Mastersizer S, Malvern Instruments, UK). Particle size and morphology was assessed by electron microscopy (Phenom, FEI, USA). Inverse Gas Chromatography (IGC: Surface Measurement Systems Ltd., UK) was used to determine the surface energies of the different particles. *In vitro* dispersion was measured by aerosolising 15 mg of formulation from a size 3 gelatin capsule (Capsulgel, Australia) in a Rotahaler device (GSK, UK), with at least 5 replicates using a twin stage impinger (TSI: Copley UK). Air flow was set to 60 L/min.

Results and Discussions:

The particle size distribution of the spray dried colistin formulation shows a dramatic shift in particle size from colistin as received to spray dried colistin (SDC), with 78% of the mass of spray dried colistin formulations being <6.4 μm (Figure 1), which indicates this percentage of SDC filled in capsules could potentially be delivered to the lower respiratory system, and similarly to stage 2 of the TSI, where cut-off is <6.4 μm at 60 L/min (8)

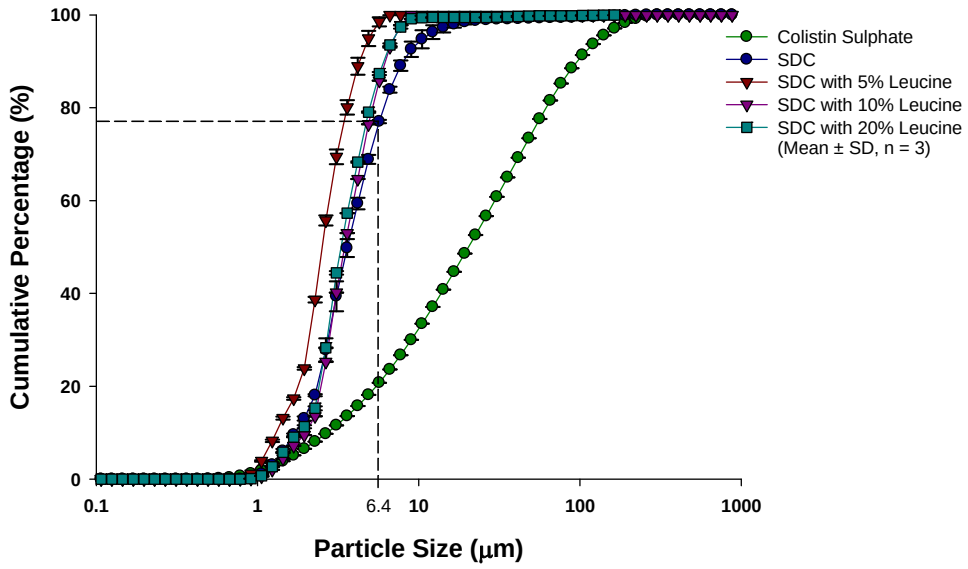

Figure 1 – Particle size distribution

Figure 2 (a) shows colistin as received to be coarse irregular particles, whereas figure 2 (b-e) shows the spray dried formulations as a mix of wrinkled 'pea shaped' particles and cenospheres (hollow particles). The wrinkled particles are proposed to form when the liquid at the surface of the droplet evaporates in the hot air from spray drying and a coherent rubbery shell forms. As drying proceeds, internal pressure causes the shell to swell, but as the internal pressure then decreases, the particle collapses on itself, forming wrinkled particles. Cenospheres appear as particles with holes formed in the shell during the drying process allowing the pressure to escape so the particles neither expand nor collapse. From these images it can be seen that after spray drying colistin, the particles are very different in morphology from the starting powder form. The particle size and morphology seems to be consistent across all the spray dried images. The spray dried SEM images are consistent with particle size distribution as seen in Figure 1, with majority of the particles being less than 10 μm.

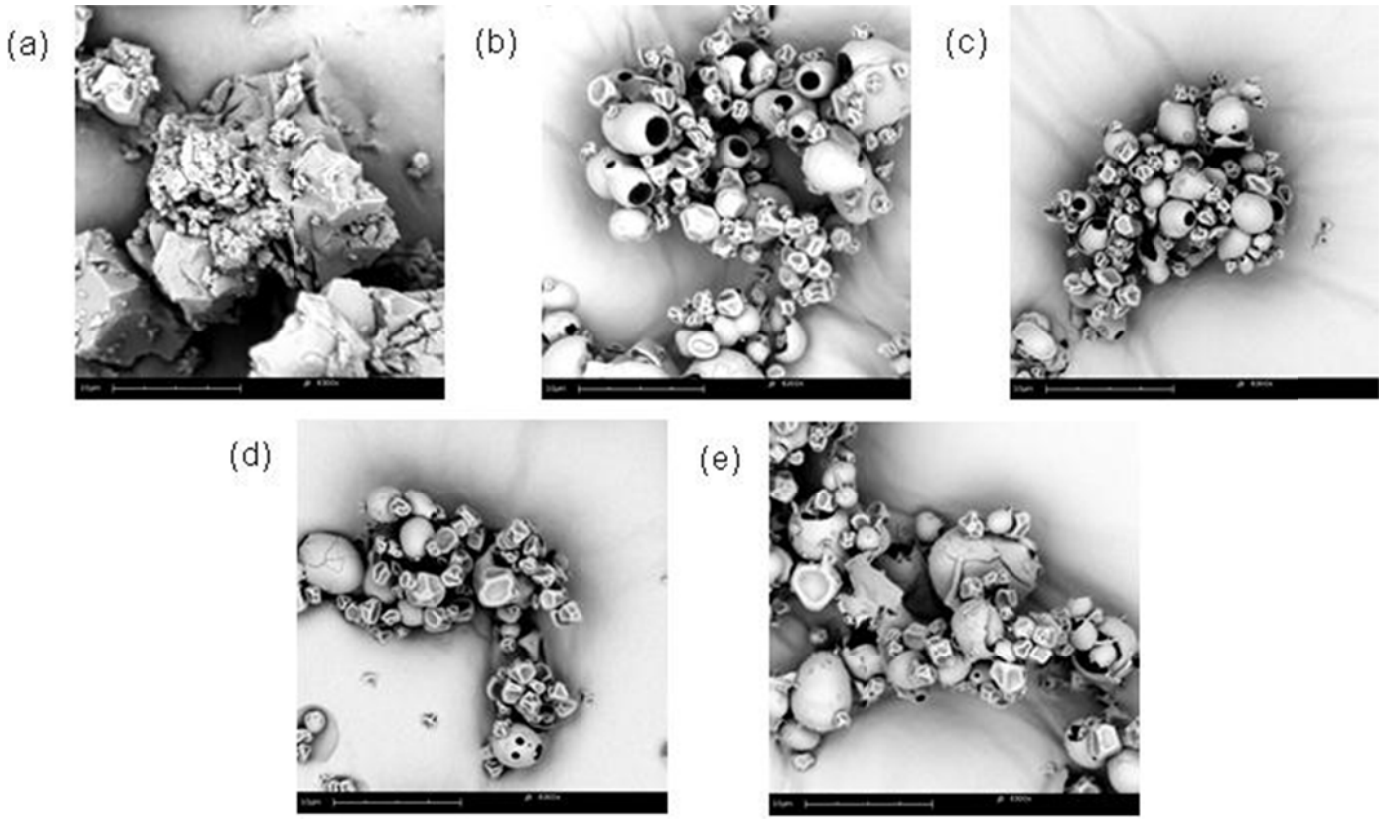

Figure 2 – SEM images of formulations (a) colistin sulphate (b) SDC (c) SDC with 5% leucine (d) SDC with 10% leucine (e) SDC with 20% lecuine.

The decrease in particle size contributed to the increase in fine particle fraction (FPF) significantly ($p < 0.001$) from 15 % to 39% when dispersed from Rotahaler (Figure 3). All spray dried formulations had a significantly higher ($p < 0.001$) fine particle fraction percentage measured by TSI, than colistin as received. When comparing the spray dried formulations to each other, there was no significant difference ($p > 0.05$).

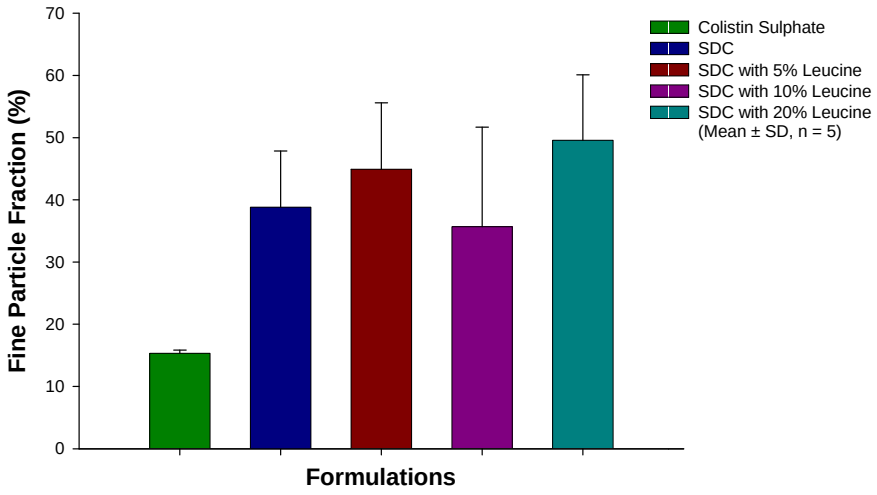

Figure 3 – FPF percentages obtained from TSI measurements

The high dispersion could be due to leucine and/or colistin having surfactant like properties, where the molecules during the drying phase are proposed to self assemble with specific orientation of the molecules at the surface where hydrophobic tails face outwards with the hydrophilic heads facing inside the droplet. As a result of the hydrophobic portion of the molecules being exposed, it causes the particles to become more non-polar and in return lowers surface energy (and may alter the surface morphology) of the spray dried powders which aids dispersion. The hypothesis of self-assembly was tested by conducting IGC surface energy measurements on each of the powder formulations. The results are shown in figure 4, where there is a significantly lower ($p < 0.001$) total surface energy after spray drying when compared to colistin as received.

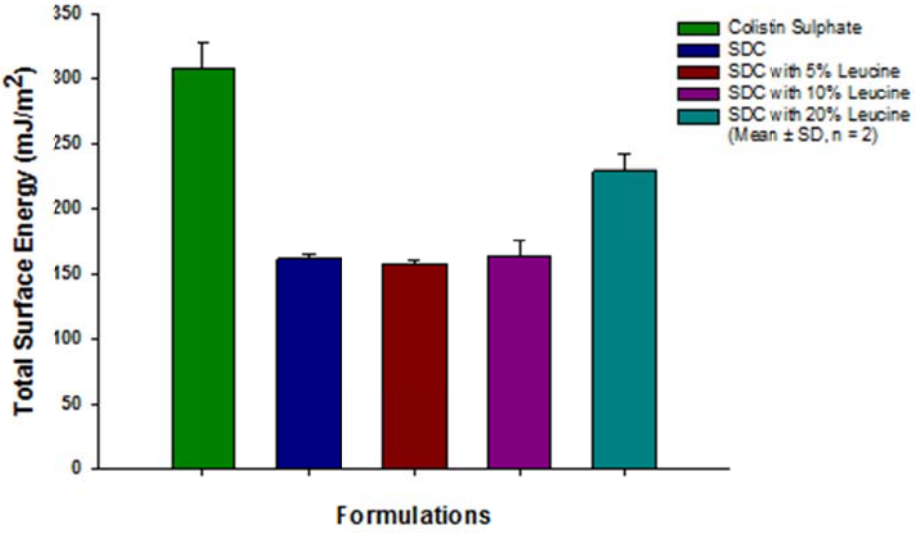

Figure 4 – Surface energies obtained from IGC measurements

These support this concept of arrangement and orientation of molecules when the formulation is spray dried. Consequently, it is proposed that there is a relationship between the total surface energy and dispersion, as the total surface energy decreases the dispersion increases. Our study also suggests that, in this case, L-leucine is not required as an additive to improve aerosolisation, and provides no further reduction in surface energy.

Conclusion:

This study has lead to the development of inhalable colistin dry powder formulations that deliver improved aerosolisation efficiency from a simple passive inhaler device. The IGC measurement provides evidence to support the surface self-assembly hypothesis for this peptide, that may account for the apparent high FPF. Particle engineering, such as altering particle size, surface morphology and surface energy, can play a significant role in the extent of drug dispersed and delivered to the lower respiratory tract.

Acknowledgements:
Teresa Jong acknowledges the scholarship support provided by Monash University and Faculty of Pharmacy and Pharmaceutical Sciences. Jian Li is an Australian National Health & Medical Research Council Senior research Fellow.

References:

1. Li, J., et al., (2006), "Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections," *The Lancet Infectious Diseases,* **6**(9), pp 589-601.
2. Lipworth, B.J., (1996), "Pharmacokinetics of inhaled drugs," *British Journal of Clinical Pharmacology*, 42(6), pp 697-705.
3. Shekunova, B.Y., et al. (2004), "Aerosolisation behaviour of micronised and supercritically-processed powders," *Journal of Aerosol Science,* 34, pp 553-568.
4. Li, H.-Y., et al., (2003), "Enhanced Dispersibility and Deposition of Spray-dried Powders for Pulmonary Gene Therapy," *Journal of Drug Targeting,* 11(7), pp 425-432.
5. Grimsey, I.M., J.C. Feeley, and P. York, *Analysis of the Surface Energy of Pharmaceutical Powders by Inverse Gas Chromatography.* Journal of Pharmaceutical Sciences, 2001. **91**(2).
6. Johnson, K.L., K. Kendall, and A.D. Roberts, *Surface energy and the contact of elastic solids.* Proc. R. Soc. Lond. A., 1971. **324**: p. 301-313.
7. Vehring, R. (2008) "Pharmaceutical particle engineering via spray drying," *Pharmaceutical Research.* 25(5), pp 999-1022.
8. Hallworth, G. and G. Westmoreland, *The twin impinger:a simple device for assessing the delivery of drugs from metered dose pressurized aerosol inhalers.* Journal of Pharmacy and Pharmacology, 1987. **39**: p. 966-972.