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Nucleation and Growth of Pharmaceutical Crystals *in situ* Using Liquid Cell Electron Microscopy

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Liquid Cell Electron Microscopy (LCEM) is a state-of-the-art technique that enables *in situ* observation of materials suspended in liquid in the electron microscope. These *in situ* observations have enabled insight into nanoparticle growth processes, battery material mechanics and electron beam-influenced decomposition.[1]

Active Pharmaceutical Ingredients (APIs) are vital products in pharmaceutical drugs and contain molecules oriented in a specific crystal structure (polymorph) that determine its bioavailability and solubility governing its effectiveness inside the body.

An API is generally the most stable crystal form of a number of putative polymorphs. However, it has been shown that by applying a magnetic field during the crystallisation process, pre-nucleation clusters are manipulated to produce a different polymorph compared to crystallization in the absence of a magnetic field; this applies for example to coronene.[2]

The vision of this project is to scrutinise the nucleation and growth of diamagnetic organic molecules currently available on the market as Non-Steroidal Anti-Inflammatory Drug (NSAID) APIs (e.g. flufenamic acid), from the moment they are exposed to the electron beam to when they form crystals.
To be able to control the crystallisation process, concerning the manifestation of polymorph growth, a thorough understanding of the preliminary stages of nucleation, regarding intermolecular forces and thermodynamic behavior.

The liquid cell holder is used to encapsulate the liquid sample from the high vacuum of the electron microscope with silicon nitride windows of 30 nm thickness and a viewing area of 30 x 30 um. The electron microscope used in UL is the double aberration corrected FEI Titan Themis equipped with a monochromator allowing fine control of dose and a Gatan OneView camera for rapid in sit video and micrograph acquisition.

Preliminary observations confirm that the selected diamagnetic organic molecules nucleate and grow, initiated by the electron beam. The observed shapes of the formed crystals in the preliminary experiments are analogous to reported polymorph geometries. These observations give an indication that there are multiple polymorphic forms of the crystal grown in situ potentially governed by a magnetic field, as is present in the TEM.

Non-crystalline entities have also been observed, suspected to be pre-nucleation clusters due to their stability. Other observed effects concerned the degradation of the crystals, predicted to arise from the production of radiolysis species from the electron beam interaction with the solvent and/or molecules.[3] Considerable experimental information has been gathered using the aforementioned equipment on the

growth of crystalline and non-crystalline species of APIs such as flufenamic acid, indomethacin and carbamazepine in a variety of solvents and dependent on their concentration. However, the role of each of the radiolysis products resulting from the electron beam interactions with the solvent/molecule requires further investigation. Establishing a deep understanding of radiolysis species behaviour is important in order to identify the effects on nucleation and crystal growth resulting from interactions with the electron beam; this requires exploring the underlying radiation chemistry.

This body of work displays results gathered in the liquid environment including; dynamic movements of non-crystalline clusters, assembly of clusters, interactions of organic clusters with other entities in the liquid cell, growth of non-crystalline clusters, diffraction tomography of organic crystals, lattice resolution imaging of organic crystals, growth and subsequent dissolution of organic crystals [4].

References:

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[4] The authors gratefully acknowledge funding from the H2020 FET-Open MagnaPharm project

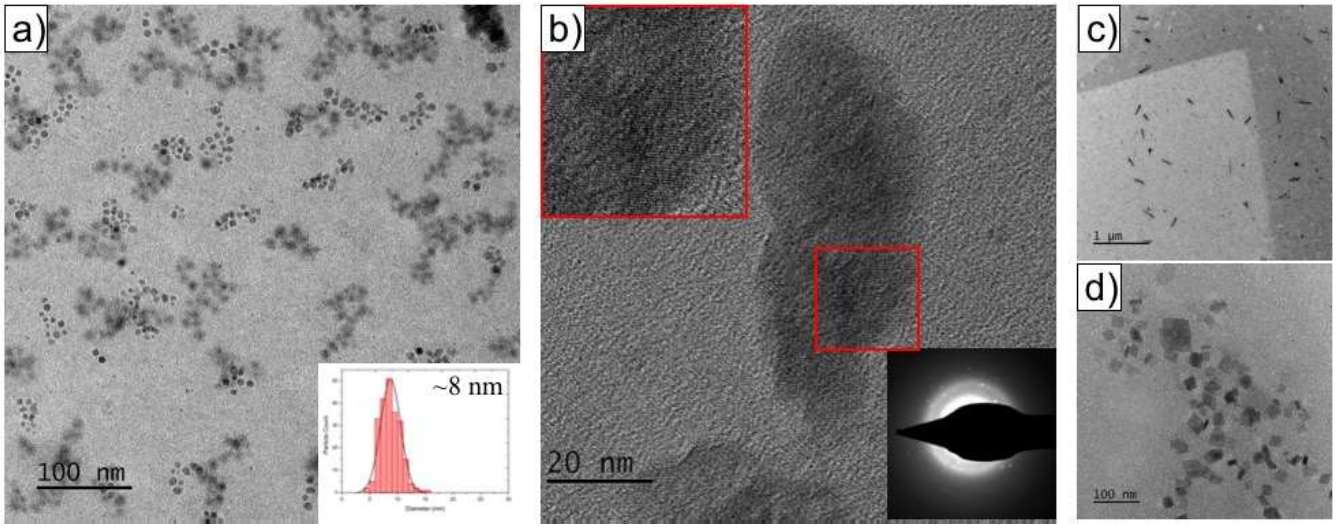

Figure 1. a) Individual particles of flufenamic acid at different z-heights and hence different focus in the liquid cell, b) lattice resolution image of a crystalline flufenamic acid particle with a close-up of the lattice in the inset, along with the corresponding electron diffraction pattern, c) needle-like and d) rhombic shaped crystals of flufenamic acid indicative of more than one polymorph grown *in situ*.

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