Kidney Stone Analysis by Nicolet FTIR Spectrometer

[a]F. KESNER, [a]I. DOMINÁK, and [b]M. LINHARTOVÁ

[a]*Nicodom, Rep. Nicolet Instrument, CZ-141 00 Prague*

[b]*Czech Geological Survey, Prague, Branch Brno, CZ-658 69 Brno*

Spectra of pure components were measured, artificially mixed and special libraries were built. Special algorithm was created to calculate the components content. Function of algorithm is described. Sample preparation methods and precision of the analysis are discussed. Examples of Kidney Stone Guide are given.

The diagnostic usefulness of information regarding the chemical composition of renal stones has been recognized since the 1950s and has been significantly improved during last years [1—13]. So it is now possible to correlate the results of every analysis with the appropriate diagnosis and therapeutic regime. Nowadays, current physical and chemical methods available for urinary stones analysis are applied. But, no method is sufficient to provide all the clinically useful information on the structure and composition of the stones [9].

A combination of refined morphological and structural examination of stone with optical microscopy [3], complemented by compositional analysis using infrared spectroscopy of the core, cross-section, and surface of calculi [14—19] provides a precise and reliable method for identifying the structure and crystalline composition and permits quantification of stone components while being highly cost-effective. Stone component may be mineral, organic, or both. More than 65 different species (including 25 ones of exogenous origin) have been found in urinary calculi.

Use of such morphoconstitutional studies leads to a classification of urinary stones in seven distinctive types and twenty-one subtypes [9] among calcium oxalate monohydrate (whewellite) and dihydrate (weddelite), phosphates, uric acid, urates, protein, and cystine (amino acids) calculi. The same chemical component may crystallize in different forms. Therefore a proper stone analysis has to identify not only the molecular species present in the calculus, but also the crystalline forms within chemical constituents. Most stones are of mixed composition and, among heterogeneous calculi, about 80 % are formed of a mixture of calcium oxalate and calcium phosphate in various proportions. By contrast, the presence of unique, but unusual compound (*e.g.* 2,8-dihydroxyadenine, xanthine, cystine, calcite) defines a specific type of urolithiasis. Quantitative evaluation of components is needed to

provide full information.

There are at least two approaches to the quantitative, or better, to the semiquantitative analysis of mixtures.

PLS techniques yield highly precise result when the composition of an unknown material with predictable components present is restricted to a reasonably well-defined range. This procedure is less well suited to this application, because the range of concentration is very wide, and an unpredictable number of components is present. This technique which requires purchase of a relatively expensive PLS Software (Nicolet TQ Analyst) would be more difficult to use and has some other disadvantages (artifacts cannot be identified).

Library Searching is the second possible method. Spectral library of real kidney stones must exist to use this method. An unknown sample spectrum is then compared to a number of library spectra and the most similar spectrum is found. The quality and quantity of the components of the most similar library spectrum is known. A match value close to 100 indicates that the sample consists of the same components in about the same ratio.

The aim of this work was to create an automated FTIR analyzer of kidney stones. The idea was to provide a qualitative and quantitative analysis in one step and connect the analysis result directly to the information about diagnosis and therapy for the kind of stone found.

EXPERIMENTAL

For 13 mm KBr pellet 0.1—0.5 mg of concrement sample and about 200 mg of dried potassium bromide (7758-02-3 KBr, Aldrich 22, 186-4 FT-IR grade) was used. The mixture was then homogenized for 2 min using a WIG-L-BUG grinding mill. A one-component stone sample was selected from the collection of human kidney stones (Motol Hospital, Prague). In a

few cases where no pure component stone was available, spectrum of minor component was subtracted. To minimize the influence of sample concentration and inhomogeneous distribution of sample particles in KBr pellet on linearity of Beer's calibration curve three independent pellets in the concentration range of 0.1—0.5 mg were prepared and measured, the spectra were appropriately weighted, baseline corrected, and the average was calculated. The KBr powder used for the pellets was dried in vacuum drier (4 h 200 °C) and stored in desiccating box over phosphorus pentoxide. The mixture was homogenized in a preheated capsule (50 °C). Spectrum of the pellet was collected immediately after preparation.

The spectra were collected on Nicolet 740 FTIR spectrometer (KBr beamsplitter, dTGS/KBr detector, resolution 4 cm^{-1}, 64 scans). When analyzing an unknown kidney stone sample, four independent samples were prepared – from the core, cross-section, surface of calculi, and a mixed sample from all parts. Stages of a stone growth can be studied in this way.

RESULTS

Creation of the Software

The Kidney Stone Library & Analysis Kit was created by spectroscopists and medical doctors to allow analysis of kidney stones using Nicolet FTIR spectrometers with OMNIC software. It consists of three parts: Kidney Stone Library – Basic (standard spectral library of about 800 spectra), Kidney Stone Analysis (advanced library of about 18000 spectra and special algorithm to work with it), and Kidney Stone Guide (additional information about kidney stone analysis).

The first step in building the software was to get the spectra of all possible kidney stone mixtures. This is theoretically possible, because the number of present components is limited and the mixtures build a finite set. However, the number of possible mixtures is too high to allow collection of real kidney stones in all the combinations. Fortunately the spectral contribution of each component is expected to be additive, so that we could take spectra of pure stones (only one-component) and artificially mix them building all theoretically possible two- and three-component mixtures. The mass fraction of the components in the mixtures ranged from 1 to 100 % with the step of 5 % for two-component mixtures and 10 % for three-component mixtures. Special software on MacroPro (Nicolet) basis was created for this purpose. More than three-component mixtures would increase number of spectra excessively. More than three components are rarely of clinical interest and this type of stones is rarely found in human kidney.

It was considered that not all components build mixtures in all possible ratios. Such combinations were

Table 1. The List of Pure Components and Library Coding

Frequent components	Rare components
0 Whewellite	13 2,8-Dihydroxyadenine
1 Weddellite	14 Hydroxylapatite
2 Cystine	15 Calcite
3 Xanthine	16 Aragonite
4 Protein	17 Gypsum
5 Dahllite	18 alpha-Quartz
6 Struvite	19 Tridymite
7 Brushite	20 N^4-Acetylsulfamethoxazole
8 Uric acid	21 Oxolinic acid
9 Uric acid dihydrate	22 Cholesterol
10 Ammonium urate	23 Whitlockite
11 Sodium urate monohydrate	24 Newberyite
12 Calcium phosphate amorphous	25 Potassium urate

excluded. Calculated spectra of mixtures were used for building two libraries to allow to provide kidney stone analysis on two levels – basic and advanced. A flexible library of about 800 most frequent mixture types was created (Kidney Stone Library – Basic). This library can be used as a standard spectral library (OMNIC – Search) to identify the major components of an unknown stone. Customer spectra can be added to this library. This library is easy to use, but the results are less precise.

Advanced library of about 18000 spectra, which includes related compounds and artifacts (like bread crust, egg shell, SiO$_2$) was also created. The library was coded to reduce the number of data and to speed up creating the library and sorting the spectra (Table 1). The advantage of this library is very high number of spectra which yields highly precise results. The disadvantage is that the spectra are very similar to each other and thus using classical SEARCH can yield match values very close to each other and results difficult to interpret.

Example of Search result **2 0 30 1 70** can be decoded as follows: **2** number of compounds, **0** code number of the first component (Whewellite), **30** mass fraction in % of the first component, **1** code number of the second component (Weddellite), **70** mass fraction in % of Weddellite. Three-component mixtures have an analogous coding.

Analyzing an Unknown Sample

To analyze the unknown kidney stone sample, OMNIC Search software can be used. The Kidney Stone Library – Basic (about 800 spectra) is recommended to be used in this case or an Advanced Library together with the decoding table is to be used. As the library spectra are very similar (Fig. 1), the match values are also very similar. In this case it is difficult to decide what result is correct. If the spectrum is slightly distorted, first hits include about the same content of major component (25 % or more) but different minor
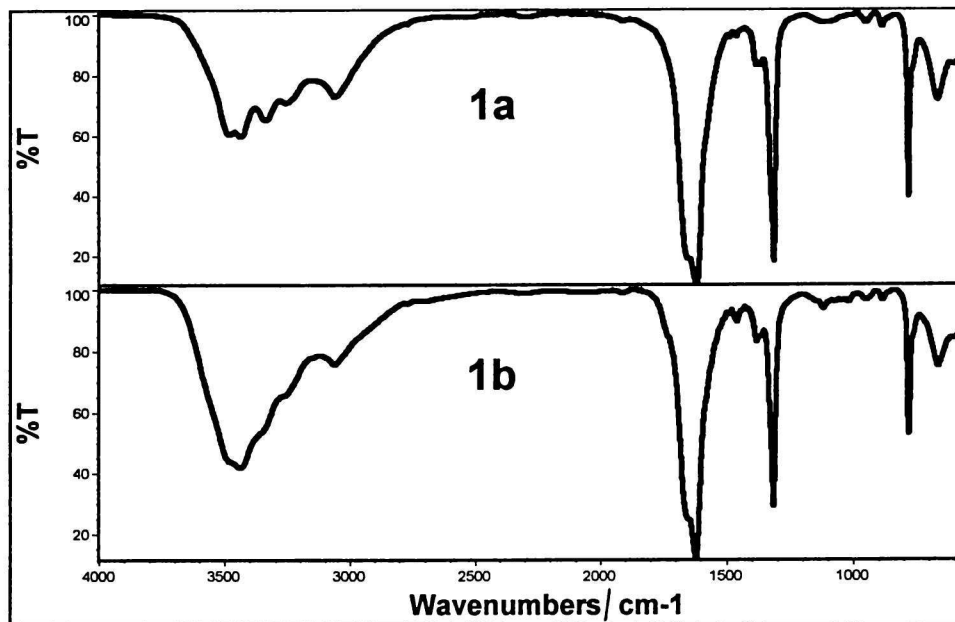
Fig. 1. Example of Kidney Stone Interpretation Guide Window: Whewellite 1a) pure chemical, 1b) real concrement.

component (about 10 %) which is not really present in the analyzed sample. As far as the minor component presence can be of crucial significance for the patient diagnosis in some cases (for infection stones like Struvite or Ammonium Urate), a more accurate result is required. Furthermore, a routine analyst expects an unambiguous result. For those reasons, we did not want to take the first hit as a correct result (although in most cases this would be sufficient) and created special algorithm.

This algorithm is a part of the Kidney Stone Analysis – Advanced software and can be activated through "Analyze" command. Correlation search is automatically selected, the analyzed spectrum is automatically baseline-corrected and checked for the highest absorbance value (an error comes if the absorbance is higher than 2.0). Then the software tries to find typical features of the spectrum. The spectrum is rejected if those features are not found. Library searching is done for the currently OMNIC active spectrum. From the first hits weighted using match values the average content of components is calculated. Using this algorithm, the unwanted minor component disappeared. The Match value of the first hit is the "reliability factor". A few other conditions were used. If Uric Acid together with Uric Acid Dihydrate are present, the result is expressed as Uric Acid content with the dihydrate content in brackets.

The software brings a message, if a rare or drug concrement or an artifact is found or a different message if similar spectrum is not found in the database. The calculated "Matrix content" is also part of the result. Matrix is a common designation for unknown organic compounds, which are always present in con-

Table 2. An Example of "Result Window" – Analyzing an Unknown Kidney Stone Sample

Advanced Analysis	
Spectrum title Sample No. 11204	
Collected Sep/16/1994, 14 : 29 : 22	
Component	mass fraction/%
Dahllite	53
Whewellite	33
Weddellite	13
Matrix (unknown matter, usually protein)	1
Reliability index 92 – Result is very reliable.	

crement samples. This is also one reason why the spectra of a real concrement differ from pure substances (Fig. 1a and b). Matrix content is usually about 5—15 % depending on the stone type. If matrix is found, the comment "Matrix (unknown matter, usually protein) is X % " appears (Table 2). Usually high matrix content (more than 20 %) signalizes that similar spectrum is probably not involved in the library.

DISCUSSION

The answer to the precision of the analysis is not simple, since achievable precision varies with the type of concrement, content of the components, baseline correction, and amount of impurities. If the content of a component is less than 10 %, the software will not detect this component. With the content about 10 % the result is not very reliable. The reproducibility of the result can also be influenced by the heterogeneity of the stone. We have optimized the described algorithm

Table 3. Characteristic Absorption Ranges for Functional Groups in Component Substances of Urinary Calculi. Stretching Vibration of Atom Group

Spectral range/cm^{-1}	Atom group
3100—3600	O—H group
3400—3600	Hydrate water
3200—3500	N—H group
3030—3300	NH_4^+ ion
2800—3100	C—H group
1600—1800	C=O group

on about 500 stones, where the components content was known from other nonspectroscopic methods.

In most cases (about 85 %) the accuracy was better than ± 5 %. According to literature [17] the error of ± (10—15 %) is not of clinical interest; thus, the accuracy seems to be sufficient. However, there were about 1—2 % of unsatisfying result which could influence the diagnosis. After that, the algorithm was slightly modified, so that no wrong results were present for the available set of spectra. Nevertheless, such a case cannot be excluded for another set of spectra, especially for complicated, more than three-component mixtures or for mixtures with a minor component of about 10 %.

For this reason, it is strongly recommended to provide a visual comparison of an unknown sample spectrum with the theoretically calculated spectrum, to use a pure components interpretation guide which is also part of the software and to study the morphological features of the sample (compared to pictures) or to use another independent reference method if the reliability factor is not very close to 100. The automated software speeds up the analysis but to get a reliable result in all cases we do not recommend exclusion of the human decision.

That is why additional information about the pure components is also available as a part of the discussed software Kidney Stone Guide. This information includes the interpreted infrared and Raman spectrum of a stone and pure chemical related, picture of the stone and methods of chemical analysis (quantitative, semiquantitative, qualitative), including the list of necessary agents and detailed description of the procedure. The software user will be informed about causes and occurrence of particular components and their optical properties. The list of characteristic IR absorption ranges (Table 3) and structural formula may be helpful for chemists interested in structural relationships.

Another aim of our work was to find the best sampling method – to decide between KBr pellet (better quantitative results but cations substitution can be generated by pressure) and diffuse reflectance (where pathlength is not defined but no cation exchange is expected). To compare both methods, the samples were collected in KBr pellets and by diffuse reflectance technique. As no substantial difference between transmission and reflectance spectra has been observed, it can be assumed that intramolecular interactions of polar groups with KBr in a KBr pellet are insignificant.

KBr pellet method can be the recommended method for kidney stone analysis. Diffuse reflectance might be used as a second method if KBr pellet technique is not available. Less precise quantitative results can be expected for this method. Therefore, this method was not used in this work.

REFERENCES

1. Schubert, G., *Z. Med. Labor. Diagn.* **22**, 33 (1981).
2. Uldall, A., *Scand. J. Clin. Lab. Invest.* **41**, 339 (1981).
3. Haber, M. H., *Farbatlas der mikroskopischen Harnanalysis.* Urban and Schwarzenberg, Baltimore, Wien, 1983.
4. Dubanský, A. and Kočvara, S., *Čas. lék. čes.* **124**, 326 (1985).
5. Louženský, G., *Čas. lék. čes.* **128**, 262 (1989).
6. Dubanský, A., Zich, M., Machovič, V., Pavlíková, H., and Linhartová, M., *Rozbory močových konkrementů.* (Analyses of Urinary Concrements.) IDVZ, Brno, 1994.
7. Křížek, V., *Cystinurie and Cystine Urolithiasis.* Státní zdravotnické nakladatelství (State Publishing House of Health), Prague, 1981.
8. Zich, M., *Urolithiasis,* Symposium of Hebrei University, Jerusalem, Israel, 1990.
9. Daudon, M., Bader, C. A., and Jungers, P., *Urinary Calculi: Review of Classification Methods and Correlations with Etiology 7 (3)*, 1081 (1993).
10. Kočvara, S., Dubanský, A., Přibyl, O., and Ždimera, A., *Rozhledy v chir.* **62**, 701 (1984).
11. Kočvara, S., Louženský, G., and Ptáček, V., *Čas. lék. čes.* **128**, 259 (1989).
12. Hesse, A., Claben, A., and Roehle, G., *Labordiagnostik bei Urolithiasis.* Stuttgart, 1989.
13. Bulková, T., Kladenský, J., Pacík, D., Štarha, M., and Linhartová, M., *Rozhledy v chir.* **71**, 120 (1992).
14. Daudon, M., Protat, M. F., Reveillaud, R. J., and Jaeschke-Boyer, H., *Kidney Int.* **23**, 842 (1983).
15. Hilgado, A., Carmona, P., Bellanato, J., Santos, M., Garcia Ramos, J. V., and Cifuentes Dellate, L., *Analisis de Calculos Urinarios por Espectroscopia Infrarroja y Raman.* C.S.I.C., Madrid, 1983.
16. Oka, T., Koide, T., and Sonoda, T., *J. Urol.* **134**, 813 (1985).
17. Hesse, A. and Sanders, G., *Atlas of Infrared Spectra for the Analysis of Urinary Concrements.* Stuttgart, 1988.
18. Linhartová, M., Bulková, T., and Vozníček, J., *Čas. lék. čes.* **130**, 233 (1991).
19. NICODOM, *Kidney Stone Analysis Kit* and *Kidney Stone Guide.* Prague, 1996.