Kidney Stone Analysis Using a Nicolet FT-IR Spectrometer

Abstract

The usefulness of FT-IR spectroscopy in the analysis of kidney stones is growing. In this note, spectra of pure components of kidney stones were measured, mixed and a set of spectral libraries were built. A special algorithm, created to calculate the content in the stone of various components, is described. Sample preparation methods and precision of the analysis are discussed, and examples of the use of the Kidney Stone Library & Analysis Kit are given.

Introduction

Mankind has always suffered from calculi in the efferent urinary tract. For example, urinary calculus was found in the pelvic area of a young man in a tomb dating back to 4800 B.C. near El Amrah, Egypt. However, it was not until the end of the 18th century that the first reports were published on the chemical composition of urinary calculi. At that time, important chemical constituents of urinary calculi were discovered, such as uric acid (Scheele 1776) and cystine (Wollaston 1810). After the systematic studies by Heller (1847) and Ultzmann (1882) characterization of urinary calculi by chemical analyses was, in principle, an established routine. The diagnostic usefulness of information regarding the chemical composition of renal stones has been recognized since the 1950s and has improved so that it is now possible to correlate the results of an analysis with the appropriate diagnosis and therapeutic regimen.

Methods of Analysis

No one method is sufficient to provide all the clinically useful information on the structure and composition of kidney stones. Methods which have been used include infrared spectroscopy, polarization microscopy, wet or dry chemical analysis, AAS, Roentgen – structural analysis, thermogravimetric analysis, porosity determination, pyrolysis gas chromatography, neutron activation analysis and solid phase NMR.

A combination of refined morphological and structural examination of kidney stones with optical microscopy, complemented by compositional analysis via infrared spectroscopy of the core, cross-section and surface of calculi, provides a precise and reliable method for identifying the structure and crystalline composition, and permits quantification of stone components while being highly cost effective.

Figure 1: Kidney stones

Composition of Kidney Stones

Stone components may be mineral, organic, or both. More than 65 different molecules (including 25 of exogenous origin) have been found in urinary calculi. Extensive analysis of morphology and composition has led to a classification of urinary stones in seven distinctive types and twenty-one subtypes, including monohydrate (whewellite) and dihydrate (weddelite) calcium oxalates, phosphates, uric acid, urates, protein, and cystine (amino acid) calculi. Confusing the matter further, the same chemical component may crystallize in different forms. Therefore, proper stone analysis has to identify not only the molecular species present in the calculus, but also the crystalline form.

Most stones are of mixed composition. About 80% are made of a mixture of calcium oxide (CaOx) and calcium phosphate (CaP) in various proportions. The presence of other compounds, like 2,8 dihydroxyadenine, xanthine, cystine, calcite, etc., places the stone into a specific type of urolithiasis. Quantitative evaluation of all components is needed to provide full diagnostic information.

Quantitative Analysis by Infrared Spectroscopy

There are at least two approaches to the quantitative or semiquantitative analysis of mixtures. Partial least squares (PLS) techniques will yield highly precise results if the composition of the unknown material is restricted to a reasonably well-defined range, with predictable components present. This procedure is not well suited to kidney stones, because the range of concentrations is wide, and an unpredictable number of components can be present. Modern PLS software (such as TQ Analyst™) can be used, but this results in greater complexity. Additionally, unmodeled artifacts cannot be identified using PLS software.

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Second, library searching or other comparative algorithms can be used. For this to work, a spectral library of real kidney stones must exist. An unknown sample spectrum is then compared to a number of library spectra and the best correlated spectrum is found. Both the constituents and their concentrations of the matched spectrum are known. A match value close to 100 indicates that the sample consists of the same components in about the same ratio.

Sample Preparation

Careful sample preparation is a key issue in kidney stone analysis. To prepare a 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 homogenized for 2 minutes using a WIG-L-BUG™ grinding mill. The one component stone samples were selected from a collection of human kidney stones (Motol Hospital, Prague). In a few cases where no pure component stone was available, spectra of minor components had to be subtracted.

To minimize the influence of sample concentration and the inhomogeneous distribution of sample particles in the KBr pellet on linearity of the calibration curve, three independent pellets in the concentration range of 0.1 - 0.5 mg concrement were produced and measured. The resulting spectra were appropriately weighted, baseline corrected and the average was calculated. The KBr pellet was free of moisture (transparent). The spectrum of the pellet was collected immediately after preparation. The spectra were collected on a Nicolet™ FT-IR spectrometer equipped with a KBr beamsplitter and dTGS detector (KBr window), using 64 scans at 4 cm^{-1} resolution.

For analysis of an unknown kidney stone sample, four independent samples were prepared – from the core, cross-section, and surface of calculi, and a mixed sample from all parts. Stages of stone growth can be studied this way.

The KBr pellet method is the recommended method for kidney stone analysis. Diffuse reflectance can be used as an alternate method if the KBr pellet technique is not available. However, less precise quantitative results can be expected for this method, and we have not tested this in the work reported here. The use of ATR and Thermo's advanced ATR correction algorithms are being studied. Infrared microscopy is a valuable method, because it combines optical microscopy and infrared spectroscopy. We have used this method when seeking pure component stones.

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 FT-IR spectrometers with OMNIC™ software. It consists of three parts: the basic kidney stone library containing approximately 800 spectra; the advanced library containing approximately 18,000 spectra and an algorithm to work with; and the kidney stone guide containing additional analysis information.

Figure 2: Main window of kidney stone analysis software

The aim of this work was to create an automated FT-IR analyzer for kidney stones, which provided a qualitative and quantitative analysis in one step, and then to connect the analysis result directly to information about diagnosis and therapy for the kind of stone found. After discussion with medical doctors, Thermo did not connect the results directly to the diagnosis text, because in diagnosis and therapy, factors other than stone composition are considered. However, this text remains a part of Kidney Stone Guide in a consultative role.

The first step in building the software was to obtain spectra of all possible kidney stone mixtures. This is theoretically possible, because the number of components is limited and the mixtures build a closed (semi-stoichiometric) set. Even so, the number of possible mixtures is too high to allow collection of real kidney stones in all combinations.

Fortunately, the spectral contribution of each component is strictly additive, so we could take spectra of single component stones and then mix them, building all theoretically possible two and three component mixtures. The concentration of the components in the mixtures ranges from 0 - 100% with the step of 5% for two component mixtures and 10% for three component mixtures. A software routine based on OMNIC Macros\Pro was created for this purpose. Consideration of mixtures with more than three components would increase the number of spectra excessively. Fortunately, stones with more than three components are rarely of clinical interest, and, more importantly, this type of stone is rarely found in humans. It is also known that not all components build mixtures in all possible ratios, so these combinations were excluded.

The calculated spectra of mixtures were used to build two libraries, for use in basic and advanced analyses.

First, a flexible, basic library of approximately 800 of the most frequent mixture types was created, which can be used as a standard spectral library in the OMNIC search routine to identify the major components of an unknown stone. Custom spectra from the end user can be added to this library. This library is easy to use, but not exhaustive.

The advanced library contains about 18,000 spectra, and includes related compounds and potential interferences (like bread crust, egg shell, SiO_2). The pure materials were coded, as shown in Table 1, which is referenced in the search routine for simplicity. This library contains a high number of similar spectra, so matching can result in high quantitative precision, but may also yield several similar match values when using OMNIC search directly, leading to ambiguous results.

NO.	COMPONENT	NO.	COMPONENT
0	Whewellite	13	2,8 -dihydroxyadenine
1	Weddellite	14	Hydroxylapatite
2	Cystine	15	Calcite
3	Xanthine	16	Aragonite
4	Proteine	17	Gypsum
5	Dahllite	18	alpha -Quartz
6	Struvite	19	Tridymite
7	Brushite	20	N4 -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 h	25	Potassium urate

Table 1: The list of pure components and library coding

Example search result: "2 0 30 1 70". This decodes as follows:

1. "2" = number of components
2. "0" = code for first component (0 is Whewellite)
3. "30" = percentage of first component
4. "1" = code for second component (1 is Weddellite)
5. "70" = percentage of second component.

Three component mixtures have the analogous coding.

Analyzing an Unknown Kidney Stone Sample

To analyze an unknown kidney stone sample, the OMNIC Search routine coupled with the basic library is recommended, as shown in Figure 3. The advanced library can be used, along with the decoding table. The results will then look as in Figure 4 (see Table 1 for decoding).
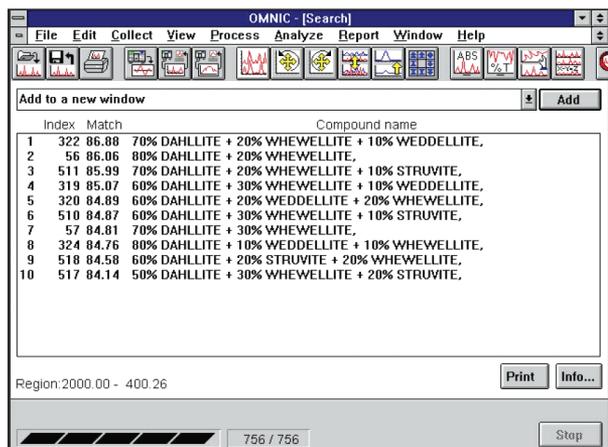
Figure 3: Searching in the basic library
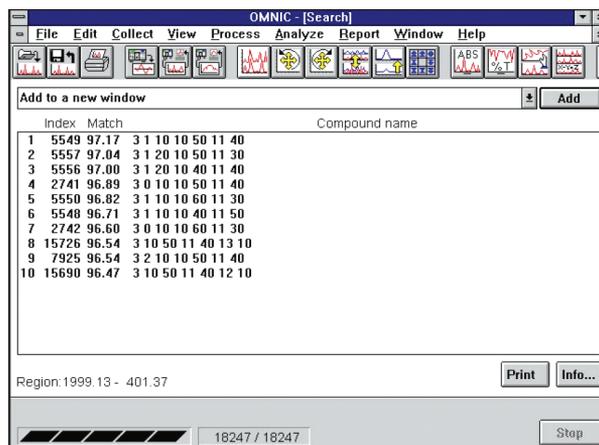
Figure 4: Searching in the advanced (coded) library

Both the library spectra and match values are similar, making it difficult to decide which result is correct. If the spectrum is slightly distorted, the list will include hits with about the same content of major component (25%) but different minor component (of about 10%) which are not really present in the analyzed sample. Since the presence of minor components can, in some cases, be of crucial significance to patient diagnosis (e.g. infectious stones, such as Struvite or Ammonium Urate), a more accurate result is required. Furthermore, routine analysis requires unambiguous results. The results should be accessible to non-spectroscopists, so they should be presented differently. For these reasons, we created a special algorithm and user interface.

This algorithm is part of the advanced kidney stone analysis library, and can be activated through the OMNIC "Analyze" command. Once "Analyze" is selected, the correlation search algorithm is selected. The unknown spectrum is automatically baseline corrected (if necessary), and checked for totally absorbing peaks (an error comes if the absorbance is higher than A = 2.0). Then the software searches for typical features of kidney stone spectra, rejecting the spectrum if these features are not found. A spectrum passing these conditions is then searched against the advanced library. An average concentration profile of the stone is calculated from the first hits, weighted using the match values. Using this algorithm, the unwanted minor components disappear. The match value of the first hit is the "reliability factor," essentially an indicator that the library describes the stone well. A few specific cases are noted, such as if both uric acid and uric acid dihydrate are present, the result is expressed as uric acid content with the estimated content of dihydrate in brackets.
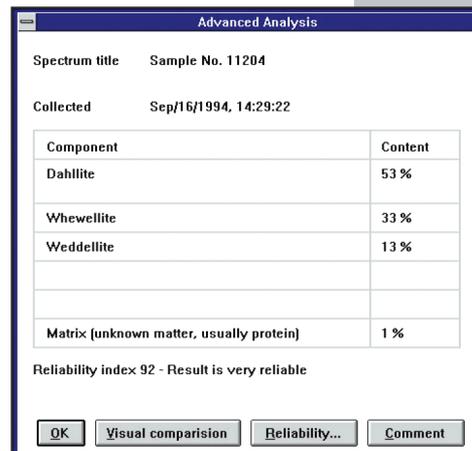
Figure 5: Result window – analyzing an unknown kidney stone sample

Error conditions are noted if either a rare or drug concrement, or an artifact is found, or if a low reliability index occurs (a similar spectrum was not found in the database).

The calculated "Matrix content" is reported. "Matrix" is a common designation for unknown organic compounds, which are always present in concrement samples. This is a major reason why the spectra of real concrements differ from pure substances or their sums. The matrix content is usually about 5 - 15%, depending on the stone type. If some matrix is identified, the comment "Matrix (unknown matter, usually protein) = X%" appears. Unusually high matrix content (more that 20%) is probably indicative that a similar spectrum is not contained in the library.

Precision of the Analysis

Any discussion of precision in this type of analysis is not simple, since achievable precision varies with type of concrement, percentages of components, baseline correction and the amount of impurities. If the content of a component is less than 10%, the software will not detect this component. If the content of a component is about 10%, the results are generally not considered diagnostically reliable. The reproducibility of the result can be also influenced by the inhomogeneity of the stone.

Thermo has optimized this algorithm using about 500 stones, where the concentration of all components was known from other methods. In most cases (about 85%) the accuracy was better than ±5%. According to the literature, an error of 10 to 15% is not of clinical interest, so the accuracy is sufficient.

Initially, about 2% of the samples gave poor results, sufficient to influence the diagnosis. The chemometrics were slightly modified to account for these samples, so that no wrong results were present for the available set of samples. Nevertheless, the occurrence of such irregularities cannot be excluded, especially for complicated mixtures with more than three components or with minor components around 10%.

Based on this experience, we recommend strongly that the user visually compare the unknown sample spectrum with the theoretically calculated spectrum. Furthermore, they should consider the pure components interpretation guide (included with the software) and study the morphological features of the sample compared to pictures. Use of an independent reference method is recommended if the reliability factor is not close to 100.

The Kidney Stone Library & Analysis Kit speeds up the analysis and can give excellent results, but a reliable analysis ultimately resides with the end user. This is why additional information is available in the Kidney Stone Guide. This information includes the interpreted infrared and Raman spectrum of a stone and related pure components, pictures of example stones, discussion of other methods of chemical analysis, discussion of the causes and occurrence of particular stones or components, optical properties, tables of characteristic peaks, structural formulas and other information. The guide provides information on the medical aspects of kidney stones, including diagnosis and therapy, however it is not intended to be definitive for making medical decisions.
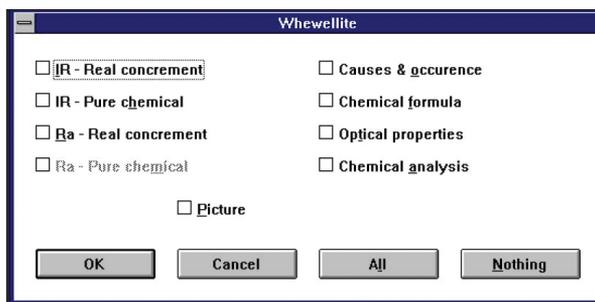
Figure 6: Example of a window with available information for each pure component
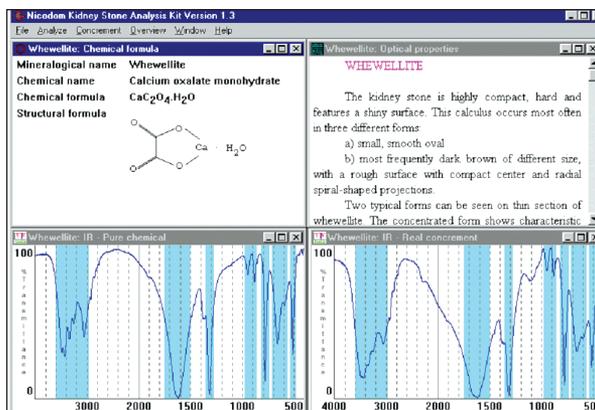
Figure 7: Example of kidney stone interpretation guide window
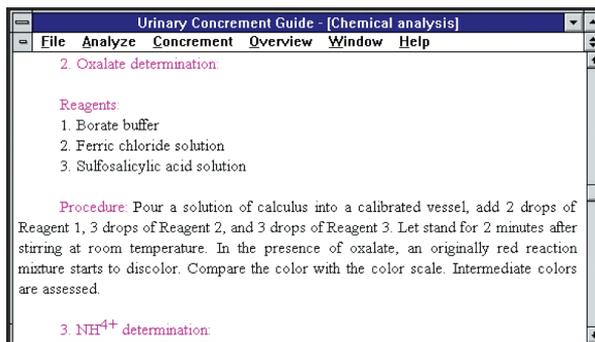
Figure 8: Example of methods of chemical analysis window
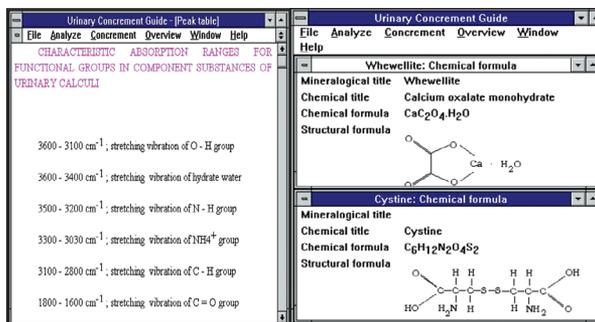
Figure 9: Example of characteristic peaks and structural formula windows

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