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Microscopy Techniques for Characterization of Hydration in Dairy Powders

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The global market for high protein powdered dairy ingredients is growing. However, poor solubility of these powders causing difficulties for manufactures and customers. Poor hydration frequently generates sediments, flecks and lumps in reconstituted dairy powders. There are two major strategies for developing highly soluble dairy powders: a) reformulation and/or b) new process technologies. This requires an in-depth understanding of the physico-chemical basis for poor rehydration. Microscopy observations facilitate individual particle imaging that can help in controlling and predicting the hydration process [1]. This communication gives a brief overview of the different microscopies techniques used to study hydration for emerging generations of dairy powders.

Bright Field (BF)/Polarized Light microscopy (PLM). BF illumination is a common imaging mode of the LM, which provides morphological characterization (particle shape, size, elongation, etc.) of dairy powders with a max practical resolution of ~ 0.5 μm (Fig. 1 a) [2]. Combining LM with image analysis software can be effectively used to measure particle size and shape. PLM consists of two polarizing plates arranged perpendicularly and is used to identify birefringent, or crystalline, materials, such as lactose crystals refract polarized light and appear bright against a dark background (Fig. 1 b, c). Conversely, isotropic substances (such as amorphous sugars) cannot rotate incident polarized light and appear dark [1, 2]. This helps distinguish between fresh (Fig. 1 a) and stored (Fig. 1 b) dairy powders, where PLM may detect lactose crystallization during water uptake [3].

Scanning Electron Microscopy (SEM). SEM has been used to characterize the surface morphology of anhydrous and humidified (equilibrated at specific relative humidity) dairy powders, such as lactose, whole milk powder, skim milk powder (SMP), whey proteins isolates, milk protein concentrates (MPC) and infant milk formulas [1, 2, 3]. In SEM, electrons rather than photons are used to visualize the sample and serve the same function as light in the LM, but electrons have much shorter wavelengths and therefore higher resolution power (~ 4 nm). Modern SEM instruments fitted with field emission electron sources can operate at significantly lower accelerating voltages (0.1 – 5 kV) compared to traditional SEM's and can be used relatively non-conducting materials to be imaged with minimal sample damage [1, 2]. Unfortunately, these types of SEM operate under high vacuum and can be used only for dry powder particles (Fig. 1 d). "Environmental" SEM's can operate at normal atmospheric pressure overcome specimen-charging effects and allows direct real-time inspection of hydration in powders [1].

High speed camera (HSC)/LM. High speed video-microscopy employs HSC fitted to an optical microscope to monitor the hydration process in real time >10,000 frames per second. The primary advantage of this technique is the possibility to extract individual images for the detailed image analysis of the powder during the early stages of rapid wetting, swelling and dispersion (Fig 1 e, f, g, h). Various image analysis software, such as ImageJ, Image Pro, ZEN blue, etc. can then be used to obtain quantitative data.

Confocal Scanning Laser Microscopy (CLSM). CLSM is a form of epi-fluorescence optical microscopy. The principal feature of CLSM is that both illumination and detection systems are focused simultaneously on a single volume element in the specimen. This provides a number of advantages compared to LM: increased resolution (~200 nm), 3D imaging of bulk samples, and sensitive simultaneous detection of two or more fluorochrome probes [1]. CLSM is a common method to visualize protein and fat distribution in dairy powders due to a single application of a dye mixture (Nile Red/Fast Green) [1] and allows mapping water migration into individual powder particle. Previously, CLSM was developed for the real-time measurement of effective diffusion coefficient (D_{eff}) with the help of fluorescent protein dyes, such as Rhodamine B. Such dyes easily penetrate into various dairy powder particles and D_{eff} values can be obtained from the analysis of CLSM images (Fig. 1 i, j, k, l) [4].

To summarize, microscopy is a powerful tool for characterization of dairy powders and their hydration process. An in-depth knowledge of microstructure is now being recognized as a necessary component for predicting functionality and properties of dairy products. This work was supported by the Food Institutional Research Measure (FIRM) project DAIRYDRY ref.15-F-679 funded by the Irish Department of Agriculture, Food and Marine.

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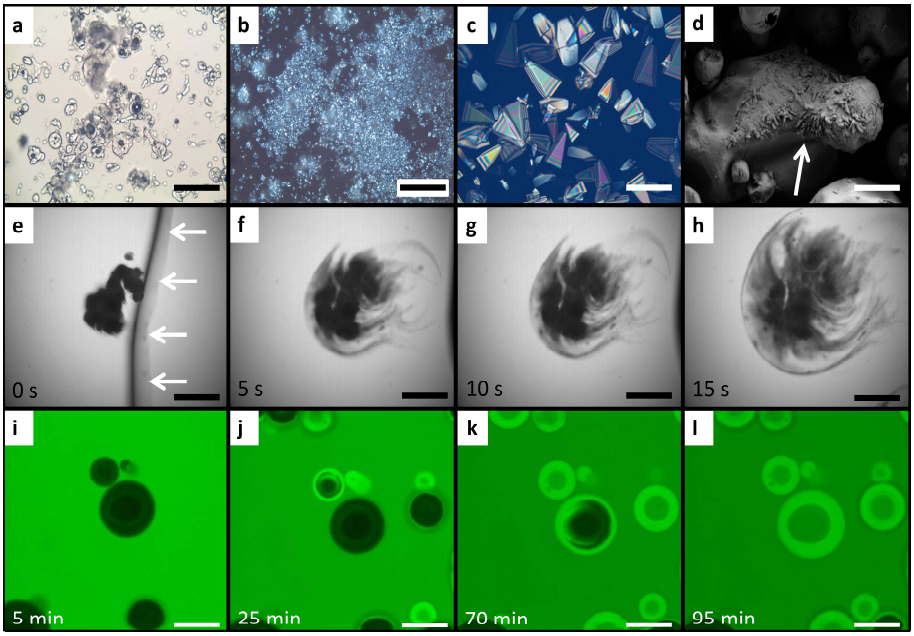

Figure 1. a) BF image of SMP (scale 200 µm); b) PLM image of SMP (scale 400 µm); c) PLM image of lactose crystals (scale 100 µm); d) SEM image of humidified (RH 65%) SMP powder, arrow indicate lactose crystals (scale 5 µm); e-h) HSC images of MPC at 0 s (e), 5s (f), 10 s (g) and 15 s (h) after hydration, arrows indicate the water front (scale 60 µm); i-l) CLSM images of MPC at 5 min (i), 25 min (j), 70 min (k) and 95 min (l) after hydration (scale 60 µm).

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