Journal of Crystal Growth #vol# (xxxx) xxxx–xxxx

Contents lists available at ScienceDirect

Journal of Crystal Growth

journal homepage: www.elsevier.com/locate/crysgro

Calcium oxalate crystal growth modification; investigations with confocal Raman microscopy

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ARTICLE INFO

Communicated by Juan Garcia-Ruiz

Keywords:
Biocrystallization
Crystal morphology
Optical microscopy
Growth from solutions
Calcium compounds

ABSTRACT

Confocal Raman Microscopy (CRM) in combination with a photophysical investigation has been employed to give insight into the interaction between calcium oxalate monohydrate (COM) and a series of tetrazole containing crystal growth modifier's (CGM's), in conjunction with characterisation of morphological changes using scanning electron and optical microscopy. The tetrazole CGM's were found to interact by surface adsorption with minimal morphological changes to the COM crystals however, significant interactions via chemisorption were observed; it was discovered that the chemisorption is sufficiently strong for aggregation of the tetrazole species to occur within the crystal during crystallisation.

1. Introduction

The formation of stones in the kidney, bladder or urinary tract [1] (urolithiasis) are undesirable biological crystallisation events that can occur within the human body; in particular, kidney stone (renal calculi) formation is very common, with 1 in 10 people affected in their lifetime [2]. The most common type of human kidney stones are calcium containing stones, the major constituent of which is COM ($CaC_2O_4 \cdot H_2O$) at 70% [3]. COM is the thermodynamically stable phase of calcium with oxalate at room temperature but has two other metastable phases, the dihydrate and the trihydrate, with the dihydrate also being commonly present in human kidney stones while the trihydrate is uncommon. Currently, few medicines used for the treatment of kidney stones do not have risks inherent with their use and thus surgical removal and other techniques including extracorporeal shockwave lithotripsy (ESWL) and medical expulsive therapy (MET) are used to combat the already grown calculi [4]. Research in the field, therefore, focuses on kidney stone prevention employing crystal growth modifiers (CGM's) as a means of controlling kidney stone formation. Those inflicted with the condition would benefit greatly from the development of therapeutics that hinder the formation of nuclei or suppresses their growth. Crystal growth modifiers recently studied include but are not limited to carboxylic acids, calixarenes, proteins and polymers [5–8].

Recently researchers have been utilising the bioisostere nature of carboxylic acids and tetrazoles [9–13] to investigate the effect the tetrazole moiety has in medicine, and more recently in the field of crystal growth modification. Before our group started investigating tetrazoles as modifiers, they had not been used as crystal growth modifiers; now crystal systems have been investigated including, barium sulfate, calcium carbonate [14], and calcium oxalate [15], all of which were discovered to be inhibited by the presence of tetrazoles during crystallisation. The tetrazole species used in this study are shown in Fig. 1. This study, in which we present our preliminary results, is a continuation and expansion of the work mentioned above to try to elucidate the mechanism of interaction between COM crystals and the tetrazole species during crystallisation. We have utilised the capabilities of Confocal Raman Microscopy (CRM) and a photophysical investigation to provide insight into the mechanism of COM modification in the presence of a series of tetrazoles which have varying degrees of impact as CGM's, the morphology changes were monitored by optical microscopy and SEM.

2. Results and discussion

2.1. CRM investigation of COM in the presence of CGM's

As has been previously reported [15], tetrazoles have been found to change the morphology of COM crystals but seemingly through different mechanisms of interaction than their carboxylic acid equivalent counter-parts. This manuscript looks at understanding the interactions with similarly sized molecules as shown in Fig. 1, with emphasis on the adsorption of the molecules to the crystal. The tetrazole species TzmeoxPh– will be used as the exemplary CGM throughout this manuscript for clarity.

Firstly, the morphology of control crystals for our system are shown

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http://dx.doi.org/10.1016/j.jcrysgro.2016.10.038

Available online xxxx

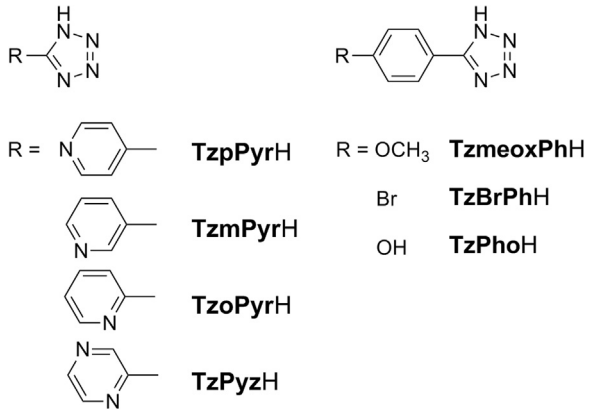

Fig. 1. Tetrazoles species structures used as CGM's (named from top left down to top right down) 5-(4-Pyridyl)–1H-tetrazole (**TzpPyr**H, 6.84 mM), 5-(3-Pyridyl)–1H-tetrazole (**TzmPyr**H, 6.84 mM), 5-(2-Pyridyl)–1H-tetrazole (**TzoPyr**H, 6.84 mM), 5-(3-Pyrizine)–1H-tetrazole (**TzPyz**H, 6.88 mM), 5-(4-Bromophenyl)–1H-tetrazole (**TBrPh**H, 4.51 mM), 5-(4-methoxyphenyl)–1H-tetrazole (**TzMeoxPh**H, 5.78 mM). 5-(4-phenol)–1H-tetrazole (**TzPho**H, 6.22 mM).

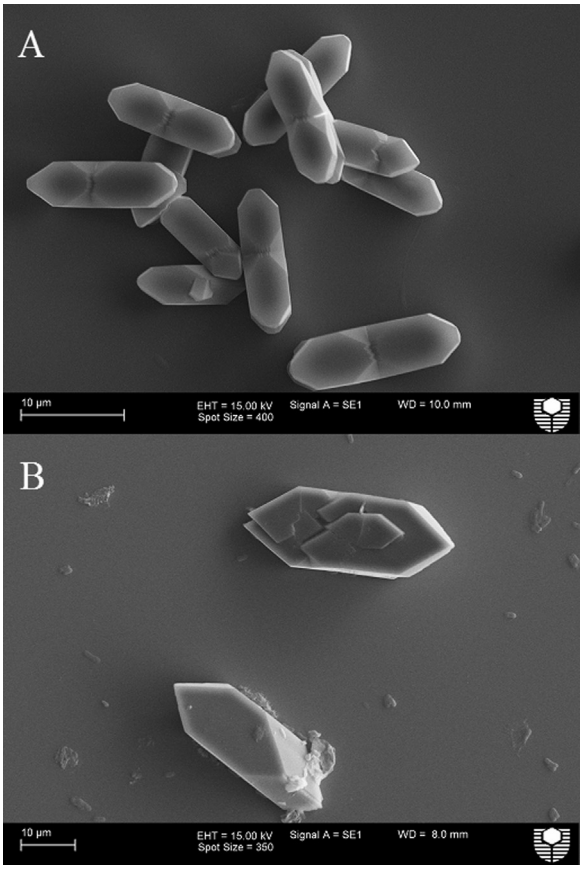

Fig. 2. SEM images of COM crystals, A) Control crystals, B) Crystals grown in the presence of **TzmeoxPh**$^-$ (5.78 mM).

in Fig. 2A, mostly twinned COM crystals between 10 and 15 µm long, ~5 µm wide, ~8 µm deep. Upon addition of all of the CGM's above in Fig. 1, reduction in the number of crystals is evident with some additives suppressing nucleation to such significant levels few particles could be found to analyse. The most potent inhibitors were **TzoPyr**$^-$, **TzBrPh**$^-$ and **TzmeoxPh**$^-$, each showing the greatest reduction of nuclei formed at their given concentrations; see Supplementary Figs. S1–S3 for SEM images of the series and Table S1 for quantitative data for the series. Fig. S3 illustrates the significant drop in the amount of particles due to the presence of many of the CGM's. However, due to the reduction in the number of nuclei during crystallisation, the particles present are subsequently larger in size than that of the control COM crystals. Interestingly single crystals now dominate instead of the more common twinned crystals shown in the control example. Negligible morphological changes were seen with this series of tetrazoles however, significant impacts on the nucleation of the crystals was clearly evidenced (Table S1).

The Raman spectra presented in Fig. 3 correspond to the COM formed under control conditions and in the presence of the **TzmeoxPh**$^-$ species. In the spectra provided the evidence of an interaction between COM and the tetrazole will be elucidated. Fig. 3A, represents the Raman signal collected at the surface of a control COM crystal, it shows the characteristic peaks given for COM from the literature [16] at ~1470 cm^{-1}, 1490 cm^{-1} and ~500 cm^{-1}. The doublet at 1470 cm^{-1} and 1490 cm^{-1} are characteristic of the monohydrate phase and not of the dihydrate or trihydrate phase for calcium oxalate. Fig. 3B, displays a typical Raman spectrum for COM grown in the presence of our tetrazole species; in this case, **TzmeoxPh**$^-$. The characteristic peaks for COM are still present but the signals are diminished in intensity due to the lowering of laser intensity to avoid exciting the tetrazole species (which fluoresces quite strongly in the regions where the characteristic peaks are visible). Fig. 3B's spectrum was taken at the surface of the crystal, which in contrast to Fig. 3C illustrates the capabilities of the CRM; this spectrum was taken a few micrometres within the crystal using the confocal ability of the instrument and allows for the observation of the fluorescence and Raman signals from the tetrazole at subsurface layers. The presence of these Raman vibrational frequencies indicates we have tetrazole species present within the crystals and not solely adsorbed to the surface, otherwise we would have observed a similar signal present for Fig. 3B. For all the Raman spectra collected containing CGM's, the main signals for the tetrazole species are found at ~1340 cm^{-1} and 1600 cm^{-1}, these peaks are believed to belong to either the C-N or N-N stretching frequencies of the tetrazole, see Fig. 3S for the Raman of **TzmeoxPh**H only, which due to the process of adsorption has characteristic peaks that are different in position to those shown here. As mentioned above, the laser intensity was reduced in order to see the structure of these bands and upon increasing the intensity of the laser, the fluorescent properties of the tetrazole species mask the structured peaks for both the tetrazole and COM. Fig. 3D fully exemplifies why CRM has been selected as the technique used to investigate the presence of the tetrazole species within the COM crystals. As presented, the Y-axis indicates the intensity of the chosen Raman signals monitored during collection of the spectrum against the distance through the crystal (in micrometres, on the X-axis). CRM allowed us to plot the intensity of the characteristic signal for the COM (blue trace −1470 cm^{-1}) and the tetrazole (red trace −1340 cm^{-1}) allowing for a comparison between the two species' intensities with respect to the position inside the COM crystal. This allows us to determine, with confidence, the location of the tetrazole species within the crystal itself. The tetrazole species have been found to reside closer to the surface of the COM crystals and relatively less found towards the centre of the crystal, the working hypothesis for this observation is that at the early stages of crystallisation i) the crystal faces of COM are not well developed for the tetrazole to adsorb onto specific faces and ii) during this time competition between the oxalate and tetrazole species occurs, which favours the oxalate species due to the initial high supersaturation. The interaction between the tetrazole and crystal is believed to occur via adsorption to the surface and subsequent growth of the COM around the tetrazole species resulting in what is observed in our system; the tetrazole species being included into the crystal itself as verified by our confocal Raman spectroscopy.