Efficient Nanoparticle Specimen Preparation for Transmission Electron Microscopy

mPrep™ System

Applications Note #504

Introduction

Nanoparticles are routinely examined with transmission electron microscopy (TEM) to measure size, determine morphology, evaluate uniformity, and elucidate aspects of function. Negative staining may be the most common preparation procedure for nanoparticles, however other protocols such as positive staining, no staining, or other treatments may be appropriate depending on the nanoparticle composition and structure.

Typically, the preparation protocol is to apply a droplet of nanoparticle suspension onto the surface of a Formvar® film-coated TEM grid [1]. After attachment of the nanoparticles to the film surface, the grid is rinsed to remove non-adherent particles, negatively stained with uranyl acetate [2, 3], rinsed a second time, blotted, and then stored in a grid box until removing the grid for TEM. Each of these steps requires use of a fine forceps to pick up the delicate grid and move it between solutions (to different reagent droplets and/or dipping into rinse or reagent baths). This extensive handling with forceps can be challenging to perform without dropping or damaging the grid or the support film. Additionally, when multiple grids are prepared at once, it is easy to inadvertently mix them up or not process them identically.

This application note demonstrates a simple methodology to prepare nanoparticle specimens on TEM grids efficiently and reproducibly using mPrep/g processing. By using mPrep/g capsules to contain the grids for all processing and storage steps, the grids are rarely handled directly, thus making the protocol easier to perform by both experienced and non-experienced lab personnel. Moreover, mPrep/g capsule processing can be used to prepare a single grid or several dozen grids with the same ease, by using common labware.

Note that different nanoparticles may require preparation modifications that can easily be accomplished using mPrep/g capsule processing described in the Sample Preparation section and the options described in the Protocol Variations section.

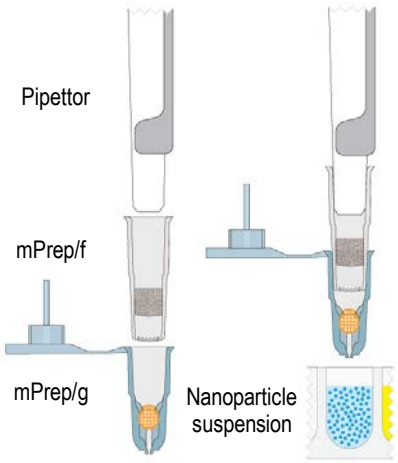

Figure 1: Setting up mPrep/g capsules with grids and application of nanoparticles. A single mPrep/g capsule containing two grids is attached to a single-channel pipettor via an mPrep/f filter coupler. In practice, identification labels are attached to each mPrep/g capsule, and multichannel pipettors can be used to process several grids.

Sample Preparation

Nanoparticle specimens were prepared for TEM using the protocol described below:

1. Formvar-filmed 400 mesh Cu grids were prepared using standard methods [1].
2. Using forceps, two Formvar-filmed grids were inserted into each labeled mPrep/g capsule.

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3. Each mPrep/g capsule containing grids was attached to an mPrep/f filter coupler and placed on a channel of a Pipetman Neo® 8-channel P200 pipettor (Figure 1).
4. 40 µl of aqueous nanoparticle suspension was pipetted into a row of 8 microplate wells to accommodate 8 grid-containing mPrep/g capsules. Note that all other reagents, deionized (DI) water for rinses and uranyl acetate solution for staining, were also pipetted into rows of the same microplate for efficient processing.
5. After adjusting the volume of the pipettor to 35 µl, the nanoparticle solution was aspirated into the mPrep/g capsules, and held inside the capsule for 30 minutes of adsorption, and then dispensed to waste (Figures 1 and 2). A lab stand was used to support the pipettor vertically in the microplate vessel.
6. Non-adherent nanoparticles were rinsed out using 8 changes (8 x 35 µl) of DI water by aspirating and dispensing to waste (Figure 2).
7. After air-drying for 5 minutes to enhance particle adsorption to the filmed grids, 35 µl of 50% ethanolic 2.5% uranyl acetate stain was aspirated into each mPrep/g capsule, held inside the capsule for 10 minutes using the lab stand support, and then dispensed to waste (Figure 2).
8. The stain was rinsed out with 8 changes of DI water.
9. Each mPrep/g capsule was then separated from its mPrep/f coupler and placed uncapped in the mPrep capsule/grid box to air-dry the grids, and then closed and stored until TEM imaging.
10. Grids were removed from the mPrep/g capsules with forceps and imaged with an FEI Tecnai™ T12 at 80 KeV and recorded to an FEI Ultrascan™ Camera.

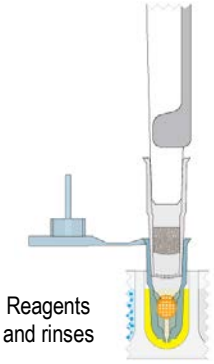

Figure 2: Positioning mPrep/g capsules in reagent vessel. Use a lab stand (not shown) to support the pipettor with the capsule(s) resting in a 96-well microplate or other vessel filled with nanoparticles, rinse, stain, or other reagent.

Protocol Variations

The general Sample Preparation protocol may be readily adapted for various types of nanoparticles, different concentrations of nanoparticles, or for other analytical or experimental requirements. Some protocol variations include:

- Filmed grids may be treated to enhance nanoparticle adsorption by aspirating appropriate reagents into the capsule to alter hydrophilic and hydrophobic properties and/or to adsorb particle-binding ligands to the grid film.
- Some nanoparticles may sediment quickly. After aspirating the particle solution, laying the pipettor on its side with the grids oriented horizontally (Figure 3) may help enhance sedimentation onto the Formvar film. For this option, the volume of the aspirated nanoparticle suspension may need to be increased to about 50 µl to insure that both grids are immersed when the pipettor is on its side. Note that this method exposes the mPrep/f filter coupler to nanoparticle contamination, so the mPrep/f coupler should not be reused.
- The number of water rinses may be reduced or eliminated for nanoparticles that adsorb poorly or are present in low concentrations.
- The particle adsorption time, staining time, and the amount of rinsing may be readily adjusted. Unlike droplet methods, the mPrep/g capsules enclose the reagent, thus making very long adsorption and reaction times feasible without concern for reagent evaporation.

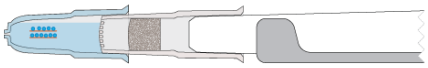

Figure 3: Enhancing nanoparticle sedimentation. Setting mPrep/g capsules horizontally can enhance sedimentation of nanoparticles onto filmed grids.

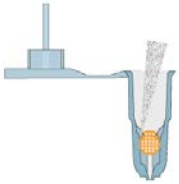

Figure 4: Blotting grids. Grids can be blotted while held inside mPrep/g capsules with a filter paper wedge or similar absorbent material.

- Negative stains other than uranyl acetate may be used [4], as can methods that use no negative stain.
- Positive stains or nanoparticle treatment solutions can be easily aspirated into the mPrep/g capsules for *in situ* processing.
- A wedge of absorbent material can be used to blot the grids. Insert a filter paper wedge or similar material into the mPrep/g capsule and touch where the grid edges are held in the capsule (Figure 4).

Results and Discussion

The overall quality of nanoparticle preparation using mPrep/g processing is comparable to conventional droplet staining and provides these advantages:

- Grid preparation is more efficient, especially for accomplishing multi-parameter analyses in a single run. Figure 5 shows three (of 8 total) preparations that were processed simultaneously by using mPrep/g capsules attached to different channels of an 8-channel Pipetman, and by pipetting all reagents directly from a 96-well microplate.
- Reduced grid handling saves time and minimizes the potential for grid loss, damage, or mix-up.
- Grids in the mPrep/g capsules are always held parallel to the fluid flow direction, exposing the film to less force than droplet methods and reducing the potential for film damage.
- Multiple grids are prepared at once, saving time and providing identical process timing.
- The method can be easily extended to prepare large numbers of grids by stacking capsules and/or using multichannel pipettors. By stacking 4 mPrep/g capsules onto a single-channel pipettor or one channel of a multichannel pipettor, 8 grids can be prepared simultaneously with the same protocol; stacking on an 8-channel pipettor enables simultaneous processing of 64 grids and a 12-channel pipettor can process 96.
- Only 20 µl of reagent is used per grid thus minimizing the amount of precious specimens and reagents.

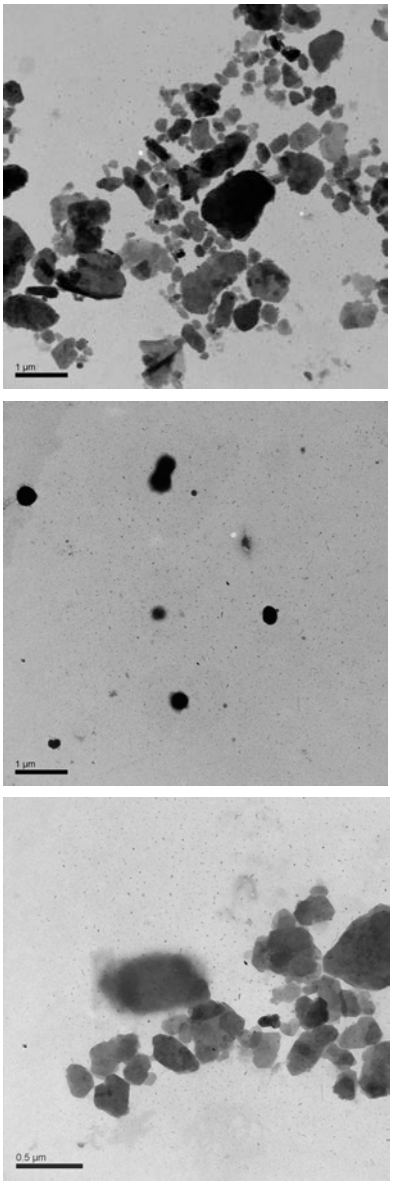

Figure 5: TEM images of three different nanoparticle preparations processed using mPrep/g capsules. All three were prepared simultaneously using the described method for mPrep/g capsules attached to different channels of an 8-channel Pipetman Neo® pipettor.

References

1. Bozzola JJ, Russell LD (1999) *Electron Microscopy*, 2nd Ed, Jones and Bartlett. Boston.
2. Hayat MA, Miller SE (1990) *Negative staining*. New York: McGraw-Hill Pub. Co.
3. Brenner S, Horne RW (1959) Negative staining method for high resolution electron microscopy of viruses. *Biochim Biophys Acta*:34:103–10.
4. Benmeradi N, Payre B, Goodman SL (2015) Easier and Safer Biological Staining: High Contrast UranyLess Staining of TEM Grids using mPrep/g Capsules. *Microsc. Microanal.* in press.

Ordering Information

Product #	Item Description/Catalog Information
G1600	16 mPrep/g capsules & 16 label sets in capsule/grid storage box
F1601	16 mPrep/f standard pore 30 µm filter couplers in capsule/grid storage box
PL96PP500	Non-sterile, PP, 96-well microwell plates, 500 µl, 10/sleeve
KIT_xxx	Starter kits with mPrep capsules and accessories including Gilson Pipetman Neo® pipettor (various custom kits available — please inquire)

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