

# Polymer Cross-Section Preparation and Analysis Application with Drug Delivery Particles

mPrep<sup>TM</sup>  
System

Applications Note #505

## Introduction

Polymer particles are ubiquitous in plastics manufacturing, pharmaceuticals, biomedical devices, and consumer products. It is common for microscopists to be called upon to analyze the chemical composition, elemental content and morphology of such materials using scanning and transmission electron microscopy (SEM, TEM), energy dispersive x-ray spectroscopy (EDS), and Fourier Transform Infrared (FTIR) and Raman spectroscopy. Before many of these analyses can be performed, the particles, polymeric coatings, thin films and other configurations require cross-sectional preparation, where the specimens must be embedded to enable microtomy.

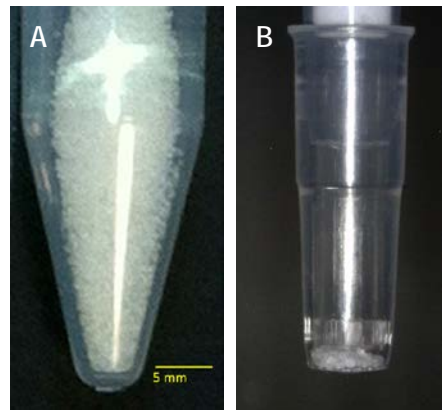
This application note demonstrates the preparation of polymeric particles (approximately 0.5 mm diameter) using mPrep/s specimen capsules for embedding and sectioning. The example shown here is a pharmaceutical hydrogel, but this applications note also illustrates how to apply these methods to thin films, fibers and other material configurations.

In most industrial lab environments, it is important that analyses be done as quickly and efficiently as possible. In pharmaceutical and medical device labs there is the additional regulatory burden of ensuring that specimens and analyses are traceable in order to meet good laboratory practice (GLP) and other documentation or regulatory standards [1, 2]. By simultaneously preparing multiple specimens with mPrep capsules and pipettor processing, the preparative process becomes very efficient. Additionally, since each specimen is always within an individually labeled capsule throughout processing and storage, this intrinsic continuous tracking greatly simplifies achieving GLP specimen traceability.

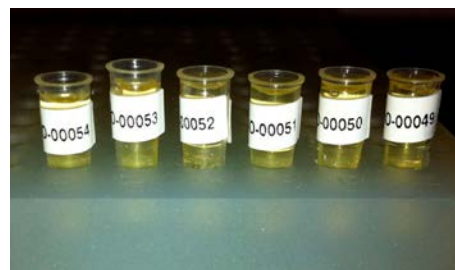
## Specimen Preparation

Four formulations of 0.5 mm diameter pharmaceutical hydrogel particles were embedded and sectioned to enable chemical analysis by transmitted FTIR imaging spectroscopy and optical microscopy, and elemental analysis by SEM-EDS. In this study, an absolutely dry state was used to prepare and examine the particles. Figure 1 shows the micro-particles as received. A small quantity of particles, roughly 10–20  $\mu$ l, were simply placed into the bottom of mPrep/s capsules and entrapped by insertion of the mPrep/s screen. Labels were then applied to each mPrep/s capsule to identify its contents (not shown).

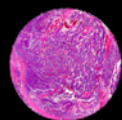
Eight mPrep/s capsules were prepared from the four particle formulations with duplicates of each. The capsules were then attached to a Pipetman Neo<sup>®</sup> 8-channel P200 pipettor (Gilson P8X200N) using mPrep/f couplers to enable



**Figure 1: Polymeric micro-particles.** Photographs show micro-particles (A) in a centrifuge tube and (B) entrapped in mPrep/s capsule by the mPrep/s screen.



**Figure 2: Epoxy-embedded specimens in mPrep/s capsules.** Several specimens in labeled mPrep/s capsules are shown in the mPrep/bench.



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simultaneous reagent delivery [2, 3]. To ensure complete dehydration and good embedding, all 8 capsules with particles were simultaneously rinsed by pipetting 3 changes of 100% acetone into the capsules from a high-density polyethylene (HDPE) reservoir. Each rinse cycle involved aspirating 100  $\mu$ l of acetone into all capsules, holding the liquid in the capsules for 3 minutes, and then dispensing the acetone to waste. After the third acetone rinse was discarded, 100  $\mu$ l of an Epon-Spurrs epoxy resin [3] was aspirated from an HDPE reservoir into the capsules, held for 10 minutes, dispensed to waste and exchanged for fresh epoxy. The 8 epoxy-filled capsules were then inserted into the wells of the silicone mPrep/bench microplate and the pipettor ejector button was pressed to transfer the epoxy-filled capsules into 8 of the wells in the mPrep/bench. The mPrep/bench sealed the bottom of the capsules to retain the fluid epoxy. The mPrep/f couplers were then removed and additional resin was used to fill each mPrep/s capsules to the top. The mPrep/bench was then transferred to a 60°C oven to cure the resin overnight. Several cured epoxy-embedded specimens in the mPrep/bench are shown in Figure 2.

After curing, the mPrep/s capsules were directly mounted in a Leica U7 microtome chuck where the capsule and epoxy were trimmed to create the mesas and block-faces suitable for semi-thin sectioning (Figure 3). Sections were then cut to a thickness of 2–3  $\mu$ m and placed with forceps onto BaF<sub>2</sub> windows for FTIR analysis. Because the particles were to be examined in a fully dry state, a dry glass knife was used. Sections were also placed onto SEM stubs using double-stick carbon tape for SEM-EDS analysis. For additional correlative analyses, several sections of each type of particle were also mounted on the SEM stubs. To prevent charging and still enable EDS analysis, the SEM stubs were lightly carbon coated. Additional correlative specimens (not shown here) included particles imaged in the hydrated state using light microscopy and by various cryo-preparation protocols for SEM.

## Results and Discussion

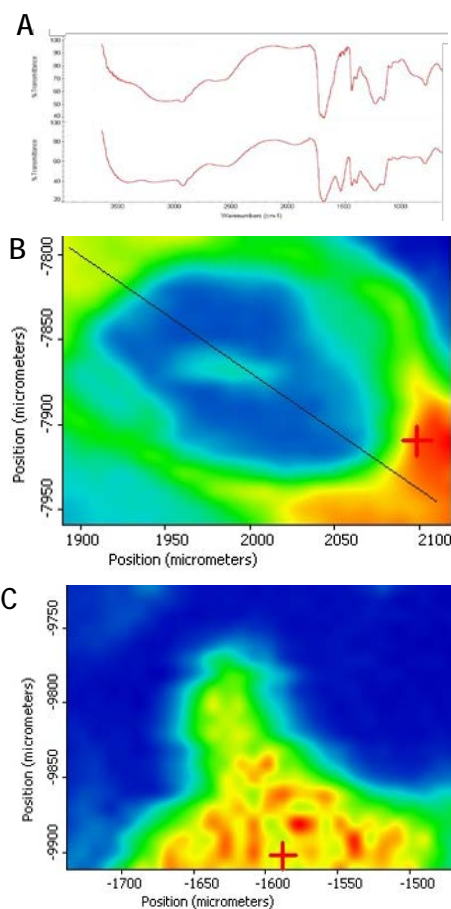
The particle sections were examined with transmitted infrared (FTIR) spectroscopy (Figure 4). The FTIR spectra (4A) and spectral maps of different particles (4B, 4C) show clear spectral differences between the different types of particles. For reasons of confidentiality, details of the particle composition and spectral analyses are not provided.

Dry sections and whole particles were examined with SEM and EDS (Figure 5). The secondary electron SEM image in Figure 5A shows section wrinkling due to the dry sectioning. SEM-EDS of this same section shows the outlines of several micro-particles due to their different elemental composition than the epoxy embedment (5B, 5C). A typical whole particle is shown by SEM along (5D) with a map for a single element (5E). Again, due to confidentiality, details of the elemental composition cannot be provided [2].

All 8 blocks were prepared simultaneously with 4 different particle formulations. Placing the particles in the mPrep/s capsules, labeling them



**Figure 3: Cross-sectioned particles.** An embedded particle specimen block is held in a microtome chuck after facing and sectioning through the mPrep/s capsule. Several visible cross-sectioned particles (arrow) can be seen in the smooth block face.



**Figure 4: FTIR spectra.** A. Two different particle types. B. FTIR spectral map of a particle. C. Spectral chemical map of the edge of a different particle.

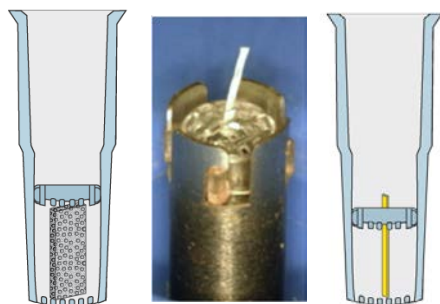
for identification, connecting them to the 8-channel pipettor and laying out the embedding reagents and labware prior to processing required less than 10 minutes. The majority of time needed to embed the samples was waiting during the three 3-minute acetone rinses, the 10-minute epoxy infiltration treatment, and the overnight curing. Actual hands-on time for all reagent exchanges and resin embedding was only a few minutes. Similarly, preparing the blocks for sectioning and then obtaining suitable sections that traversed multiple particles (ideal for microscopy and spectroscopy) was quick because the particles were entrapped in high concentration near the tip of the mPrep/s capsule, requiring only minimal trimming and sectioning.

### Summary and Conclusions

The mPrep/s capsule method provided easy, rapid, and identical sample preparation, with minimal hands-on time enabled by using simultaneous 8-channel pipetting steps. Because mPrep/s capsules provide a means for labeling specimens throughout all processing steps, GLP-level documentation was enabled, which is important in pharmaceutical and medical device microscopy [1, 2].

### Additional and Related Applications

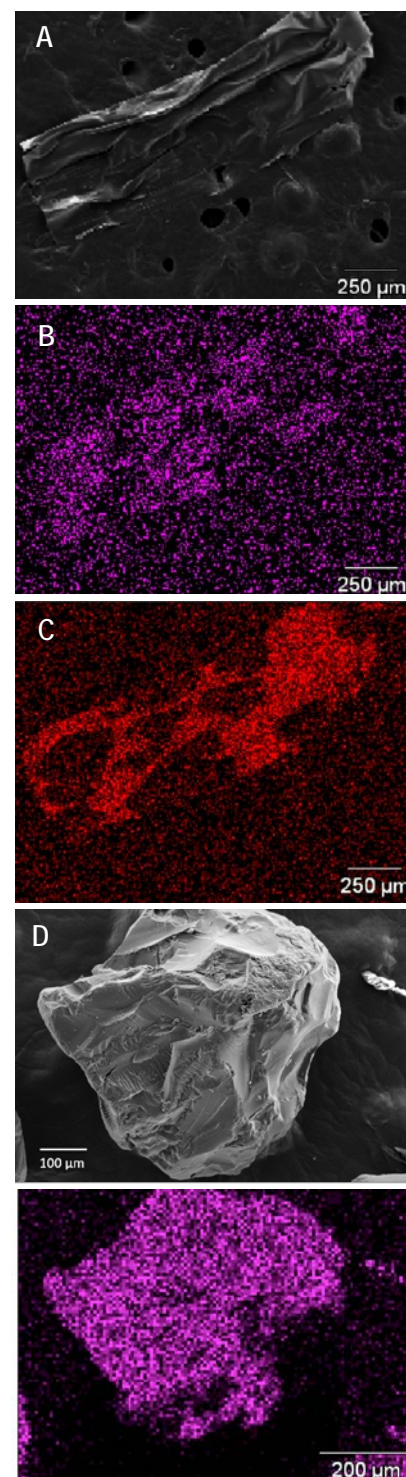
While the method here demonstrated sample preparation of particles of approximately 0.5 mm diameter, this type of preparation can be readily applied to prepare other materials for similar cross-sectional analysis. For example, sufficiently rigid polymers, films and coated substrates can be simply cut to fit across the inside diameter of the mPrep/s capsule (4.3 mm) and then be entrapped with the mPrep/s screen (Figure 6A). With the mPrep/s Workstation, it is also easy to clamp fibers, small segments of films and many other materials in the mPrep/s screen for positioning in the capsule for embedding (6B, 6C). Note that embedding materials other than the Epon-Spurr's resin, such as epoxies, acrylics, waxes, and other thermal melting embedding materials, can be used.



**Figure 6: Multiple specimen types can be oriented in mPrep/s capsules for embedding and sectioning.** A) Diagram of thin film specimen cut to fit across capsule diameter. B) Photo of nano-fiber bundle clamped in mPrep/s screen and held with Workstation prior to sliding into capsule. C) Diagram of fiber or other specimen held by mPrep/s screen within capsule.

### References

1. SL Goodman, JJ Edwards (2014) Patent Litigation and the Power of Correlative Microscopy. *Microsc Microanal*, 20 (suppl 3) (2014) 1416–7.



**Figure 5: SEM and EDS analysis of particles.** A cross-section through multiple particles of the same type embedded in an epoxy block imaged by Secondary SEM (A) and SEM-EDS (B, C) single element maps. A whole particle imaged with SEM (D) and an EDS single element map (E).

2. SL Goodman (2015) Efficient and Documented Preparation of Pharmaceutical Particles for Correlative Microscopy Analyses using mPrep Capsule Processing. *Microsc Microanal*, (2015), in press.
3. SL Goodman, KD Wendt, MS Kostrna, C Radi (2015) Capsule-Based Processing and Handling of Electron Microscopy Specimens and Grids. *Microscopy Today*, in press.

Ordering Information

<b>Product #</b>	<b>Item Description/Catalog Information</b>
S0812	8 mPrep/s capsules, 12 screens, 8 label sets in capsule/grid storage box
F1601	16 mPrep/f standard pore 30 µm filter couplers in capsule/grid storage box
B96S	mPrep/bench Model 96S silicone rack for mPrep capsules, 96-well
TL100	mPrep/s screen insertion tool
R1550	15ml reagent reservoirs, non-sterile, HDPE, 50/PK
WS100	mPrep/s Workstation for orienting specimens
KIT_xxx	Starter kits with mPrep capsules and accessories including Gilson Pipetman Neo® pipettor (various custom kits available — please inquire)