Guidance for sample preparation and analysis of small particles suspensions on the Morphologi® G3

Introduction

To analyze suspensions of small <15 µm particles on the Morphologi G3, care needs to be taken when preparing the samples.

This technical note is intended to act as a guide for sample preparation for such an application.

Sample preparation

Figure 1 shows the equipment that is required for preparing a sample of suspended material for presentation to the Morphologi® G3. Essentially a drop of sample is placed onto a microscope slide and a cover slip is placed on top to disperse the sample.

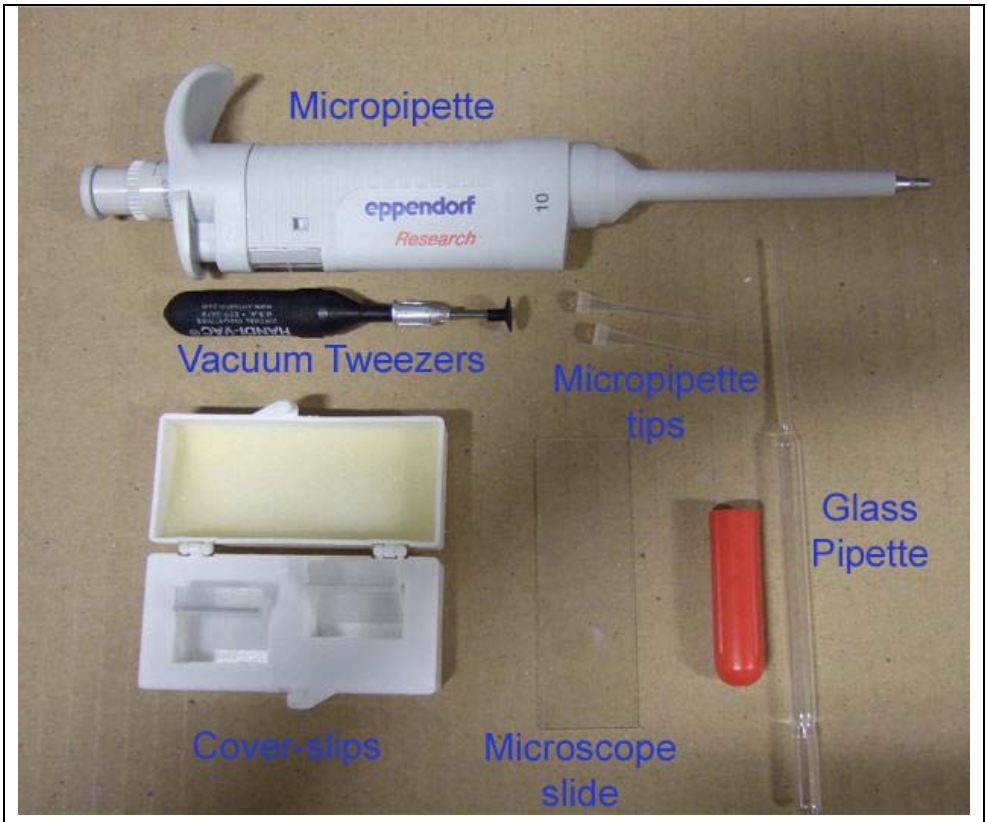

Figure 1: Requirements for preparing a sample of nasal formulation for presentation to the Morphologi G3.

It should be noted that analysis on the Morphologi G3 is carried by static image analysis this means the particles need to be stationary. If the suspension media is constantly evaporating, even if slowly, the smaller particles are likely to move. It is therefore advised to ensure a seal is formed around the edge of the coverslip to form an air tight environment to prevent such evaporation.

Due to the particle size a 20 X or 50 X magnification will be required. Due to the small depths of field of these objectives there may be issues for focusing. Therefore, it is necessary to prepare the sample in as thin a layer as possible. For this a micropipette is essential. Experience shows that 5 µl of sample for a 22 mm x 22 mm cover-slip will be a good starting point but +/- 1 or 2 µl may be required. Figure 2 depicts the sample preparation described below. Note it is important to shake the sample container vigorously before taking the aliquot of sample to ensure good sampling.

A) A microscope slide is placed onto the Morphologi 4-slide holder.

B) The appropriate volume of formulation is placed onto the slide using a micropipette

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C) A cover-slip is placed gently over the sample using vacuum tweezers. It is important to prevent, as far as possible, the formation of bubbles.

D) The vacuum tweezers are used to apply gentle pressure to the cover-slip to encourage the sample to spread out evenly under the cover-slip and to minimize air bubbles

E) The volume of sample required should be enough to wet the entire area under the cover-slip but with no significant excess at the edges

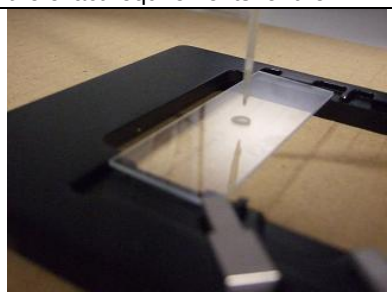
F) Mineral oil or nail vanish is used to seal the edges of the cover-slip to prevent evaporation.

SOP setting guidance

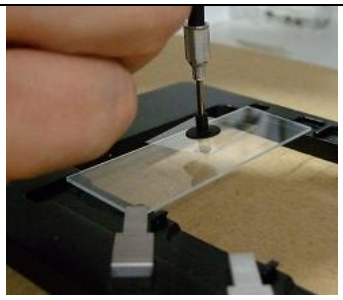
Measurements may be required using either the 20 X or 50 X magnifications. The main advantage of 50 X magnification is improved image definition for the fine particles, and the main disadvantages are the time taken to measure and sensitivity to focus position. For the 20 X the main advantages are the relative speed of measurement and ease of focusing and the main disadvantage is lower image fidelity for the fine particles. Figure 4 illustrates this point showing a field of view image of a well dispersed nasal formulation at both the 20 X and 50 X magnifications. Therefore, it is important to establish the exact requirements for the application when setting the SOP and choosing which magnification to use.

Images of touching particles should be filtered out from the result using a filter, for example of solidity < 0.9. It is also advisable if using the 50 X magnification, to filter out images with less than 100 pixels from the results whether interest is in size or shape. This will also allow results obtained with the 50 X and 20 X magnifications to be compared in similar size range: 100 pixels is equivalent to a particle of approximately 0.6 μm with the 50 X lens – the smallest particle imaged with the 20 X (10 pixels trash size) is approximately 0.5 μm. However if using the 20X magnification and shape is of interest a 100 pixel filter should still be applied in this case as well.

A) Place slide on 4-slide holder



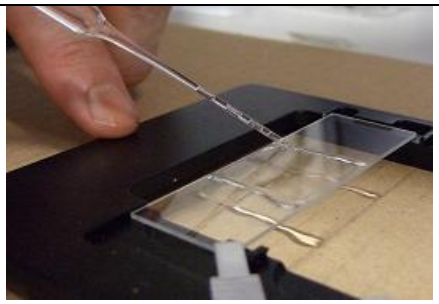
B) Place 5 µl drop of suspension on the slide



C) Place a cover-slip over the suspension using vacuum tweezers

D) Apply pressure to the cover-slip to encourage the suspension to spread out and to minimize air bubbles

E) Suspension spreading out under cover-slip. Volume to be sufficient to wet entire area of cover-slip with no significant excess at the edges.



F) Seal the edges of the cover-slip with mineral oil (or nail varnish) to prevent evaporation.

Figure 2: Nasal formulation sample preparation

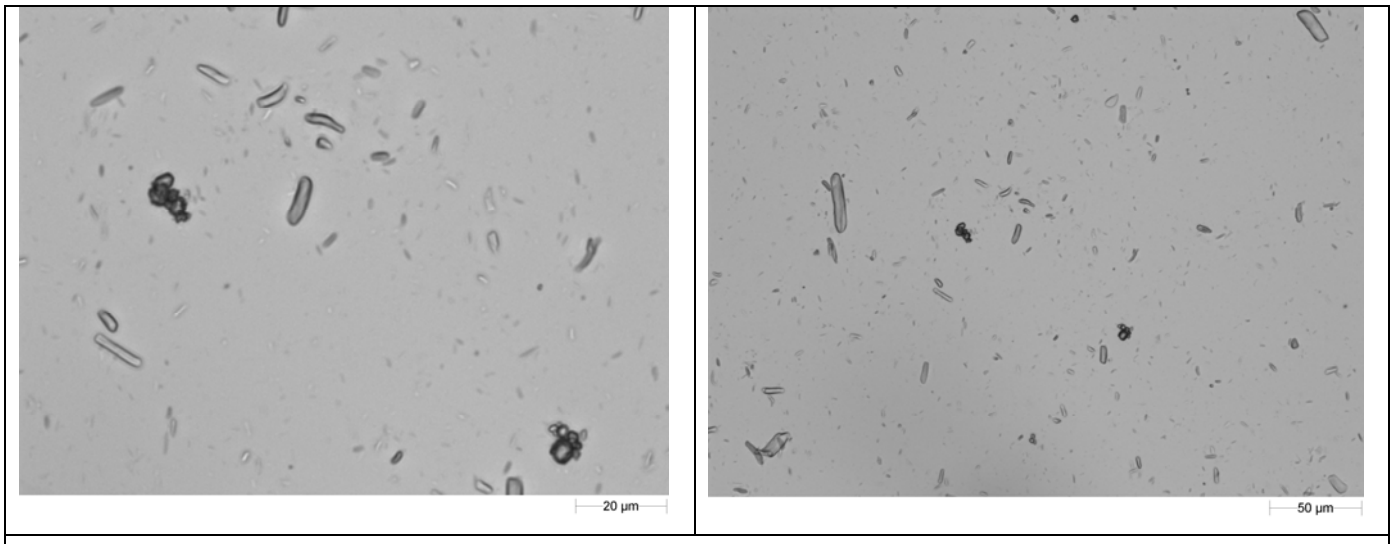


Figure 4: Images of a typical nasal spray dispersion taken at 50 X (left) and 20 X (right) magnifications

It is important to check the coverslip box in the sample carrier page of the SOP, as shown in Figure 3, so that the system knows one is present when the plate tracking is performed.

Figure 3: Sample carrier page of the SOP where the cover slip option should be checked.

Running the analyses

When running the analyses it is best to avoid fixed focus for this type of application. This is because the fixed focus offset position will be relative to the top of the cover-slip and not the microscope side and any slight variation in thickness of sample (even though a precise amount has been used) will affect the offset and thus the focus position. For this reason it is also not possible to set up four samples to run from the same SOP as the offset focus position would be based on the first sample only. It is better to use the manual focus and to set it for each sample. When using the 50 X magnification especially, the small depth of field can make it difficult to focus. When setting the focus position it is necessary to focus on the smallest particles of interest to ensure they are not missed during the analysis..

Finalizing the SOP

Repeat analyses should be performed to verify reproducibility. If required, classification based on size, shape intensity or particular parts of the distributions can be further developed and classification reports in terms of charts or tables showing the proportion of API particles in specific size classes can be presented.

Additional Considerations

Depending on the suspension media and the particles themselves it may be possible that particles will undergo Brownian motion and will not be stationary and may therefore be difficult to analyze as they might be counted more than once.

If the odd large particle is present in the suspension it can cause problems as the coverslip will sit on top of the large particle and then may not be parallel with the slide. It may be possible to over come this by using a double sided sticky gasket between the slide and coverslip as shown in Figure 5.

Figure 5: Slide with a gasket (sticky on both sides) placed on it. The coverslip would be placed on top.

If the suspension media is rather viscous, fine particles may be held at different heights within the oil layer and thus at different positions in the objective's depth of field. This means some fine particles may be out of focus making measurements difficult as illustrated in Figure 6. Sometimes the problem can be overcome by allowing the particles to settle to the bottom.

Additionally if large particles are present care must be taken not to sub sample as they may fall out of suspension more quickly than the small particles.

Larger suspended particles >15 µm can also be measured on the Morphologi but it is likely that preparation using the Morphologi Wet Cell would be more appropriate in this case.

Summary

Wet suspensions of small particles can be analyzed on the Morphologi G3 providing care is taken with the sample preparation to ensure the particles are static and well sampled.

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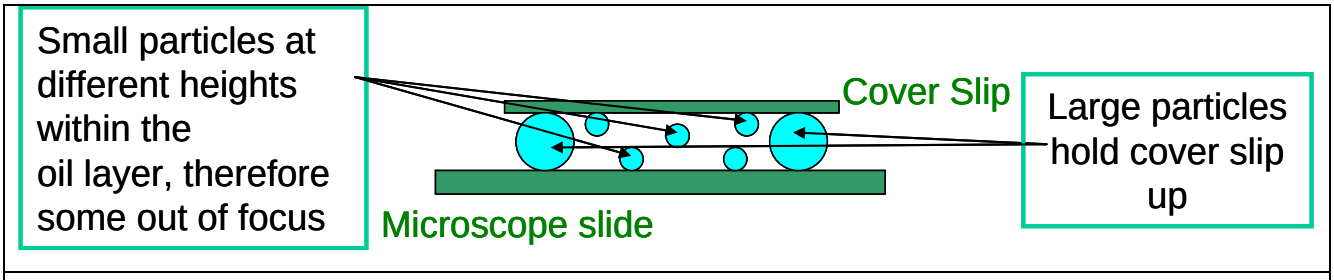

Figure 6: Illustration of the problem when larger particles are present in the suspension.

Malvern Instruments Ltd
Enigma Business Park • Grovewood Road • Malvern • Worcestershire • UK • WR14 1XZ
Tel: +44 (0)1684 892456 • Fax: +44 (0)1684 892789

Malvern Instruments Worldwide
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