

# Transmission electron microscopy of a model crystalline organic, theophylline

J Cattle<sup>1</sup>, M S'ari<sup>1</sup>, N Hondow<sup>1</sup>, P Abellán<sup>2</sup>, A P Brown<sup>1</sup>, R M D Brydson<sup>1</sup>

<sup>1</sup>Institute for Materials Research, School of Chemical and Process Engineering, University of Leeds, Leeds, LS2 9JT, United Kingdom

<sup>2</sup>SuperSTEM, STFC Daresbury Laboratories, Keckwick Lane, Warrington, WA4 4AD, United Kingdom

E-mail: sm09jec@leeds.ac.uk

**Abstract.** We report on the use of transmission electron microscopy (TEM) to analyse the diffraction patterns of the model crystalline organic theophylline to investigate beam damage in relation to changing accelerating voltage, sample temperature and TEM grid support films. We find that samples deposited on graphene film grids have the longest lifetimes when also held at -190 °C and imaged at 200 kV accelerating voltage. Finally, atomic lattice images are obtained in bright field STEM by working close to the estimated critical electron dose for theophylline.

## 1. Introduction

It is well known that organic and biological materials are particularly sensitive to electron beam exposure [1]. This makes transmission electron microscopy (TEM) of such materials a challenge, but not entirely impractical. If damage can be mitigated, TEM analysis may be used to acquire useful structural, compositional and even chemical information [2, 3]. Additionally, TEM analysis of certain samples can be used to fill gaps in knowledge left by techniques more typical for organic materials analysis (X-ray diffraction (XRD), polarised light microscopy, atomic force microscopy etc.) For example, conventional TEM (CTEM) was recently used to identify a previously unidentified form of theophylline [4], which only exists as a minor phase (<1%) of form II of theophylline. The ability of TEM to be used for the analysis of individual crystals of material with wide ranges in size is one of its biggest advantages for pharmaceutical product analysis.

There are numerous strategies that can be used to limit the effect of the electron beam on the sample. Generally, the simpler option is to control spot size and other TEM parameters to reduce the intensity of the beam, though there will be a point where the signal will be so low that navigation and imaging of the sample becomes impractical. Here, we present a set of experiments on theophylline aimed at improving its resistance to beam damage without altering the electron beam fluence rate. Theophylline was chosen as a model material for this study. It is a xanthine derivative with the chemical formula  $C_7H_8N_4O_2$ . It bears many similarities to caffeine and is used as a treatment for conditions such as asthma, chronic obstructive pulmonary disease and sleep apnea [5]. There are many polymorphs of theophylline, with form II being used in this study as it is easily reproduced and readily identifiable by crystal morphology and electron diffraction [4, 6]. Here we perform a thorough test of parameters including accelerating voltage, sample temperature and support film type on theophylline in order to identify the effects of each in electron beam-induced damage.



## 2. Experimental

A theophylline solution was prepared by dissolving powder form theophylline (purchased from Sigma-Aldrich) in nitromethane. Samples for use in TEM were prepared by pipetting a droplet of the theophylline saturated nitromethane solution on to carbon coated copper TEM grids followed by heating to  $\sim 50$  °C to evaporate the nitromethane. Large, thin, triangular crystals of form II theophylline (typified in Fig. 1 (a)) were deposited on the grids, with the thinnest axis laying flush with the carbon film, leaving them in an ideal orientation for electron beam analysis.

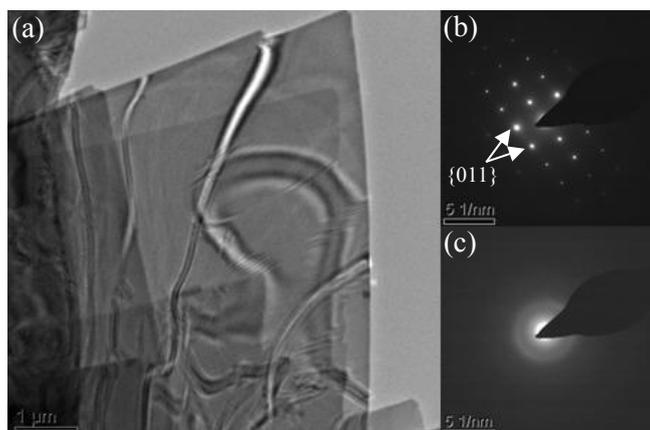
Analysis was carried out using a FEI Tecnai F20 field emission TEM operated between 80 kV and 200 kV as required, with a tip extraction voltage of 4.5 kV and with an attached Gatan Orius SC600A CCD camera. Gatan DigitalMicrograph software was used for image capture and analysis. Electron fluence rates were calculated using conversion formulae provided by FEI and were dependent on magnification, beam spread, camera emulsion setting and fluorescent plate exposure time. Magnification (2,500 times) and beam spread (0.08 m diameter at the viewing screen) were kept constant, with changes to spot size being used to alter the electron fluence rate. This was kept in a typical range of  $\sim 10$  to  $\sim 20$   $\text{e}^- \text{nm}^{-2} \text{s}^{-1}$ . Beam damage was analysed by locating a fresh area of crystal in bright field mode followed by capture of diffraction patterns at set time intervals (typically 30 s) until all diffraction spots in the pattern had faded. Post-capture, these series of diffraction patterns were analysed by measuring the intensity of pairs of Bragg spots (typically the  $\{011\}$  spots, indicated in Fig. 1b) at each time interval and relating that to the total electron fluence at that time. Intensities were averaged and normalized against the highest intensity. Critical electron dose values were obtained by measuring the fluence at  $1/e$  of the highest recorded intensity.

Standard conditions used were: 200 kV accelerating voltage at an ambient temperature with the sample on a copper grid with a continuous carbon film (Agar Scientific). Variations on these standard conditions were investigated which included using 80 kV accelerating voltage, cooling the sample to -190 °C and using a holey carbon film and graphene film coated copper TEM grid (Graphene Supermarket).

## 3. Results and Discussion

### 3.1. Analysis under standard conditions

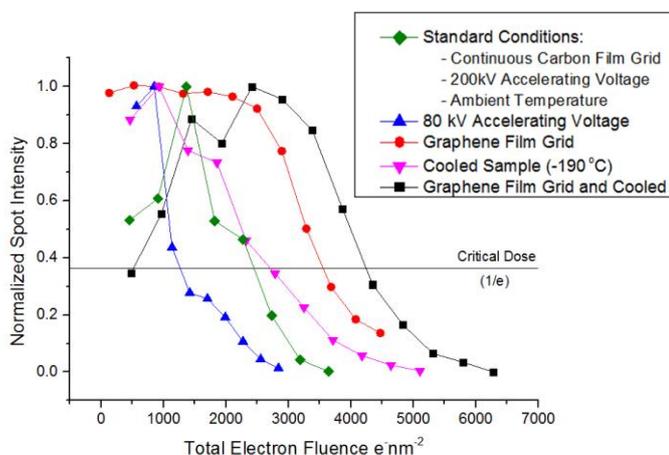
Initially, the standard conditions were used to acquire bright field images of theophylline and to produce a benchmark set of diffraction patterns and spot intensity data. A typical image of theophylline form II exhibits the expected triangular plate morphology of the crystals, as well as showing numerous bend contours (Fig. 1a). Such contours indicate crystallinity; they shift over the surface of the crystals as damage or reorientation occur, before disappearing completely upon loss of crystallinity. Fig. 1b shows a typical diffraction pattern ( $[100]$  zone axis) for form II theophylline and Fig. 1c shows how the pattern appears after exposure to  $\sim 4500$   $\text{e}^- \text{nm}^{-2}$  total electron fluence. The  $\{011\}$  spots from the  $[100]$  pattern are typically used for intensity measurements. The critical dose for theophylline form II under standard conditions is determined to be  $\sim 2500$   $\text{e}^- \text{nm}^{-2}$  (Fig. 2, data set in green). Fluctuation in this and some of the other data sets has been attributed to reorientation or tilt of the crystals as damage progresses, causing spots to brighten or dim.



**Fig. 1:** (a) Bright field TEM image of crystals of theophylline, displaying characteristic triangular, plate-like morphology and multiple bend contours. (b) A typical diffraction pattern along the [100] axis of an unaltered theophylline form II crystal. (c) The same pattern after exposure to  $\sim 4500 \text{ e}^- \text{ nm}^{-2}$ .

### 3.2. Sample cooling

Analysis of theophylline at  $-190 \text{ }^\circ\text{C}$  was carried out using a Gatan cryo-holder, with other variables remaining as per the standard conditions, resulting in a slight increase in critical dose to  $\sim 2800 \text{ e}^- \text{ nm}^{-2}$  (Fig. 2, data set in pink). The lower temperature helps to prevent sample heating, immobilises atoms/molecules and decrease ambient thermal energy that may contribute to bond breaking [1,7].



**Fig. 2:** Graph of normalized {011} spot intensities versus total electron fluence for theophylline samples under a variety of microscope and specimen conditions.

### 3.3. 80 kV analysis

The result of dropping the accelerating voltage to 80 kV (other variables as per standard conditions) is a significant decrease in critical dose to  $\sim 1400 \text{ e}^- \text{ nm}^{-2}$  (Fig. 2, data set in blue). Reducing the accelerating voltage causes an increase in the beam-sample interaction, most notably the ionisation cross-section, thus decreasing the damage threshold [8].

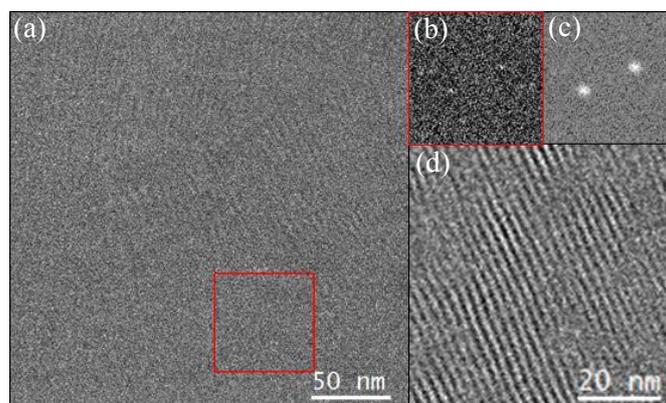
### 3.4. Graphene film analysis

Using the standard conditions and theophylline deposited over grids with holey carbon and graphene films resulted in an increase in critical dose to  $\sim 3600 \text{ e}^- \text{ nm}^{-2}$  (Fig. 2, data set in red). Graphene is well known as an electronic and thermal conductor [9], and its effect here is to improve conduction away from the theophylline crystals, preventing heat and charge build up to improve theophylline's stability

under the electron beam. Analysis was also carried out on a sample of theophylline on a graphene film at  $-190^{\circ}\text{C}$  to test if the effects of these would be compounded and resulted in a larger critical dose of  $\sim 4250\text{ e}^{-}\text{nm}^{-2}$  (Fig. 2, data set in black).

### 3.5. Prospects for direct lattice imaging of theophylline

Scanning TEM (STEM) has previously been shown to be a viable method of obtaining lattice images of sensitive materials [10], as the scanned nature of the focussed probe mitigates beam damage better than the parallel illumination of CTEM. Fig. 3 shows a high resolution bright field phase contrast STEM image of theophylline recorded using a Nion UltraSTEM 100 at 100 kV and a total fluence ( $\sim 9300\text{ e}^{-}\text{nm}^{-2}$ ) three times higher than that usable in CTEM. (100) lattice fringes are barely visible in the raw image, but are more clearly seen in the Fourier filtered image (Fig. 3d).



**Fig. 3:** (a) Bright field phase contrast STEM image of a theophylline crystal. (b) Fourier transform (FFT) of highlighted area in (a). (c) The same FFT with a mask applied to increase the (100) spot intensities by a factor of 10. (d) The inverse of (c), showing the (100) lattice spacings.

## 4. Conclusions

These results have confirmed the dominance of radiolysis as the main damage mechanism affecting crystals of theophylline when exposed to an electron beam. It has also been shown that this damage can be mitigated by increasing the accelerating voltage where possible, use of a graphene film coated TEM grid and use of a liquid nitrogen cooled sample holder. Bright field STEM shows distinct potential for direct lattice imaging of electron beam sensitive materials.

## Acknowledgments

The authors would like to thank Mark Eddleston and Bill Jones (University of Cambridge) for their input and discussion regarding this study.

## References

- [1] Egerton R F 2013 *Ultramicroscopy* **127** 100
- [2] Brydson R et al. 2014 *Journal of Physics: Conference Series* **522** 012060
- [3] Fryer J R and Holland F 1984 *Proceedings of the Royal Society of London A* **393** 353
- [4] Eddleston M D et al. 2013 *Chemistry A European Journal* **19** 7883
- [5] Schultze-Werninghaus G and Meier-Sydow J 1982 *Clinical Allergy* **12** 211
- [6] Seton L et al. 2010 *Crystal Growth & Design* **10** 3879
- [7] Sader K et al. 2009 *Ultramicroscopy* **110** 43
- [8] Williams D B and Carter C B 1996 *Transmission Electron Microscopy: A Textbook for Materials Science* vol 2 (New York: Springer)
- [9] Pantelic R S et al. 2011 *Nano Letters* **11** 4319
- [10] Haruta M and Kurata H 2012 *Scientific Reports* **2** 252