Urol Res (2008) 36:247–249
DOI 10.1007/s00240-008-0148-2

ORIGINAL PAPER

X-ray diffraction analysis of urinary calculi: need for heat treatment

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Received: 27 February 2008 / Accepted: 8 August 2008 / Published online: 3 September 2008

Abstract Although X-ray diffraction (XRD) is the most reliable method for analysis of urinary stones, it has its specific limitations. It fails to detect amorphous phases, cannot distinguish between chemically different phases having identical lattice geometry (e.g., brushite $CaHPO_4 \cdot 2H_2O$ and gypsum $CaSO_4 \cdot 2H_2O$) and may miss some phases (e.g., apatite and calcium urates) due to peak overlaps. XRD of urinary stones was performed using a DRON 2.0 diffractometer with CuKα radiation and repeated after calcining the sample, preferably with weighing. XRD of the calcined samples enabled detection of amorphous magnesium phosphates, poor crystallized apatite mixed with struvite, weddellite and/or organic matter, hidden organic calcium salts mixed with uric acid; unambiguously discriminated between brushite and gypsum, struvite and its potassium analogue; confirmed presence of quartz in one stone. Statistical study of 341 samples from Rostov region has shown that three-phase mixtures are most frequent (32.3%). Redoing XRD phase analysis after heat treatment, preferably at 500 and/or 900°C, considerably enhances capabilities of the method due to (i) avoiding peak overlaps; (ii) crystallization of amorphous phases; (iii) concentrating minority inorganic components in organic stones; (iv) different decomposition products from indistinguishable phases; (v) semi-quantitative information from the weight loss data.

Electronic supplementary material The online version of this article (doi:10.1007/s00240-008-0148-2) contains supplementary material, which is available to authorized users.

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Keywords Urinary stones · X-ray diffraction · Heat treatment · Apatite · Brushite · Amorphous phases

Introduction

X-ray diffraction (XRD) and infrared spectroscopy (IRS) are now routine tools for identification of urinary calculi. Each of the two methods, although much more reliable than chemical analysis [1, 2], is still subject to errors. Like any other experimental technique, XRD has its specific limitations:

1. It fails to detect amorphous phases.
2. It cannot distinguish between chemically different phases having identical lattice geometry. The most striking example is an isoelectronic and structurally related pair, brushite $CaHPO_4 \cdot 2H_2O$ and gypsum $CaSO_4 \cdot 2H_2O$. Their XRD patterns are very similar (actually, there are more differences between the patterns of brushite found in the Powder Diffraction File PDF-2 [3]). In urinary calculi, such a pattern is usually attributed to brushite, and possible presence of gypsum is ignored. Patterns of struvite $NH_4MgPO_4 \cdot 6H_2O$ and its potassium isomorph are also similar.
3. Minority phases may be missed due to peak overlaps, a problem common for most instrumental methods. XRD patterns for calcium urates in the PDF-2 are of low quality and resemble uric acid. Thus, determination of calcium urates in the presence of uric acid is questionable. Apatite in urinary stones is usually poor crystallized, with very weak and broad reflections, and strongest of them ($2\Theta = 31.80$, 32.22 and 32.93)[1]

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[1] In this paper, all reflections are reported as 2Θ values for CuKα radiation, $\lambda = 1.5418$ Å.

overlap with reflections (or their shoulders) from weddellite $CaC_2O_4{\cdot}2H_2O$ (31.79 and 32.26) and struvite (31.94 and 32.90). Thus, apatite may be hidden (note that IRS may also miss apatite even at a 40% level [2]). Similarly, two of the three strongest reflections from whewellite $CaC_2O_4{\cdot}H_2O$ (14.94 and 30.13) overlap with reflections from struvite (15.00 and 30.21), and the strongest peak from weddellite (14.33) is masked by struvite, too (14.42).

These limitations sometimes lead to incorrect diagnoses and generalizations; e.g., some authors report most calculi to be phase-pure according to IRS [4], or XRD [5] or microscopic [6] analyses, whereas others, combining different methods, report that most calculi are mixed phases [7–9].

This work suggests a simple modification to the routine analysis: XRD patterns should be taken not only for native samples but also after their calcination.

Materials and methods

The samples were fresh urinary calculi (gone spontaneously or after lithotripsy or obtained surgically) mostly from “Zdorov’e” medical centre in Rostov-on-Don, Russia. They were ground and mounted onto petrolatum. XRD scans were performed using a DRON 2.0 diffractometer with Ni-filtered CuKα radiation between 4° and 34–39° (2Θ). In some instances, higher-angle scans (30–70°) with an internal standard were used to refine lattice parameters. Another portion of the sample was placed into a porcelain crucible, heat-treated in air and X-rayed. If the amount of the sample was insufficient, the same portion was used for both experiments. When possible, weight loss on calcination was measured with an analytical balance. Phase analysis was done with the aid of PDF-2 [3].

Results and discussion

Examples of the XRD patterns taken before and after heat treatments are shown in Supplementary Figs. 1–8. 15–20 min calcination at 500–550°C is usually sufficient for burning out all organics and complete conversion of organic calcium or sodium salts (oxalates or urates) to carbonates. Only large organic samples (1 g or more) need longer durations or higher temperatures. Under these conditions, poor-crystallized apatite does not recrystallize (Supplementary Fig. 1), brushite $CaHPO_4{\cdot}2H_2O$ and struvite $NH_4MgPO_4{\cdot}6H_2O$ turn amorphous (Supplementary Figs. 1, 2), whereas gypsum $CaSO_4{\cdot}2H_2O$ and $KMgPO_4{\cdot}6H_2O$ transform into well-crystallized $CaSO_4$ and $KMgPO_4$, respectively.

The decomposition of oxalates and hydrous phosphates, together with burning out organics, eliminates peak overlap and increases concentration of apatite and other thermally stable phases and, thus, enables their detection (Supplementary Figs. 2–4). Considerable amounts of poor crystallized apatite (up to 30–40%) might remain hidden in pristine samples but were found after the heat treatment (Supplementary Fig. 2). Owing to calcination, as low as 0.1% calcium phosphate and/or calcium carbonate could be unambiguously determined in large uric acid or cystine stones. For samples rich in calcium oxalates, temperatures higher than 550°C are unfavorable due to formation of CaO, since its strong reflection at $2\Theta = 32.23°$ may mask apatite again.

At higher temperatures, 800–900°C, amorphous phosphates crystallize (struvite and brushite produce $Mg_2P_2O_7$ and $Ca_2P_2O_7$, respectively) or react with other components (Supplementary Figs. 1, 2, 4–6) providing additional information. E.g., Supplementary Fig. 6 shows that poor crystallized apatite transformed into whitlockite whose lattice constants indicate ca. 13% Mg substitution according to the plot from [10], in contrast to the sample shown in Supplementary Fig. 1, with lattice constants characteristic of Mg-free $Ca_3(PO_4)_2$.

Supplementary Fig. 2 is especially instructive. The pristine sample seemed to be pure struvite, with no signs of calcium salts, but the two heat treatments revealed that the sample contained much calcium phosphate and some oxalate or urate (decomposed to calcite), and, according to the pattern taken after 890°C treatment, calcium compounds were dominating.

Occurrence of crystalline SiO_2 in urinary calculi [8] seemed strange; however, three weak reflections attributable to quartz were detected in one of our samples, too. Presence of quartz received strong confirmation when, after the treatment at 550°C, these reflections were retained and even enhanced.

Some new constituents of urinary calculi were detected. One of the samples, consisting mostly of uric acid, showed weak reflections from unidentified phase, which, upon calcination, turned into $CaCO_3$ (Supplementary Fig. 7). Presumably, it was some organic calcium salt, different from the known oxalates and urates. Supplementary Fig. 8 illustrates appearance of a face-centred cubic phase with $a = 7.41(1)$Å after burning uric acid. This phase resembles, but not coincides with, both $Na_{3-x}(PO_4)_{1-x}(SO_4)_x$ (PDF 36–551) and $Na_{1-x}Fe_{1-x}Si_xO_2$ (PDF 48–80). Its exact composition and origin remain unknown.

Supplementary Tables 1 and 2 summarize statistical results of this work. According to literature data, at least 40 crystalline phases may occur in urinary stones. Of these, only 16 phases were unambiguously identified in our 341 samples from 336 patients (where male to female ratio was unusually low, 1.26). Supplementary Table 1 shows that XRD, when repeated after heat treatment, is capable to detect up to four or five phases in an urinary stone, and, in contrast to many previous studies, only 32.0% stones are single-phase, whereas three-phase stones are most frequent (32.3%). In agreement with all previous works, whewellite is the most abundant phase. Apatite and weddellite are the second and the third most frequent phases, respectively, but they rarely dominate in a stone, as opposite to anhydrous uric acid and struvite which are relatively rare but, when present, usually dominate in a stone.

Only 215 of our 341 samples have been calcined. Of these, the heat treatments have revealed hidden constituents (mostly apatite, $CaCO_3$, Mg in calcium salts and unknown phases) in 88 cases (41%) (Supplementary Table 2). In other 59% cases, absence of hidden phases has been established and identity of struvite or brushite has been confirmed.

The suggested protocol for XRD of urinary stones is as follows.

1. Examine the sample visually. If there are parts essentially different in appearance, they should be treated separately. Grind the sample with a mortar and pestle and take an XRD pattern of its representative part. The reminder (if any) should be left to dry at room temperature (when quantitative analysis is planned).
2. If amount of the sample is extremely low and the XRD pattern shows strong reflections from organic phase(s) (uric acid, ammonium urate, cystine, etc.), then the heat treatment is of no use because the mass after the calcination will be much smaller (if any) and nothing will be detected.
3. If amount of the sample is sufficient, calcine a weighed portion of the dry powder in an open crucible or dish at 500–550°C until the carbon black disappears. This takes from 15 min. to an hour or two, depending of amount of organics. The remainder should be white, yellow or gray, not black. Then weigh it and take its XRD pattern to find possible apatite and/or $CaCO_3$ (from decomposition of oxalates, urates or other organic salts) and to discriminate between brushite and gypsum, struvite and its potassium isomorph. This step may be excluded, if the above problems have been resolved by the first XRD pattern; apatite and calcium oxalate or urate have been found, brushite and struvite have not. If quantitative analysis is not planned, then complete elimination of the carbon black is unnecessary and calcination time may be shorter.
4. Calcine a weighed portion of the dry sample (or reminder from the step 3) at 850–900°C for 10–20 min, weigh it and take its XRD pattern to reveal possible crystallization of amorphous phosphates or their reaction with other components.

In conclusion, redoing XRD phase analysis after heat treatment considerably enhances capabilities of the method:

- Reveals phases hidden due to peak overlap (e.g., poor-crystallized apatite becomes visible when weddellite transforms to calcite or struvite transforms to amorphous phase);
- reveals amorphous phosphates;
- concentrates minor inorganic components due to burning organics and decomposition of hydrates;
- discriminates between X-ray similar phases such as brushite and gypsum, struvite and its potassium analogue;
- enables semi-quantitative estimates based on weight loss.

Providing more detailed information about composition of urinary stones will be, of course, beneficial to diagnostics and metaphylaxis of stone disease.

Acknowledgments The work was supported by “Zdorov’e” medical centre, Rostov-on-Don, Russia.

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