Establishing Composition and Structure of Intact Urinary Calculi by X-Ray Coherent Scatter for Clinical Laboratory Investigations

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Purpose: Current urinary stone analysis techniques are limited in their abilities to simultaneously characterize composition and structure. Laboratory techniques such as IRS and x-ray diffractometry require small powdered samples for analysis, rendering composition results dependent on the choice of sample and its preparation. We investigated the application of x-ray CS analysis to identify topographic urinary stone composition ex vivo. CS is essentially a transmission based x-ray diffractometry method that depends on molecular structure and, therefore, can distinguish different compounds. Diagnostic x-ray equipment facilitates the examination of structural arrangements of minerals in intact calculi.

Materials and Methods: Tomographic images of intact calculi CS properties were acquired with a purposely built scanner. Composition is extracted from CS by fitting a library of common pure stone component CS signatures to those of the unknown sample in each image pixel. Two zones per stone were isolated (powdered) for IRS composition analysis for comparison with CS maps at these locations. Each stone was also independently analyzed for bulk composition by IRS analysis.

Results: CS composition maps revealed the spatial arrangement of minerals in intact calculi. IRS results showed good agreement with CS in the selected regions of interest. Bulk composition by IRS was noted to miss some important stone components, indicating that the choice of sample may skew composition results.

Conclusions: CS from diagnostic x-rays can be used to identify structure and composition in mixed urinary calculi nondestructively. The tissue specific CS images presented support the development of CS analysis as a means of identifying stone composition characteristics in the laboratory.

Key Words: urinary calculi; lithotripsy; x-ray diffraction; scattering, radiation

Kidney stones are a common urological disorder with a reported incidence of 13% in North America, which is continually increasing.¹,² The determination of stone composition is important for identifying the need for further investigations and preventive therapies to avoid subsequent interventional procedures. The current standards for stone analysis are XRD and IRS. However, they are destructive in nature and only analyze the chemical structure of small powdered fragments. Many stones are of mixed composition with different compositions in the core and surfaces.³ Therefore, an accurate analysis of stone composition is only achieved if representative zones of the stone are sampled.⁴ Inadequate sampling may affect the decision of which preventive strategy would benefit the patient or whether further metabolic testing is necessary.

Although various radiographic imaging modalities have attempted to achieve comprehensive stone analyses, at present all lack the ability to fully characterize composition based on radiographic contrast. Zarse et al used micro CT to compare high resolution images of urinary calculi with surface Fourier transform, infrared microspectroscopy measurements.⁵ They found nonoverlapping HU values in regions of pure COM, COD, CP, MAP, UA and CYS but they did not identify CPD. On helical CT the overlap of density distributions of some calcium components prevents a unique solution.⁶,⁷ In addition, multiple components in regions smaller than the spatial resolution make it impossible to determine the concentration of individual components. This is most severe with CT⁶,⁷ but it may also be a practical limitation with micro CT.⁵

While the contrast in conventional radiographic imaging techniques is generated by x-rays transmitted through the specimen, imaging with coherently scattered x-rays provides contrast based on interactions with the specimen at the atomic level. At diagnostic energies (less than 120 kVp) low angle x-ray scatter (less than approximately 10 degrees) is principally coherent⁸ and it gives rise to tissue specific diffraction patterns. While the diagnostic x-ray spectrum must be narrowed to amplify CS characteristics, each pure chemical sample or stone type produces a distinct CS pattern, indicating that CS analysis can be used as a basis for classifying urinary calculi by composition. This CS analysis technique has been used to accurately identify pure powdered chemical compounds and ex vivo stone types (COM, COD, CP, CPD, MAP, UA and CYS).⁹
CS patterns arising from composite materials such as mixed urinary stones show the characteristics of each individual component. The CS pattern for a composite is a linear combination of the individual CS patterns of its components because x-rays scattered from different tissues in a material do not interfere with each other. Thus, unlike transmission-based radiographic measurements, CS-based characterizations are unaffected by the presence of more than one component and they allow the analysis of regions containing multiple components. Moreover, CS is a nondestructive technique that preserves structural information. Our diagnostic x-ray coherent scatter analysis technique can discriminate common stone components (COM, COD, CP, CPD, MAP, UA, and CYS) and identify their distributions nondestructively.

MATERIALS AND METHODS

Instrumentation
A diagnostic XRlI based system developed by our group has been used to acquire coherent scatter images (fig. 2). Diagnostic x-rays operating at 70 kVp are filtered by 0.30 gm/cm² gadolinium to decrease the spectral blur of the beam, thus, improving the angular resolution of the measured CS patterns. Following this filtration the x-rays are then collimated to a 1 mm² pencil beam. Collimation is achieved with a triple aperture, parallel plate collimator to eliminate the contribution of scatter from this aperture to the measured signal. Samples are mounted on a stage located in front of the collimator that has the ability to translate, rotate, and elevate during acquisition. This permits the acquisition of CS data from x-ray projections through the sample and from tomographic slices in 2 and 3 dimensions. Low-angle scatter from the sample is monitored through the sample and from tomographic slices in 2 and 3 dimensions. Following this filtration the x-rays are then collimated to a 1 mm² pencil beam. Collimation is achieved with a triple aperture, parallel plate collimator to eliminate the contribution of scatter from this aperture to the measured signal. Samples are mounted on a stage located in front of the collimator that has the ability to translate, rotate, and elevate during acquisition. This permits the acquisition of CS data from x-ray projections through the sample and from tomographic slices in 2 and 3 dimensions. Low-angle scatter from the sample is monitored through the sample and from tomographic slices in 2 and 3 dimensions. Following this filtration the x-rays are then collimated to a 1 mm² pencil beam. Collimation is achieved with a triple aperture, parallel plate collimator to eliminate the contribution of scatter from this aperture to the measured signal. Samples are mounted on a stage located in front of the collimator that has the ability to translate, rotate, and elevate during acquisition. This permits the acquisition of CS data from x-ray projections through the sample and from tomographic slices in 2 and 3 dimensions. Low-angle scatter from the sample is monitored through the sample and from tomographic slices in 2 and 3 dimensions. Following this filtration the x-rays are then collimated to a 1 mm² pencil beam. Collimation is achieved with a triple aperture, parallel plate collimator to eliminate the contribution of scatter from this aperture to the measured signal. Samples are mounted on a stage located in front of the collimator that has the ability to translate, rotate, and elevate during acquisition. This permits the acquisition of CS data from x-ray projections through the sample and from tomographic slices in 2 and 3 dimensions. Low-angle scatter from the sample is monitored through the sample and from tomographic slices in 2 and 3 dimensions.

Chemical Reference Library
Reference scatter patterns were acquired from pure chemical samples (Sigma-Aldrich, St. Louis, Missouri) for all major stone components (COM, CP, CPD, MAP, UA, and CYS) except COD since the latter is not readily available in pure chemical form. The reference pattern for this material was derived from a powdered calculus identified by IRS as containing only this material. Each of these chemical samples was interrogated with 70 kVp x-rays for 4 seconds at 200 mA to generate high-quality reference CS patterns.

Intact Human Urinary Calculi
CT images of CS properties were acquired from intact urinary calculi. A subset of 5 calculi of mixed composition were chosen for demonstration purposes because all 7 primary stone components were identified in these specimens. For each 2-dimensional slice scatter patterns were acquired at 0.25 mm increments as the intact stone was translated through the x-ray beam operating at 70 kVp and 200 mA for each of the 90 angular views. Coherent scatter CT images were reconstructed and tissue composition maps were produced using the chemical reference library. For each stone 2 zones were sampled (powdered) for IRS analysis characterizations. IRS composition results were then compared to those obtained by CS. Composition results from CS in these 2 regions were obtained from the average contribution of each material in a region of interest in composition maps. Each stone was also analyzed for bulk composition independently by IRS analysis. As routinely done in patient calculous analysis, the sample from these stones was chosen by the IRS technician to avoid any bias in sample selection.

RESULTS

Figure 3 shows the composition maps derived from CS patterns for 5 stones of mixed composition. Although each stone was tested for the 7 common stone components (COM, COD, CP, CPD, MAP, UA, and CYS), only those identified as being present are shown. Each set of composition maps shows the structural arrangement of components through a cross-sectional view of all 5 stones. While a single tomographic slice per stone sample is shown for demonstration purposes, CS images can be acquired in 3 dimensions.

The 2 regions of each stone that were subsequently powdered for characterization with IRS analysis are indicated in the stone photographs (fig. 3). They were chosen to represent areas of differing composition in the sample, as identified by CS analysis. Components identified in these regions via CS

![Fig. 1. Scatter patterns from composite materials such as urinary calculi show characteristics of each individual component. CS pattern measured from mixed stone is simply linear sum of component CS patterns, weighted by their concentration. Mixed stone CS pattern shown has features of each constituent mineral (COM, CP and UA), as expected. Angular scatter range of CS patterns is 0 to 10 degrees with approximately 1.5-degree angular resolution.](image)
FIG. 3. CS composition maps and associated composition analyses by IRS for 5 urinary calculi (I to V). Composition maps only include materials identified by CS in stone sample. Two samples were obtained per stone from regions indicated (A and B, respectively), powdered and characterized by IRS. Bar graphs indicate composition analysis results from CS and IRS for these 2 regions. As routinely done clinically, bulk composition assessment by IRS is also shown on same graph for comparison.
analysis were also determined by IRS. It is not surprising that
the component percents did not exactly match because IRS
measurements were done in a small subset of prepared pow-
dered samples from each region. The independent identifica-
tion of similar compounds in each stone region by IRS validates
the CS composition maps and provides evidentiary support that
CS can be used as a basis for classifying urinary calculi by
composition.

Figure 3 also shows the results of the analysis of bulk
composition by IRS on the bar graphs for each stone sample, as
routinely done for clinical purposes. While these measure-
ments generally identified the primary stone components,
some secondary components were missed. These composition
analyses demonstrate that an accurate estimate of components
can only be obtained by techniques requiring small powdered
samples, such as IRS and XRD, if several zones in the stone are
sampled.

DISCUSSION

As currently assessed via conventional techniques (IRS and
XRD), stone composition is often used to devise patient spe-
cific preventive strategies. IRS and XRD are restricted to
surface measurements of small powdered samples, render-
ing them inappropriate for nondestructive stone analyses. In
these routine laboratory analyses stones are crushed into a
fine powder and mixed, and subsequent characterizations
are performed in small subsets of these powdered samples.
The pulverization of stone fragments results in a loss of
structural information pertaining to the order of component
deposition. Also, as stones increase in size, the probability of
detecting secondary components with XRD or IRS decreases
unless multiple sections of the stone are sampled.13,14
Moreover, it has been documented that certain stone constituents
may be chemically altered upon powdering.13,14

The diagnostic x-rays used in CS operate at a higher
energy than conventional XRD analysis and allow the inves-
tigation of intact calculi since they can be transmitted
through much larger specimens. This technique can provide
regional distributions of components and, thus, it is insen-
sitive to specimen sampling or preparation errors. Such
detail may provide clinical information that is not available
otherwise. An example is in the case of stones containing the
MAP (struvite) component, indicative of infection. A patient
with an existing urinary infection is at risk for MAP stones.
However, any type of stone can also trigger infection due to
its presence in any portion of the urinary tract. Under these
conditions MAP crystals may be deposited on the stone
peripheral layers. Although there is infection in each case,
the stone formation mechanisms can be different, leading to
different prevention and treatment options.

The identification of stone component distributions may
also aid in enhancing the efficacy of prevention efforts. It is
generally accepted that composition is a key indicator of etio-
logical processes for noncalcium stones because their condi-
tions for formation are less multifactorial than those of calcium
containing stones.15 However, Trinchieri et al provided recent
evidence that knowledge of calcium crystal types and minor
components of calcium stones also provides insights into treat-
ment planning.16 While certain conditions in the urinary en-
vIRONMENT, such as pH, supersaturation of chemicals, etc, favor
the growth of certain crystal types, they are not a requirement
when a number of crystals with similar lattice dimensions are
present in urine. Crystals can be deposited on the surface of
others with similar dimensions. This process, known as epi-
taxy, allows crystallization to occur in environments that have
not met critical supersaturation conditions. The most common
type of stones, that is calcium oxalate, often have a minor UA
component. UA crystals can lead to heterogeneous nucleation
and promote calcium oxalate crystallization. The regional dis-
tribution of these 3 components may provide insights into the
role of epitaxy in the formation of such stones. While control-
ling crystal saturation levels in the urine is an important factor
for preventing stone formation, a therapy aimed at eliminating
epitaxial factors may further help prevent crystallization. In
the case of calcium oxalate stones of this nature the elimina-
tion of UA through allopurinol or dietary restrictions of protein
and purine intake can in part inhibit their formation.

The current study indicates that CS analysis resolves key
components in intact stones. The potential application of
this method is demonstrated with postoperative intact uri-
inary stones. The particular stone samples shown contain
varying amounts of the 7 common stone components. By
producing composition maps of stones using CS analysis we
can evaluate component distributions nondestructively. The
composition maps derived from CS tomographic measure-
ments of mixed stones were validated through comparisons
with IRS analysis in regions of interest. The 2 techniques
identified similar components in these regions. Each of the 7
common stone components was successfully identified via
CS analysis. The discrepancies between associated compo-
nent proportions measured by each technique highlight the
dependence of composition assessment by IRS on the choice
of sample. Only a small subset of the powdered specimen
submitted for IRS analysis could be practically analyzed
and, thus, this may have led to misrepresentations in com-
ponent percents. Further evidence of the sampling problem
is demonstrated by the original analysis of bulk stone com-
position by IRS. We observed that, although IRS identified
the primary stone components via such an analysis, some
stone components were missed.

As in IRS, the analysis algorithm used in CS analysis
depends on a spectral library of known combinations. The
comparison between unknown CS cross sections and the
spectral library must be optimized to generate the best pos-
sible match. However, such algorithms cannot always guar-
anteed that the combination of materials found through
analysis represents the true composition with absolute cer-
tainty. Therefore, our current study includes the examina-
tion of a larger set of calculi with different pure component
combinations to determine the accuracy of the CS analysis
technique when multiple components are present.

This study addresses stone composition in a laboratory
setting. However, the CS approach may have the potential
for in situ analyses. Knowledge of stone composition in vivo
may affect the choice of therapy because some hard stones
are not amenable to extracorporeal shock wave litho-
tripsy.17,18 As discussed, tomographic acquisitions of CS
signals are impractical for use in patients due to the large
dimensions of the abdomen. However, even if only material
identification was possible using CS analysis through the
use of projection based measurements of CS, this may still
have diagnostic potential. The implementation of such a
technique could be achieved using lithotripsy units equipped
with standard x-ray tubes and image intensifiers. After the
stone is localized through stereotactic means a pencil beam
of x-rays shaped with appropriate collimation could be directed toward this target through the shortest possible path through the body. The resulting CS signals could be recorded via the lithotripter x-ray detection system (image intensifier) and material analysis could be performed to extract the composition. The success of such a clinical analysis technique hinges on the assumption that the intensity of scatter arising from tissues surrounding the stone does not completely overshadow that of stone components. To demonstrate the feasibility of making CS based measurements of urinary stone composition in vivo the examination of signal-to-noise characteristics of stone CS signals must be examined in the presence of realistic amounts of surrounding tissue. To determine optimal parameters for making these measurements stones can be scanned in varying sizes of water or gel matrix baths to simulate soft tissue. Also, the degradation of CS signals due to multiple scatter events must be explored. It may be possible through judicious post-patient collimation to largely eliminate multiple scatter contributions to CS signals. Thus, while the use of a standard clinical diagnostic x-ray source can allow in situ measurements, further studies must be done to explore the in situ applicability of this technique.

CONCLUSIONS

CS analysis provides detailed information not available through current stone analysis techniques. The examination of intact calculi by CS analysis reveals the presence of minerals and their spatial arrangement in composition image maps. These CS composition maps also demonstrate that misrepresentations or oversights can occur due to inadequate sampling in IRS characterizations. Knowledge of the interrelationship between these stone features and other important mechanistic properties can impact clinical decisions aimed at preventive and curative therapies.

Abbreviations and Acronyms

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<thead>
<tr>
<th>Abbreviation</th>
<th>Corresponding Term</th>
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<tr>
<td>COD</td>
<td>calcium oxalate dihydrate</td>
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<tr>
<td>COM</td>
<td>calcium oxalate monohydrate</td>
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<td>CP</td>
<td>calcium phosphate</td>
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<tr>
<td>CPD</td>
<td>calcium phosphate dihydrate</td>
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<td>CS</td>
<td>coherent scatter</td>
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<td>CSCT</td>
<td>coherent scatter CT</td>
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<td>CT</td>
<td>computerized tomography</td>
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<td>CYS</td>
<td>cystine</td>
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<td>IRS</td>
<td>infrared spectroscopy</td>
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<tr>
<td>MAP</td>
<td>magnesium ammonium phosphate</td>
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<td>UA</td>
<td>uric acid</td>
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<td>XRD</td>
<td>x-ray diffraction</td>
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<td>XRII</td>
<td>x-ray image intensifier</td>
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