Introduction

Product innovation in areas such as drug delivery, personal care products and agrochemicals, for example insecticides or bio-stimulants, is increasingly incorporating nano-sized systems to add a functional benefit to the products. Pharmaceutical formulation scientists may explore a potential improved bioavailability for the pharmaceutical active ingredient (API) through nanoencapsulation. Insecticides have been formulated with the active ingredient encapsulated to achieve a more targeted or controlled release over time. Cosmetics have been developed to achieve optical effects through formulations of nanosized polymer entities or silica particles to give a “flawless” or “brightened” appearance. In other personal care applications the inclusion of nanoliposomes help achieve a controlled release of a fragrance or a moisturizer and in some case help improve product stability.

Regulatory developments such as the European Commission (October 2011) recommendations\(^1\) for a definition of the term nanomaterial and the Cosmetics Regulation\(^2\) (EC Regulation No 1223/2009) - which has a requirement for the identification of any nano-sized systems where one or more external dimensions or internal structure is in the size range of 1nm to 100nm - drives the demand for a better understanding of micro and nanostructures in products.

When studying these types of products directly by electron microscopy (EM) to explore the size of structures present, the formation of ice crystals during conventional plunge freezing process disrupts the system’s structure. By applying high pressure freezing (HPF) and in situ sublimation it is possible to preserve the nanoformulation structure for subsequent observation by Cryo-Scanning Electron Microscopy (Cryo-SEM). Several cosmetics or healthcare products were examined in the course of this particular study and the images were examined to determine the presence of nano-sized systems.
CRYOPRESERVATION

The temperature and nature of the cryogen and the plunging conditions can all adversely impact the samples micro- and nano-structure. The use of low temperature techniques (cryopreservation) and in particular HPF with liquid nitrogen overcomes many of these problems.

The Leica EMPACT High Pressure Freezer precisely controls the temperature / pressure during the freezing event (~60ms). The combination of high pressure and rapid freezing encourages vitreous ice formation.

- Leica EMPACT HPF ~200MPa (2000 bar)
- \( \frac{dT}{dt} \sim 9000^\circ \text{s} \)
- Able to handle 200 μm in thickness and

Conventional plunge freezing can lead to the formation of ice crystals which disrupts any micro or nanostructure in the formulation.

Fracture

Post HPF the sample planchet / ‘freezer hat’ is cleaved under vacuum in the GATAN Alto 2500.

Sublimation

The temperature of the sample is raised from -196°C to -89°C under vacuum, water sublimes (solid / gas) revealing the microstructure.

Pt / Pd Coating

To facilitate SEM, the sample is coated in Pt/Pd prior to examination.

Transfer

Finally the sample is transferred under vacuum into a pre-cooled sample stage within the Hitachi S4700.

Direct observation of the preserved structure is achieve via a FEG-SEM (Hitachi S4700) Secondary and backscatter electrons can be imaged, together with elemental analysis using Energy-dispersive X-ray spectroscopy (EDX).

Gatan Alto 2500

The Gatan Alto 2500 enables precise control of temperature / pressure. This allows the use of sublimation to extract the water from the matrix leaving the microstructure exposed for coating and subsequent imaging.
Example 1

Several cosmetics products were examined in the course of this particular study and the images were examined to determine the presence of nano-sized systems. A selection of cosmetics that had been purposely developed to achieve a “flawless” skin or “brightened” skin appearance were examined. Some products showed some evidence of quite complex structures with entities of different sizes. Some of these structures were under the 100nm in dimension. In these product different ingredients are used to achieve this visual effect - for example polymer or silica based.

Some products showed a “continuum” of sample matrix indicated by the dark spaces matrix (Figure 1) but contained very little structure with evidence of the occasional emulsion droplet.

![Figure 1 A skin brightening face cream](image1.png)

Example 2

A topical product consisting of an emulsion containing a suspension of polymer based entities was studied. Figure 2 shows large spherical entities which appear to demonstrate a structured surface. This is evidence for sub-micron particulate matter bound around the larger structure which are are suspected to be polymer based and are of approx size range of 1-3 μm while the sub-micron material observed appears to fall in the range below 100nm.

There is evidence observed for particulate agglomerates, apparently bound by a component of the formulation which is probably polymer based.

![Figure 2 A topical product containing a suspension of polymer based species](image2.png)

Example 3

For delivery of actives to the skin, lipid-based delivery systems such as solid lipid nanoparticles and liposomes have been considered as they offer high affinity to the skin and high active ingredient encapsulation ability. Lipid systems of under 100nm were observed in sample 3 (Figure 3). These are thought to act as delivery of moisturizers or fragrances and perhaps have beneficial effect on product stability too.
How do you define a nanomaterial?

In October 2011 the European Commission published its recommendations (1) on a definition of the term nanomaterial for regulatory applications. The definition is as follows “A nanomaterial means a natural, incidental or manufactured material containing particles in an unbound state or as an aggregate or as an agglomerate and where for 50% of the particles in the number size distribution, one or more external dimensions is in the size range of 1nm to 100nm.”

Conclusion

With the recent advances in cryotechnologies such as HPF, the level of nanostructure preservation attainable in electron microscopy has increased. It is now possible to capture dynamic time sensitive events and to place them in their ultrastructural context. The cryopreserved structure dimensions were measured. The majority were found to contain a range of structures of different sizes. Some of these structures did indeed have dimensions of <100nm.

In this era of product innovation in sectors such as healthcare and personal care products, nano-sized systems are increasingly incorporated into formulations to provide a functional benefit such as active delivery or optical effects.

Regulatory drivers such as the Cosmetics Regulation (into force on 11th of July 2013) mean that it is now more important than ever to establish and explore approaches to study and measure structural properties. Under the Regulation there is a requirement for those products with structures found to be <100nm, confirming the presence of nano-sized systems, if marketed in Europe would have to be clearly labelled with a list of ingredients followed by the word ‘nano’ in brackets. There is also a requirement for specifications such as particle size, physical and chemical properties to be measured and defined.

Whilst microscopy approaches are possibly the only way to directly characterise these systems, other approaches must also be considered in order to obtain information on number size distributions and chemical properties. Surface area determination approaches such as BET, light scattering techniques such as Dynamic Light Scattering (DLS) and other techniques could be applied, as appropriate, to the nanoformulation sample.
Intertek Analytical Expertise

Intertek provides expert analytical services at both R&D level or to GLP/cGMP if required for the chemicals, pharmaceutical and beauty product industry. Services include:

- Product characterisation
- Method Development and Validation
- Extractables and Leachables
- Dissolution & Physico-Chemical Properties
- Elemental Analysis and Trace Metals
- Formulation Support Analysis
- Quality Control Testing
- Batch Release Testing
- Reference Standard Materials Programs
- Investigation Analysis
- Patent, Legal Case, Counterfeit Analysis Support
- Impurities and Degradation Product ID

Physical characterization expertise in particular provides data to assist with formulation or process development, regulatory submission data, QC testing, GMP lot release and manufacturing troubleshooting.

The wider Intertek physical characterization capabilities include microscopy (SEM, EDX, TEM and LM), X-Ray Powder Diffraction, (XRPD), thermal analysis (DSC and TGA) and particle size technology:

- Surface area & porosity
- Particulate size, distribution and shape
- Zeta potential
- Powder flow characteristics
- Polymorph analysis
- Microstructure analysis e.g. encapsulation
- Thermal properties
- Physico-chemical properties

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References


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