

TOF-SIMS analysis of kidney stones possibly induced by the ingestion of melamine-containing milk products

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Ingestion of melamine contaminated food likely product kidney stones and may leads to acute kidney failure in mammals. In this study, ToF-SIMS has been applied to imaging the micro structure of kidney stone samples, revealing information of the micro structure of different stone samples. Our data indicate that the melamine- induce stones were formed by different mechanisms. However, information obtained using other techniques is also required to correlate the detailed differences in the microstructure of the stone samples with the formation mechanism. Copyright © 2010 John Wiley & Sons, Ltd.

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Introduction

Melamine (1,3,5-triazine-2,4,6-triamine, C₃H₆N₆) is a raw material widely used in the chemical industry. Because it gives analytical characteristics of protein molecules, melamine has been deliberately used by unscrupulous manufacturers to adulterate foods in order to artificially inflate the values of protein levels. Studies, using desorption electrospray ionization/surface desorption atmospheric pressure chemical ionization mass spectrometry (DESI/SDAPCI-MS)^[1] and extractive electrospray ionization mass spectrometry (EESI-MS)^[2] on dairy products and egg samples^[3] revealed the presence of melamine in the positive ion mass spectrum. The presence of melamine affects the kidneys resulting in the production of kidney stones and also acute kidney failure. Initial studies on kidney stones by DESI/SDAPCI-MS have thus far failed to detect melamine in most cases, which is not surprising due to its probable association with other components/crystals within the stone. However, it is thought that melamine-induced stones are very different from the more natural nonmelamine-induced stones in that they are more fragile and could possibly have a different microstructure.

In this work, the composition and microstructure of kidney stones believed to be melamine associated and obtained from several infants are examined using TOF-SIMS to see whether the technique can ascertain any differences. Initial spectra taken on the surface of the stones show the presence of nitrogen-containing species, as well as phosphate ions, and so forth. However, as with much the DESI/SDAPCI-MS, there is no satisfactory evidence of melamine, which, as stated above, is of no surprise. Since kidney stones can be induced by other species, polished cross-sections of the above stones are compared with those obtained from adults, which are unlikely to be melamine induced. The TOF-SIMS spectra/images of these surfaces obtained in both high mass and spatial resolution modes using a Bi cluster source are compared to ascertain whether differences in composition, crystal/micro-structure and spatial distribution can be determined.

Materials and Methods

Samples

Samples were obtained from six patients. Four samples were taken from infants with a 1.5–2.5-year period of drinking commercial milk products which might contain melamine, whilst two samples were taken from adults with no history of drinking powdered milk. Figure 1 summarizes the clinical data. Preliminary work using ESI-MS/MS^[4] indicated the presence of uric acid in the extracted solution of Sample 2 (Fig. 2), whilst melamine was detected in Sample 3 (Fig. 3). The details of these ESI mass spectra will be discussed elsewhere.^[4] Thus, for this TOF-SIMS study, Samples 2 and 3 (child, kidney pelvis) were compared with Sample 6 (adult, kidney pelvis).

Sample preparation and analysis

Samples were prepared by mounting in epoxy and polished down to 3 μm (diamond paper with water as lubricant) using the polishing wheel of Leica EM TXP target sectioning system.

The TOF-SIMS spectra were obtained on an ION-TOF GmbH (Münster, Germany) TOFSIMS IV located at the University of Toronto, and fitted with a Bi liquid metal ion gun. In order to maintain molecular information, no sputter cleaning of the sample was performed. Mass spectra/images were obtained using both the high spatial and high spectral resolution modes of the instrument.^[5] In each case, the Bi₃⁺⁺ primary ion was used as

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Clinical Data of Human Kidney Stone Samples

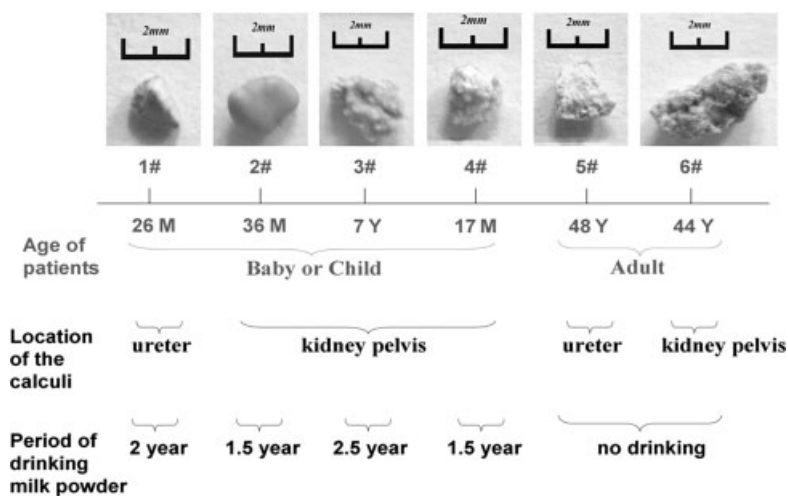


Figure 1. Clinical data for the 6 kidney stones.

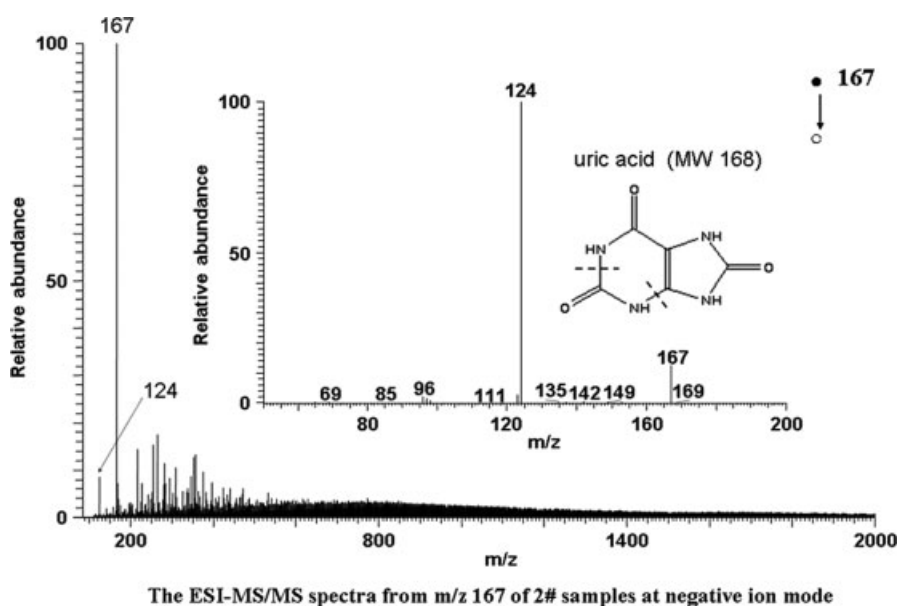


Figure 2. The ESI-MS/MS spectrum of deprotonated uric acid (m/z 167) detected in Sample 2.

opposed to Bi^+ or Bi_3^+ as it provided a better secondary ion yield with better spatial and mass resolution. Charge neutralization was achieved utilizing the electron flood-gun supplied with the instrument. Both positive and negative spectra were collected.

The ESI-MS experiments were carried out using a commercial linear ion trap mass spectrometer (LTQ-XL, Finnigan, San Jose, CA) installed with an electrospray ionization source. The ESI-LTQ mass spectrometer was set to work in positive/negative ion detection mode for the analysis of the stone solution samples. The nebulizing gas (N_2) pressure was 1 MPa, the ESI high voltage was $+4.5/-3.5$ kV, the temperature of the heated capillary was 350°C . Other parameters were default values of the instrument and no further optimization of the ESI-MS was performed. Tandem mass spectrometry experiments were performed with 20–37% collision energy (CE), in which the parent ions were isolated with a mass-to-charge window width of 1.5 Da.

Results and Discussion

Figures 4–6 show the distribution of selected fragments for Samples 2, 3 and 6, respectively, collected in a high spatial resolution mode. Images for both positive and negative spectra are shown. To highlight distributional differences a color overlay is also shown (online version). For the positive, $-\text{K}$ is shown in red, CH_4N in green and Ca in blue, whilst for the negative, $\text{PO}_2 + \text{PO}_3$ is shown in red and $\text{CN} + \text{CNO}$ in green. It can be seen that the three different stones produce very different distributions of the species.

Visual evidence (Fig. 1) shows that the appearance of the six kidney stones is quite different. Some seem angular, such as that in Sample 6, whilst others seem smoother. These differences in structure would probably arise from the different composition and make-up of the stones. From the ESI-MS/MS spectrum of Sample 2 (Fig. 2), direct evidence is obtained for uric acid content where the

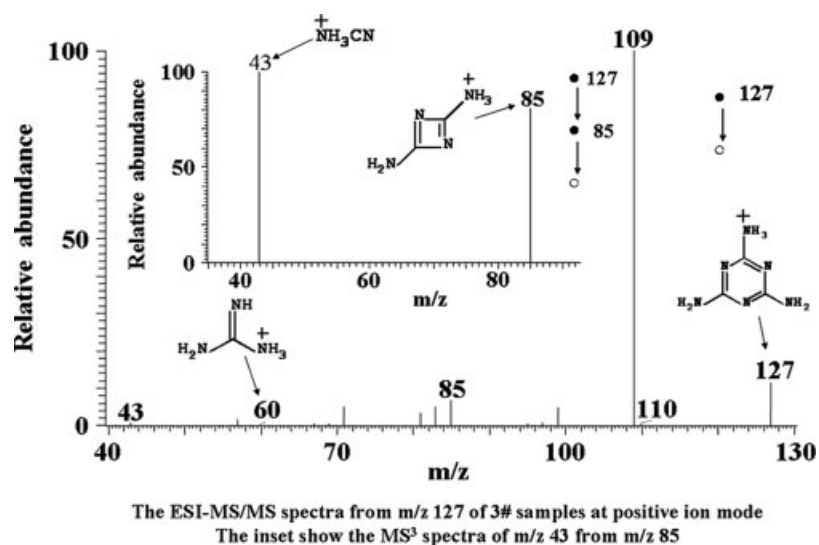


Figure 3. The ESI-MS/MS spectrum of protonated melamine (m/z 127) detected in Sample 3.

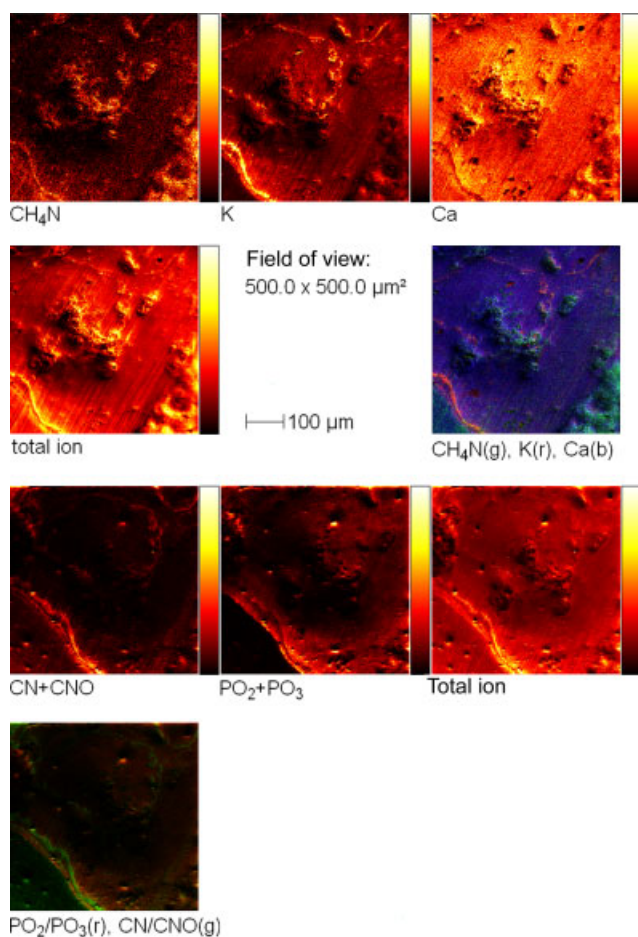


Figure 4. TOF-SIMS images for Sample 2. Positive ions, upper 2 rows, and negative ions, lower 2 rows.

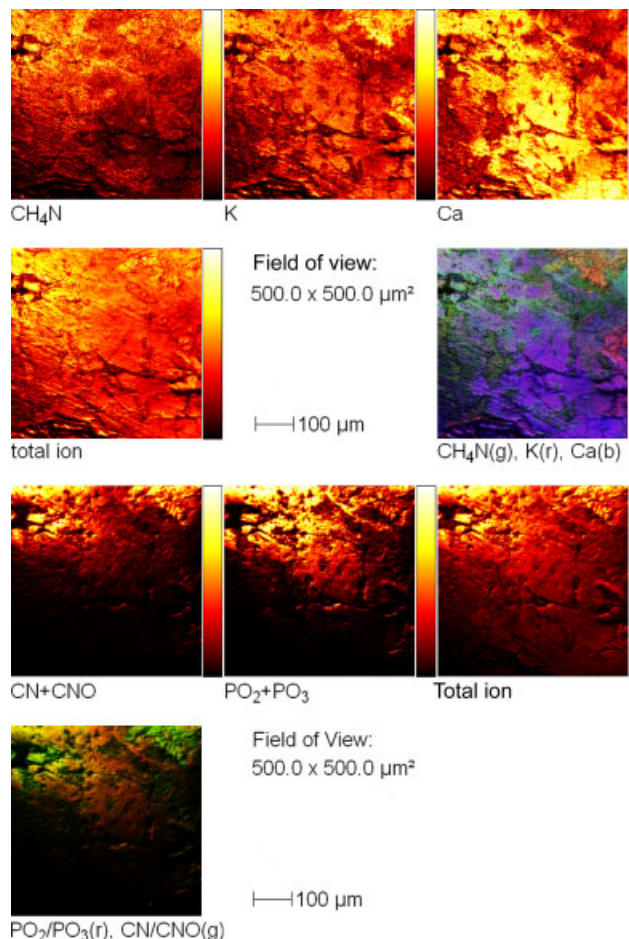


Figure 5. TOF-SIMS images for Sample 3. Positive ions, upper 2 rows, and negative ions, lower 2 rows.

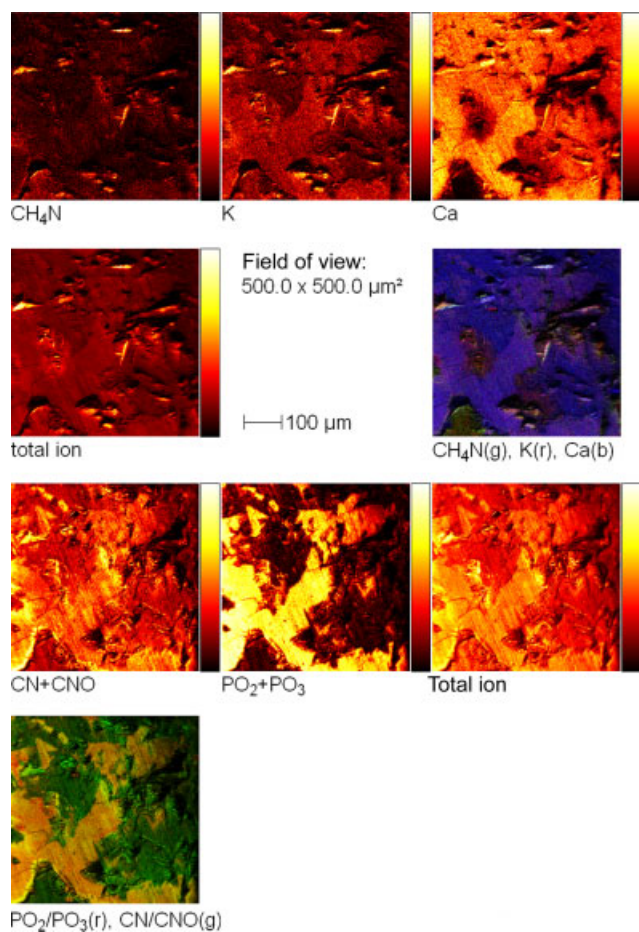


Figure 6. TOF-SIMS images for Sample 6. Positive ions, upper 2 rows, and negative ions, lower 2 rows.

characteristic fragment (m/z 124) was obtained from the m/z 167 peak in negative ion mode corresponding to deprotonated uric acid molecule. Conversely, using the positive ion detection mode, characteristic fragments (m/z 110, 85 and 60) were detected in the ESI-MS/MS spectra of the m/z 127 peak which corresponds to the protonated melamine molecule for Sample 3 (Fig. 3). This is consistent with the fact that Sample 3 was collected from the left kidney pelvis of a seven-year-old child who regularly consumed 150 g of milk powder every day for over 2.5 years, and is thus more likely to contain more melamine.

The TOF-SIMS results provide very complimentary information to that obtained from other techniques such as ESI and DAPCI.^[1–3] While not being able to directly detect fragments associated with uric acid and melamine, the presence of which were shown by these techniques, TOF-SIMS showed very different

distributions of the species. Of note, is an increase in N content at the 'inter-granular' boundary of Sample 2 which is clearly seen as a line in Fig. 4. Conversely, Sample 3 shows larger areas of N-containing species amongst the Ca distribution (compare overlays). In essence, this implies that the uric acid component is more prominent on the surface of the grains which constitute the kidney stone and, upon cross-sectioning, would appear more at the boundaries, whilst the melamine appears to be more part of the structure of the stone. The smoothness of the Sample 2 may arise from its uric acid content.

The structure of the adult kidney stone is very different from that of Samples 2 and 3. Unlike Sample 2, its appearance is very granular, however, the distribution of the Ca signal, like Sample 2, is much more uniform on the cross-section further supporting the influence of melamine in stone formation in Sample 3.

This study is very much work-in-progress. However, there is clear indication that differences in the formation of the stones can be detected by TOF-SIMS.

Conclusion

It is seen that TOF-SIMS is able to show the distributional differences associated with the different stones which may be related to how they are formed. However, the results do have to be tied in with information from other techniques in order to correlate the differences in structure with the N-containing species present. That being said, the TOF-SIMS results do indicate that a different mechanism for stone formation occurs in the presence of melamine.

Acknowledgements

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