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Infrared and Raman studies on renal stones: the use of second derivative infrared spectra

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Abstract Urolithiasis is a very frequent illness found in Argentina; however, stone analysis is not routinely performed. In this work, 86 renal calculi (from cities of Luján and San Antonio de Areco, State of Buenos Aires, Argentina) have been analyzed by infrared and Raman spectroscopies, and the results correlated with the main possible pathological conditions. Calcium oxalate monohydrate (whewellite) and mixtures of calcium oxalate monohydrate and carbonate apatite were the most frequent compounds found in our samples. In order to improve the identification of different phases present in calculi [particularly whewellite and weddellite (calcium oxalate dihydrate)], the second derivative spectra were used. The FTIR spectroscopy together with the second derivative analysis provides the urologist with a good method for calculi component determinations.

Keywords Infrared spectroscopy · Raman spectroscopy · Second derivative spectra · Weddellite · Whewellite · Calcium phosphate · Carbonate apatite · Uric acid · Calcium oxalate

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Introduction

Urinary calculi are the symptom of many health disorders, which lead to the formation of precipitated solids in the urinary tract. Knowledge of their composition is an important, and sometimes the only tool to identify disorder origin [1]. So, calculus composition is relevant information to have in order to determine and prevent recurrent urolithiasis and to choose appropriate treatments. Therefore, the analysis must be performed under reliable analytical methods [2–4].

Many different methods for the analysis of urinary stones have been reported [2–6]. Among them, X-ray diffraction and Fourier transforms infrared (FTIR) and Raman spectroscopies are now most widely used because they have many advantages over chemical analysis or other instrumental methods such as thermal analysis or scanning electron microscopy [5]. X-ray diffraction is used for the analysis of calculi since early 1940s [7–11]. According to several authors [12–14], X-ray diffraction technique is the most reliable method used for urinary analysis if constituent shows a crystalline phase. But it has its specific limitations, fails to detect amorphous phases, cannot distinguish between chemically different phases having identical lattice geometry (e.g. $CaHPO_4 \cdot 2H_2O$ and $CaSO_4 \cdot 2H_2O$) and may miss some phases (e.g. apatite and calcium urates) due to peak overlaps [5, 11].

For the above reasons, the use of IR spectroscopy increased from 8% in 1980 to 81% in 2005 [2, 4, 6, 15, 16]. A combination of spectroscopies techniques (Raman and FTIR) provides complementary information data about stone components, enabling more reliable and efficient

treatment of individual patients. Furthermore, these techniques allow not only rapid qualitative as well as semi quantitative determinations, but also analysis of different stone layers by slicing small amounts of sample [5, 15, 16].

However, in spite of the advantages of vibrational spectroscopies in the studies of renal stones, some difficulties are presented to discriminate between weddellite ($CaC_2O_4 \cdot 2H_2O$) and whewellite ($CaC_2O_4 \cdot H_2O$) when the calculi are composed of a mixtures of them. Solving these problems is important because whewellite and weddellite are the most common constituents of kidney stone. When one of them is a single component of calculi, this is a symptom of hyperoxaluria, but if a mixture of oxalates is present, an hypercalcuria should be considered as the origin of the renal lithiasis [17, 18]. Most of the chemical methods are unable to distinguish between whewellite and weddellite [5, 11].

The most complete analysis of renal calculi should be a combination of methods (perhaps the best should be spectroscopic and X-ray diffraction), but not all instruments are always available in a laboratory. However, as mentioned above, X-ray diffraction is not suited when the calculus is amorphous.

Derivative spectra (2DR) are a powerful analytical tool successfully used in many inorganic and organic systems and in some spectroscopies [19–21]. The application of the 2DR calculation yields negative bands whose peaks widths at half height are decreased by approximately a factor of three [22]. Therefore, the advantage of the second derivative spectra is essentially to enhance spectral resolution because its lineshape is intrinsically narrower than that of the absorption line. This enables the locations of peaks which are too closely spaced in the absorption spectrum to be resolved (shoulders or overlapped bands). Hence, two or more bands included in a broad one can be elucidated by this method. Furthermore, they also eliminate difference in background offsets and slope. Recently, we have used 2DR in the study of apatitic compounds [21]. To our knowledge, even though the second derivative spectra have been used widely for a long period of time, there was no report of their use in the study of renal calculi compositions.

Other different methods of stones analysis were developed for FTIR. These include the use of computerized libraries and the application of partial least-squares regression (PLS) and artificial neural network analysis (ANNs) [2, 23–25, 30, 33, 34]. Most of them were used for determining the qualitative and semi-quantitative composition using databases of infrared spectra of known constituents.

The aim of this work was to extend the analytical potentiality of vibrational spectroscopy to the study of renal calculi composition. For this purpose, we analyzed the composition of 86 calculi by FTIR and Raman

spectroscopy including the analysis of second derivative of infrared signals when it was required.

Samples studied in this paper were from patients of Luján and San Antonio de Areco cities (located in Buenos Aires province, Argentina), where urolithiasis incidence is unknown.

Materials and methods

Synthesis of calcium oxalate compounds

Calcium oxalates monohydrate and dihydrate were prepared as previously described [26] which were used as standards for infrared and Raman spectra. In the synthesis, all the solutions were made from reagent grade chemicals using double distilled water.

Synthesis of calcium oxalate monohydrate (whewellite)

Seven liters of distilled water was placed in a crystallizer and heated to 70°C. Solutions of $Na_2C_2O_4$ (7.5×10^{-3} M) and $CaCl_2$ (7.5×10^{-3} M) were dropped simultaneously at the same rate (250 ml/h). The slurry was filtered, and the crystals were washed with water and ethanol, and then dried at 50°C under vacuum for 36 h [26].

Synthesis of calcium oxalate dihydrate (weddellite)

Calcium ions solution (50 ml) was added dropwise to 200 ml of solution containing the other reagents [8.71×10^{-3} mol (M) $NaH_2PO_4 \cdot 2H_2O$, 1.22×10^{-3} M $Na_2HPO_4 \cdot 12H_2O$, 4.61×10^{-2} M NaCl, 7.82×10^{-4} M $Na_3C_6H_5O_7 \cdot 2H_2O$ (sodium citrate), 6.83×10^{-3} M Na_2SO_4, 1.42×10^{-3} M $MgSO_4 \cdot 7H_2O$, 1.73×10^{-2} M NH_4Cl, 3.2×10^{-2} M KCl, 3.73×10^{-4} M $Na_2C_2O_4$] and kept at 4°C. After 1 h, the slurry was filtered, and the crystals were washed and dried [26].

The prepared samples were characterized by infrared and X-ray diffraction. In the case of weddellite, the presence of small amount of phosphate was detected by infrared and XRD.

Samples characterization

The mineral composition of 86 renal stones was analyzed by infrared and Raman spectroscopies. All calculi were collected by variable surgical interventions throughout the urinary tracts of patients from La Plata and Lujan hospitals. Before the analysis, the stones were washed with distilled water, air dried and stored in a sterile container.

For IR absorption analysis, about 1 mg of the powdered sample was mixed with 300 mg of KBr (infrared grade).

The mixture was carefully milled in an agate mortar and pellized under vacuum. The FTIR spectra were recorded at 4 cm^{-1} resolution with a Bruker 66 spectrometer in the range 4,000–400 cm^{-1} (typically 100 scans were averaged to obtain a good signal–noise ratio).

Second derivative spectra were calculated based on the Stavitzky and Golay (S–G) method [27], which provides a combined approach to smoothing and derivatization. The absorption data (converted from transmittance spectra), of all samples studied, were treated with a 9-point S–G smooth [21]. The derivative algorithms were performed with the Opus (Bruker) software.

The Raman spectra (at room temperature) of these compounds were recorded using a Bruker IFS-66 (3,500–100 cm^{-1}, 4 cm^{-1} resolution) instrument provided with a Raman FRA-106 accessory and Nd: YAG laser (NIR range 1,064 nm).

For calculi with different appearance in several parts, it was necessary to record infrared and Raman spectra for each one.

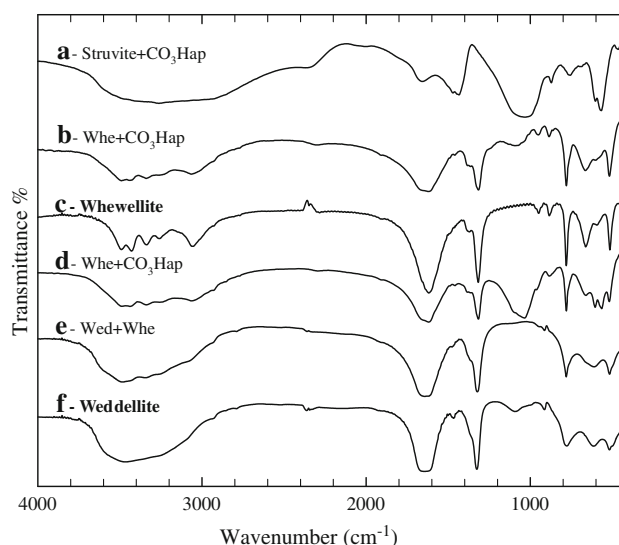## Mixture of whewellite and weddellite phases and derivative analysis

In order to validate the analysis of second derivative calculations applied to infrared spectra of calculi composed of mixtures of calcium oxalates phases, five samples were prepared using standard crystals in the following proportions (whewellite/weddellite): 1:4, 1:3, 1:1, 3:1 and 4:1 wt/wt. Second derivatives were applied to spectra of all the samples prepared.

Results

Figure 1 shows *selected* FTIR spectra of the 86 analyzed renal stones. The spectra of calcium oxalate monohydrate (**c** whewellite) and calcium oxalate dihydrate (**f** weddellite) were included for comparison because they are the main components in our samples. Whewellite and weddellite (see "Materials and methods" for preparations) were used as standard crystals. Wavenumbers and relative intensity bands for spectra **b**, **c**, **d** and **e** look very similar in some spectral regions.

The infrared spectra of pure weddellite and whewellite differ in the OH (3,600–3,000 cm^{-1}), CO stretching and fingerprint (below 1,000 cm^{-1}) regions. Infrared spectrum of whewellite shows OH stretching bands at 3,496, 3,429, 3,341, 3,269 and 3,065 cm^{-1}, while weddellite shows one broad band with two features at 3,467 and 3,266 cm^{-1}.

The C–O antisymmetric stretching of oxalate ions (B$_{2u}$ mode) appears in the region of about 1,600 cm^{-1} [28, 29]. This mode is observed in the infrared spectra at 1,644 cm^{-1}

Fig. 1 FTIR spectra of selected samples of renal stones (in all cases see "Results" and "Discussion"). **a** Struvite and carbonate apatite, **b** mixture of whewellite and carbonate apatite, **c** calcium oxalate (type whewellite), **d** mixture of whewellite and carbonate apatite, **e** mixtures of oxalates whewellite and weddellite, and **f** calcium oxalate (type weddellite)

for weddellite and at 1,619 cm^{-1} for whewellite. The infrared spectra of oxalates compounds show quite intense bands centered at about 1,300 cm^{-1}. These bands have been assigned to B$_{3u}$ OCO by stretching mode C$_{2v}$ symmetry species [29]. These bands were observed at 1,327 and 1,318 cm^{-1} for weddellite and whewellite, respectively.

In the region from 1,000 to 500 cm^{-1}, two (or one) weak bands appear at about 949 and 885 cm^{-1} attributed to a combination modes $v_s(CO) + \delta(O–C=O)$ [30]. The most intense band in this region is observed at about 780 cm^{-1}. This mode is assigned to $\delta(O–C=O) + v$ (M–O); the water librations appear at 660 cm^{-1}. Finally, a strong band at 516 cm^{-1} can be observed due to $v(M–O) + v(C–C)$ combinations modes [29].

According to the band description discussed above and from comparison of spectra of Fig. 1, it should be concluded that calcium oxalate monohydrate (whewellite) is the main component of calculi corresponding to spectra **b** and **d**. In the case of spectra **e**, preliminary analysis of band profile is apparently very similar to weddellite, but fine structure observed for the region 3,600–3,000 cm^{-1} (resemble the whewellite spectrum) require additional discussion using second derivative analysis (see below).

Up to now we are considering only mixtures of oxalates; however, if an additional band at about 1,050 cm^{-1} is observed (as in the spectra **b** and **d**), there is evidence that the calculi are composed of a mixture of oxalate and phosphate.

To elucidate among phosphates species (struvite, hydroxyapatite, carbonate apatite, whitocklite and other) is

very important for understanding the lithogenic process factors involved in stone formation. For example, crystallization of carbonate apatite does not require previous urinary infection, but in vitro physicochemical studies and clinical investigations have demonstrated that crystallization of these compounds together with struvite is favored under alkaline urine induced by urea-splitting bacteria [18]. In such case, the carbonate phosphate ratio of carbonate apatite is higher than that found in metabolic condition.

Differentiation between struvite [$Mg(NH_4)PO_4 \cdot 6H_2O$] and carbonate apatite is rather difficult due to the overlapping modes of $\delta(NH_4)$ and $v_3(CO_3)$ [15, 16, 21]. The spectrum of struvite shows a wide peak in the region about $1,000$ cm^{-1}, suggesting the presence of a mixture of phosphate phases. Nevertheless, the weak band $v_1(PO_4)$ may help for the assignment because this mode appears at 940 cm^{-1} for struvite and 960 cm^{-1} for carbonate apatite. The absence of absorption bands at 960 cm^{-1} and the low intensity band at about $1,400$ cm^{-1} are evidences that these calculi are composed mainly of struvite.

Therefore, for the reason discussed above, the calculi b and d (Fig. 1) are composed of a mixture of oxalate (type of whewellite) and carbonate apatite.

In the spectrum **a**, Fig. 1, many overlapped bands appear in the region of $3,400$–$2,800$ cm^{-1} due to $v(OH)$ of water and $v(NH)$ of ammonium. Bands at $1,600$ and $1,400$ cm^{-1} correspond to deformation modes of water and ammonium, respectively. The presence of all these bands and the absence of bands in $1,320$ cm^{-1} (C–O stretching of oxalate) evidence that the calculi is formed mainly by a struvite ($Mg(NH_4)PO_4 \cdot 6H_2O$).

The weak band observed at about 950 cm^{-1} was assigned to symmetric stretching modes of PO_4^{3-}. Finally, the asymmetric stretching vibration v_3 is expected in the region about $1,020$–$1,100$ cm^{-1} [21, 31]. The absence of absorption bands at $1,040$ and 960 cm^{-1} is considered evidence that these calculi are not a mixture of phosphate phases.

Raman spectra of the selected calculi presented in Fig. 1 are shown in Fig. 2. The composition of oxalate calculi obtained by infrared was confirmed by Raman spectroscopy based on the position of C–O stretching mode (v_1). This mode appears in infrared as a single band at $1,318$ cm^{-1} for calcium oxalate monohydrate (whewellite) and at $1,327$ cm^{-1} for calcium oxalate dihydrate (weddellite). The Raman spectrum (**c**) of calcium oxalate monohydrate (whewellite) shows a characteristic doublet due to the stretching vibration v_1 of C–O at about $1,465$ and $1,492$ cm^{-1}, while calcium oxalate dihydrate shows a single band at $1,476$ cm^{-1}. Therefore, whewellite and weddellite could be distinguished by simple inspection of the Raman spectrum [5, 15]. In consequence, it is clear that the spectra **b** and **d** (Fig. 2) correspond to a calculus type
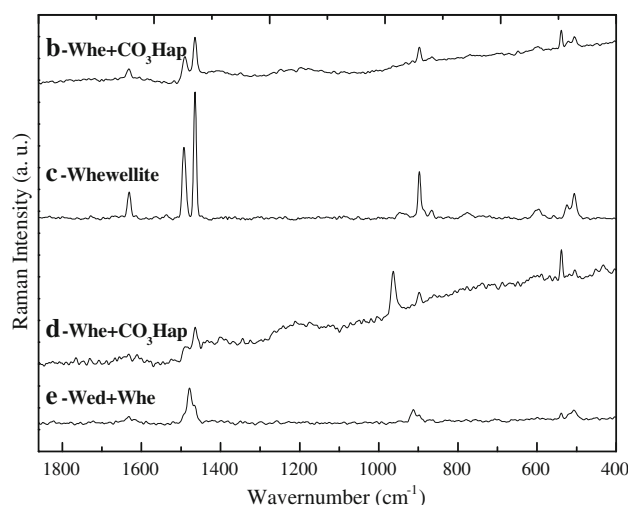
Fig. 2 FT-Raman spectra of some renal stones of Fig. 1: **b** calculi containing whewellite and carbonate apatite, **c** whewellite (standard), **d** calculi composed of whewellite and carbonate apatite, and **e** mixture of oxalates: whewellite and weddellite

whewellite. Moreover, the presence of bands at 965 cm^{-1} in spectrum **d** corresponds to the stretching vibration P–O (v_1), evidencing phase mixtures in this calculi. A band at 960 cm^{-1} in Raman and at $1,015$ cm^{-1} in infrared spectra (characteristic of carbonate apatite) and the absence of bands at 940 cm^{-1} (Raman) and $1,005$ cm^{-1} (infrared) evidence that this calculus is composed of a mixture of carbonate apatite and oxalate phase.

In the spectra **e** (Fig. 2), three features are observed at about $1,500$ cm^{-1}: one intense band at $1,478$ cm^{-1} and two shoulders at $1,465$ and $1,492$ cm^{-1} (very weak). This result suggested that the calculi should be composed of a mixture of oxalates. As discussed above, some assignments doubts arise when mixtures of oxalate phases are present in a sample.

In order to improve the analytical potentiality of spectroscopic techniques, the second derivative of infrared spectra was applied to selected renal calculi. This technique was successfully used before in proteins mixtures, polymer analysis and biomaterials characterization [19, 21, 22], between other, but was not previously used with analytical purpose in urinary stones.

The mathematical process of second derivative yields negative bands whose peak widths at half height decreases in one-third. This enables the location of peaks that are too closely spaced in the absorption spectrum to be resolved by the instrument. As shown in Fig. 3, the sharpened features in the spectrum facilitate the detection of a characteristic mixture of oxalate in the calculi on the basis of intensity of the bands.

To validate the application of second derivative analysis in the particular case of oxalate stones, the infrared spectra of whewellite and weddellite and mixtures of them in
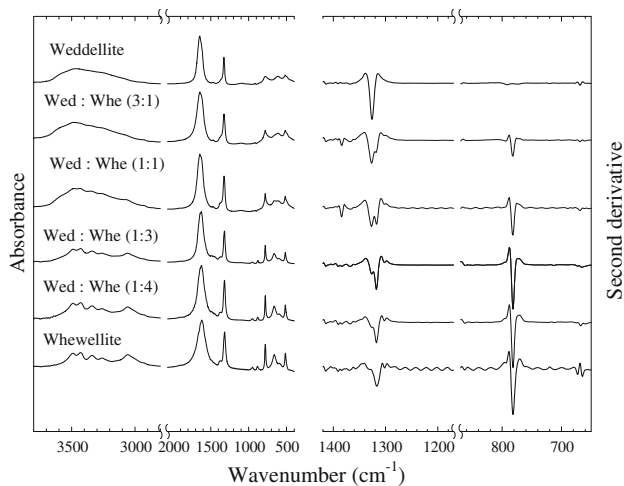
Fig. 3 Infrared spectra in absorbance of whewellite, weddellite and mixtures in known proportions (*left*). Second derivative of absorbance spectra are shown at *right*
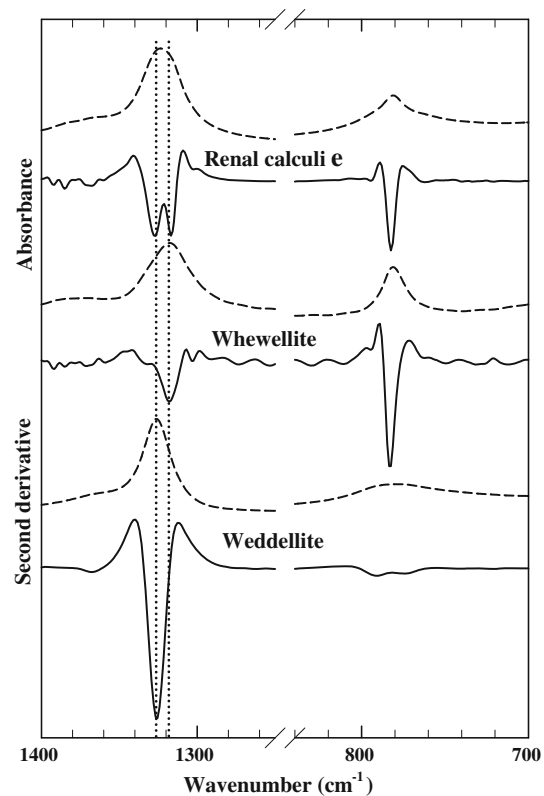

Fig. 4 Infrared spectra in absorbance (*dashed line*) of calculi **e** (Fig. 1), whewellite and weddellite and the corresponding second derivative spectra (*solid line*). *Dotted lines* indicate peak absorption bands of whewellite and weddellite at 1,318 and 1,327 cm^{-1}, respectively

convenient proportions (in weight) were recorded (see "Materials and methods"). The second derivative spectra were calculated with the method described above. Figure 3 compares the infrared (left) and the second derivative spectra (right) of standards and mixtures for a wavenumber regions. The infrared spectra of mixtures does not show a detectable shift of band at about 1,320 cm^{-1}, but the second derivate spectra distinguishes two bands for the mixtures (whewellite and weddellite) in intensity proportional to the corresponding concentration.

The region about 1,300 cm^{-1} is the adequate region to focus the treatment because the bands at 1,327 and 1,317 cm^{-1}, corresponding to OCO stretching modes, are quite narrow and intense in both standards. In contrast, bands at 3,600–3,000 are very wide and not very well resolved to be distinguished by the second derivative method (see Supplementary Figure 1).

Moreover, bands at 781, 663 and 517 cm^{-1} in whewellite spectrum are adequate for second derivative analysis, but in the case of weddellite these bands are very wide. Therefore, the second derivatives obtained are very weak to be distinguished from whewellite derivatives (see Fig. 4).

According to the experience obtained from the preparations of oxalates mixtures, minor component should be detected if the concentration is larger than 20% wt/wt. For lower concentration of any of the components, the signal:noise ratio should be comparable.

Figure 4 shows an application of the second derivative spectra to the specific case of urinary stone composed of oxalates mixture. Spectra **c** (whewellite), **f** (weddellite) and **e** Fig. 1 were depicted in this figure in absorbance mode, for selected regions: 1,400–1,200 and 900–700 cm^{-1}. The corresponding second derivative spectra were included in

this figure. Shape and bands positions for all absorbance spectra look very similar in the represented regions. The second derivative spectra of renal calculi, however, shows a doublet for the band centered at 1,320 cm^{-1} and a singlet for standards. This result reveals that the sample corresponding to spectra stone is composed of a mixture of oxalates of about similar concentration due to similar peak intensity. The double peaks centered at about 1,327 and 1,318 cm^{-1} correspond to the wavenumbers observed for weddellite and whewellite phases, respectively. This conclusion can be reached only by the application of second derivative calculations.

The results obtained here confirm that the second derivative analysis is very useful for renal stone compositions determination, especially in the case of oxalate mixtures.

After the analysis of 86 renal calculi studied by FTIR, Raman and second derivative of infrared spectra, the composition results obtained are classified into eight main groups collected in Table 1.

Whewellite is the most frequently found phase in our samples (almost 70%; see Table 1). Very often whewellite forms a single phase (36.1%) or makes mixtures with

Table 1 Stone composition with respect to gender and pathophysiologic conditions

Components	Percentage of stones	Percentage of males	Percentage of females	Pathophysiologic conditions
Weddellite $Ca(COO)_2 \cdot 2H_2O$	5.8	3.5	2.3	Hypercalciuria deficit of urinary crystallization inhibitors
Whewellite $Ca(COO)_2 \cdot H_2O$	36.1	19.8	16.3	Hyperoxaluria, in some case it could include hyperuricosuria
Mixture of oxalate (whewellite and weddellite)	9.3	5.8	3.5	Hypercalciuria and hyperoxaluria
Mixture of phosphate and oxalate (whewellite)	24.4	15.1	9.3	Hypercalciuria and hyperoxaluria
Carbonate apatite	2.3	2.3	–	Hypercalciuria
Uric acid	5.8	3.5	2.3	Hyperuricosuria, low urinary pH
Struvite	7	1	6	Infection with urease-producing organisms
Mixture of struvite and carbonate apatite	9.3	3.5	5.8	Infection with urease-producing organisms

Sex	Number of stones	Percentage	Ratio (M/F)
Sex-wise distribution of stone formers			
Male	48	55.8	4:3
Female	38	44.2	

carbonate apatite (24.4%). These groups were associated to hypercalciuria and hyperoxaluria. Note that struvite (7%) and mixture struvite and carbonate apatite (9.3%) were more frequent than weddellite (5.8%). It is interesting to note that carbonate apatite is the second phase most frequent found in our samples (35.0%). It is mainly present as mixtures of two phases: with whewellite 24.4% and struvite (9.3%) but only 2.3% as simple phase (Supplementary Figure 2 is especially clear).

Furthermore, it should be noted that weddellite (5.8%) and uric acid were found with low frequency in our samples in comparison with other statistics [32–34]. Particular interest is the second because uric acid is the result of metabolism of meat which is one of the most consumed foods in Argentina.

For additional information, the distribution of stones sort by gender is included at the end of Table 1. Data presented in Table 1 (correlations between columns 1 and 2, 3 and 4) are reliable, but correlations between column 1 and 5 are only tentative because without blood and urine analysis the pathologies cannot be identified as implied in this table. The relationship presented in these two columns is based on previous studies [17, 18].

Discussion

Each method described in the introduction for calculi analysis has some advantages for determination of urinary stone components, but none provides complete information about composition, especially in the case when calculi is integrated by a mixture of several compounds.

Nevertheless, in the case of mixture of oxalates compounds, many techniques, including infrared, are unable to detect phases when one of them is the mayor or similar constituent in the mixture. In this case, the second derivative method of infrared spectra can be applied with good success.

The second derivative method improve the results obtained by infrared and Raman spectroscopy, without the requirements of electronic spectra libraries and the application of partial least-squares (PLS) regression and artificial neural network analysis, which in principles are expensive and only available in specialized laboratories.

In the particular case of oxalate mixtures, the best region for the application of second derivative method is about $1,320 \text{ cm}^{-1}$. The *single* bands located at $1,327 \text{ cm}^{-1}$ for weddellite and $1,317 \text{ cm}^{-1}$ for whewellite are narrow and intense; therefore, the resulting second derivatives are strong, without considerable noise. The limitations of the method in the case of oxalate mixtures are reduced with respect to other substances because the region selected for the analysis is clean and does not show overlapping bands.

The presence of both oxalates can be demonstrated by the position of CO stretching band in the second derivative spectra.

A broad infrared absorption band at about $1,320 \text{ cm}^{-1}$ is resolved in a single component in the second derivative spectra when only one oxalate is present in the calculi. However, in the case of a mixture of oxalates, the broad

infrared absorption band is resolved into two components at about 1,318 cm^{-1} for whewellite and 1,327 cm^{-1} for weddellite.

The relative compositions of each oxalate should be estimated by the peak area of second derivative spectra. Therefore, the second derivative of absorption spectra should be considered a semi-quantitative identification method.

We analyzed 86 urinary stones from patients of Lujan and San Antonio de Areco cities (state of Buenos Aires, Argentina) using FTIR and FT-Raman spectroscopy, and second derivative calculations were used to resolve infrared spectrum of mixture of oxalate calculi (see Table 1). 51% of the analyzed stones (44 calculi) were composed of calcium oxalate (pure) or a mixture of oxalate and apatite (24.4%). These data compare favorably to those found in other countries [6, 32–34]. Furthermore, whewellite was the most frequent compound found in the analyzed samples, in agreement with the statistics found in Germany [32]. In contrast, low frequency was found for uric acid stones in our samples, in comparison with other results reported from Germany and Sudan [32, 33]. However, the distribution among males and females are quite similar.

Interestingly, ammonium urate was not found in the analyzed samples. The cases of renal lithiasis in children were not analyzed in this study, but we did not exclude the possibility to find these stones in Lujan and Areco cities.

It is interesting to note that struvite calculi (pure or in a mixture with apatite) in our samples were found more frequent (16.3%) than in Europe [32]. In agreement with the results reported in [33], struvite is more frequent in women than in men (see Supplementary Figure 3). This fact was explained by other authors due to the higher incidence of urinary tract infections in woman than in men.

In this paper, the renal stone compositions were identified using spectroscopic methods, but the process followed in this work does not distinguish if calcium oxalate monohydrate is formed directly or mediated the transformation of calcium oxalate dihydrate stones by urine. If this transformation is feasible, an overestimation of the percentage of whewellite calculi should be reported.

Finally, in the Table 1, we also correlated the calculi composition obtained from our vibrational studies with the specific pathological conditions. As mentioned before, these correlations were based on classification given in literature data, mainly from Refs. [17, 18].

Several authors have argued that renal lithiasis is a multifactored disease in which several factors are implied [1, 5, 17, 18, 21, 32–36]. Therefore, it is clear that not all people with hypercalciuria, hyperuricuria and others can induce renal calculi. It must also be considered for additional factors when a particular renal stone is generated.

Nevertheless, such alterations, combined with others, would generate a favorable situation for stone formation.

Conclusions

The combined FTIR and Raman spectroscopies are very useful for calculi characterization. However, in the case of oxalate mixtures combined spectroscopy methods do not show satisfactory results for compounds identifications. In this case, the second derivative of infrared spectra should be used.

Therefore, a combination of infrared and second derivative allows to the urologist detailed information about stone components, without the requirements of an additional instrument like Raman spectrometer, reducing analytical charge. Consequently, based on a correct analysis, the urologist can correlate the specific etiological conditions for a better diagnosis.

The second derivative methods must be considered as an additional tool in some cases for which infrared or Raman spectra are not easy to interpret. But in comparison with other methods described in "Introduction", the application of the second derivative method is seemingly straightforward and does not require the use of expensive spectra libraries as well as the complicated regression analysis. Therefore, diffusion of the application of second derivative method should be useful, especially in the case of professional non-specialized in spectroscopy.

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