Application Note

CRYO-SEM WORKFLOW OF OIL BASED SAMPLES

related instruments: EM ICE, EM ACE900, EM VCT500 & EM VCM
CRYO-SEM WORKFLOW OF OIL BASED SAMPLES

Application Note for High Pressure Freezer EM ICE, Freeze Fracture System EM ACE900 and Vacuum Cryo Transfer System EM VCT500 with Vacuum Cryo Manipulation System EM VCM

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Freeze fracture is a powerful method to investigate the internal structures of different sample types like cells and tissues but also suspensions and emulsions in their native, watery state. To keep the samples as native as possible, a small portion of the sample is high pressure frozen in between two specimen carriers. To “open” the sample for further investigation in the cryo SEM, the carriers are subsequently forced apart in a freeze fracture machine. The fractured surface is coated with platinum and carbon before imaging to avoid any sample charging during SEM investigation. Advantage of that workflow is the high sample throughput and resolution of the internal structure of the sample. Because the samples are still in their native state, the distribution of the different components like oil and water can be easily analysed. This makes this technique very interesting for quality analysis in the pharmaceutical or food industry. In this article we will show the power of this technique by using classic food mayonnaise as a sample.

Specimen: Mayonnaise
Desired preparation: Cryo-SEM workflow
Objective of observation: Freezing quality of oil sample

Procedure
High pressure freezing
Samples were added to the 3 mm interlocking carriers for freeze fracture. Sample carriers were slightly overloaded to prevent any air inclusion inside the carrier. Subsequently samples were directly frozen with the EM ICE and stored in liquid nitrogen for further processing.

Sample transfer to EM ACE900 via EM VCT500
To prepare the samples for cryo SEM investigation, the samples need to be freeze fractured and freeze etched. To do that under cryogenic conditions, the samples must be loaded into a pre-cooled EM ACE900. This is done with the EM VCT500/ EM VCM. After liquid nitrogen storage the samples are transferred to the EM VCM and loaded onto a VCT freeze fracture holder. For loading into the EM ACE900, the sample holder is transferred with the EM VCT500 shuttle. For this purpose, the EM ACE900 has to be equipped with an EM VCT500 dock.

Freeze fracture and etching
The samples were loaded in the EM ACE900 at a stage temperature of -160 °C. The samples were fractured at a temperature of -145°C after reaching an equilibrium (wait 10-15 minutes). The freeze etching was processed for 1 min at -110°C. The last step, the coating, was performed with electron beam evaporation and a mixture of platinum and carbon (Pt/C coating with multi angle rotary shadowing). In this way a 4 nm thick layer was coated on top of the samples.
Sample transfer to cryo-SEM
To keep the samples under optimal conditions the samples were directly transferred with the EM VCT500 shuttle from the EM ACE900 to the JEOL JSM 7800F Prime equipped with the EM VCT500 cryo stage.

Results
The imaging proves the nicely frozen quality of the mayonnaise samples. A very well fractured plane is visible. The large “round” structures can be identified as fat globules held in a water system as an emulsion. The fat globules have a well-defined round shape as well as no visible structure inside, showing exactly what is expected. Also in the surrounding emulsion, no ice crystal formation is visible. Furthermore, an additional regular structure in between the fat globules can be detected. This may be some additional phase separation, occurring due to air expose of the mayonnaise over a period of time.

![SEM image of mayonnaise sample](image1)

![SEM image of mayonnaise sample](image2)
Sample preparation process time with Leica solution
EM ICE: 1hr total process time. User interaction time 30 minutes (high pressure freezing).
EM ACE900: 1hr total process time. User interaction time 30 minutes (freeze fracturing and etching).
EM VCT500: 15 minutes total process time. User interaction time 10 minutes (sample transfer).

Instruments used

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