



A closer look at PLGA microspheres

David McCarthy

PLGA (poly lactic-co-glycolic acid) is a biodegradable and biocompatible copolymer that is widely used in the pharmaceutical industry as a drug delivery vehicle.

As a microscopist, I am frequently asked to image microspheres/microparticles (and nanospheres/particles) that are formulated from PLGA using the Scanning Electron Microscope (SEM), for both size distribution and shape. Most researchers are concerned with obtaining a formulation that will yield a uniform particle size, which is within a desired range, and that SEM images also match data from the mean particle analyser that uses light scattering techniques. In addition, sometimes it is necessary to image fractured particles to determine if they are solid or hollow.

Fig. 1. Pseudo coloured image of the original microsphere in Fig. 2, shown overleaf.

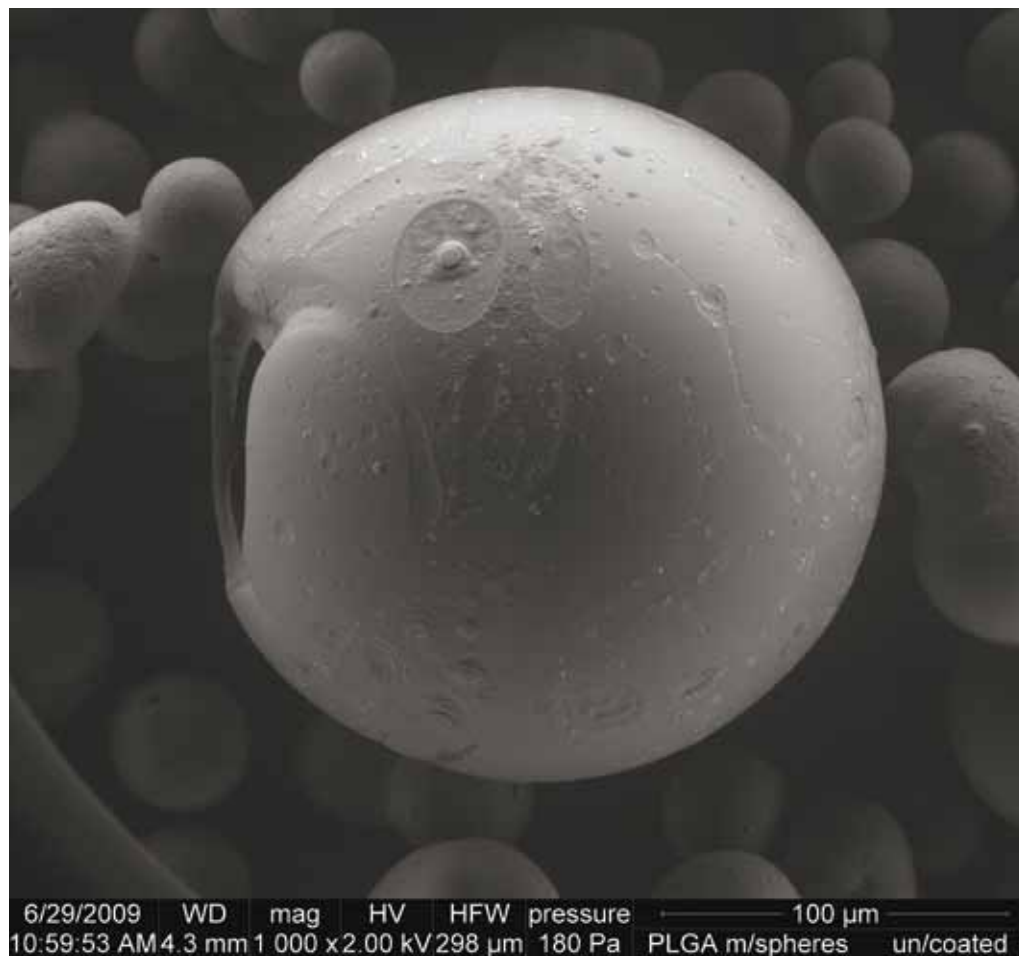


Fig. 2. PLGA Microsphere, this is imaged uncoated, low KV, low vacuum and short working distance. The final image capture was 2048 x 1768 resolution with a 30 ms dwell time.

Samples of PLGA microspheres are usually dried for SEM imaging. Freeze drying or spray drying are the most common methods, the samples are then routinely gold coated (Sputter Coating) prior to viewing. After coating, surface charge is sometimes experienced even at low accelerating voltages due to the nature of the material. Some formulations are thermally sensitive to the beam and also clumping of particles is common; these clumps will break up under the beam and thus the surface conduction from the coating is broken, resulting in movement of particles and further charging (I suspect this is not unique to PLGA microspheres). This can be overcome with patience, or another cycle in the

sputter coater. However, too much gold coating can mask any surface ultra structure and render the spheres clean and smooth. To address these issues, I have shown a comparison between uncoated, low vacuum imaging (Figure 2), with routine SEM imaging (Figure 5) as well as stereo (Figure 6) and pseudo colouring (Figure 1) on the same microsphere.

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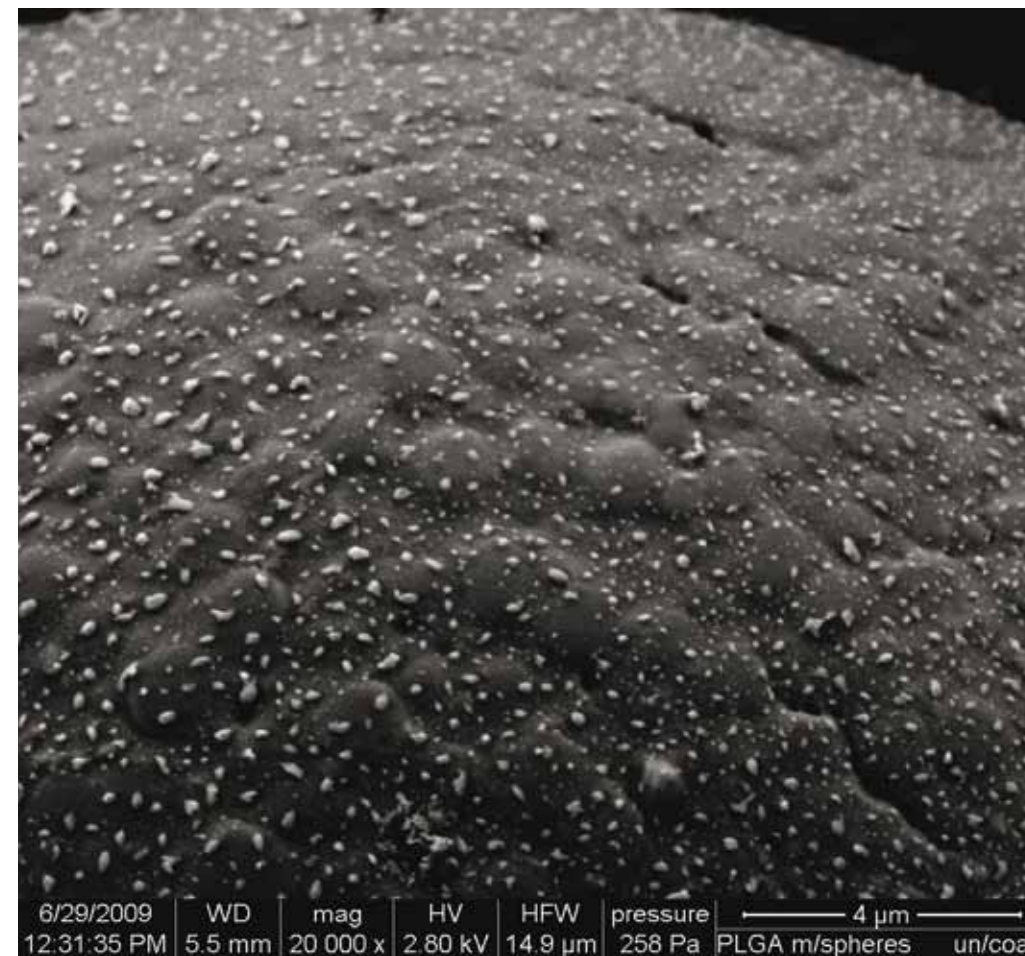


Fig. 3. PLGA Microsphere, high magnification image of the surface of a smaller sphere, good resolution as the sample is uncoated and taken under with a low KV, low vacuum, but a short working distance. However, some depth of field is lost.

Low Vacuum/uncoated SEM imaging (Large Field Detector)

PLGA microspheres were attached to a SEM stub with a carbon adhesive disc and transferred using a squirrel hair brush. Excess sample was removed with a gentle spray of compressed air and then placed into the FEI Quanta FEG 200 for imaging. To enhance surface morphology of the spheres, a low accelerating voltage of 2KV seems to be the optimum for this kind of sample with a chamber pressure of 180 Pascal's, see Figure 2. In addition, to gain maximum resolution at this low KV, the sample working distance was raised to 4 mm. The final image capture was 2048 x 1768 resolution

with a 30 ms dwell time. Figure 3 is taken at a higher magnification of one of the smaller PLGA microspheres that shows more surface detail. In Figure 4, a TV rate view of the sample showing considerable noise, but with information and detail present, achieved with a very slow scan and long dwell time.

SEM imaging at High Vacuum (EHT detector)

The stub containing the PLGA microspheres was then taken out of the microscope, transferred to the sputter coater, an Emitech K550 and coated with Gold/Palladium for one minute at 20mA, then

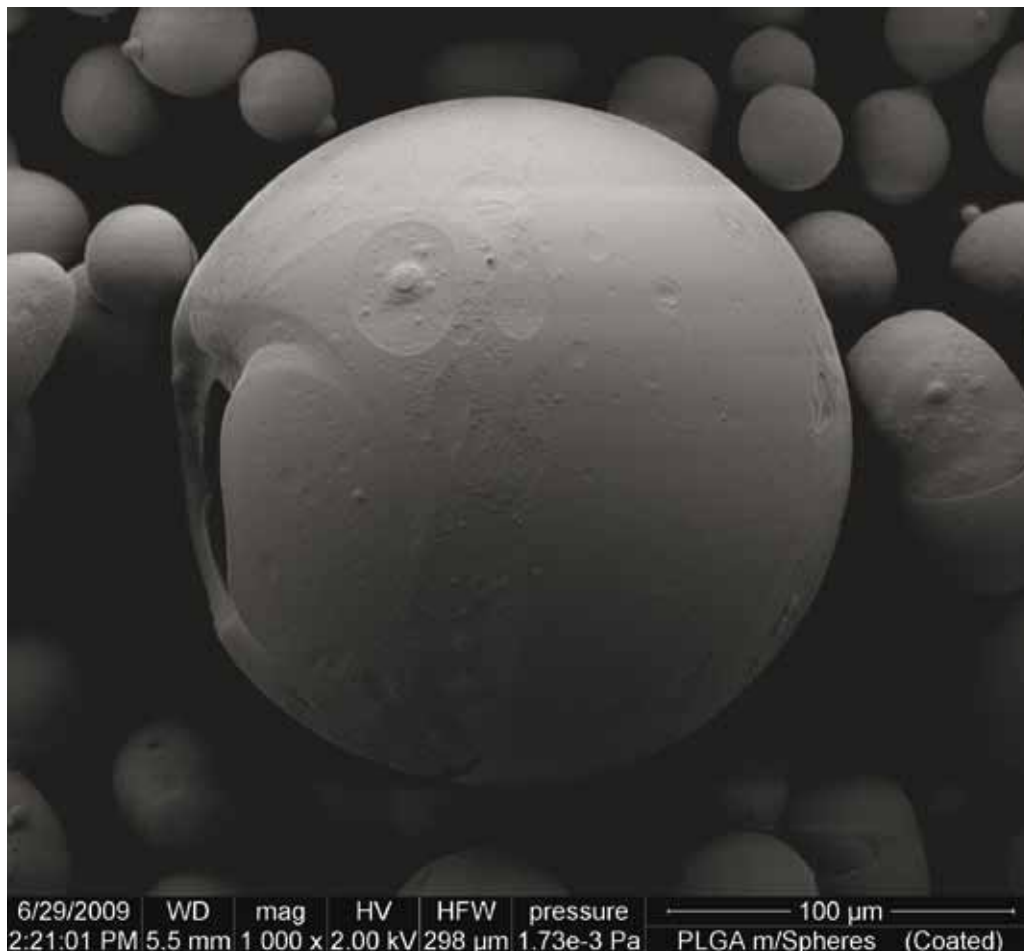


Fig. 5. PLGA Microspheres, Gold/Palladium coated (1 minute at 20mA) and imaged in high vacuum at low KV, short working distance, okay but showing some charge.

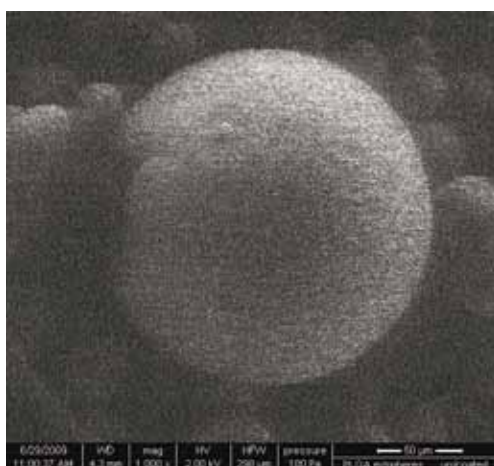


Fig. 4. PLGA Microsphere, imaged uncoated, low KV, low vacuum, short working distance and using a Rapid Scan to show operating noise levels.

back to the microscope for imaging. To compare the image with the previous imaging mode, 2KV was selected (see Figure 5). This image shows good morphology but a careful look will reveal some charging. Although this is at an acceptable level, it is always best to minimise or eliminate charging totally.

Conclusion

The advancement of technology in high resolution, low vacuum, low KV SEM imaging, coupled with the Large Field secondary electron Detector (LFD) on uncoated samples, is now a reality. No sputter coating means, not only are we imaging the true surface, but any change in surface detail from heat generated by the coater is completely avoided

as well as masking any surface detail with metal. The short working distance necessary to achieve optimum resolution does limit the ability to tilt specimens but, in Figure 6, a working distance of 10 mm was used to perform a stereo image, with no significant loss in image quality (although with some tweaking). In Figure 7, a high stage tilt of 72 degrees was applied and some charging occurred under these conditions so a further 2 minutes of coating

was performed. Figure 1 shows how much more impact an image can have by being pseudo-coloured.

Acknowledgments

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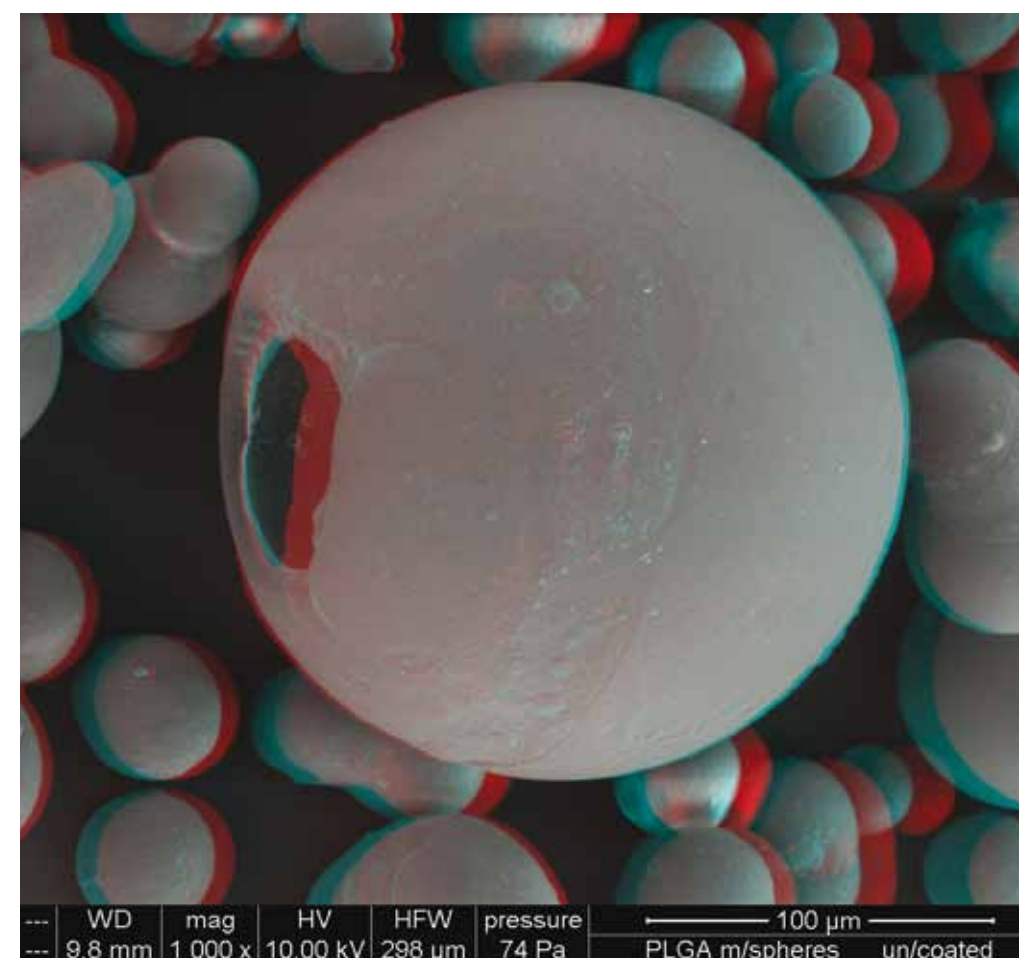


Fig. 6. PLGA Microspheres, Stereo pair (anaglyphs). Higher KV to compensate the longer working distance of 10 mm (eucentric height). Stereo Glasses required when viewing this image.

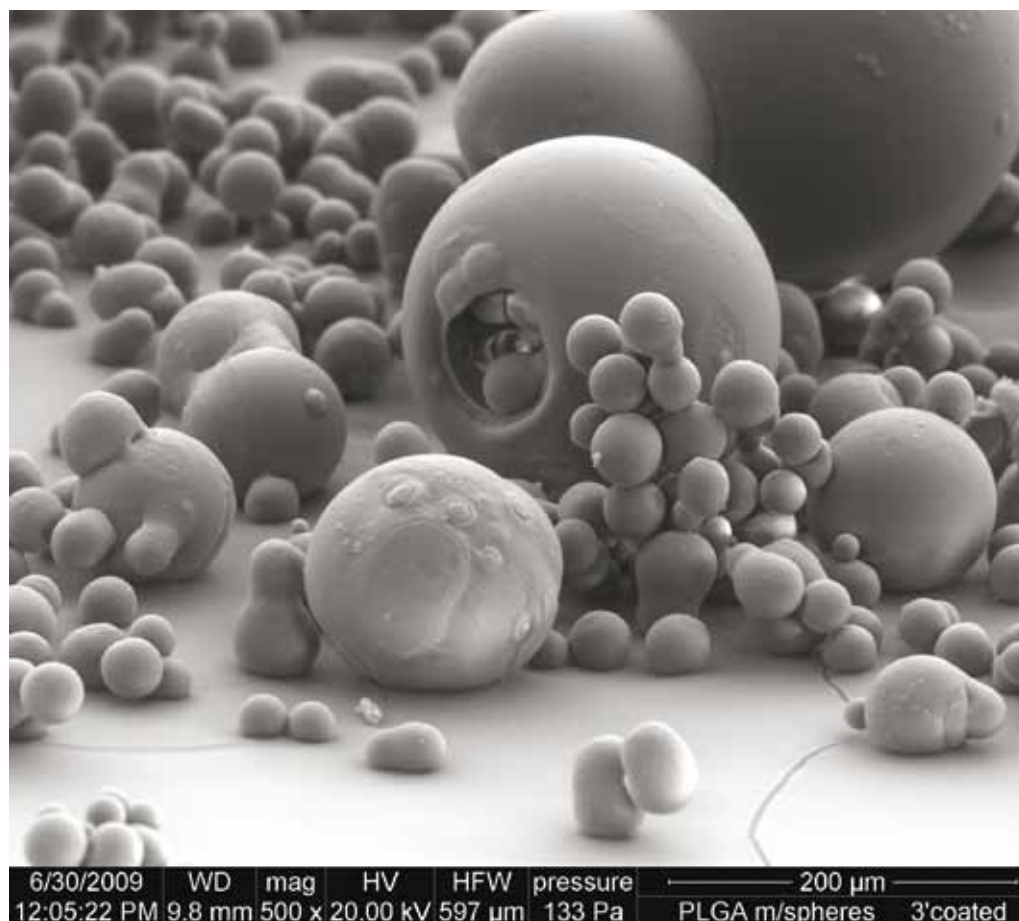


Fig. 7. PLGA Microspheres. This sample was imaged in the Low vacuum mode, but has been coated for 3 minutes. I feel showing some loss of surface detail. A higher KV was used to compensate the longer working distance and a high stage tilt angle of 72 degrees.

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David set-up the EM unit in The School of Pharmacy in January 1977 and has imaged a wide variety of samples using the TEM, SEM and optical microscopes, including cell cultures, crystals and most forms of drug delivery vehicles, from liposome's and polymers to carbon nanotubes. The unit currently has a FEI CM120 Biotwin TEM with an AMT digital camera, a FEI Quanta FEG ESEM and a Nikon Microphot optical microscope.

David has been a microscopist for 37 years and has won many awards for images, including 10 'Images of Excellence' from the Wellcome Trust (2007/8/9), First prize for the UK Micro and Nanotechnology Network Image Competition (2006), Overall winner in the 'Visions of Science' competition in both 2004 and 2005 and 5 prizes from the RMS International Micrograph Competition (including two Firsts). David is also honorary secretary of the Society of Electron Microscope Technology (SEMT) from 2000 and since 2005 sits on the RMS EM section committee.

