doi:10.1017/S1431927621000945
Microsc. Microanal. 27 (Suppl 1), 2021


# Significance of Cryogenic Broad Ion Beam Milling in Evaluating Microstructures of PLGA-based Drug Products

Youlong Ma[1], Jing Liang[2], Jiwen Zheng[2], Yan Wang[3], Muhammad Ashraf[1] and Charudharshini Srinivasan[1]

[1]Division of Product Quality Research, Office of Testing and Research, Office of Pharmaceutical Quality, Center for Drug Evaluation Research, US Food and Drug Administration, Silver Spring, MD, USA, United States, [2]Division of Biology, Chemistry and Materials Science, Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, US Food and Drug Administration, Silver Spring, MD, USA, United States, [3]Division of Therapeutic Performance, Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, MD, USA., United States

Poly Lactic-co-Glycolic Acid (PLGA)-based microspheres are being explored as a controlled-release strategy for delivering a variety of active pharmaceutical ingredients (APIs) because of its biocompatibility and biodegradability. Currently, there is no generic PLGA-based drug product [1]. Development of PLGA-based generic drug product remains challenging since subtle differences during manufacturing steps may result in significant changes in physicochemical properties, and subsequently affect the safety, stability and release characteristics of the drug product [2; 3]. Therefore, studies to characterize microstructural equivalence (Q3) among the PLGA-based drug products are of critical importance. The aim of this work is to characterize the internal microstructures and composition of PLGA-based microspheres. Towards that end, as an exploratory investigation of PLGA-based microspheres, a robust method has been developed to assess the native morphology and drug distribution within these microspheres. In this work, a cryogenic broad ion beam (BIB) milling system (TiC-3X, Leica) was utilized for high-quality sectioning of PLGA-based microspheres (i.e., ARESTIN®, minocycline hydrochloride microsphere). The BIB milling system assisted with liquid nitrogen (LN2) cooling during the ion milling was used to prepare cross-sectional samples. These samples of PLGA-based microspheres were prepared at 2kV acceleration voltage and 10 hours of milling time at -160°C. The cross-sections of microsphere were gold coated prior to imaging. The imaging and elemental analysis of samples were conducted using a field emission scanning electron microscope (FE-SEM, TESCAN Mira3) equipped with energy dispersive X-ray spectrometer (EDS, Oxford Max-80 SDD). To demonstrate the maintenance of native microstructures of these microspheres, samples prepared with BIB milling system were compared with blade cut samples as shown in Figure 1. Blade cut microspheres show significant damage and uneven cross-section (Figure 1A), while BIB milling induced minimal damage to the cross-sections (Figure 1B). Microspheres were further characterized individually to examine the microstructures (Figure 2A). The gray scale image of microspheres presents the two-phase structure comprising bright regions of API particles (minocycline hydrochloride) within a darker PLGA matrix. A pseudo-color image was obtained by overlaying the original micrograph with the post-processed binary image (Figure 2B and 2C). The API particles which were found embedded within the matrix were further analyzed using EDS to confirm its chemical composition. The EDS spectra (Figure 2D) provided confirmation for API composition with nitrogen (N) corresponding to minocycline hydrochloride, while the composition of the matrix had carbon (C) and oxygen (O) which corresponds to PLGA microspheres. Our results show the importance of utilizing cryogenic BIB milling in microstructural imaging and analysis. BIB technique facilitated the imaging of the API distribution within the microspheres with high-quality cross-sectional samples with no damage preserving the native morphology, compared to conventional blade cutting. The BIB method developed in this study may be used as a prospective tool to investigate the equivalence of microstructural properties between the Reference Listed Drug and generic drug product.

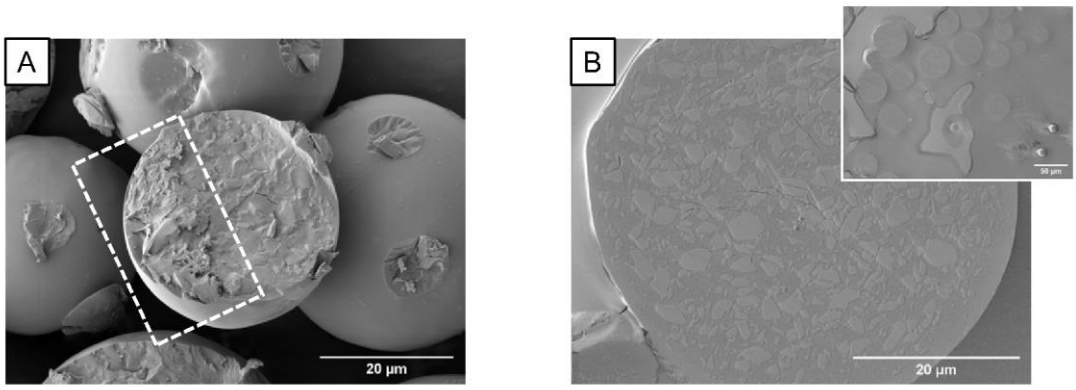


**Figure 1.** SEM micrographs of PLGA microspheres (ARESTIN®). (A) Blade cutting of microspheres show significant smear (white dashed box) and uneven cross-section. (B) SEM micrograph of a single microsphere processed using the broad ion beam milling reveal microstructures with no damage. The inset shows group of sectioned microspheres at lower magnification.

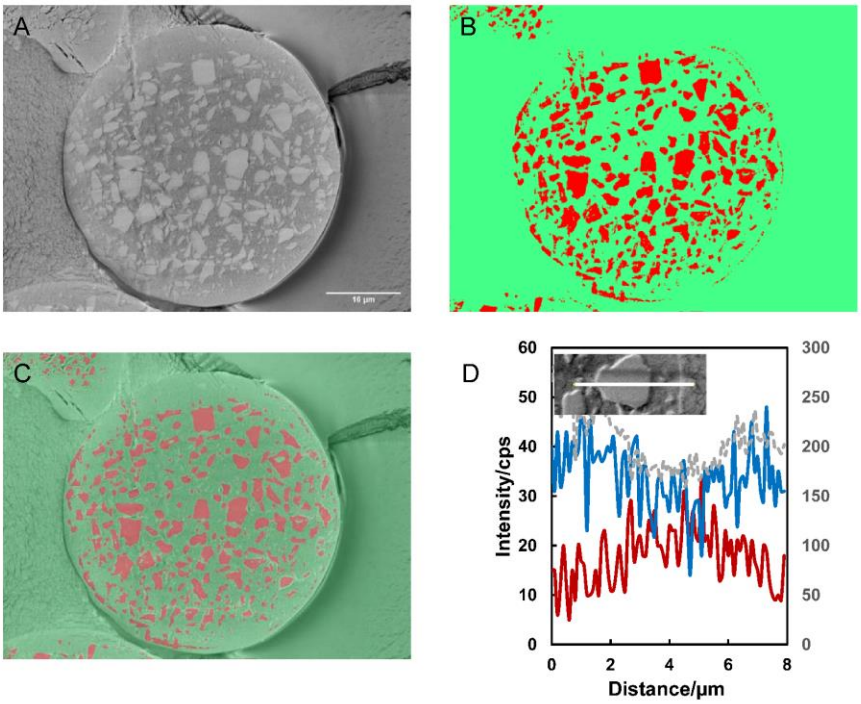


**Figure 2.** Figure 2. SEM micrograph showing drug distribution within the PLGA microspheres (ARESTIN®) with EDS analysis. (A) The gray scale micrograph reveals API particles (bright region) embedded within the PLGA matrix. (B) Post-processed binary image using ImageJ presents API particles in 'Red' with a green background. (C) Overlay of A and B micrographs. (D) API particles analyzed with EDS to determine the composition, including Carbon (Gray), Oxygen (Blue) and Nitrogen (Red).

References

[1] Yan Wang, et al. American Pharmaceutical Review **19** (2016), p. 5-9.
[2] John Garner, et al. Journal of pharmaceutical sciences **107** (2018), p. 353-361.
[3] Chengqian Zhang, et al. International Journal of Pharmaceutics **585** (2020), p. 119441.

https://doi.org/10.1017/S1431927621000945 Published online by Cambridge University Press