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Advances in the identification of calcium carbonate urinary crystals

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INTRODUCTION

Crystalluria is the result of urine supersaturation with different types of salts the most frequent of which are calcium oxalate, calcium phosphate, and uric acid [1,2]. In normal subjects, supersaturation can transiently occur due to non-pathological high solute concentration, while in stone formers supersaturation is frequent and persistent over time [1,2]. Various conditions can cause crystalluria, including infection [3], acquired or inherited metabolic diseases [4] or, more often, dietary habits [5]. In all these conditions a correct identification of crystals is mandatory, this being achieved with the combined and matched knowledge of: urinary pH, crystal morphology, and birefringence features under polarized light [6]. When this approach is insufficient, more sophisticated techniques, such as infrared spectroscopy [6-8], scanning infra-red and Raman vibrational spectroscopy [9] are needed.

In this paper, we report the case of a woman whose urine contained three different types of crystals with unusual morphology which, with the use of advanced infrared spectroscopy techniques, were identified as variants of calcium carbonate (Ca-carb) crystals.

CASE REPORT

On July 4, 2016 a 64-year-old woman was seen in the outpatient clinic of the Rheumatology Department of the University Hospital at Liège (Belgium) for a periodic evaluation of her psoriatic arthritis, for which she was under treatment with methotrexate. Co-morbidities included arterial hypertension, hypothyroidism and dyslipidemia, which were treated with nifedipine, L-thyroxine, and rosuvastatine respectively. Laboratory investigation was normal but for a reduced e-GFR-MDRD (51 ml/min/1.73 m², normal value: >90 mL/min/1.73 m²). Urinalysis, performed with dipstick 30 minutes after micturition on the second urine of the morning, collected 2 hours after getting up with the patient under fasted conditions, showed: pH 7.5, specific gravity 1.025, and negative albumin, hemoglobin, leukocyte esterase, and nitrites.

The bright field microscopic examination of the centrifuged urine sample showed a few bacteria, a few squamous epithelial cells, and a massive crystalluria. With the additional use

of polarized light, three different types of crystals could be seen: 1. Flattened rectangles slightly birefringent under polarized light; 2. Non birefringent “daisy-like” crystals, identical to those described elsewhere [10,11]; 3. Strongly birefringent sticks and needles with well-defined edges (**Figures 1 and 2**).

Due to the unclear nature of crystals, the remaining aliquot of urine was filtered and dried and the sediment was submitted to advanced infrared spectroscopy investigation in the *Service des Explorations Fonctionnelles Multidisciplinaires, Hôpital Tenon, Paris* (**see below**).

Of note, crystalluria was not found in any of the urinary samples examined subsequently.

Infrared microspectroscopy and attenuated total reflectance (ATR) imaging allowed to identify the flattened rectangles as calcite (**Figure 3**). Raman spectroscopy with spatial resolution under 1 micron identified “daisy-like” crystals as vaterite, and sticks/needles crystals as aragonite (**Figure 4**). Energy dispersive X-ray spectroscopy (EDX), performed on glass slide, showed a homogeneous spatial repartition of calcium in all crystals (**Figure 5**). Thus, we could identify the observed crystals as polymorphs of anhydrous Ca-carb.

DISCUSSION

Different types of Ca-carb exist. The most stable ones are: the anhydrous (CaCO_3) i.e., calcite, aragonite, and vaterite; the hydrated i.e., monohydrocalcite ($\text{CaCO}_3, \text{H}_2\text{O}$), and ikaite ($\text{CaCO}_3, 6\text{H}_2\text{O}$); the amorphous, which may contain water molecules [12,13]. Such biominerals are the most common types present in mollusk shells or pearls, eggshells, corals [14,15] and cyanobacteria [16].

Ca-carb has a pivotal role in biomineralisation processes in a large number of animal species [17] and microorganisms, while it is infrequent in humans, with the exception of physiological crystals in the inner ear and pathological calcifications in pancreas and

gallbladder, where the three polymorphs of Ca-carb calcite, aragonite and vaterite are often observed [18]. Moreover, it has been reported that, in the thin descending limb of the Henle's loop, urine is supersaturated not only with calcium phosphate but also with Ca-carb [19]. The clinical meaning of Ca-carb crystals in the urine of humans is unclear at the moment, even though a possible association with a diet rich in vegetables has been hypothesized for some subjects whose urine contained daisy-like crystals [10,11].

The Ca-carb described in this paper are very unusual and differ from those reported in textbooks and atlases mostly as dumbbells, granules and, less frequently, as ovoids, spheres, or tetrads [20-28], which are birefringent under polarized light [20, 26-28]. Microscopic infrared spectroscopy and Raman spectroscopy show that these crystals are anhydrous Ca-carb polymorphs and EDX analysis confirmed the high calcium content. Similarly to previous cases with "daisy-like" crystals [10-11] also in the present subject Ca-carb crystals were not found in subsequent urine samples. This fact suggests that supersaturation with Ca-carb, the cause of which is unclear, was transient.

The difference in morphology between these three Ca-carb crystals is due to their crystalline structure: trigonal for calcite, orthorhombic for aragonite and hexagonal for vaterite. We can explain the differences between the three polymorphs from the different arrangement of the carbonate ions in their crystal structures. Many force fields have been developed to describe the Ca-carb interactions [29,30]. Other empirical models have been used to explore the bulk and surface properties of Ca-carb at ambient conditions [30,31]. Environment factors influence the structure that the crystal lattice takes during its formation: humic acid, presence and/or association of magnesium, sulfate, potassium, sodium and the rate of calcium [32]. The complexity of the urinary environment and its ionic balance could explain the presence of these three types of Ca-carb in this patient. Furthermore, it has been underlined that several

kinds of amorphous carbonate exist, their stability depending on the presence of other compounds such as phosphates [33].

In conclusion, with the use of advanced infrared spectroscopy techniques we have identified for the first time with certainty three different types and morphologies of calcium carbonate in human urine. However, the relationship between urinary environment, concentration between different ions and the formation of calcite, aragonite and vaterite, cannot be still answered in this study. Moreover, our patient and all patients except one in the series reported by Fogazzi *et al.* were females [10,11]. No clear explanation can be provided to explain the high prevalence of female gender and alkaline urine among patients who form vaterite in urine. Further investigation is required.

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Legends to figures

Figure 1. Crystals as seen with bright field microscopy: 1. flattened rectangles; 2. “daisy-like”; 3. stick/needle -like; 4. Agglomerate of “daisy-like” crystals.

Figure 2. Crystals as seen with polarized light microscopy: 1. slightly birefringent flattened rectangles; 2. non birefringent “daisy-like”; 3. strongly birefringent stick-needle like.

Figure 3. Infrared spectroscopy peaks (1797, 1413, 875 and 711 cm^{-1}) of calcium carbonate (calcite).

Figure 4. Typical RAMAN spectra of vaterite (daisy like crystals) and aragonite (sticks and needles).

Figure 5. Energy dispersive X-Ray spectroscopy (EDX) spectra showing a presence of calcium in all crystals (calcite; aragonite and vaterite). The urine sediment is deposited on a glass slide.

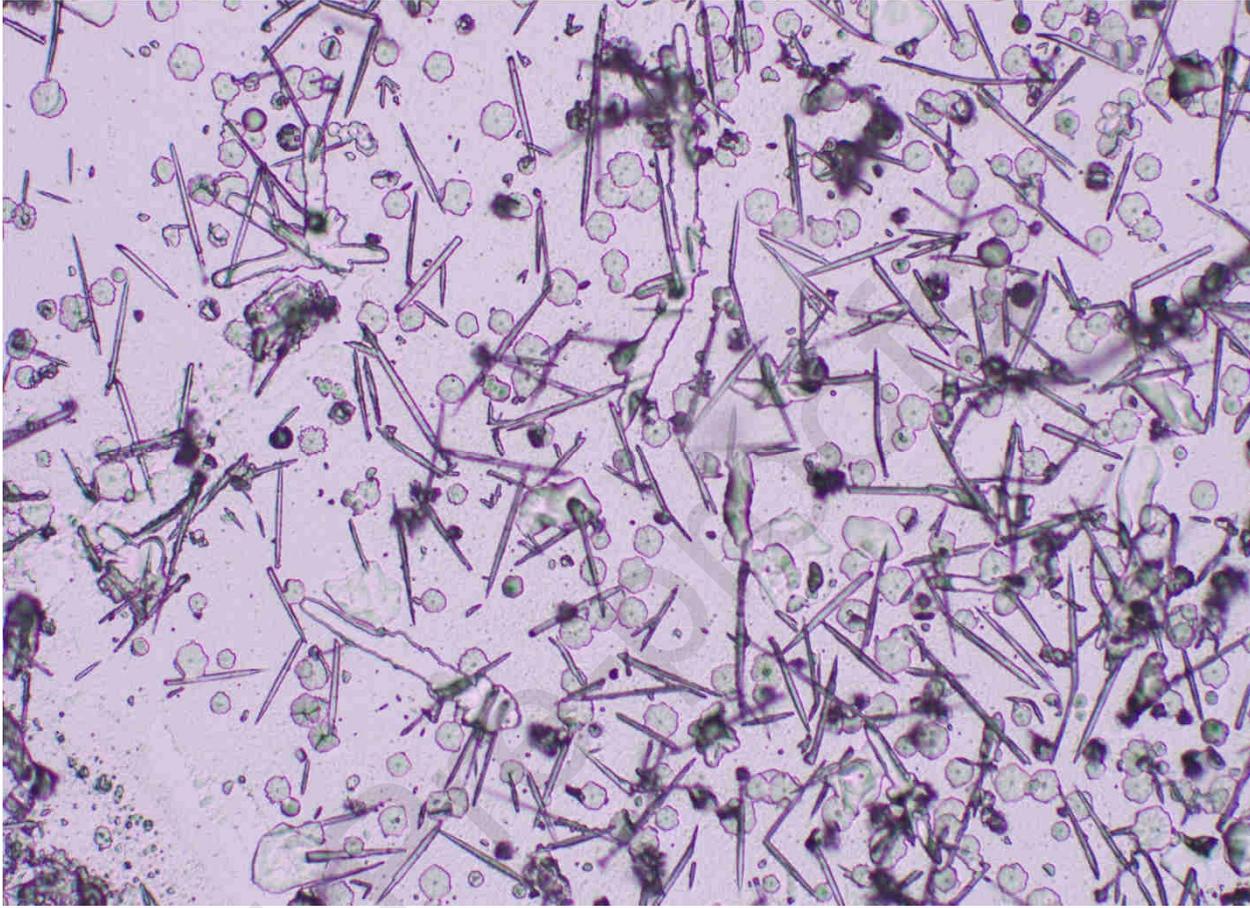


Figure 1

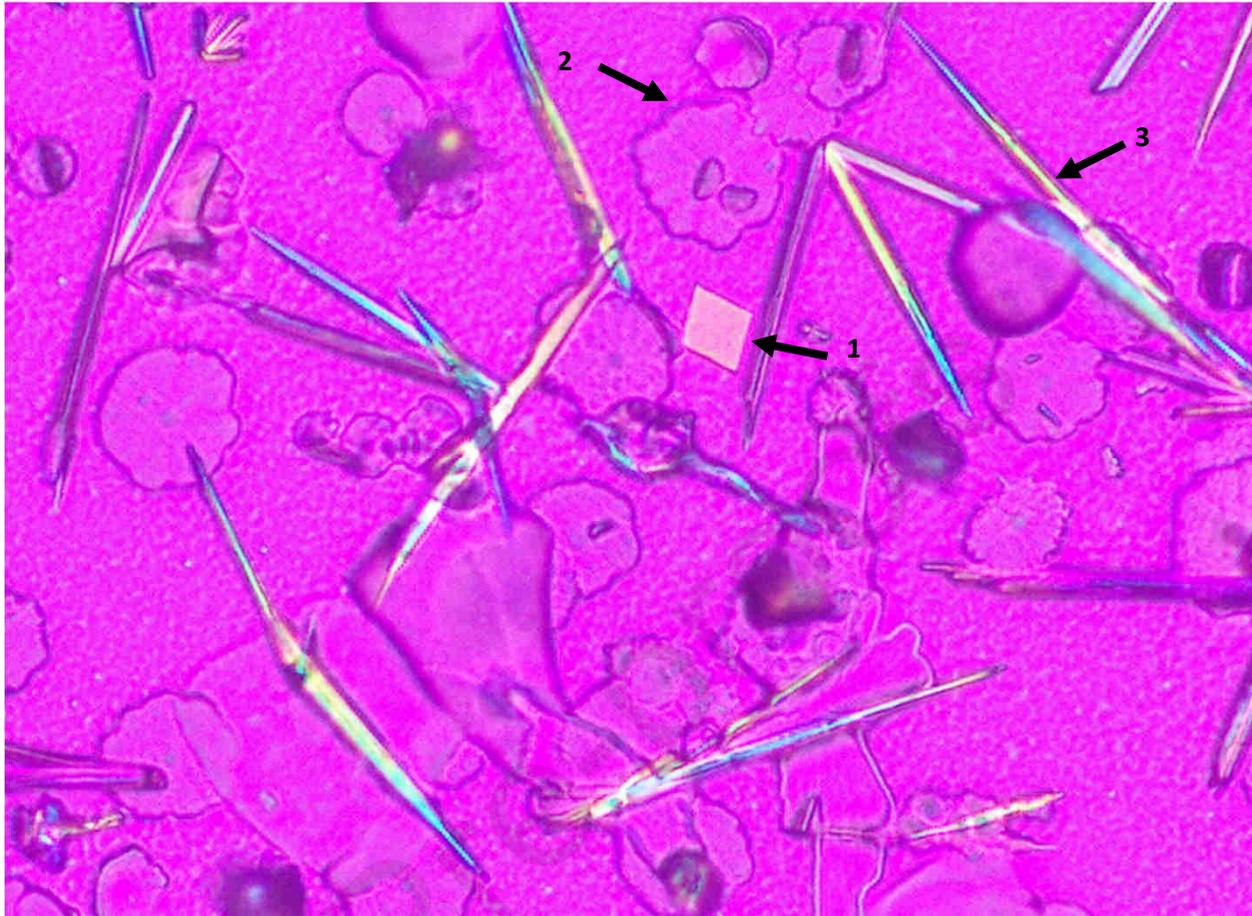


Figure 2

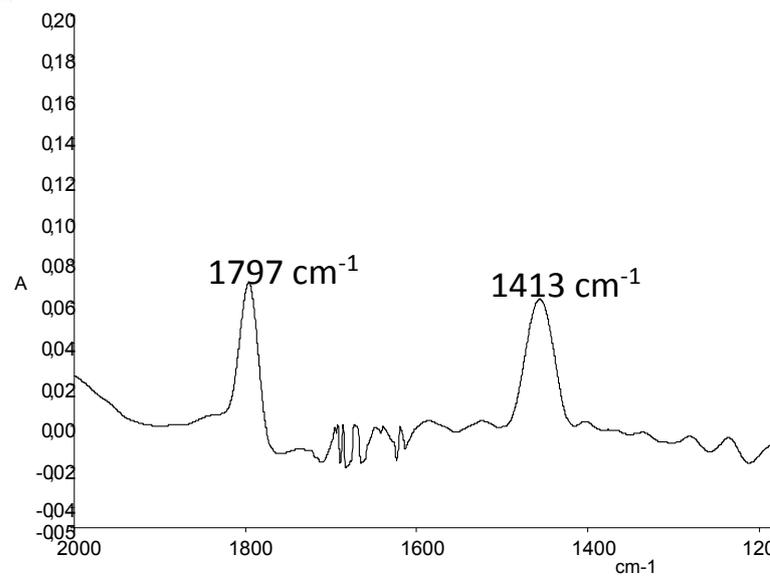
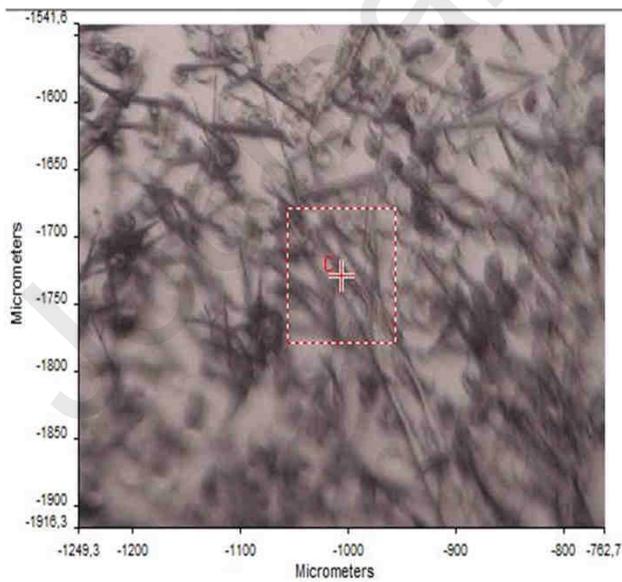


Figure 3

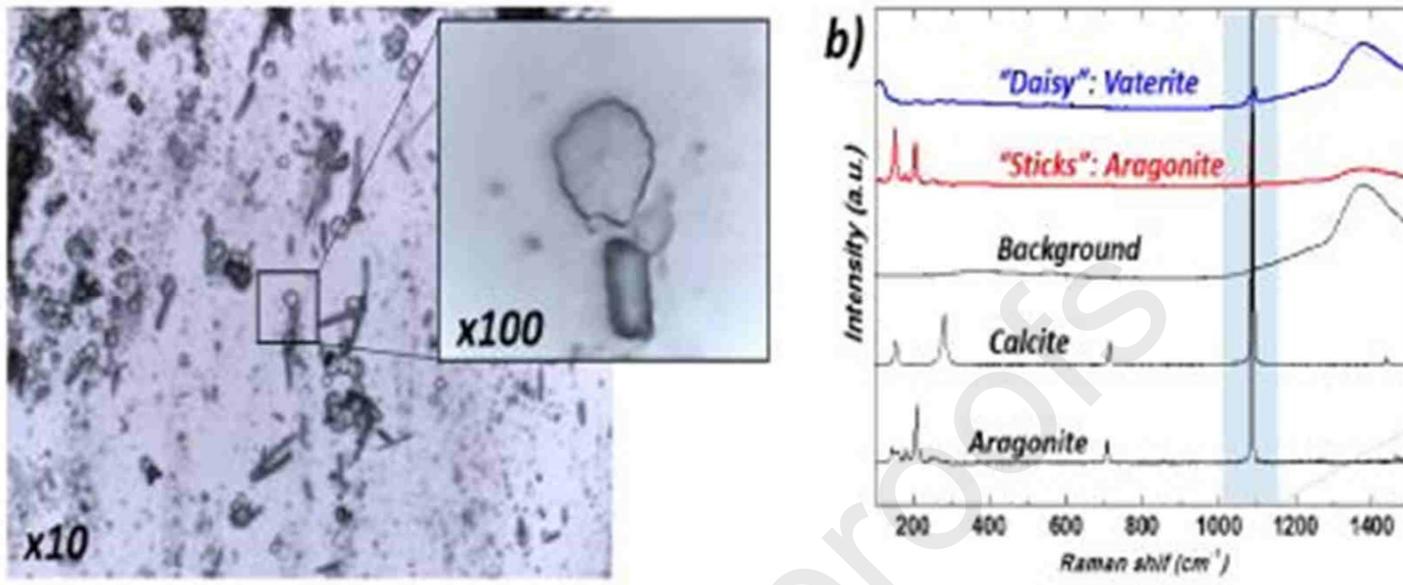
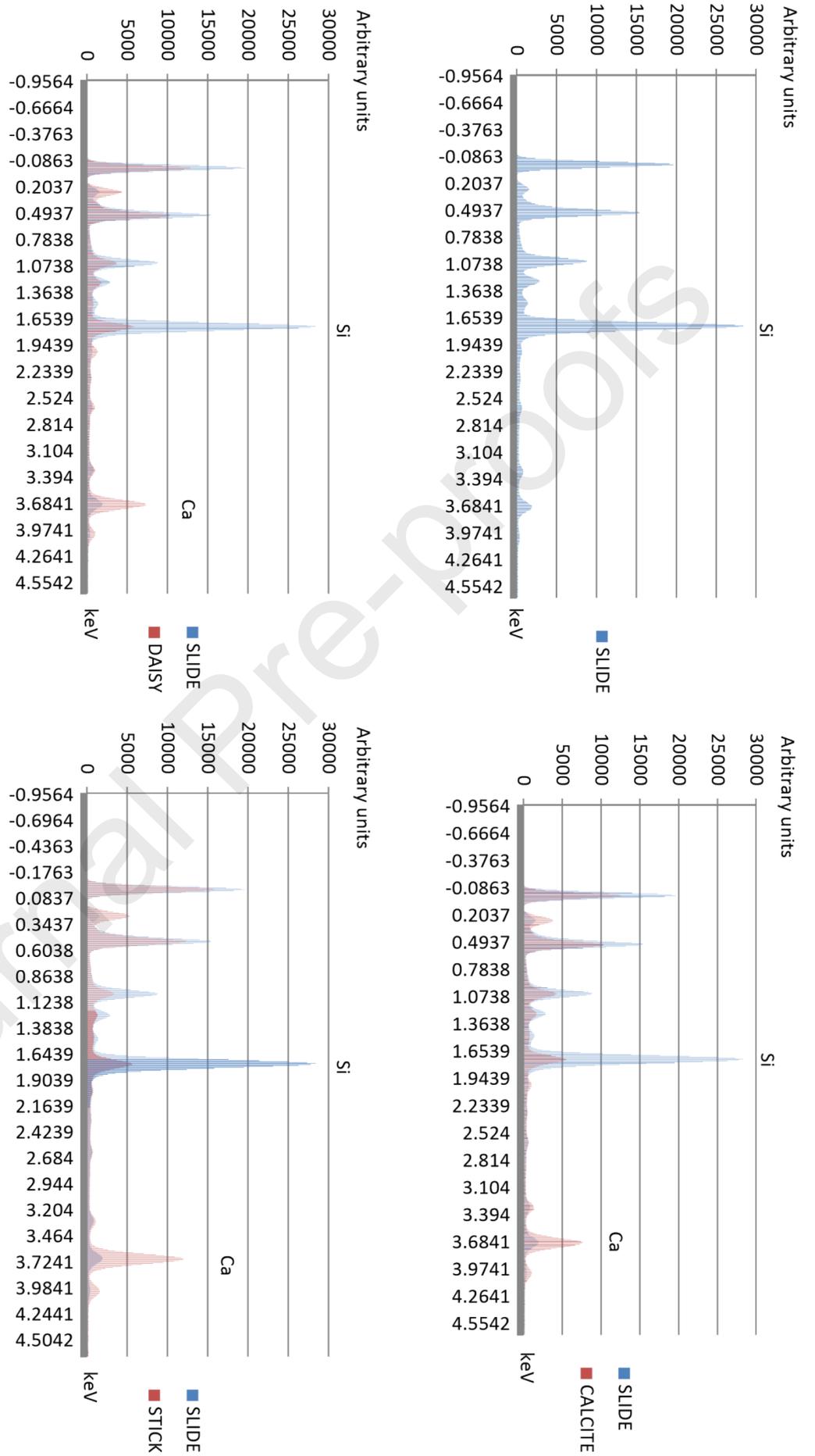


Figure 4

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KEY WORDS

Urinary sediment

Calcium carbonate crystals

Infrared microspectroscopy

Raman spectroscopy

Energy dispersive X-Ray spectroscopy

ABSTRACT

The examination of the urinary sediment of a 64-year-old woman showed the presence of three different types of crystals, all with unusual morphology, which could not be identified with bright field microscopy, polarized light, and the knowledge of urine pH (7.5). The use of microscopic infrared spectroscopy, Raman spectroscopy and energy dispersive X-ray spectroscopy led to the identification of the three types of crystals as calcite, vaterite and aragonite, which are all variants of calcium carbonate crystals. This paper confirms the complex morphology and nature that urinary crystals may at times have and the utility of advanced infrared spectroscopy techniques for their identification.

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