Kidney Stone Analysis by Nicolet FTIR spectrometer

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Abstract

The importance of kidney stone analysis by IR spectroscopy is growing. Spectra of pure components were measured, artificially mixed and spectral libraries were built. Special algorithm was created to calculate the components content. Function of this algorithm is described. Sample preparation methods and precision of the analysis are discussed. Examples of Kidney Stone Guide are given.

Introduction

Mankind has always suffered from calculi in the efferent urinary tract. For example, an urinary calculus was found in the pelvic area of a young man in a tomb near El Amrah (Egypt) dating back to 4800 BC. However, it was not until much later, at the end of the 18 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 /16/.

The diagnostic usefulness of information regarding the chemical composition of renal stones has been recognized since the 1950s and has significantly improved during last years, so it is now possible to correlate the results of every analysis with the appropriate diagnosis and therapeutic regimen.

Methods of Analysis

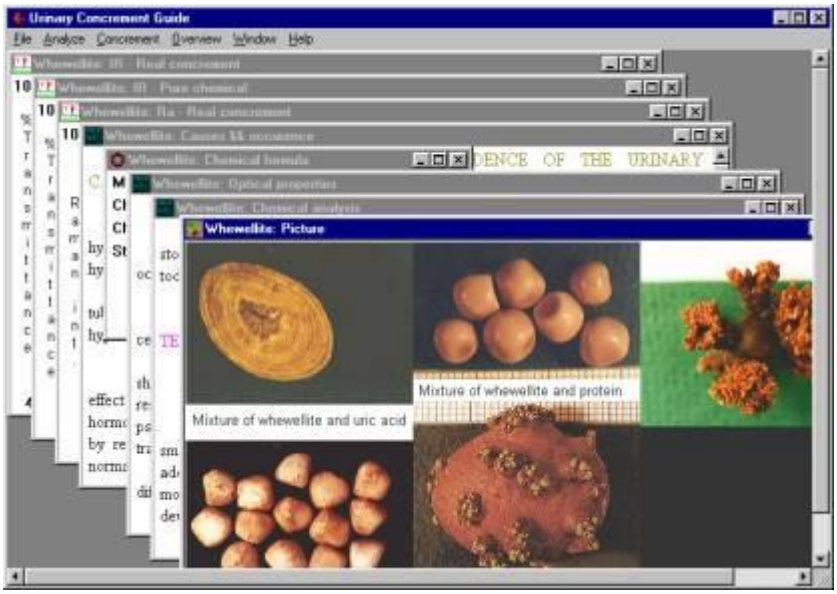
Current physical and chemical methods available for urinary stones analysis are critically reviewed. No one method is sufficient to provide all the clinically useful information on the structure and composition of the stones /7/.

Several methods can be used for this type of analysis. Infrared Spectroscopy, Polarization Microscopy, wet or dry chemical analysis, AAS, Roentgen - Structural Analysis, Thermogravimetric Analysis, Porosity Determination, Pyrolysis Gas Chromatography, Neutron Activation Analysis, Solid Phase NMR are here an example /28/.

A combination of refined morphological and structural examination of stone with optical microscopy, complemented by compositional analysis using 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.

Picture 1

Example of a Kidney Stone picture



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.

Using such morphoconstitutional studies leads to a classification of urinary stones in seven distinctive types and twenty-one subtypes among monohydrate (whewellite) and dihydrate (weddelite) calcium oxalates, phosphates, uric acid, urates, protein, and cystine (amino acids) calculi.

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 forms within chemical constituents.

Most stones are of mixed composition and, among heterogeneous calculi, about 80% are made of a mixture of CaOx and calcium phosphate (CaP) in various proportions. By contrast, the presence of unique, but unusual compound (e.g. 2,8 dihydroxyadenine, xanthine, cystine, calcite, etc.) defines a specific type of urolithiasis. Quantitative evaluation of components is needed to provide full information.

Quantitative Analysis by Infrared Spectroscopy

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

PLS techniques will yield high precision results if the composition of the unknown material is restricted to a reasonably well-defined range, with predictable components present /26/. This procedure is less well suited to this application, because the range of concentration is very wide, and an unpredictable number of components will be

present. This technique requires purchase of a relatively expensive PLS Software (e.g. TurboQuant), would be more difficult to use and has some other disadvantages (e.g. 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 than 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.

Sample Preparation

A careful sample preparation is a key issue in kidney stone analysis. For 13 mm KBr pellet a 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 2 minutes using WIG-L-BUG grinding mill. The one component stone sample was selected from a collection of human kidney stones (Motol Hospital, Prag). In a few cases where no pure component stone was available, spectrum of minor component had to be subtracted.

To minimize the influence of sample concentration and non homogenous 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 produced and measured, the spectra were appropriately weighted, baseline corrected and the average was calculated. The KBr pellet was free of moisture (transparent). Spectrum of the pellet was collected immediately after preparation.

The spectra were collected on Nicolet 740 spectrometer, KBr beamsplitter, dTGS/KBr detector, resolution 4 cm-1, 64 scans.

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

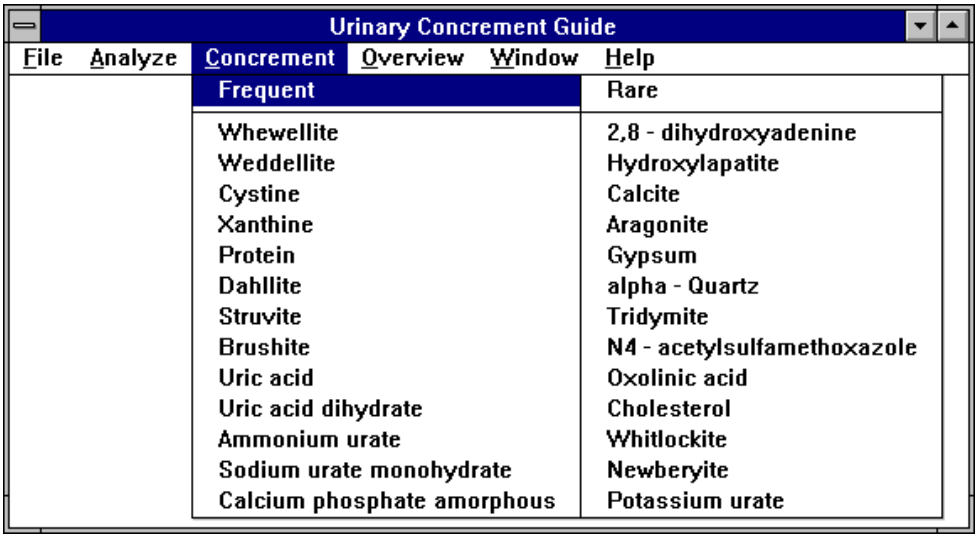
KBr pellet method is the recommended method for kidney stone analysis. Diffuse reflectance might be used as a second method if KBr pellet technique is not available. Less precise quantitative results can be expected for this method. We did not use this method in our work.

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

Picture 2

Main Window of Kidney Stone analysis 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 18.000 spectra and a special algorithm to work with it) and Kidney Stone Guide (additional information about kidney stone analysis).

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. But consulting that with medicals, we did not connect the results directly to the texts, because for diagnosis and therapy also other factors are important than only stone composition. Anyway, the texts remained a part of Kidney Stone Guide.

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 closed set. However 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 pure stones (only one component) and artificially 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. Special software on MacrosPro 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 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 18.000 spectra, which includes also related compounds and artifacts (like bread crust, egg shell, SiO2), was created. The library was coded to reduce the number of data and to speed up creating of the library and sorting of the spectra. The advantage of this library is a very high number of spectra which yields high precision 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.

Table 1

The list of pure components and library coding

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 amorphous"**	**25**	**"Potassium urate"**

Example of Search result **2 0 30 1 70**

1. number (2) - number of components
2. number (0) - code number of (Whewellite)
3. number (30) - percentage of first component
4. number (1) - code number of second component (Weddellite)
5. number (70) - percentage Weddellitu

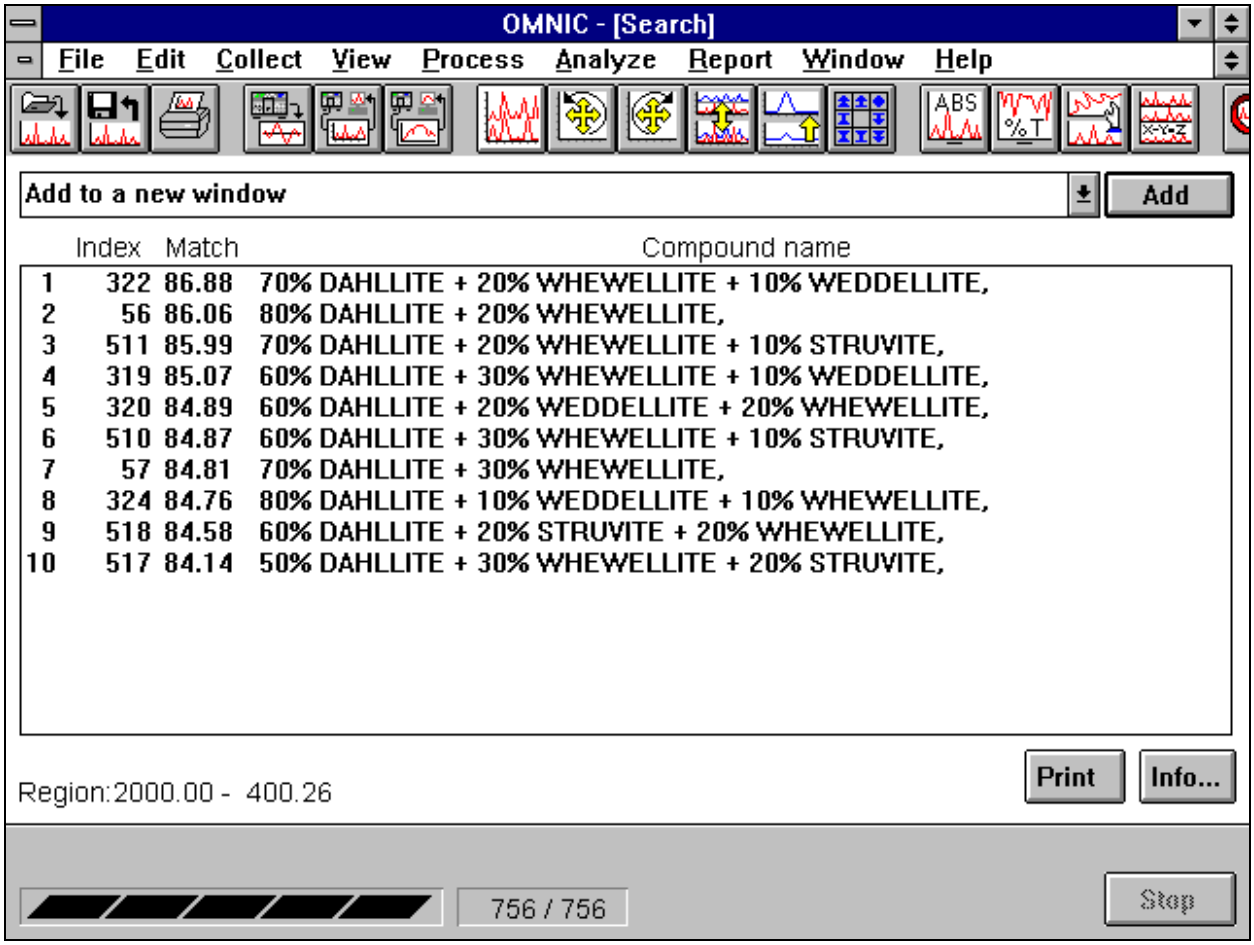
Three component mixtures have the analogous coding.

Analyzing an Unknown Kidney Stone 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 can be used. The results will then look as in Picture 4 (see also Table 1 for decoding):

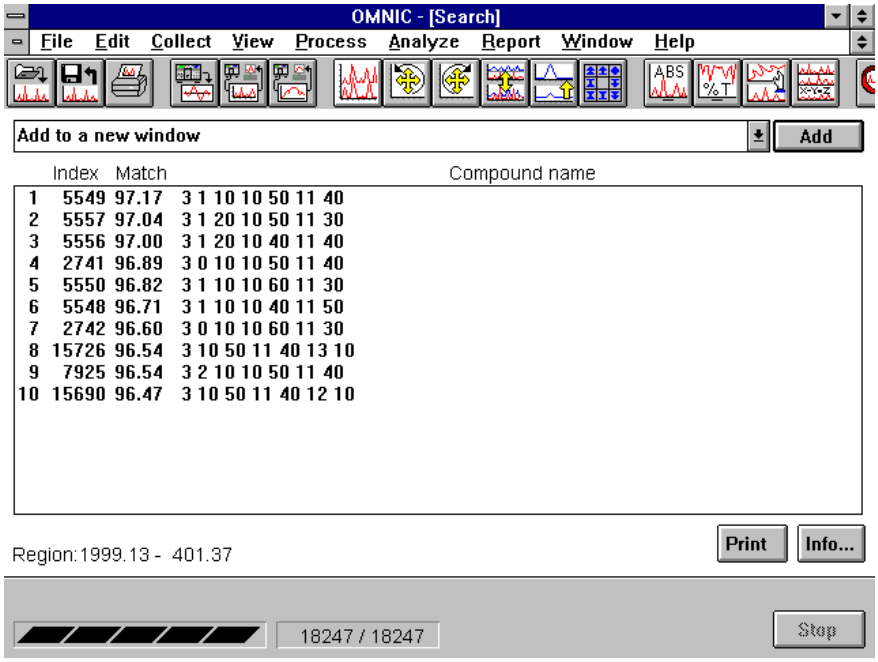
Picture 3

Searching in the Basic library



Picture 4

Searching in the Advanced (coded) library



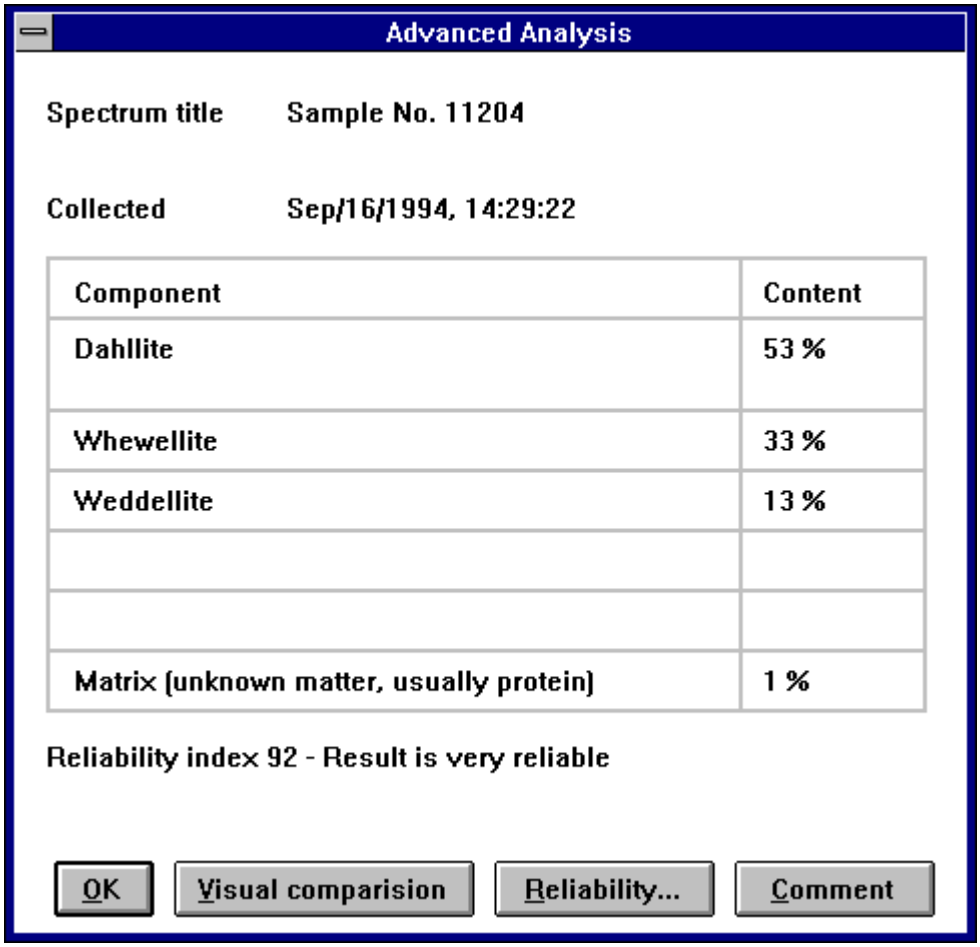
As the library spectra are very similar, the match values are also very similar. In this case it is difficult to decide what result is correct. If the spectrum is slightly distorted, first hits include about the same content of major component (25% or more) but different minor component (about 10%) which 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 (e.g. for infection stones like Struvite or Ammonium Urate), a more accurate result is required. Furthermore, the routine analyst requires result with unambiguous components content and different look of the result.

From 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 the "Analyze" command. With an "Analyze" command, correlation search is selected. The analyzed spectrum is automatically baseline corrected if necessary, checked for the highest absorbance value (an error comes if the absorbance is higher than A = 2.0). Then the software tries to find typical features of kidney stone spectrum, an error comes and 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, e.g. if Uric Acid together with Uric Acid Dihydrate are present, the result is expressed as Uric Acid content with the estimated content of its dihydrate in brackets.

Picture 5

Result window - Analyzing an unknown Kidney Stone sample



The software brings a message, if a rare or drug concrement or an artifact is found and a different message if similar spectrum was not found in the database.

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

Precision of the Analysis

Precision of the analysis is an often discussed question. The answer is not simple, since achievable precision varies with type of concrement, contents of the component, baseline correction and 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 not very reliable. The reproducibility of the result can be also influenced by the inhomogenity of the stone.

We have optimized the described algorithm on about 500 stones, where the components content was known from other non spectroscopic methods.

In most cases (about 85%) the accuracy was better than +- 5%. According to literature /16/ the error of +- 10-15% is not of clinical interest, so the accuracy seems to be sufficient.

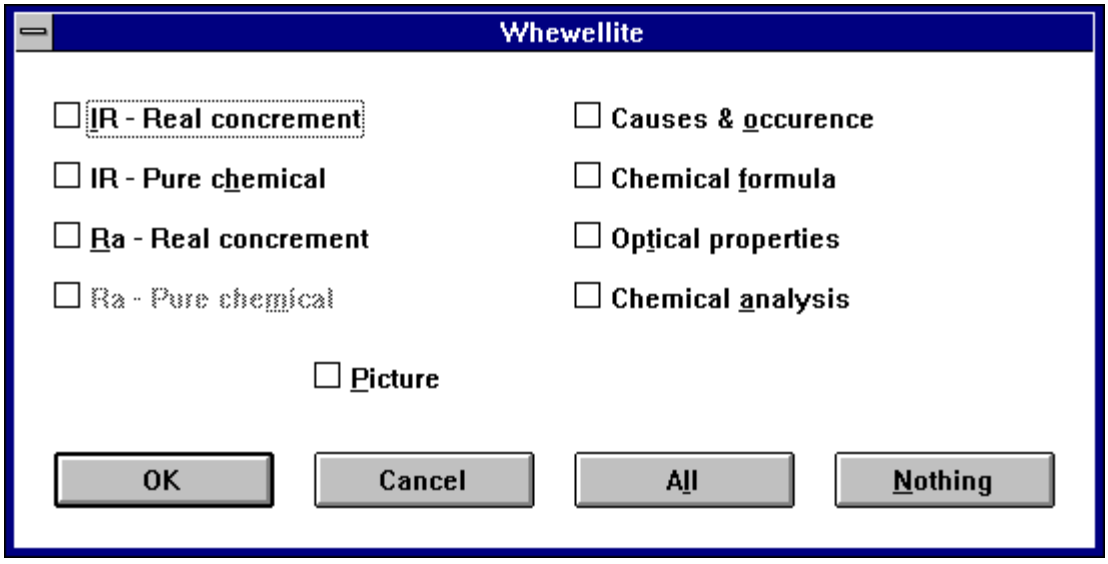
However, there were about 1-2% of unsatisfying results which could influence the diagnosis. After that, the algorithm was slightly modified, so that no wrong results were present for the available set of spectra. Nevertheless, such a case cannot be excluded for another set of spectra, especially for complicated more than three component mixtures or for mixtures with minor component content of about 10%.

From 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, other methods of chemical analysis (quantitative, semiquantitative, qualitative), causes and occurrence of the component, optical properties, table of characteristic peaks, structural formula and other information. This Guide also gives to the routine chemist a brief information about medical aspects of kidney stone analysis, like diagnosis and therapy. This brief information is not dedicated to medical doctors.

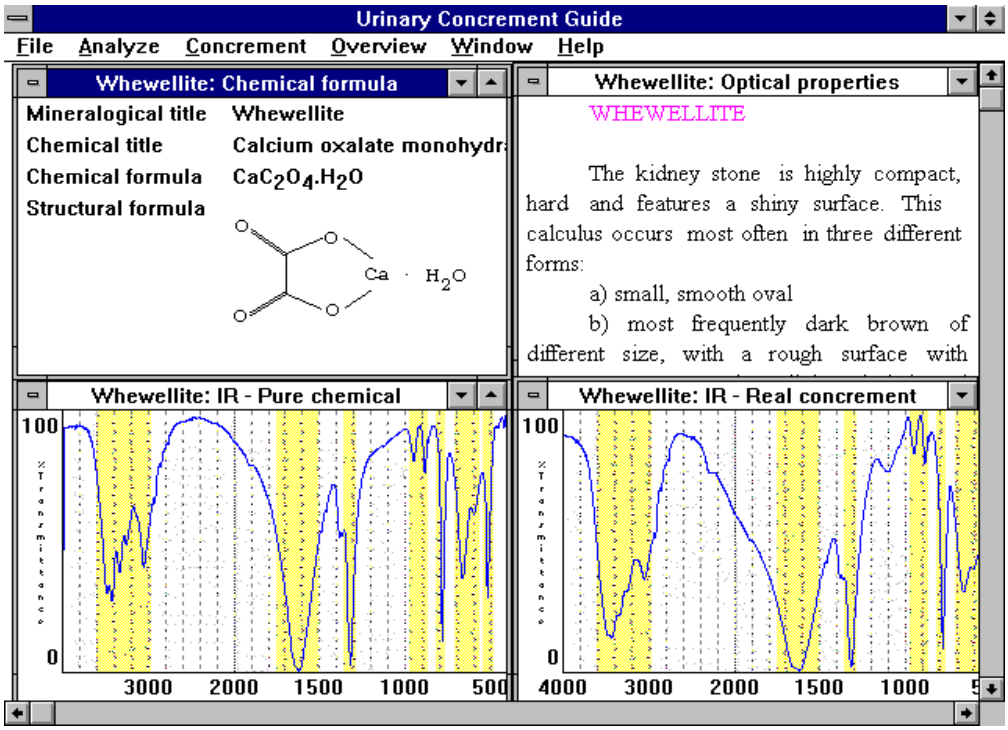
Picture 6

Example of a window with available information for each pure component



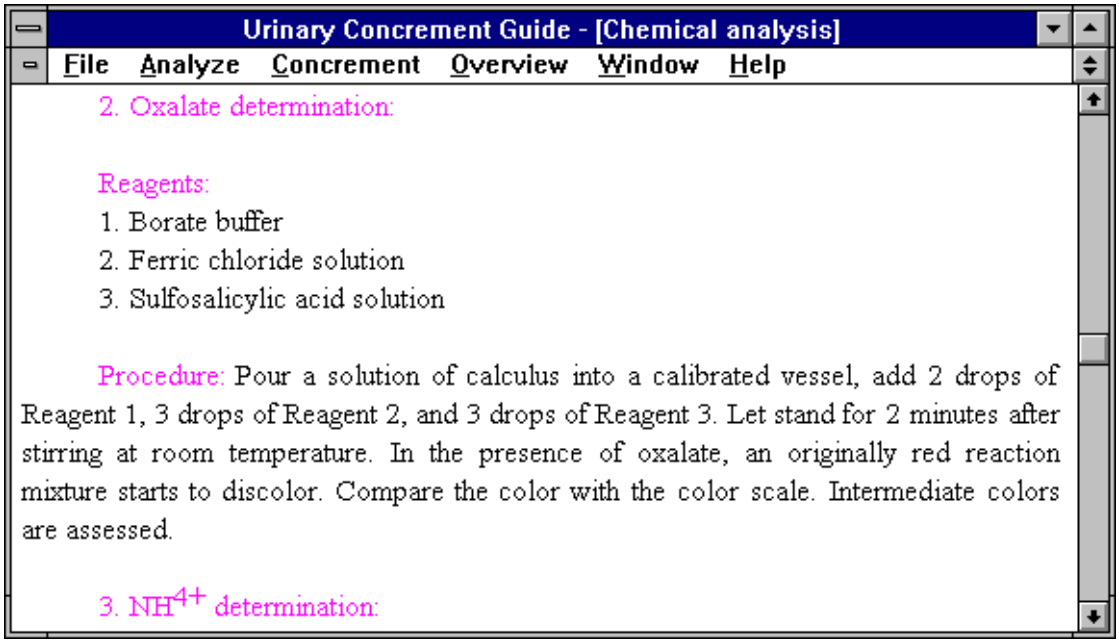
Picture 7

Example of Kidney Stone Interpretation Guide window



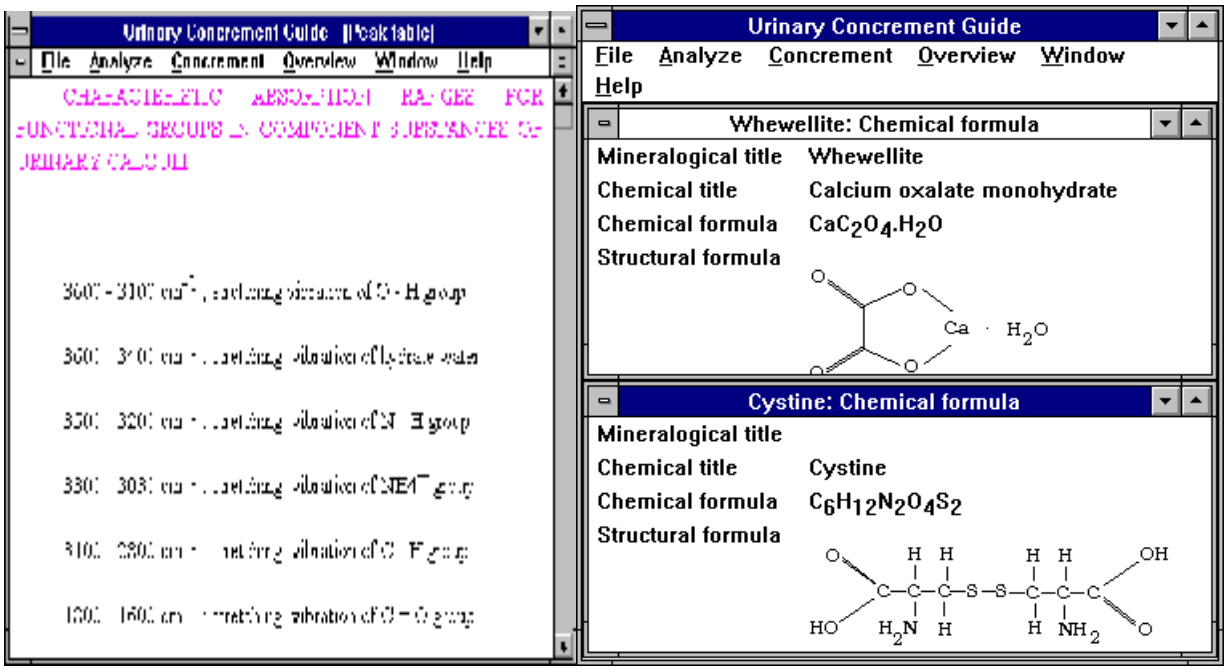
Picture 8

Example of Methods of Chemical Analysis window



Picture 9

Example of Characteristic peaks and Structural formula windows



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